Introduction to Clinical Microbiology

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BASIC PRINCIPLES OF SPECIMEN COLLECTION

The laboratory can make accurate and useful determinations only if a specimen has been collected properly. The specimens to be analyzed are likely to contain living organisms

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

The following basic principles of specimen collection are fundamental to ensuring appropriate specimen management:

• 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 and avoid hazards that result from leakage.
• Label the specimen accurately with the specific anatomic site and the patient informations.
• Transport the specimen to the laboratory promptly or make provisions to store the specimen in an environment that will not degrade the suspected organism(s).

Collection Procedures

- Specimens for microbiology cultures should be collected in sterile containers, Except for stool specimens, which can be collected in clean, leakproof containers.

- In general, swabs are not recommended for collection because they do not provide sufficient quantity, are easily contaminated, and can become dried out, leading to a loss of organisms.

- Swabs are appropriate for specimens from the upper respiratory tract, external ear, eye, and genital tract.
- The tips of swabs may contain cotton, Dacron, or calcium alginate. Cotton-tipped swabs tend to have excessive fatty acids, which may be toxic to certain bacteria.

- Swab collection systems are available that provide **transport media** and protect the specimen from drying.

- Lesions, wounds, and abscesses present many problems to the microbiology laboratory. The specimen is collected from the advancing margin of the lesion and should be collected by needle aspiration rather than by swab. Before the specimen is collected, the area should be cleansed to eliminate as much of the commensal flora as possible. Aspirated material should be placed into a sterile tube or transport vial and not "squirted" onto a swab.

Specimen Collection Guidelines

- **Blood culture** Disinfect skin with alcohol and iodine, Blood culture media set (aerobic and anaerobic bottles) or Vacutainer tube with SPS (*SPS,* sodium polyanethol sulfonate). The volume of blood collected /**adults, 20 ml**; **children 5 to 10 ml**

- **Body fluids** (abdominal, amniotic, ascites, bile, joint, pericardial, pleural), Disinfect skin before needle aspiration Sterile, screw-cap tube , volume is ≥1 ml
- **Catheter tips, IV** (Foley catheters not cultured), Disinfect skin before removal Sterile, screw-cap container

- **Cerebrospinal fluid** Disinfect skin before aspiration, use screw-cap tube. For 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; use swab if eardrum ruptured.
2. Outer ear: Remove debris or crust from ear canal with saline moistened swab; rotate swab in outer canal. use Transport system

- **Eye**

1. Conjunctiva Sample both eyes; use separate swabs moistened with sterile saline
Swab put in transport system

2. Corneal scrapings with local anesthesia, scrape with sterile spatula and inoculate directly to agar. Agar available at bedside

- **Feces** Collect directly into container, avoid contamination with urine, Clean, leak proof

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.

- **Fungal scrapings** Wipe nails or skin with alcohol Clean, screw-cap container

a) **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** (male) : Flexible swab inserted 2-4 cm into urethra for 2-3 sec or collect discharge, Swab transport system or JEMBEC transport system

- **Respiratory tract: LRT**

Bronchial specimens

- 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 lower respiratory tract infection. If fungal or mycobacterial infections are possible, three separate early morning specimens (collected on successive days) are appropriate.

- These 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 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, Anaerobic transport system or sterile screw-cap container

- **Urine**

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, 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, screw-cap container or urine transport kit

3. Indwelling catheter, Disinfect catheter collection port, aspirate 5-10 mL with needle and syringe Sterile, screw-cap container or urine transport kit

4. Suprapubic aspirate, Disinfect skin, aspirate with needle and syringe through abdominal wall into full bladder, Sterile, screw-cap container or anaerobic transport system

Shipping Infectious Substances

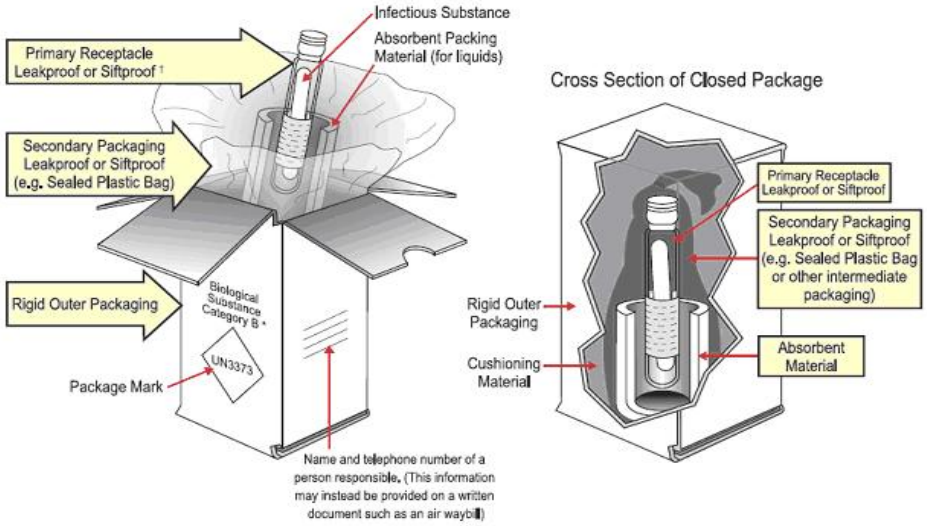
- The shipment of clinical specimens and cultures of microorganisms is governed by a complex set of national and international guidelines issued by the U.S. Department of Transportation (DOT) and the U.S. Postal Service.
- An infectious substance is assigned to a risk group with a number from 1 (low risk) to 4 (high risk).
- Patient specimens or culture isolates must be triple packaged before being shipped. Specific instructions must be followed for labeling the container as "**Hazardous Material**."

Types of Anticoagulants

- Anticoagulants are used to prevent clotting of specimens, including blood, bone marrow, and synovial fluid. The type of anticoagulant used and the concentration are important because some anticoagulants have antimicrobial properties.

- **Sodium polyanethol sulfonate (SPS)** is the most common anticoagulant used for microbiology specimens.
- **Heparin** is another acceptable anticoagulant and is often used for viral cultures and for isolation of *Mycobacterium* spp. from blood.

- **Citrate and ethylenediamine tetracacetic acid (EDTA)** should not be used for microbiology specimens.



* *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 RECEIPT AND PROCESSING

Specimen Priority

Appropriate specimen management should include guidelines for prioritizing the handling of specimens. A four-level scheme of prioritization may be used based on the critical nature of the specimen or potential for specimen degradation.

Lists clinical samples and the ways each can be prioritized in a four-level system.

Level 1: Critical/invasive (Amniotic fluid, Blood, Brain Cerebrospinal fluid, 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)

Specimen Preparation

- Most specimens arrive in the laboratory in one of three forms: swab, tissue, or fluid. Specimens such as sterile body fluids, pus, urine, and sputum are inoculated directly onto selected media.

- Large volumes of sterile body fluids (peritoneal, pleural, continuous ambulatory peritoneal dialysis [CAPD] are concentrated to increase the recovery of bacteria. The specimen can be centrifuged for 20 minutes at 3000rpm . The sediment is then used to inoculate media and to prepare smears.

- If the specimen consistency is thin enough to avoid filter clogging, filtration with a Nalgene filter unit can be performed.

- Some laboratories place the swab into 0.5 to 1.0 mL of broth or saline and then vortex the specimen to loosen material from the swab and produce an even suspension of organisms.

- Tissues can be prepared for culture by **homogenization,** in which the tissue is ground in a tissue grinder. Because homogenization can destroy certain organisms, in some situations the tissue is minced with sterile scissors and forceps into small pieces suitable for culture.