Subject/Title: **Specimen Collection and Transportation of Microbiology Specimens**	*Approved By and Title:* Bill Pettross, M. D. Director of FRHG Laboratories
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PRINCIPLE

It is critical that the laboratory provide complete guidelines for the proper collection and transport of specimens to ensure quality patient care. All diagnostic information from the microbiology laboratory is contingent on the quality of specimen received. Consequences of a poorly collected and/or poorly transported specimen include failure to isolate the causative microorganism and recovery of contaminants or normal microbiota, which can lead to improper treatment of the patient. Often, direct specimen smears are utilized to determine the quality of the specimen, to provide rapid information for diagnosis and therapy, and to allow the physician to determine if additional, better-quality specimens should be collected. This procedure addresses instructions for physicians, nurses, and laboratory assistants on collecting and transporting samples.

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SPECIMEN

1. Specimen Safety considerations

a. Follow universal precaution guidelines. Treat all specimens as potentially bio-hazardous**.**
b. Laboratory workers should use appropriate barrier protection (such as gloves and laboratory coat or gown) when collecting or handling specimens. If splashing may occur, protective eyewear, face masks, and aprons may be necessary.
c. Do not contaminate the external surface of the collection container and/or its accompanying paperwork.
d. Minimize direct handling of specimens in transit from the patient to the laboratory. Use plastic sealable bags with a separate pouch for the laboratory requisition orders or transport carriers (for example, small buckets with rigid handles).

NOTE: Specimens obtained by a physician using needle aspiration should be transferred to a sterile tube or anaerobic transport vial prior to transport of the specimen to the laboratory. If there is little material in the syringe, the physician should draw a small amount of sterile nonbacteriostatic 0.85% NaCl or sterile broth through the syringe and then transfer the specimen to a sterile tube. Alternatively, and only if the specimen will be compromised by transferring it from the syringe, a small amount of sterile 0.85% NaCl or broth may be drawn into a syringe prior to removal of the needle. The physician should use a protective device while removing the needle to avoid injury and should cap the syringe with a sterile cap prior to transporting it to the laboratory.

2. General guidelines for proper specimen collection

a. Collect specimen before administering antimicrobial agents when possible.
b. Collect specimen with as little contamination from indigenous microbiota as possible to ensure that the sample will be representative of the infected site.
c. Utilize appropriate collection devices. Use sterile equipment and aseptic technique to collect specimens to prevent introduction of microorganisms during invasive procedures.
d. Clearly label the specimen container with the patient's name and identification number or date of birth (DOB). Always include date and time of collection and your initials. LINK
e. Collect an adequate amount of specimen. Inadequate amounts of specimen may yield false-negative results. LINK
f. Develop an understanding of the microbiology laboratory's source identification schemes. Know when to include "rule-out" request. For example, the laboratory may routinely screen for *Shigella*, *Salmonella*, and *Campylobacter* species in stool cultures but not for *Yersinia* or *Vibrio* species.
g. Consider geographic location and season when notifying the laboratory of rule-out requests. For example, Coccidioides immitis is endemic in the southwestern United States, and rotaviruses are more commonly found in infants and children in winter.
h. Identify the specimen source and/or specific site correctly so that proper culture media will be selected during processing the laboratory.

i. If a specimen is to be collected through intact skin, cleanse the skin first. For example, use 70% alcohol followed by iodine solution (1 to 2% tincture of iodine or 10% solution of povidone-iodine). Prevent burn by tincture of iodine by removing excess after specimen has been collected.
j. Before collection the specimen, consider the risk/benefit ration of the collection procedure to the patient.
k. Collect specimens in sturdy, sterile, screw-cap, leak proof containers with lids that do not create an aerosol when opened.

3. General guidelines for proper specimen transport

a. Transport all specimens to the laboratory promptly.

PLACING ORDERS

1. Placing inpatient orders

a. An order must be placed in the HIS (Hospital Information System). A requisition is generated. When the specimen is collected on the floor a verification slip is generated. The specimen with a separate collection verification form for each test ordered must be transported to the lab as soon as collection has been verified. The verification form should include the following information: Refer to Acceptance or Rejection of Samples and Requisitions for additional information.

- Patient name
- Patient age and sex
- Patient room number
- Physician name
- Specific anatomic culture site
- Date and time of specimen collection
- Initials of person obtaining specimen
- Antimicrobials, if any, patient is receiving
- When appropriate, include clinical diagnosis, special culture request, relevant patient history
- Test or procedure requested

b. A separate order is needed for each test, including anaerobic cultures. For example, if sputum is collected for AFB, Fungus and routine culture, a total of three orders must be entered: one for AFB, one for fungus, and one for routine microbiology.
c. Special requests for culture of unusual isolates (i.e., *C. diphtheriae*, *Leptospira*, *Actinomyces*, *Nocardi*a, *Brucella*, *Hemophilus ducreyi*, *B. pertussis*, etc.) require prior notification of the laboratory in addition to ordering the tests.

2. Placing Outpatient orders

a. Orders are placed and received in the LIS (Laboratory Information System) using function RE. Refer to the Inpatient order guidelines for details.

LABELING

1. Each sample must have a label firmly attached to the specimen container bearing the following information: Refer to Acceptance or Rejection of Samples and Requisitions
    - Date & Time of Collection
    - Patient name
    - Hospital number or DOB
    - Culture site
    - Initials of Collector

SPECIMEN COLLECTION (ACCEPTANCE AND REJECTION CRITERIA)

1. All clinical specimens must be collected in clean sterile containers, which must be properly sealed. The outer portion of the container must not be contaminated.
2. Optimal specimens are aseptically obtained fresh pus, fluid, or tissue that is rapidly and safely transported to the laboratory. Direct aspiration into a syringe is recommended. Swabs should not be used if fluid can be obtained.
3. Swabs without transport medium are not satisfactory since they allow drying of the specimen and loss of viability. All specimens should be transported to the laboratory in a sealed zip lock bag.
4. The following are reasons for rejection of specimens. For additional information refer to previous link to Acceptance or Rejection of Samples

PROBLEM	ACTION
Unlabeled or improperly labeled specimen	Laboratory personnel can handle minor outpatient corrections such as, date, time of collection, source and site with a call to the doctor's office. For inpatients; a nurse must come to the laboratory and identify the specimen before it is processed. Specimens that are easy to obtain should be recollected. Invasive specimens (e.g. CSF) should be processed but do not publish results until doctor has been consulted. If the problem can not be resolved with the nursing staff, the doctor should be called.
Prolonged transport: Urine – 1 hr at rm temp Stools for trophozoites – 1 hr since collection for soft formed, ½ hr since collection for fluid specimens Gonorrhea specimens – ½ hr without transport Medium	Alert submitter of the discrepancy and request a repeat collection of specimen. Note problem in the LIS: "Received after prolonged delay"

