



Microbiology Laboratory · Procedure
830 BAYOU PINES WEST · LAKE CHARLES, LA. 70601

[SPECIMEN COLLECTION]

Procedure Number: MC-76

Adopted Date: [121107]

Revised/Approved Date: 03/18/2008, 05/11/2010, 07/27/10

Approved By _____

Intended Use: To define the proper procedures for specimen collection to ensure the quality and integrity of the specimen.

Precautions and Warnings: Practice Standard (Universal) Precautions when handling biohazardous specimens.

Procedure:

Every specimen that is brought into lab is checked against the requisition. The requisition is compared to the labeled specimen to make sure the name matches. If the specimen is received unlabeled or with improper label **or without TWO identifiers:**

1. Call the Dr's office
2. Have a person responsible for the collection identify and place a proper label upon the specimen. Send the specimen back to the account
3. Reject and recollect if there is any doubt about positive identification or specimen label does not match requisition

Refer to Rejection of Microbiology Specimens Procedure MC-13. Document in LIS how problem was resolved.

Once identity of the specimen is established, the data entry clerk or tech enters requisition information into the laboratory computer system. Demographic information should be entered for new patients in computer system from the information on the

requisition. Patients that are already in system will be retrieved when date of birth or social security number is entered. The test is ordered in the system at this time. A barcode label will then be generated.

If a microbiology specimen is to be collected in house, the patient should have a requisition. The front desk secretary identifies the patient by asking his name. The request is checked that verbal and written name on request is the same. The patient will then be asked other demographic information. The test requested is ordered in computer under patients name.

The phlebotomist will retrieve labels from label printer and call patient by name into phlebotomy room. Once the patient is in the room, the phlebotomist asks for the patient's date of birth or address and compares this to information on label and requisition. Once the specimen is collected, the phlebotomist labels specimen with barcode label for that patient and brings specimen to microbiology laboratory.

All specimens whether collected in-house by laboratory personnel or off site should be collected, stored, and transported using the following the guidelines listed below.

Collection, Transport, and Storage Guidelines for Microbiology Specimens:

Abscess(also lesion,wound,pustule,ulcer)for aerobic culture

Wipe area with sterile saline or 70% alcohol and swab along leading edge of wound with Stuart's or Amie's culture swabs. Store specimen at room temperature and transport to lab within 48 hours. (07/27/10 AS)

Anaerobic culture

Proper collection and transport of specimens for anaerobic culture cannot be overemphasized. Because indigenous anaerobes are often present in large numbers as normal flora on mucosal surfaces, even minimal contamination of a specimen can give misleading results. The specimen should be taken from site of infection and precautions should be taken to avoid contaminants and aeration of specimen. For anaerobic conditions to be maintained in transport to laboratory, inoculate BBL Amies's cultureSwab Plus. This swab has gel in bottom that provides an oxygen free environment. Send to lab within 48 hours at room temperature. (07/27/10 as)

Specimens acceptable for anaerobic culture:

- Body fluids – Ascitic, cerebrospinal, pericardial, pleural, prostatic, seminal, synovial, thoracentesis, bile, bone marrow transudate
- Exudates – Aspirated pus from wounds or abscesses, or if “sulfur granules” are present
- Genital specimens
 - Female – material from placenta, glands, culdocentesis, endometrium, fallopian tube

- Male – prostatic or seminal fluids
- Lesions – material from gall bladder, etc.
- Surgical specimens
- Respiratory – transtracheal aspirate
- Urine – suprapubic aspirate

Blood or bone marrow

Disinfect venipuncture site with 70% alcohol and disinfectant such as betadine. Draw blood at time of febrile episode into blood culture media set(aerobic and anaerobic bottle or one pedi bottle for pediatric patients). Phlebotomists must be trained in blood culture collection before drawing blood cultures. Refer to Blood Culture Collection procedure. Draw two sets, one from right and one from left arm. Do not draw more than three sets in a 24-hour period. Bone marrow specimens should be submitted in Vacutainer tube containing anticoagulant. (07/19/10 AS) Transport to lab within two hours at room temperature. Bone marrow specimens can only be collected by physicians.

