BASIC PRINCIPLES OF SPECIMEN COLLECTION

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The goal of the specimen collector must be to maintain the viability of the organisms with minimal contamination

**Basic principles of specimen collection are**

- If possible, collect the specimen in the acute phase of the infection and before antibiotics are administered.
- Select the correct anatomic site for collection of the specimen.
- Collect the specimen using the proper technique and supplies with minimal contamination from normal biota (normal flora).
- Collect the appropriate quantity of specimen.
- Package the specimen in a container designed to maintain the viability of the organisms.
- Label the specimen accurately with the specific anatomic site and the patient informations.
- Transport the specimen to the laboratory promptly.
SPECIMEN COLLECTION GUIDELINES

**BLOOD CULTURE** Disinfect skin with alcohol and iodine, blood culture media set (aerobic and anaerobic, bottles) or vacutainer tube with SPS (sodium polyanethol sulfonate) /adults, 20 ml per set; children 5 to 10 ml per set

**BODY FLUIDS** (Abdominal, amniotic, ascites, bile, joint, pericardial, pleural), disinfect skin before needle aspiration sterile, screw-cap tube, ≥1 ml

**CEREBROSPINAL FLUID** Disinfect skin before aspiration, use sterile screw-cap tube/bacteria ≥1 ml, fungi, ≥2 ml, AFB ≥2 ml, virus ≥1 ml

**EAR**
1. **Inner ear**: clean ear canal with mild soap, aspirate fluid, with needle if eardrum intact; Or use swab if, eardrum ruptured, sterile, screw-cap tube or anaerobic transport system

2. **Outer ear**: remove debris or crust from ear canal with saline moistened swab; rotate swab in outer canal, swab transport system
**FECES SAMPLES**
- Collect directly into container, avoid contamination with urine, clean, leakproof container or enteric transport system.

- A rectal swab can be submitted for bacterial culture but it must show feces. A single specimen is not usually sufficient to exclude bacteria or parasites.

- If a bacterial infection is suspected, three specimens should be collected, one a day for 3 days.

- If parasites are suspected, three specimens collected within 10 days should be sufficient for microscopic detection of ova and parasites.

- The newer methods detect parasite antigens, and one sample is usually sufficient.

- Commercial systems are available with preservatives for bacteria and parasites. The appropriate ratio of stool to preservative is 1:3.
FUNGAL SCRAPINGS
- Wipe nails or skin with alcohol, use clean, screw-cap container.
  A) hair/nails/skin hair: 10-12 hairs with shaft intact
  b) nails: clip affected area
  c) skin: scrape skin at outer edge of lesion

GENITALIA
A. CERVIX/VAGINA: Remove mucus before collection; do not use lubricant on speculum; swab endocervical canal or vaginal mucosa, swab transport system or JEMBEC transport system

B. URETHRA: Flexible swab inserted 2-4 cm into urethra for 2-3 sec or collect discharge, swab transport system or JEMBEC transport system

LESION/WOUND/ABSCESS: Wipe area with sterile saline or alcohol, superficial swab along outer edge, swab transport system, deep aspirate with needle and syringe, anaerobic transport system
RESPIRATORY TRACT: Lower bronchial specimens
- sputum, rinse mouth or gargle with water, instruct to cough deeply into container. Or the patient should rinse the mouth with water and expectorate with the aid of a deep cough directly into a sterile container (expectorated sputum).

- Patients with dentures should remove the dentures first. A single specimen should be adequate for detection of bacterial LRT infection. If fungal or mycobacterial infections are possible, three separate early morning specimens (collected on successive days) are appropriate.
- Specimens may be collected through aerosol-induction in which the patient breathes aerosolized droplets of a solution that stimulates cough reflex (induced sputum).
RESPIRATORY TRACT: URT

1. **NASAL**: Insert premoistened swab with sterile saline 1 inch into nares, swab, use transport system
2. **NASOPHARYNX**: Insert flexible swab through nose into posterior nasopharynx, rotate for 5 sec, swab transport system or direct inoculation to media
3. **THROAT SWAB**: Posterior pharynx, tonsils, and inflamed areas, swab transport system

**TISSUE**: Disinfect skin; do not allow tissue to dry out; if necessary, moisten with sterile saline, use transport system or sterile screw-cap container
Collection of throat swab

Rub the swab across the tonsillar areas and the posterior pharynx, specifically targeting any inflamed areas.
Lumbar puncture for CSF collection

- The best site for puncture is inter space between 3 and 4 lumbar vertebrae
  (Corresponds to highest point of iliac crest)

The Physician should wear sterile gloves and conduct the procedure with sterile precautions. The site of procedure should be disinfected and sterile occlusive dressing applied to the puncture site after the procedure.
URINE SAMPLES
1. **CLEAN-CATCH MIDSTREAM**: Clean external genitalia; begin voiding and after several ml have passed; collect midstream without stopping flow of urine, sterile, screw-cap container, collect 2-3 ml. The first portion of the urine flow washes contaminants from the urethra, and the midstream portion is more representative of that in the bladder.