PROBLEM	ACTION
Improper container (nonsterile)	Do not process. Call submitter and request repeat specimen
Leaking container	Do not process sputum, blood, and viral specimens. Call submitter for repeat specimen collection and dispose of the leaking one. Other specimens – call submitter and ask for repeat a collection. Hold specimen.
Oropharyngeal contaminated sputum	See respiratory culture quality assessment procedure.
Obvious foreign contamination	Alert submitter of discrepancy. Request repeat specimen collection.
Duplicate specimens submitted at the same time	Select the one of best quality for culture. Report by note in the LIS
Duplicate specimens on same day for the same request (except blood)	Place specimen in refrigerator. Call submitter and indicate duplicity. Culture only one specimen.
Specimen unsuitable for culture request; i.e. anaerobe request from aerobic transport	Call submitter, indicate discrepancy. Request proper specimen for the work requested.
Quantity not sufficient (QNS)	Blood: If less than 16ml from adult, inform submitter and request another specimen. Process, note problem on report. Body Fluids: If QNS for multiple requests, call doctor and determine priority of testing.
Collection/Verification sheet not completely filled out or patient information doesn't match sample.	Hold specimen. Return slip to floor with note: "This specimen will be further processed when this requisition is completely filled out and returned to the laboratory".

COLLECTION OF ANAEROBIC SPECIMENS

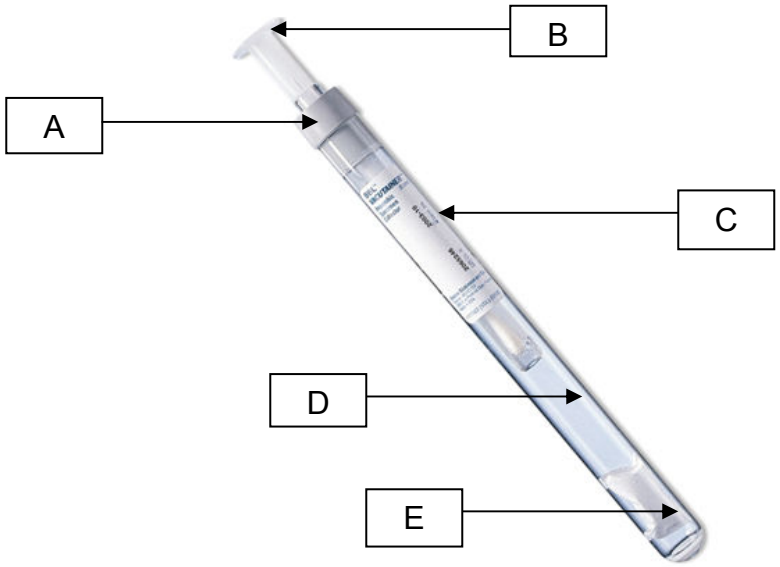
1. Anaerobic cultures are best collected by aspirating abscess fluid with a sterile syringe and needle. Syringes can be capped and submitted, or the aspirated fluid can be injected into a vacutainer anaerobic specimen collection. The submission of swabs for anaerobic culture is discouraged, but if swabs must be used, they should be placed immediately into anaerobic transport tubes.
2. Anaerobic bacteriology is time-consuming and expensive. Thus, it will be done only on appropriate and properly handled specimens. Specimens should be delivered to the lab ASAP.
3. Through the HIS. Order "anaerobic" culture. Be sure to specify the source. When both the anaerobic and aerobic cultures are to be ordered on one specimen, two separate orders must be placed through the HIS, one request for anaerobic culture and a second order for aerobic culture must be place. Anaerobic cultures are not routinely set up unless specifically ordered.

APPROPRIATE SPECIMENS FOR	INAPPROPRIATE SPECIMENS FOR

ANAEROBIC CULTURE	ANAEROBIC CULTURE
PULMONARY: Percutaneous transtracheal aspirate, thoracentesis fluid, direct needle puncture aspirate of lung infiltrate or abscess, and bronch brush (Bartlett cath) collected through plugged double lumen catheter	Expectorated sputum, tracheal tube suctioning, bronchoscopic aspirate or wash, N-P, throat or mouth swab.
URINARY: Percutaneous suprapubic aspirate of urine, nephrostomy specimens	Voided urine or catheterized specimen
ABSCESS: Needle and syringe aspirate of closed abscess after decontamination of surface	Swab from surface of abscess or swab after incision and drainage

APPROPRIATE SPECIMENS FOR ANAEROBIC CULTURE	INAPPROPRIATE SPECIMENS FOR ANAEROBIC CULTURE
UTERINE: Culdocentesis after decontamination of vagina with povidone iodine or aspirate into syringe through IV type catheter passed through the cervical opening under direct visualization	Vaginal or cervical swabs
OTHER: Joint fluid, spinal fluid, blood and biopsy tissue collected with ordinary care	Superficial wound, feces, or rectal swab.

4. Instructions for Vacutainer anaerobic specimen collector:



- Do not use if package is damaged.
- Do not use if indicator (E) is pink.
- Do not remove stopper (A) during collection.

5. Anaerobic Collection by swab

a. Peel apart package and remove specimen collector.
b. Remove plunger with sterile swab attached (B).
c. Obtain specimen.
d. Replace swab through hole and into inner tube (C).
e. Press down on disc portion of plastic plunger (B) until disc rests against top of rubber stopper (A) forcing the inner tube (C) into outer tube (D). Hold tube at a 45^0 angle while depressing plunger.
f. Hold tube at $10^0 – 30^0$ angle to floor and rotate with a swirling motion.

6. Collection of liquid or purulent specimens
   a. Collect specimen with sterile syringe and needle. Air trapped in syringe should be expelled by holding syringe and needle upright.
   b. Remove swab plunger unit (B) and expel material into inner tube (C).
   c. Proceed with step 5 and 6 as stated above.
   d. Transport upright if liquid specimen.