Body fluids(amniotic,aqueous/vitreous,abdominal,acites(peritoneal), bile,CSF,joint(synovial)pericardial,pleural)

Physician should disinfect skin before aspirating specimen with needle aspiration. Fluid should be sent to lab immediately at room temperature in a sterile, screw cap tube, tightly sealed container or syringe as long as needle is not attached.

Bone

Physician should disinfect skin before surgical procedure and take sample from affected area for biopsy. Send specimen to lab immediately at room temperature in a sterile, screw-cap tube.

Ear

Inner ear – Physician should clean ear canal with mild soap solution before myringotomy (puncture of the ear drum). Aspirate material from behind drum if eardrum is intact. Use Stuart's or Amie's culture swab to collect material from ruptured ear drum. Store swab at room temperature and transport to lab within 48 hours. (07/27/10 AS)

Outer ear - Wipe away crust with sterile saline. Use Stuart's or Amie's swab to collect and firmly rotate swab in outer canal. Store swab at room temperature and transport to lab within 48 hours. (07/27/10 AS)

Eye

Conjunctiva –Sample both eyes with Stuart's or Amie's swab premoistened with sterile saline. Store specimen at room temperature and transport to lab within 48 hours. (07/27/10 AS)

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Corneal scrapings – Physician should instill local anesthetic before collection. Inoculate thioglycolate broth at bedside. Specimen should be sent to lab immediately at room temperature.

Foreign bodies (IUD, IV catheters, pins, prosthetic valves)

Physician should disinfect skin before removal. Once removed, place into a sterile, screw-cap container. Send to lab immediately at room temperature.

GI tract

Stool culture – It is recommended that no more than two specimens per patient for routine bacterial stool culture be ordered without prior consultation with an individual who can explain the limited yield provided by additional specimens. Collect specimen and place into a clean, leak proof, tightly sealed container. Store specimen at 2-8°C and send to laboratory within 2 hours. (07/28/10 AS) If longer than 2 hours, specimen must be placed in Modified Cary-Blair transport container. Routine culture will include testing for *Salmonella*, *Shigella*, *Campylobacter* and Enterohemorrhagic *E.coli*. Specify on requisition if testing for *Vibrio*, *Aeromonas*, *Plesiomonas*, or *Yersinia*, is needed.

OCP (Ova, cysts, and parasites) – It is recommended that no more than three specimens per patient for OCP be ordered without prior consultation with an individual who can explain the limited yield provided by additional specimens. For outpatients, specimens should be spaced a few days apart to assure recovery of parasitic elements that are passed intermittently. For hospitalized patients, specimens can be collected for a designated length of time to avoid prolonging the hospital stay and should not be collected after fourth day without prior consultation. (Revised 06/24/08 AS) Specimens can be sent to lab in clean, leak proof, tightly sealed containers within one hour or stored at 2-8°C for up to 24 hours. If sent in ecofix vials, store at room temperature. Wait 7-10 days if patient has received antiparasitic compounds, barium, iron, Kaopectate, metronidazole, Milk of Magnesia, Pepto-bismol, or tetracycline.

Stool pH, Stool reducing substances, Crypto/Giardia - Freshly collected stool specimen in leak-proof, tightly sealed container. Send to lab as soon as possible.

***Clostridium difficile* Toxins A and B**- The stool specimen should be transported in an airtight container and stored at 2-8°C until testing is performed.

Occult Blood - Patients that use the DEVEL-A-TAB Sampler will collect specimens at home over the course of two consecutive bowel movements. Using a ballpoint pen, write your name, age, address, phone # and date of each bowel movement on card. Prior to defecation: Flush the toilet to clear bowl. For ease of collecting specimen, place tissue provided onto water in bowl. Lift front flap on test slide. After defecating

into bowl, using applicator stick, stab stool in 4 different sites of the bowel movement and apply to circle # 1. Close flap. Discard stick in trash. DO NOT FLUSH APPLICATOR STICK. Repeat the same procedure for the next bowel movement and apply to circle #2. Place the card into the provided mailing envelope, seal by removing tape strip and fold as indicated. Mail or return to the laboratory. IT IS AGAINST POSTAL REGULATIONS TO MAIL SPECIMEN IN A STANDARD PAPER ENVELOPE.

