

I. Purpose:

Proper specimen collection is vitally important to the success of all cultures. Guidelines regarding timing and the steps to minimize contamination must be followed closely. Rapid detection and recovery of organisms is critical to the management of the septic patient.

CAUTION: All specimen material should be considered potentially hazardous and thereby handled according to practices of Universal Precautions. Use of proper personal protective equipment (i.e. Lab coats, gloves) must be used for procedures in which exposure to blood or other potentially infectious material is reasonably anticipated to occur.

II. Responsibility:

All healthcare workers collecting cultures.

III. Procedure:

Patient Identification

All specimens must be labeled at the patients' bedside after proper patient identification. Each specimen must contain the zebra label or patient's addressograph label, date and time of collection, collector's initials and the source of the specimen.

Urine Specimens – clean catch (See dirty urines for Chlamydia/GC DNA testing)

1. Instruct patient to wash hands well with soap and water.
2. Tell the patient not to touch the inside of the cup, cap, or straw on the inside of the cap. Take the cap off of the cup and place it on the counter with the straw facing upward.
3. Have the patient cleanse themselves with the enclosed towelette then collect the urine specimen.
4. **Female**
 - a) Sit comfortable on the toilet seat with your knees as far apart as possible.
 - b) Spread labia with one hand and wipe inner fold from front to back. Discard towelette and repeat with second and third.
 - c) Keeping labia separated, begin to pass the urine into the toilet.
 - d) Without stopping flow, collect urine into cup.
 - e) Finish voiding into toilet
 - f) Replace cap tightly on cup, making sure not to touch inside of rim of cup.

Male

- a) Wipe head of penis in a single motion with first towelette.
- b) Repeat with second towelette. If not circumcised, hold foreskin back before cleansing.
- c) Start urinating into toilet.
- d) Without stopping flow, collect urine into cup.
- e) Finish voiding into toilet

- f) Replace cap tightly on cup, making sure not to touch inside of rim of cup.
5. Label specimen according to protocol. Indicate type of specimen.
6. To place urine into appropriate transport tubes.
 - a) Peel back the label on top of urine collection cup to expose the integral sampling device.
 - b) Place the small gray vacuum tube in the holder portion with the stopper down
 - c) Push the tube down to pierce the stopper. Hold in position until the urine stops flowing into the tube. Remove the tube from the device and shake vigorously to mix contents.
 - d) Repeat with large cherry red/yellow marbled vacuum tube for routine urinalysis.
7. Label tube(s) with:
 - a) Patient's name
 - b) Identification number
 - c) Date and time of collection
 - d) Collector's initials
 - e) Indicate specimen is "clean catch" urine
8. Discard lid into sharps container. Flush remaining contents of cup into toilet then place the cup into container for contaminated specimens.
9. Place urine tube(s) into a biohazard transport bag and transport to laboratory.

Urine from Foley Catheter

1. Wash hands and put on gloves.
2. Never collect specimen from the collection bag!
3. Pinch tubing below aspiration port, allow urine to collect in the aspiration port area.
4. Clean aspiration port with alcohol pad.
5. Insert needle into port and withdraw 5ml for culture and 10ml for urinalysis.
6. Transfer the urine sample to the small gray (boric acid) vacuum tube for the culture and to the large cherry red/yellow marble vacuum tube for the urinalysis; make sure to use a transfer safety device.
7. Label specimen according to protocol. Indicate type of specimen and source. i.e. Foley

Stool Specimens

- 1 Collect the stool specimen in a clean bedpan or other clean, dry large mouthed container. It is preferable to use the outpatient stool collection kit. Do not pass the stool specimen into the toilet.
 - a) Do not allow urine to be passed into the collection container.
- 2 Transfer the specimen, especially areas that are bloody, watery, or mucous-like, into the appropriate transport device(s) for the tests ordered.
 - a) Use a separate transport device for each test. Do not pour any of the contents (preservative) out of the collection device.
 - b) The stool transport devices contain a spoon in the lid to pick up the stool specimen and place it into the container. Pick up small pieces of the stool at a time.
 - c) Place spoonfuls into the transport device, mixing the stool with the preservative.
 - d) Fill the container to the **red line**.
 - e) Do not overfill the container because inadequate preservation of the specimen will affect the result.