BLOOD, EAR, AND EYE SPECIMENS

1. Blood Specimens: For systemic and localized infections the following is recommended.
   a. In suspected acute sepsis, meningitis, osteomyelitis, arthritis, or acute untreated bacterial pneumonia, obtain two blood cultures (from two separate venipuncture sites) before starting therapy.
   b. For fever unknown origin (e.g., occult abscess, typhoid fever, or brucellosis), obtain two separate blood cultures initially; 24 to 36 hours later, obtain two more just before the expected (usually afternoon) temperature elevation. The yield beyond four cultures is virtually nil.
2. For suspected infective endocarditis the following is recommended:
   a. Acute – Obtain 3 blood cultures with 3 separate venipunctures during the first 1 to 2 hours of evaluation and begin therapy.
   b. Subacute – Obtain 3 blood cultures on day 1 (ideally 15 minutes or more apart); if all are negative, 24 hours later, obtain three more. From undiagnosed patients who have received antimicrobial agents in the week or two before admission, obtain two separate blood cultures on each of 3 successive days.
   c. The major pitfall in interpretation of blood cultures is their contamination by microbial flora of the skin. The site of the venipuncture should be swabbed with 70% alcohol followed by 2% tincture of iodine for children 2 months of age and younger or ChoropPrep Frepp for adults, swabbed concentrically, starting at the center. The disinfectant should be allowed to dry before blood is aspirated. If further palpation of the vein is necessary during aspiration, the finger must be similarly disinfected. Draw patient specimens aseptically into a sterile syringe. 16 – 20 ml of inoculum should be obtained from adult patients. 1 to 5 ml should be obtained from pediatric patients.
   d. For adult patients, prepare one aerobic Bactec Plus aerobic/F vial and one Bactec lytic/10 anaerobic/F vial by removing the plastic flip cap from each vial and cleaning the exposed rubber septum with 70% isopropyl alcohol. Inoculate the Bactec Plus aerobic/F vial with 8 – 10 ml of blood. Inoculate the Bactec lytic

vial with 8 – 10 ml of blood. Always note the volume of blood inoculated into the vial.

e. For pediatric patients use the Bactec Peds Plus/F vials. Inoculate with 1 – 5 ml of blood. Always note the volume of blood inoculated into the vial.
f. Label all vials with the patient's name, ID number, date and time drawn. Transport to the laboratory immediately.
g. Order blood culture through the HIS for outpatients. Receive all cultures in the LIS Refer to Receiving Specimens Through LIS in Microbiology . Routine blood cultures are held for 5 days with preliminary reports daily. All positive blood cultures are phoned to the physician.
h. Bottles are held longer in some situations if the laboratory is notified, e.g.; brucellosis, Myc*obacterium avium-intracellular* and systemic fungal infections.
i. A second order must be placed when these organisms are to be cultured.

EYE

Do not touch external skin. Obtain maximum material. Culture both eyes. Use Star swabs with modified Stuarts.

EAR

For otitis media aspirate from tympanocentesis. For external ear, clean the external ear surface and swab drainage. Star swab with modified Stuarts.

GENITAL SAMPLE

1. Routine and GC cultures
   a. In general, vaginal cultures are of minimal value. Cultures for gonorrhea should be obtained directly from the uterine cervix. Anaerobic cultures should not be performed except on abscess fluid aspirated by syringe and needle from a paravaginal abscess. Other infections such as trichomonas, candidiasis, or those caused by Gardnerella vaginalis may be diagnosed by direct wet mounts. Wet mounts should be submitted in a 3 ml re cap vacutainer tube containing 0.5 ml saline. Keep warm and transport immediately to the lab. Presence or absence of yeast, trichomonas, clue cells and amine odor will be reported.
   b. All genital cultures should be directly inoculated onto a prewarmed Jembec MTM agar. Place the Jembec into the provided ziplock bag and drop the tablet into the well in the dish.
   c. **Transport it and the swab immediately** to the laboratory. N. gonorrhoeae is sensitive to cold and needs 5 – 10% CO_2 soon after collection. Alternately use Star swab modified Amies charcoal swab.
   d. Order either "genital culture" or GC screen only. Gram stains are not routinely performed on "GC only screens". Order separately and send swab.

e. Preparation of patient and collection:
   1. Cervix – wipe the cervix clean of vaginal secretion and mucus. Use a speculum without lubricant. Under direct vision, gently compress cervix with blades of speculum and use a rotary motion with a swab – obtain exudates from endocervical glands.
   2. Urethra – collect specimen one hour or more after urinating. Wipe urethra clean with sterile gauze or swab. If discharge cannot be obtained by "milking" the urethra, use a swab to collect material from about 1 cm inside the urethra.
   3. Vagina – use a speculum without lubricant. Swab mucosa high in the vaginal canal under direct visualization.
   4. Throat for GC – order GC screen; send swab and inoculated Jembec plate.
   5. Anal for GC – order GC screen; send swab and inoculated Jembec plate.

2. Chlamydia Detection By PCR

   a. Specimen Collection

      Note: Handle all specimens as if they are capable of transmitting infectious agents.
      1. If a urine specimen is to be collected, the patient must not have urinated for the last two hours.
      2. The only acceptable specimens are: urine specimen collected in a clean, polypropylene container without preservatives; endocervical and urethral swab specimens collected and transported in acceptable media.
      3. The test is not intended for use with throat, rectal, or other types of specimens.
      4. Urine specimens: Collect 10 to 50 mL of the first catch urine (first part of the stream) into a clean, polypropylene container without preservatives. Seal the specimen container. (Foley cath and midstream urines are unacceptable)
      5. Swab specimens: Collect and transport endocervical or urethral swab specimens in M4 Culture Transport Medium. Use recommended methods to sample columnar and squamo-columnar cells after removing cervical mucus. Use only dacron, rayon, or calcium alginate tipped collection swabs with plastic or non-aluminum wire shafts. Do not use collection swabs with wooden or aluminum shafts. Leave one swab in the transport media after collection. Do not place the swab used to clean the mucus in the transport media.

   b. Specimen Transport
      1. Urine specimens may be transported at 18–25°C. They are stable for 24 hours at this temperature.
      2. Urine specimens that require shipment to off-site testing centers must be shipped overnight with guaranteed delivery within 24 hours. In this case, the specimen may be shipped at 18–25°C. If the specimen will not reach the testing site within 24 hours, it should be stored at 2–8 °C until it is shipped to ensure that the specimen storage time at 18–25°C does not exceed 24 hours.

   c. Specimen Handling and Storage by laboratory personnel

1. Urine specimens that will not be processed within 24 hours of collection must be stored at 2–8°C. Storage of urine specimens at 18–25°C for more than 24 hours may result in specimen degradation. These specimens should not be used for testing.
2. Specimens stored at 2–8°C must be processed within seven days of collection. Specimens that cannot be processed within seven days may be stored at –20°C or colder, and may be stored this way for up to two months.
3. Swab specimens that will not be processed upon receipt at the laboratory must be stored at 2–8°C and should be processed within seven days. Specimens that cannot be processed within seven days may be stored at –20°C or colder, and may be stored this way for up to 30 days.

d. Other sources for Chlamydia culture (sent to reference Laboratories)
   1. Use a Dacron or rayon tipped swab with a plastic or aluminum shaft. Inoculate multimicrobe test media as stated as previously stated. Sent to reference lab.
   2. Genprobe/Pace (specimen collection and handling of endocervical specimens for N. gonorrhoeae and/or C. trachomatis testing. Store at 2° to 25°C and test within 7 days of collection. Sent to reference laboratory.)