2. **CATHETER CLEAN URETHRAL AREA**, Insert catheter, and allow first 15 ml to pass; then collect remainder sterile, use screw-cap container or urine transport kit

3. **INDWELLING CATHETER**, Disinfect catheter collection port, aspirate 5-10 ml with needle and syringe, use screw-cap container or urine transport kit

4. **SUPRAPUBIC ASPIRATE**, Disinfect skin, aspirate with needle and syringe through abdominal wall into full bladder, use sterile, screw-cap container or anaerobic transport system
Patient specimens or culture isolates must be **TRIPLE PACKAGED BEFORE BEING SHIPPED.**

The material is placed into a **Primary receptacle** that must be watertight. Absorbent material is placed around the primary receptacle, and it is then placed into a **Secondary container** that is also watertight. The secondary package is sealed and placed into a sturdy **Outer container** constructed of fiberboard. Specific instructions must be followed for labeling the container as **“hazardous material.”**
The proper shipping names "Biological Substance, Category B"; "Clinical Specimen"; and "Diagnostic Specimen" are authorized until December 31, 2006. From January 1, 2007 only the proper shipping name "Biological Substance, Category B" will be authorized.

† If multiple fragile primary receptacles are placed in a single secondary packaging they must be either individually wrapped or separated to prevent contact.

Note: Follow package manufacturer's closure instructions.
**SPECIMEN PRIORITY**

A four-level scheme of prioritization may be used based on the critical nature of the specimen or potential for specimen degradation.

**LEVEL 1:** Critical/invasive (amniotic fluid, blood, CSF, heart valves, pericardial fluid)

**LEVEL 2:** Unpreserved (body fluids, bone, drainage from wounds, feces, sputum, tissue)

**LEVEL 3:** Quantitation required (catheter tip, urine, tissue for quantitation)

**LEVEL 4:** Preserved (feces in preservative, urine in preservative, swabs in holding medium, (aerobic and anaerobic)

Specimens should be transported to the laboratory ideally within 30 minutes of collection, preferably within 2 hours.
MACROSCOPIC OBSERVATION

Notations from the macroscopic observation should include the following:

• stool consistency (formed or liquid)
• blood or mucus present
• volume of specimen
• fluid: clear or cloudy
  areas of blood and mucus are selected for culture and direct microscopic examination.

Anaerobic cultures may be indicated if gas, foul smell, or sulfur granules are present.

The diagnosis is evident if adult helminths or tapeworm proglottids are present in the specimen.
**MICROSCOPIC OBSERVATION**

A direct microscopic examination is a useful tool that provides rapid informations.

(1) it can be used to determine the quality of the specimen. Sputum specimens that represent saliva rather than lower respiratory secretions can be determined by the quantitation of WBCs or epithelial cells.

(2) it can give the indication of the infectious process involved. Gram stain of a sputum specimen revealing WBCs and Gram-positive diplococci is indicative of *streptococcus pneumoniae*.

(3) the routine culture workup can be guided by the results of the smear.

(4) it can dictate the need for nonroutine or additional testing. The presence of fungal elements in a specimen for bacterial culture will alert the technologist to notify the physician to request a fungus culture.
CULTURE MEDIA SELECTION

- The selection of media to inoculate is based on the type of specimen submitted and the organisms likely to be involved in the infectious process.

- Specimens in which fastidious pathogens are more likely involved require media with appropriate nutrients to aid in their recovery.

- Specimens that are collected from a site containing normal biota will require types of media to diminish the normal biota while allowing the pathogens to be detected.

  **The routine primary plating media include**

- Nonselective agar plate
- Enriched medium for fastidious organisms
- Selective and differential medium for enteric gram negative bacilli for most routine bacterial cultures
- Selective medium for gram-positive organisms for specimens in which mixed gram-positive and gram negative bacteria are found