- f) Tightly recap the container.
 - g) Thoroughly shake the vials containing liquid preservative to complete mixing process.
 - h) Label specimen according to protocol. Indicate type of specimen.
3. Transport the devices to the laboratory as soon as possible. The orange and the white-capped vials must arrive in the lab within 24 hours of collection. The grey and pink capped vials are stable for several days at room temperature.
 4. Stools for routine bacterial cultures: No more than 2 specimens/patient without consultation, culture not acceptable on inpatients after the third hospital day without consultation.
 5. Stool testing for *Clostridium difficile* is not recommended unless the patient has been hospitalized for 3 days or more **OR** if patient has a history of previous antibiotic exposure.

Appropriate Containers for Stool Tests

Tests	Container & Description	Storage
Ova & Parasite (OP) (both vials required)	Para Pak ZN-PVA & Grey cap Para Pak 10% Buffered Pink cap Neutral Formalin	Room Temperature
Giardia & Cryptosporidium Antigens (GIACRYAG)	Para Pak 10% Buffered Pink cap Neutral Formalin	Room Temperature
Stool Culture + Shiga toxin (STCP)	Para Pak C&S Orange Cap	Room Temperature
Clostridium difficile DNA Amplification (CDDNA)	Para Pak clean Vial White Cap (or sterile container) ** Liquid Stool or specimen that takes the shape of the collection container.	Room Temperature -good for 24 hr Refrigerated 2-8C- good for 5 days
Fecal WBC (SWBC)	Para Pak clean Vial White Cap (or sterile container)	Room Temperature
Rotavirus (ROTOVIR)	Para Pak clean Vial White Cap (or sterile container)	Room Temperature

Respiratory Specimens

Sputum – Non AFB cultures

1. Remove dentures or other obstructions from mouth.
2. Have the patient rinse mouth or gargle with water prior to specimen collection.
3. Instruct the patient to cough deeply.
4. Remove the cap of the sterile cup being careful not to touch the inside of the container or lid.
5. Ask the patient to cough up the material produced and place into the container.
6. The patient should **not** spit into the container.
7. Recap tightly, Label specimen according to protocol. Indicate type of specimen.

Sputum –AFB cultures – Recommend collection times three, 24 hours apart, early morning.

1. Remove dentures or other obstructions from mouth.
2. Do not rinse mouth.
3. Instruct the patient to cough deeply.
4. Remove the cap of the sterile cup being careful not to touch the inside of the container or lid.

5. Ask the patient to cough up the material produced and place into the container.
6. The patient should **not** spit into the container.
7. Recap tightly, Label specimen according to protocol. Indicate type of specimen.
8. Place specimen in a sealed biohazard transport bag.
9. Send to Lab immediately, refrigerate at 2-8°C if delayed.

Throat – For Streptococcus Group A – order Strep A screen or Strep A DNA probe.

For override, physician should call lab and lab will order a THC (throat culture in Horizon). Please specify organism the physician is looking for.

1. Peel open the package containing the swabs and transport media being careful not to contaminate the swabs. Snap off and discard the cap on the tube transport.
2. Have patient tilt head back, breathe deeply, open mouth wide and say "Ah". This serves to lift the uvula and aids in reducing the gag reflex.
3. Use tongue depressor to gently depress the tongue and look for areas of inflammation (redness) and exudate (pus).
4. Pull the red capped -2 swabs out of the package.
5. Guide the swabs over the tongue into the posterior pharynx (back of throat).
***Every effort should be made to avoid touching the swabs to the tongue, teeth, and roof of the mouth or the inside of the cheeks.**
6. Carefully but **firmly rub** the swabs over several areas of pus or inflammation, over the tonsils (or tonsillar crypts if tonsils have been removed), and over the posterior pharynx.
7. Remove swabs carefully from the mouth, again avoid touching the swabs to the tongue, teeth, roof of the mouth or the inside of the cheeks.
8. Insert swabs completely into the tube transport.
9. Label specimen according to protocol. Indicate type of specimen.