3. GC detection by PCR:

a. Specimen Collection
   Note: Handle all specimens as if they are capable of transmitting infectious agents.
   1. If a male urine specimen is to be collected, the patient must **not** have urinated for the last two hours. The only acceptable specimens are: male urine specimen collected in a clean, polypropylene container without preservatives; endocervical (symptomatic or asymptomatic) and male urethral (symptomatic only) swab specimens collected and transported in acceptable media.
   2. Note: The test is not intended for use with throat, rectal, or other types of specimens.
   3. Urine specimens: Collect 10 to 50 mL of the first catch urine (first part of the stream) into a clean, polypropylene container without preservatives. Seal the specimen container. (Foley cath and Midstream urines are unacceptable)
   4. Swab specimens: Collect and transport endocervical or urethral swab specimens in M4 Culture Transport Medium. Use recommended methods to sample columnar and squamo-columnar cells after removing cervical mucus. Use only dacron, rayon, or calcium alginate tipped collection swabs with plastic or non-aluminum wire shafts. Do not use collection swabs with wooden or aluminum shafts. Leave swabs in the transport media after collection.

b. Specimen Transport
   1. Urine specimens may be transported at 18–30°C. They are stable for 24 hours at this temperature.
   2. Urine specimens that require shipment to off-site testing centers must be

shipped overnight with guaranteed delivery within 24 hours. In this case, the specimen may be shipped at 18–30°C. If the specimen will not reach the testing site within 24 hours, it should be stored at 2–8°C until it is shipped to ensure that the specimen storage time at 18–30°C does not exceed 24 hours.

3. Collect and transport endocervical or urethral swab specimens in M4 Culture Transport Medium. The swab should be left in the media to provide visual evidence of specimen inoculation.
4. Swab specimens may be transported at 18–30°C only if the total transport and testing time is less than one hour. Transport the specimens at 2–8°C if testing will be more than one hour from time of collection.
5. Swab specimens that require shipment to off-site testing centers should be shipped at 2–8°C and should be shipped as soon as possible after collection.

c. Specimen Handling and Storage
   1. Urine specimens that will not be processed within 24 hours of collection must be stored at 2–8°C. Storage of urine specimens at 18–30°C for more than 24 hours may result in specimen degradation. These specimens should not be used for testing.
   2. Specimens stored at 2–8°C must be processed within seven days of collection. Specimens that cannot be processed within seven days may be stored at –20°C or colder, for up to thirty days.
   3. Swab specimens that will not be processed upon receipt at the laboratory must be stored at 2–8°C and should be processed within seven days. Specimens that cannot be processed within seven days may be stored at –20°C or colder, and may be stored this way for up to thirty days.

4. Herpes Detection
   a. Obtain cellular scrapings from the base of vesicles with a scalpel, swab or applicator stick.
   b. Rupture a young vesicle, absorb vesicular fluid with a swab and scrape the base of the lesion. Do not draw blood. Do not prepare the specimen collection site with alcohol or iodophors.
   c. If pus is present, clean lesion and discard swab prior to taking the specimen. Place the scrapings or break the swab off into a vial of viral transport medium (blue cap tube labeled "viral transport" or multi microbe media.
   d. Replace cap so swab stick will insert into enter of hole of cap. Return to laboratory. Specimens are processed at an outside reference lab.

RESPIRATORY CULTURES

1. Expectorated sputum
   a. All sputum samples are contaminated to varying degrees with Oropharyngeal secretions. Mechanical rinsing of the mouth immediately before expectoration will reduce the number of contaminating bacteria.
   b. The patient should rinse his mouth with water. The patient should be instructed to cough up material from deep in the lungs and expectorate a single bolus into a sterile, wide-mouth container. This should be done under direct supervision,

preferably when the patient first wakens in the morning. If unable to get specimen by expectoration, contact respiratory therapy. Methods used to facilitate an adequate specimen from patients with non-productive coughs include: ultrasonic nebulization with 10% saline, hydration, chest physiotherapy and postural drainage.

c. Cap the container and deliver to the laboratory immediately, as there is no effective transport medium. Order resp culture. If ASAP results are desired on the Gram stain, it must be noted in the comment section (a call to the lab is helpful).

d. Upon receipt in the laboratory, a Gram-stained preparation is examined microscopically for the presence of inflammatory and epithelial cells. Specimens with $\geq$ 10 squamous epithelial cells are rejected for culture. The nursing unit will be notified by telephone when the sample is unacceptable so that another can be collected without delay.

2. Bronchoscopy, Endotracheal aspirates, and transtracheal aspirates

a. Bronchoscopy specimens include brushing, transbronchial biopsies, or bronchial secretions that are aspirated through the inner channel of the bronchoscope with or without an irrigating solution. In the transtracheal aspiration procedure, a large-bore intravenous catheter is inserted through anesthetized skin and the cricothyroid membrane into the trachea. After the catheter is advanced several centimeters into the trachea, the needle is carefully withdrawn leaving the catheter in place. Material is obtained by applying suction to the catheter with a syringe.

b. Specimens will be cultured regardless of cellular components. Anaerobic culture will be set up on properly collected and transported transtracheal aspirate specimens. A separate order should be entered for an anaerobic culture.

c. Clearly indicate all other collection methods (e.g., transtracheal aspirates, etc.) These will be prompted for when an order for respiratory culture is ordered.

THROAT CULTURES

1. Generally, throat cultures will be routinely processed for the recovery of beta hemolytic Strep only. Bacteria other than beta hemolytic Streptocci do not cause primary acute pharyngitis. Staphylococci may cause tonsillar abscesses. *H. influenzae* constrictive epiglottis and *Corynebacterium diphtheriae* will cause a membranous pharyngitis
2. A well taken throat swab is essential. Use the Star swab with modified Stuarts. The tongue should be depressed while the swab is rubbed vigorously over each tonsillar area and the posterior pharynx. Any exudates should be touched, and care should be taken to avoid the tongue and uvula. Do not break the transport media ampule.
3. The laboratory offers two screening tests for beta Strep detection. If rapid results are required (within ½ hour), order the "Rapid Beta Strep screen". Since only a 70% sensitivity rate is achieved with the rapid test, all negative screens will be backed up by the culture method. All other Strep screens will be done by the culture method. Results are available within 24 – 48 hours.