If not using the DEVEL-A-TAB Sampler for collection, collect stool specimens at home over the course of two consecutive bowel movements. Pass specimen directly into a wide mouth leak proof container with a tight fitting lid. Store specimens in refrigerator. Bring both specimens to laboratory.

Do not collect specimen during or until three days after a menstrual period, or while you have blood in urine or bleeding from hemorrhoids or dental work.

Enterohemorrhagic E. coli (EHEC) – Stool specimens should be refrigerated and sent to lab within two hours of collection or put in Modified Cary-blair transport media container. (07/28/10 AS)

Campy EIA – Specimens should be submitted in an airtight container and can be stores up to 96 hours at 2-8°C. (Revised 05/11/10 AS)

Genital tract

Female

Bartholin cyst – Disinfect skin before collection. Aspirate fluid and send in sterile, screw cap container or inoculate aerobic/anaerobic swab (swab with gel in bottom). Store at room temperature and send to lab within 24 hours if collected with swab. If fluid is sent, transport to lab immediately.

Cervix – Remove mucus before collection of specimen. Do not use lubricant on speculum. Swab deeply into endocervical canal using Amie's or Stuart's culture swab. In addition, use viral transport media to collect if herpes or viral testing is needed. Store specimen at refrigerated (2-8°C) temperature Inoculate Gen-probe vial if GC/Chlamydia testing is ordered. Transport to lab within 24 hours at room temperature.

Cul-de-sac – Submit aspirate in sterile, screw cap, tightly sealed container or inoculate aerobic/anaerobic swab (swab with gel in bottom). Transport to lab within 24 hours at room temperature. If fluid is sent, transport to lab immediately.

Urethra – Remove exudate from urethral opening. Collect discharge by massaging urethra against pubic symphysis or insert flexible swab 2-4 cm into urethra and rotate swab for 2 seconds. Inoculate Stuart's or Amie's culture swab. Store at room

temperature and transport to lab within 24 hours.

Vagina – Remove exudate. Swab secretions and mucous membrane of vagina with Stuart's or Amie's culture swab. Store at room temperature and transport to lab within 24 hours.

Vagina-rectal culture for Group B Strep – Swab the lower vagina (vaginal introitus), followed by the rectum (i.e., insert swab through the anal sphincter) using the same swab or two different swabs. Store at room temperature and transport to lab within 48 hours. (07/27/10 as)

Wet prep - Collect vaginal and urethral discharges, prostatic secretions or urine sediment and send to lab in sterile container. If a swab is used to collect, place swab in sterile container or tube with a couple drops of saline. The specimen should be sent to lab as soon as possible so that the trichomonas organism will be actively motile if present.

KOH prep - Any scrapings or exudates to be examined for fungal elements are acceptable.

BD AFFIRM VPIII (CANDIDA, GARDNERELLA, AND TRICHOMONAS detection) – Open the seal on outer plastic pouch of **Affirm VPIII** Ambient Temperature Transport System and remove all components [each plastic pouch contains enough material for the collection and transport of one vaginal specimen]. Tear open the foil pouch and remove the ATTS Reagent Dropper. Break ampule in ATTS Reagent Dropper by firmly squeezing vial with finger and thumb. Dispense reagent from ATTS Reagent Dropper into Sample Collection Tube. Peel wrapper to expose patient swab. Remove swab. Discard wrapper.

Place the patient in position for a pelvic examination. Insert an UNLUBRICATED speculum (WITHOUT JELLY OR WATER) into the vagina to permit visualization of the posterior vaginal fornix. Using the sterile polyester-tip swab, obtain a sample from the posterior vaginal fornix. Twist or roll the swab against the vaginal wall two or three times, ensuring the entire circumference of the swab has touched the vaginal wall.

Swab the lateral vaginal wall while removing the swab.

Immediately place the patient swab in the Sample Collection Tube containing the ATTS Reagent. Break swab shaft at pre-scored line just above the top of the tube. Discard remaining shaft into an infectious waste container. Place the Sample Collection Cap over the exposed end of the swab and firmly press the cap onto the Sample Collection Tube. The cap will 'snap' onto the tube when it is properly seated. Label the Sample Collection Tube with patient/lab identification information. Include date and time that sample was taken.