Nasal Wash

1. Draw approximately 2mL of the sterile phosphate buffered saline into bulb syringe. (Use smaller volume for neonate)
2. Insert the bulb syringe into the nostril and expel the saline.
3. Immediately expand the bulb to collect the wash.
 - a. RSV Antigen detection only: Expel collected nasal wash specimen into the sterile cup or vial.
 - b. Viral culture only: Expel collected nasal wash specimen into the viral transport medium vial, 1 ml of fluid minimum.

Nasopharyngeal Swab

1. Remove excess secretions or exudates from the anterior nares.
2. Use flexible fine-shafted swab to collect specimen. (recommend flocced swabs)
3. The wire swab can be bent to pass through the nasal passage and down to the nasopharynx. Work carefully but quickly to minimize discomfort, avoid touching swab to proximal portion of nasal passage or throat.
4. Rotate swab gently a few times.
5. Remove the swab being careful not to touch the interior surface of the nares.
6. Place wire swab into transport tube.

Nasal Aspirate Procedure

1. Use mucous collection device to collect specimen.
2. Insert appropriate size catheter nasally into posterior nasopharynx.
3. Apply suction, using intermittent suction as catheter is withdrawn.
4. Wash aspirate through tubing with 5-8 mL of sterile phosphate buffered saline.
5. Transfer material from tray to sterile container.

Wounds and aspirates

Tissue or aspirated fluids are superior to swab specimens. Swabs are inferior specimens and are discouraged.

Tissue or biopsy material - Submit as much tissue as possible.

1. Clean the surface of the site to be sampled with soap and water, rubbing alcohol, surgical scrub solution.
2. Collect the tissue according to surgical protocols.
3. Place the sample obtained into sterile container with enough sterile saline to keep moist, or into a Port-A-Cul anaerobic jar.

**** Anything other than culture on tissue or biopsy contact your site Pathology Laboratory for transport requirements.**

Closed wound or abscess

1. Clean the surface of the site to be sampled with soap and water, rubbing alcohol, or surgical scrub solution.
2. Aspirate abscess wall material with needle and syringe.
3. Aseptically transfer all material into Port-A-Cul anaerobic vial or to sterile plain top fluid tube. Do not send syringe.

Open wound

1. Clean the surface of the site to be sampled with soap and water, rubbing alcohol, or surgical scrub solution.
2. Aspirate material with needle and syringe, if possible, or pass swab deep into the base of the lesion.
3. Aseptically transfer all material into Port-A-Cul anaerobic vial or to sterile plain top fluid tube. Do not send syringe.

Surface lesion

1. Clean the surface of the site to be sampled with soap and water, rubbing alcohol, or surgical scrub solution.
2. Open lesion and sample the advancing edge by aspiration or using swabs, firmly rub over the leading edge expressing any purulent exudate onto aerobic culture swabs. Surface lesion samples are unsuitable for anaerobic culture.

Drainage

1. Send drainage material in a sterile tube or specimen cup.
2. Be sure to secure the lid tightly.

Genital Specimens for Chlamydia trachomatis and Neisseria gonorrhoeae DNA:

Endocervical/ Urethral swab specimens- Females

1. Call laboratory at 585-LABS for appropriate collection kit.
2. Collect and transport endocervical or urethral swab specimens in **3 mL M4RT Remel Culture Transport Media**.
3. Use only dacron, rayon, or swabs included in the collection kit with plastic or non-aluminum wire shafts. Do NOT use collection swabs with wooden or aluminum shafts.
4. First remove excess mucus, blood, and examination lubricants with the large sterile swab.
5. Then use the small sterile swab to collect specimen using recommended methods to sample columnar and squamo-columnar cells.
6. Insert swab into M4RT Remel Culture Transport Media tube, break shaft and close securely.
7. Label specimen with patient identification information, date, time, and initials of the person who collected the specimen.
8. Transport specimen at room temperature to the laboratory as soon as possible. The pneumatic tube system may be used for transportation of specimens.