4. Cultures for recovery of *Corynebacterium diphtheriae* require special media. Arrangements must be made with the laboratory before ordering this culture. Culture recovery of *Bordetella pertussis* requires prior laboratory notification. Nasopharyngeal swab is recommended instead of a "cough plate". Regan Lowe plates must be inoculated immediately at bedside.

THROAT FOR GC – see Genital

SPINAL FLUID SPECIMENS

1. Surgical prep and collection by physician required. Lumbar puncture must be performed under conditions of strict asepsis. Specimens are transported in sterile screw-cap tubes. Handle as Emergency specimen: hand carry to laboratory.
2. At least one tube (second or third collection) must be sent to bacteriology first, before other studies such as cell count and chemistries are done. Obtain "as much as possible": 4 – 5 ml is optimal for adults, 0.5 – 1.0 ml in children (additional fluid may be required if other tests are ordered).
3. Order routine culture and specify CSF as the source. A Gram stain will be done STAT on all spinal fluids.
4. If cryptococcal antigen or viral studies are desired, order the appropriate tests.

STERILE BODY FLUID SPECIMENS

1. Skin contamination for all specimens obtained by needle aspiration should follow the procedure outlined for blood culture.
2. Fluids which collect in pericardial, pleural, peritoneal and synovial spaces must be aspirated with the utmost precaution to avoid introducing microorganisms and to avoid contamination of the specimen. Direct aspiration into a syringe is recommended. If the material cannot be aspirated into a syringe, it should be placed into a sterile tube or container. Labeled specimens must be transported without delay to the laboratory.
3. Order the aerobic culture. Clearly indicate the specific source. Anaerobic culture will be performed only upon request. A second order for the anaerobic culture must be entered if anaerobic culture desired. A STAT Gram stain will be done on all normally sterile body fluids.

STOOL SPECIMENS

1. For culture
   a. Feces should be passed directly into a clean, wide mouth container with a tight, leak-proof cover. Feces may also be collected from a sterile bedpan; however, the specimen is unsatisfactory if there is any contamination with urine or water.

Those portions of the stool which contain pus, blood, or mucus should be submitted for examination. Fecal specimens should be at least 50 grams (walnut sized) if solid, 20 ml if fluid. Transport feces in a screw-capped specimen cup.

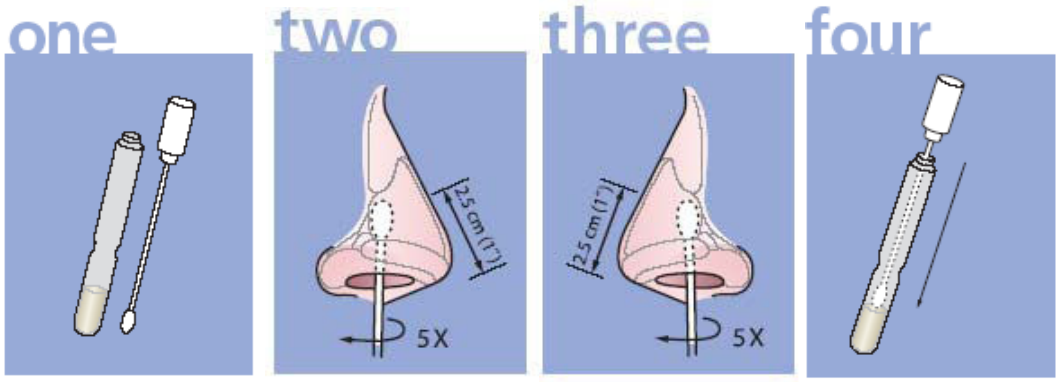
b. If the specimen is collected off site, it should be transferred immediately to a Cairy Blair vial.
c. If stool is not readily obtainable, a rectal swab may be submitted. The swab is passed beyond the anal sphincter, carefully rotated and withdrawn.
d. Order a stool culture. If a stool for WBC is needed, a separate order must be entered.
e. If a *Clostridum difficile* toxin test is ordered, it must also be ordered separately.
f. Stools will be cultured for the presence of *Salmonella sp*., *Shigella sp*., *Campylobacter sp*., *Aeromonas, Plesiomonas* and *Escherichia coli* H7:0157. Stool will not be checked for *Yersinia enterocolitia* or vibrios unless specifically requested. If other organisms are suspected, that fact must be noted under comments so appropriate techniques can be employed.
g. Stool specimens for culture should be transported immediately at room temperature to the laboratory.
h. It is recommended no more than one specimen per day be submitted for culture. It is not recommended to culture stool specimens from patients who have been hospitalized for more than 3 days.

ANAL SWAB FOR GC – see GENITAL CULTURE

VRE SCREEN

Obtain rectal swab with aerobic collections kit.

MRSA Screen



1. Use aerobic culture collection kit
2. Carefully insert the swab into the patient's nostril. The swab tip must be inserted up to 1 inch from the edge of the nares or until resistance is met. (this might be slightly less for children). Roll the swab 5 times.
3. Insert the same swab in the second nostril and repeat the sampling as described

above

4. Return swab to the container. At the patients bedside label the container with a patient label. Write the date and time of collection, and your initials on the label.