This system has been qualified for transport use at ambient conditions (15 – 30°C) and refrigerated conditions (2 – 8°C) for up to 72 h.

Male

Prostate – Clean glans with soap and water before collection. Collect secretions with Stuart's or Amie's swab or send in sterile, screw cap tube. Store at room temperature and transport to lab within 24 hours or immediately if sent in tube.

Urethra – Use Stuart's or Amie's culture swab to collect. Insert flexible swab 2-4cm into urethra and rotate for 2 seconds. Store at room temperature and transport to lab within 24 hours.

Hair, Nails, or Skin Scrapings (for fungal culture) – If collecting nails or skin scrapings, wipe with 70% alcohol before collection. Hair: collect hairs with intact shaft. Nails: send clippings of affected area. Skin: scrape skin at leading edge of lesion. Send specimen in sterile, screw top tube or container and transport to lab at room temperature within 24 hours. If KOH is also needed be sure to order on requisition.

Respiratory tract

Lower

Bronchial alveolar lavage, bronchial brush, bronchial wash – Submit specimen in sterile, screw-top, tightly sealed container and transport to lab at room temperature within 24 hours. Anaerobic culture is appropriate only if sheathed (protected) catheter is used.

Sputum, tracheal aspirate (suction) – For sputum culture, patient should be instructed to rinse or gargle with water before collection. Have patient collect from deep cough. Induced sputa on pediatric or uncooperative may be watery because of saline nebulization. Specimen should be sent to lab in a sterile, tightly sealed container. Specimen should be stored at 2-8°C and sent to lab within 24 hours.

Upper

Nasopharynx – Insert flexible swab through nose into posterior nasopharynx and rotate for 5 seconds. This is the specimen of choice for *Bordetella pertussis* culture. Submit swab moistened with Stuart's or Amie's medium to laboratory. Store at room temperature and transport to lab within 48 hours. (07/27/10 as)

If testing for **Influenza A and B** viral antigens, only the swab that comes with kit can be used for collection. Collection will be performed by Pathology Laboratory personnel.

***The Microbiology department should be notified if *Corynebacterium diphtheriae* or *Bordetella pertussis* is suspected before arrival of the specimen. The State lab or reference lab should be consulted for proper collection, handling, and transport.

Pharynx(throat) – Swab posterior pharynx and tonsils with Stuart's or Amie's culture

swab. Store at room temperature and transport to lab within 48 hours. (07/27/10 as)

Tissue

Disinfect skin before collection. Send specimen in sterile, screw-cap container or anaerobic swab (gel at bottom of swab) if anaerobic culture is ordered. If sent in container, do not allow specimen to dry out. Moisten specimen with sterile, distilled water if not bloody. Store at room temperature and transport to lab within 2 hours.

Urine

Clean-voided midstream urine – Females: clean external genitalia with soap and water, then rinse with water; hold labia apart and begin voiding in commode; after several milliliters have passed, collect midstream. Males: Clean glans with soap and water, then rinse with water; retract foreskin; after several milliliters have passed, collect midstream. Specimen should be sent to lab in sterile, screw cap, tightly sealed container and stored at 4°C. Send to lab within 24 hours.

Straight catheter (in and out) – Clean urethral area with soap and water and rinse with water. Insert catheter into bladder and allow 15mL to pass then collect remainder into sterile, screw cap, tightly sealed container. Transport to lab within 2 hours at room temperature or 24 hours if refrigerated.

Indwelling catheter (Foley) – Disinfect catheter collection port. Aspirate 5-10mL of urine with needle and syringe. Urine should be put in sterile, screw-cap, tightly sealed container and store at 2-8°C. Transport to lab within 24 hours at room temperature.

Suprapubic aspirate – Disinfect skin. Physician should perform needle aspiration above the symphysis pubis through the abdominal wall into the full bladder. Urine may be sent in sterile, screw cap, tightly sealed container or sent in syringe as long as needle is not attached. Transport to lab immediately at room temperature.

All specimens should be received in laboratory in tightly sealed containers and free of external spillage.

References:

Bailey and Scott's Diagnostic Microbiology, 11th edition, 2002, Forbes, Sahm, and Weissfeld
Pages 3-17



I have performed the annual review of this Procedure

Procedure MC-76

Signature_____ Date_____