Urethral swab specimens- Males

1. Call laboratory at 585-LABS for appropriate collection kit.
2. Collect and transport urethral swab specimens in **3 mL M4RT Remel Culture Transport Media**.
3. Use only dacron, rayon, or swabs included in the collection kit with plastic or non-aluminum wire shafts. Do NOT use collection swabs with wooden or aluminum shafts.
4. Use the small sterile swab to collect specimen using recommended methods.
5. Insert swab into M4RT Remel Culture Transport Media tube, break shaft and close securely.
6. Label specimen with patient identification information, date, time, and initials of the person who collected the specimen.
7. Transport specimen at room temperature to the laboratory as soon as possible. The pneumatic tube system may be used for transportation of specimens.

Urine Specimens for Chlamydia trachomatis and Neisseria gonorrhoeae

DNA: Dirty urine collection

Urine specimen Male (CTNGUR) or Female (DNACH) -

1. Urine specimens may be used for Chlamydia trachomatis DNA ONLY testing for females; Chlamydia trachomatis and Neisseria gonorrhoeae DNA testing can be performed on males. However, the patient must NOT have urinated for the last two hours.
2. Collect 10 to 50 mL of the first catch urine into a clean, polypropylene container without preservatives.
3. Urine specimens in cups must be transported to the laboratory by hand and are **Not** allowed to be placed in the pneumatic tube system.
4. Urine specimens in cups can be transferred to a 10mL plain yellow, no additive tube. The plain yellow tube can be sent to the laboratory in the pneumatic tube system.
5. Label specimen with patient identification information, date, time, and initials of the person who collected the specimen.

6. Transport specimen at room temperature to the laboratory as soon as possible.

Other Specimens for Chlamydia trachomatis: Rectal, Throat or eye specimens-
order as Chlamydia culture (DNA probes not available on these sources)

1. Call laboratory at 585-LABS for appropriate collection kit.
2. Collect and transport swab specimens in **3 mL M4RT Remel Culture Transport Media**.
3. Use only dacron, rayon, or swabs included in the collection kit with plastic or non-aluminum wire shafts. Do NOT use collection swabs with wooden or aluminum shafts.
4. First remove excess mucus, blood, and examination lubricants with the large sterile swab.
5. Then use the small sterile swab to collect specimen using recommended methods to sample columnar and squamo-columnar cells.
6. Insert swab into M4RT Remel Culture Transport Media tube, break shaft and close securely.
7. Label specimen with patient identification information, date, time, and initials of the person who collected the specimen. Label with SOURCE.
8. Transport specimen at room temperature to the laboratory as soon as possible. The pneumatic tube system may be used for transportation of specimens.

Genital Specimens for Bacterial, Bacterial vaginosis, Group B Strep Screens, or Yeast Cultures

1. For Neisseria gonorrhoeae culture (GCC) a charcoal swab is preferred.
2. For genital cultures in which GC is not suspected, Yeast cultures, or if a Gram stain is requested, use a blue-capped gel swab.

Bacterial vaginosis DNA test - BVDNA

1. Call laboratory at 585-LABS for appropriate collection kit.
2. Open the seal on the outer plastic pouch of AFFIRM VPIII ambient temperature transport system and remove all components. One per patient. Other swabs are NOT acceptable.
3. Break the ampule in the ATTS reagent dropper by firmly squeezing the dropper close the center – do this only once.
4. Dispense reagent into sample collection tube.
5. Peel wrapper to expose patient swab, remove swab, discard wrapper.
6. Collect patient specimen.
7. Insert the culture swab into the posterior vaginal fornix.
8. Rotate the swab for 2 to 3 times against the vaginal wall to ensure adequate sampling.
9. Swab the lateral vaginal wall while removing the swab.
10. Immediately place the swab into the sample collection tube containing the ATTS reagent.
11. Break the swab shaft at the pre-scored line just above the top of the tube. Discard the remaining shaft into an infectious waste container.
12. Place the sample collection cap over the exposed end of the swab and firmly press the cap onto the tube, the cap will snap when properly seated.
13. Label specimen with patient identification information, date, time, and initials of the person who collected the specimen.
14. Transport specimen at room temperature to the laboratory as soon as possible. The pneumatic tube system may be used for transportation of specimens.