OVA AND PARASITE EXAM

1. Procedures for recovery of intestinal parasites should always be performed before barium is used for radiological examination. Stool specimens containing barium are unacceptable for examination. There are also certain substances and medications that interfere with the detection of intestinal protozoa, such as mineral oil, bismuth, antibiotics, antimalarials, and nonabsorbable antidiarrheal preparations. After administration of any of these compounds, parasitic organisms may not be recovered for a week to several weeks.
2. Fecal specimens should be collected in a clean container. The specimen should not be contaminated with water or urine because water may contain free-living organisms that can be mistaken for human parasites and urine may destroy motile organisms.
3. ParaPaks are supplied by the laboratory. Collect stool specimen as described above. Collect stool specimens with the spoon, especially from an area that is bloody or slimy, and place stool specimen in vials until liquid reaches the "fill line". Mash the specimens in the vial until well mixed with the fluid. Replace the cap and spoon on vials. Be sure caps are tight. Shake hard until mixture looks like soup.
4. Examination of liquid specimens should occur within 30 minutes of passage. If this is not possible, then the specimen should be placed in the ParaPak. Soft specimens should be examined within approximately one hour of passage or placed within the ParaPak.
5. It is recommended that normal examination for stool parasites before therapy include three daily specimens. When a patient is suspected of having intestinal amebiasis, six specimens may be recommended. The number of specimens recommended for post-therapy examination is also three. However a patient who has received treatment for a protozoan infection should be checked 3 – 4 weeks after therapy and those treated for Taenia infections, 5 – 6 weeks after therapy.
6. A series of three specimens, as indicated above, should be submitted on separate days, every other day if possible. It is inappropriate for multiple specimens to be collected on the same patient on the same day.
7. The following summary is a guide to the types of samples which may be helpful in specific situations.
    a. Amebiasis – Loose, watery, mucous or bloody stool for Entamoeba histolytica requires immediate examination. Promptness is important because amoebic trophozoites lose motility progressively and degenerate within a short time. No specimen older than one hour will be examined for motile amoebae. Minimum number of adequate examination is 3 stool samples over a six day period.
    b. Cryptosporidium – Must be specifically requested. Use ParaPak kit.
    c. Duodenal Aspirate – For giardiasis or strongyloidiasis: Specimens must be kept warm and sent to the laboratory immediately. Prior notification is requested.
    d. Giardia – Minimum – 6 stool samples over a 4 week period. Use ParaPak kit.
    e. Helminths except Pinworm – Minimum – 3 stool samples over a 6 day period.

f. Pinworm prep – Obtain a pinworm prep kit from the laboratory. Collect in early AM before rising. Place sticky paddle over perianal (anal folds) region with adhesive side facing skin. Place exposed paddle in specimen container, label specimen and transport to lab. The sticky paddle collects the eggs deposited by the adult pinworm (Enterobius vermicularis) while the patient sleeps. Use gloves and wash thoroughly after collecting because the eggs are viable.
g. Sigmoidoscopic material – Collect in trap. Keep warm. Submit to laboratory immediately.

URINE CULTURES

1. Voided urine is often contaminated by bacteria from the urethra and from external genitalia. However, carefully collected, midstream, voided specimens from uninfected patients generally contain fewer than 104 bacteria per ml, whereas bacteria counts of 105 or more organisms per ml of urine are usually associated with infection.
2. Urine specimens may be collected by clean-voided midstream technique, by diagnostic catheterization, by suprapubic aspiration, or from indwelling catheter.
3. Urinary catheter tips are not cultured because the tip is contaminated as it is removed from the urethra. Urine samples collected from indwelling catheter bags are not accepted for culture. Stagnant urine in a catheter bag will be overgrown with bacteria, making culture results misleading and insignificant.
4. Anaerobic cultures will not routinely be set up on clean-catch urine specimens. Urinary tract infections are rarely caused by anaerobic bacteria. If anaerobic infection is suspected, a suprapubic bladder aspiration should be performed.
5. Preparation of Patient and Collection
   a. Indwelling catheter – The catheter port on tubing should be cleaned with an alcohol pad. Collect using a sterile syringe and needle. Place in a sterile urine container. Label and deliver immediately to the laboratory. Order a urine culture using the collection code for catheter.
   b. Clean Voided Urine – Collection must be the responsibility of an adequately trained individual. The cleansing procedures must remove contaminating organisms from the vulva, urethra meatus, and related perineal area so that bacteria found in urine can be assumed to have come from the bladder and urethra only.
6. Collection Instructions
   a. General instructions
      1. Obtain midstream collection kit. (BD vacutainer with gray tube kit)
      2. Cleanse the penis or vagina area with both of the enclosed two towelettes.
      3. Use one forward to backward stroke per wipe and discard.
      4. Retract foreskin on males. Spread the labia on females and keep spread apart until urine is voided.
      5. The patient now voids.
      6. After the first 20 – 25 ml has been passed, a specimen (20 – 25 ml) is caught directly in the sterile container without stopping the stream.
      7. Transferring the urine to gray top preservative tube.
         - Peel back label on cap to expose the integrated sampling device

- Place the tube into the cavity on the cap with the stopper down. Advance tube over the puncture point to pierce the stopper. Fill the gray top tube to the fill line (3ml).
- Hold tube in position until filled. The tube vacuum will fill the tube.
- Replace label over the integrated sampling device entrance hole and reseal.
- Label sample with patient identifiers, date & time of collection, method of collection (e.g. cath, midstream) and your initials.

8. Order a urine culture. Transport the specimen immediately to the laboratory.

b. Instructions for Infants
   1. Plastic bags may be attached after careful preparation, as above.
   2. The bags should then be attached so that the urine specimen can be collected immediately after it is voided.
   3. If the patient has not voided within 30 minutes after the collection apparatus has been attached, it should be removed, the patient rescrubbed and a new collection device attached.
   4. Order a urine culture. Indicate the specimen was collected using a urine bag – transport immediately to the laboratory.

Note: All urine specimens for culture must be delivered to the laboratory immediately. If transport is delayed place on ice or refrigerate.

WOUND CULTURES

Surface Lesions

a. To remove accumulated drainage and transient skin flora, which may lead to ambiguous results, cleanse wound with sterile saline or water. It is imperative that the surface lesion be opened and the advancing edge of the lesion firmly sampled. If wound is dry, swabs may be moistened with sterile saline. Never submit a dry swab that has been carelessly rubbed over a surface lesion.

b. Please submit an additional swab for optimum Gram stain preparation. Replace swabs in culturette tube. Swabs are to be kept at room temperature and delivered to the lab with a minimum of delay. Order an aerobic culture. Note specific anatomic site on requisitions. Surface lesions are unsuitable for anaerobic studies.

RSV (Respiratory Syncytial Virus)

Specimen a. Nasopharyngeal Wash (2 – 3 ml volume)

b. Nasopharyngeal Swab in saline (use flexible rayon tipped applicator)

Influenza A & B **(refer to pages 22-27 for nasopharyngeal collection instructions)**

Specimen
a. Nasopharyngeal Wash (2 – 3 ml volume)
b. Nasopharyngeal Swab in saline (use flexible rayon tipped applicator)

AFB SAMPLES

1. Sputum
   Collect only material brought up from the lungs after a productive cough. Do not collect sputum immediately after mouth wash. A series of 3 – 6 single early morning specimens collected on successive days. Immediate submission to the laboratory after collection is recommended. 5 – 10 ml volume is adequate.
2. Urine
   Entire first morning midstream specimens are recommended. Instruct patient to wash genital region well prior to collection of specimen. Urine for AFB should only be submitted weekdays before 11 am.
3. Surgically collected specimens
   Submit spinal fluids in sterile lumbar collection tubes, other fluids in sterile containers. Pieces of tissue or swabs must be kept in sterile containers without preservatives or fixatives.
4. Gastric specimens
   Prior laboratory notification is necessary. The specimen must be processed promptly because mycobacteria die rapidly in gastric washings. Gastric specimens will routinely be accepted only on weekdays and must be received in the laboratory before 2 pm.
5. Ordering all the above types
   Order AFB culture. Note the source of the specimen. Transport promptly to the laboratory. In house AFB smears are read and reported within 24 hours. AFB cultures are sent out to a reference lab.
   Order blood AFB – this requires prior notification of the laboratory since special vials are required.