Group B Streptococcus screens – DNA amplification

1. Screening for GBS (Group B Streptococcus) colonization in antepartum women between 35

and 37 weeks' gestation, with collection of both vaginal and rectal swabs. Eswabs, Red top or blue top swabs may be used. Charcoal swabs may NOT be used.

2. Vaginal and rectal specimens may be collected using the same swab or two different swabs, combined in the same transport system. Cervical, perianal, perirectal specimens are not acceptable; a speculum should not be used for sample collection.
3. Label specimen with patient identification information, date, time, and initials of the person who collected the specimen.
4. Transport specimen at room temperature to the laboratory as soon as possible. The pneumatic tube system may be used for transportation of specimens.

Endocervical Specimens

15. Remove excess mucous from the cervical os and surrounding mucosa using a swab.
16. **Discard the Swab.**
17. Insert the culture swab into the endocervical canal.
18. Rotate the swab for 10 to 30 seconds in the endocervical canal to ensure adequate sampling.
19. Withdraw the swab carefully; avoid any contact with the vaginal mucosa.
20. Immediately place the swab into the culture transport tube.

Vaginal Specimens (Not suitable specimen type for Chlamydia)

1. Wipe away any excessive amount of secretion or discharge and discard.
2. Sample the secretions from the mucosal membrane of the vaginal vault with a sterile swab.
3. Place the swab(s) into the transport tube.

Male Urethral Specimens

1. Patient should not have urinated for at least one hour prior to sample collection.
2. Insert the swab 2 to 4 cm into the urethra using a rotating motion to facilitate insertion.
3. Once inserted, rotate the swab gently while using sufficient pressure to ensure the swab comes into contact with the urethral surfaces. Allow the swab to remain inserted for 2 to 3 seconds.
4. Withdraw the swab.
5. Immediately place the swab into the transport tube.

Ocular Specimens

Conjunctiva

1. Clean away any crusty exudate material prior to collecting specimen.
2. Peel open the package containing the swabs and transport, being careful not to contaminate the swabs. Snap off and discard the cap on the tube transport.
3. Gently pull the lower eyelid down to expose the conjunctiva.
4. Use swab to rub over the surface of the conjunctiva, touching inflamed areas and collecting exudates. Repeat with 2nd swab provided in the package
5. Insert swabs completely into the tube transport.
6. Label specimen according to protocol. Indicate type of specimen.

Note: If physician inoculates the medium directly, place the inoculum in "S8" shapes to indicate "conjunctiva".

Corneal Scrapings

1. Collected by the physician according to standard practices.
2. Place the scrapings directly onto the culture medium obtained from the laboratory.
3. Make "C" shapes to indicate "cornea" when inoculating the surface of the medium

Eyelid

1. Collected by the physician according to standard practices.
2. Place the scrapings directly onto the culture medium obtained from the laboratory.
3. When inoculating the surface of the medium directly, make "RL" shapes to indicate "right eyelid" or "LL" shapes to indicate "left eyelid"

IV. References:

1. Shea, Yvonne, "Specimen Collection and Transport" in Clinical Microbiology Procedures Handbook, ASM Press, Washington 1992.
2. Miller, J. Michael, Specimen Management in Clinical Microbiology, ASM Press, Washington, 1999.
3. Vacutainer Brand Urine Collection Kit for Midstream Specimens, July 1995.
4. BD Affirm VPIII Package insert Loo5510 2011/01
5. Department of Health and Human Services, Centers for Disease Control and Prevention. Prevention of Perinatal Group B Streptococcal Disease, Revised Guidelines from CDC, 2010. MMWR 2010;59