MYCOLOGY SAMPLES

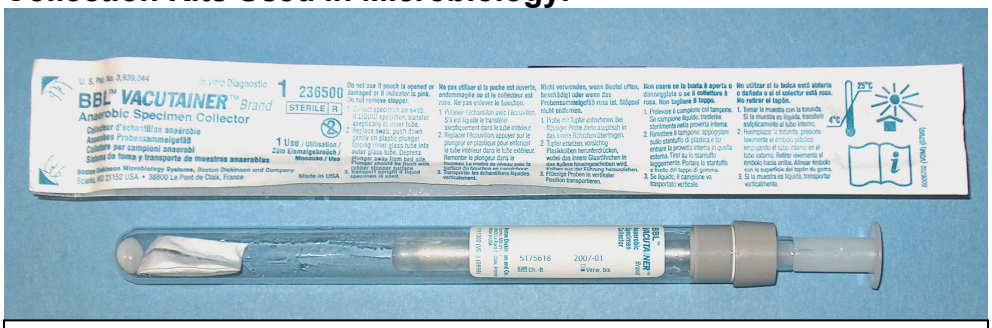
1. Skin - Clean site with 70% ethanol to help eliminate surface contaminants. Using a scalpel, skin scrapings should be made from the active periphery of the lesion. Submit scrapings in a sterile Petri dish or container.
2. Nails - Clean site with 70% ethanol. Collect shavings and material under the nail plate. Scrapings should be deep enough to assure acquiring recently invaded tissue. Submit nail clippings and scrapings in a sterile Petri dish or container.
3. Hair - Use forceps to pluck involved hairs from the edges of the patches. Submit hair, including shaft, in a sterile Petri dish or container.
4. Other - Collect and submit specimens as described for specific type. Specimens associated with the systemic and deep seated mycoses are obtained from a wide variety of sources. They should be obtained, whenever possible, under aseptic conditions and in sufficient quantity for both microscopic and cultural examinations.

5. Blood - This requires prior laboratory notification since additional media must be inoculated at bedside. Order blood fungus culture.

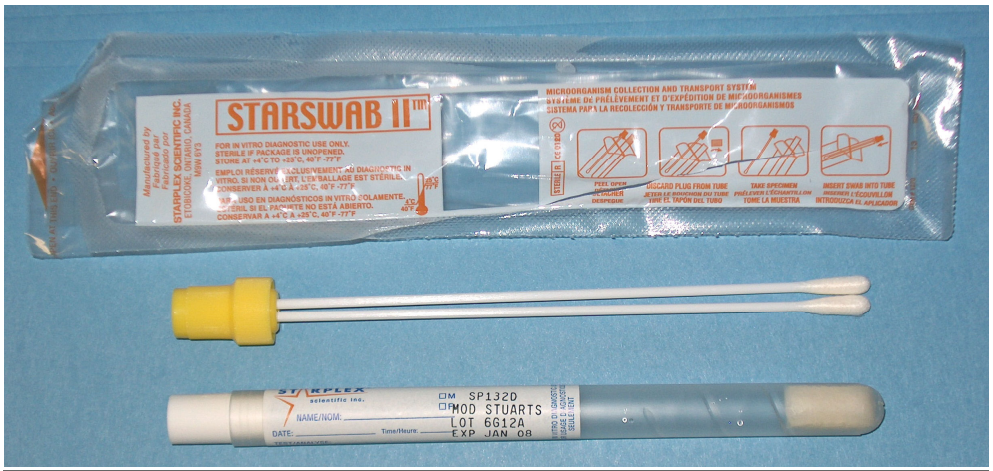
ROUTINE SUSCEPTIBILITY TESTING

1. MIC panels are performed on the common, rapidly growing pathogens including the Enterobacteriaceae, Psuedomonas spp. and other miscellaneous Gram-negativebacilli, Staphylococcus aureus, other Staphylococcus spp. when appropriate and Enterococcus spp. A quantitative microdilution technique is used, and requires 18 – 24 hours once an isolate is obtained in pure culture.
2. Haemophilus influenzae is tested by the Kirby-Bauer method against a limited panel of antibiotics. All Haemophilus influenzae, Neisseria gonorrhoeae, and Enterococci from critical isolates are tested for production of beta lactamase. All Streptococcus pneumoniae isolates are routinely screened for penicillin susceptibility. Strep pneumoniae isolates from CSF and blood are also tested against 3rd generation cephalosporins.

Collection Kits Used in Microbiology:



Anaerobic Culture Collection Kit



Aerobic Culture Collection Kit:
Routine, Throat, Strep Screen A & B, Rapid Beta Strep, Genital

Aimes/Charcoal Swab:
Genital & GC Cultures

CVM Transport: Viral Collections

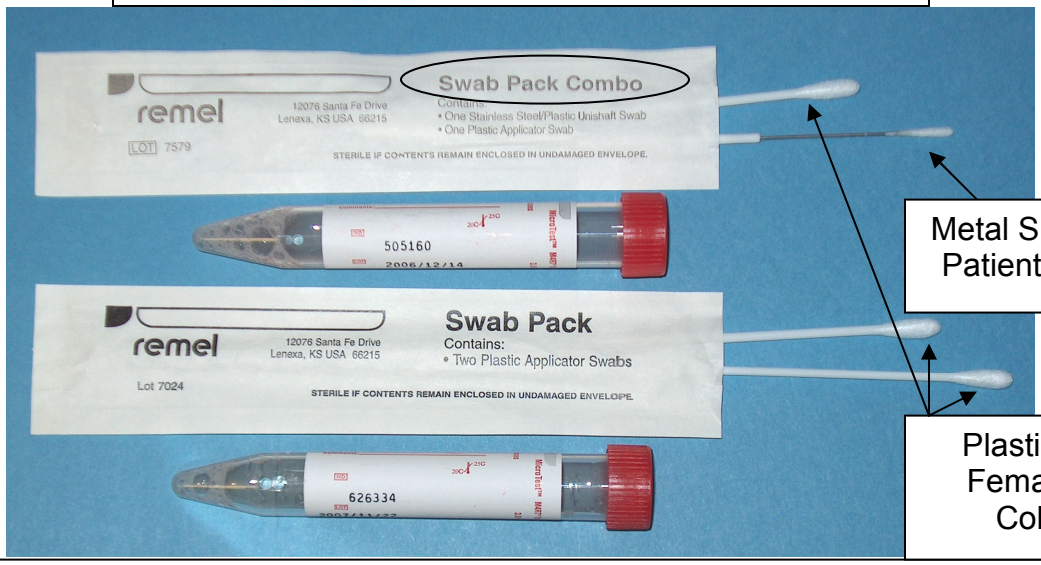
Specimen Collection and Transportation of Microbiology Specimens

Sterile Saline:
Wet Mount Collections

Gray and Pink Topped Cups for O&P analysis

Orange Topped Cup for Stool Culture

Stool Collection Kit:
Ova & Parasite and Stool Culture

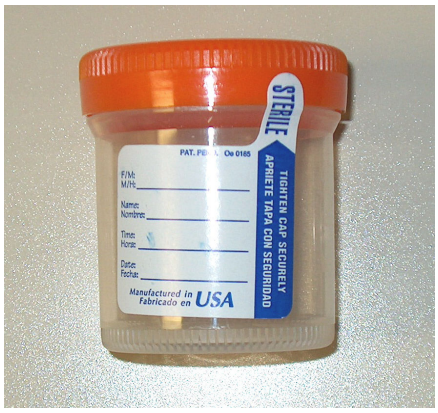


Metal Shaft for Male Patient Collections

Plastic Shaft for Female Patient Collections

M4RT Transport Media for Chlamydia Collections, Male and Female Patients
Note: Red Capped media tubes are stored at room temp and blue at refrigerated temp. After collection, both are refrigerated.

Urine Cup Kit with C&S Preservative Tube for Midstream Specimens



Sterile Specimen Collection Cup

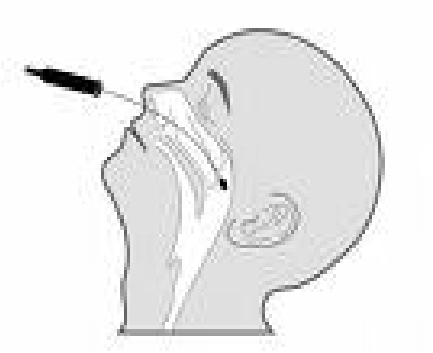
eSwab Collection kit

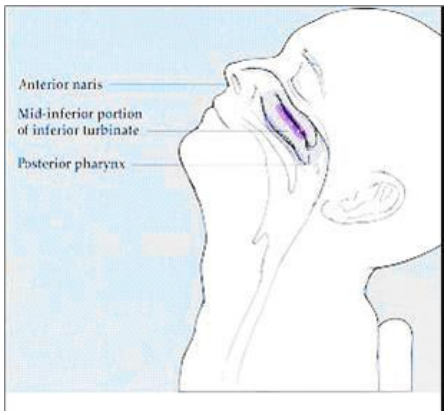
1. Open eSwab sample collection kit.
2. Open swab pouch and collect sample
3. Aseptically unscrew cap from tube.

4. Insert swab into tube and break the swab shaft at red line.
5. Replace cap securely
6. Label tube properly with patient information.
7. Send to lab with proper orders.

NASOPHARYNGEAL SPECIMEN COLLECTION- TO BE DONE BY TRAINED PERSONNEL UNDER NURSING DEPARTMENT SUPERVISION

A. Nasopharyngeal Swab Method





(Patients head should be inclined back as shown above)

Materials for Nasopharyngeal Swab Collection

- Flexible ,soft or aluminum wire nasopharyngeal with synthetic tip
- 1 ml saline tube (for Rapid Influenza A & B)
- M4 Viral Transport Media (for viral culture or H1N1)

Procedure:

1. Insert swab into one nostril.
2. Rotate swab over surface of posterior nasopharynx.
3. Withdraw swab from collection site; insert into saline transport tube or M4
4. Repeating procedure for the second nostril will deliver optimal combined sample.
5. After collection, immediately transport specimen to the laboratory for viral testing and viral antigen detection. If transport is delayed, place specimen on ice or in refrigeration.

B. Nasopharyngeal Wash

Materials:

- 3-5ml syringe with 22 inch sterile NG tube 8-french (length and diameter of syringe and tubing as appropriate for infant, child or adult.)
- Viral transport M4 media for H1N1, or saline for RSV
- Specimen container

Procedure:

1. Fill syringe with saline; attach tubing to syringe tip.
2. Quickly instill saline into nostril.
3. Method A - Aspirate the recoverable nasopharyngeal specimen. (Recovery must occur immediately, as the instilled fluid will rapidly drain.)
4. Method B – (alternate) In appropriate cases, patients may tilt head forward to allow specimen to drain into suitable sterile container.
5. (if aspirated) Inject aspirated specimen from syringe into sterile specimen container.
6. Repeating procedure for the second nostril will deliver optimal combined sample.
7. Label specimen and transport to Laboratory immediately. If transport is delayed refrigerate or place on ice.

C. Vacuum-assisted Nasopharyngeal Aspirate method

Materials:

- Suction outlet
- Sterile suction catheter
- Mucus trap (i.e., Lukens tube)
- Viral transport M4 for H1N1, or saline for RSV or INFAB

1. Attach mucus trap to suction outlet and catheter, leaving wrapper on suction catheter; turn on suction and adjust to suggested pressure.

Patient age	Catheter size (French) **	Suction Pressure
Premature infant	6	80-100mmHg
Infant	6	80-100 mmHg
Toddler/Preschooler	8	100-120 mmHg
School age	8	100-120 mmHg
Adolescent/Adult	8	120-150 mmHg

** To determine length of catheter tubing, measure distance from tip of nose to external opening of ear.

2. Without applying suction, insert 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 naris and external opening of the ear.
3. Apply suction. Using a rotating movement, slowly withdraw catheter. **NOTE**: Catheter should remain in nasopharynx for a minimal period of time, not to exceed 10 sec.
4. Hold trap upright to prevent secretions from going into pump.
5. Rinse catheter (if necessary) with approximately 2.0 ml M4 media.
6. After collection, immediately transport specimen to the laboratory. Place specimen on ice if delayed.

Reference:

BD Diagnostics insert: 2-2452 February 2005

Affected Departments: Laboratory Services, Laboratory Services