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Specimen Collection and Handling

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Cytology is often performed in the investigation of patients with suspected infection. This is because cytology provides a safe, rapid, and cost-effective means to diagnose many infections, allowing correct management to be instituted. Moreover, cytology permits appropriate ancillary investigations to be undertaken either at the time of specimen procurement (e.g., triage of material for microbiology culture) (Fig. 2.1) or after material is processed (e.g., special stains for microorganisms using cell block sections). The use of ancillary investigations (e.g., cell block preparation, special stains, immunocytochemistry, molecular studies) in cytopathology is covered in Chap. 14. Appropriate specimen collection and handling are essential to the diagnosis of infectious diseases. Sample collection for pathogens needs to be coordinated. This requires effective communication with clinicians, radiologists, and microbiologists, particularly if an uncommon infectious process is suspected. Universal precautions must be followed when handling specimens.

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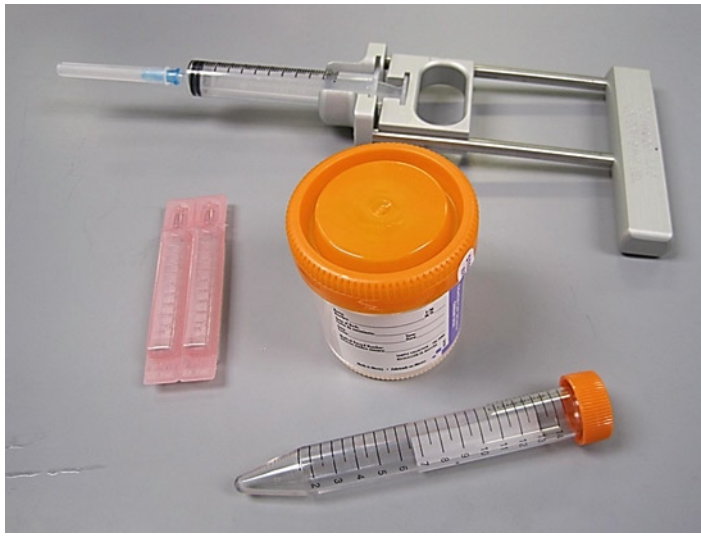

FIG. 2.1. Aspirated material obtained by FNA can be submitted by the cytologist for microbiology studies such as culture using suitable sterile containers or tubes (*orange* capped examples shown), along with some sterile saline (two *pink* sterile saline solution packs shown) or placed on moistened filter paper. Bacteriostatic saline or formalin should not be used. Drying of specimens during transport may compromise the recovery of viable organisms. Alternatively, sterile culture media can be used at the time of specimen collection to directly transfer specimens obtained by FNA.

- *Virology.* Methods used to diagnose viral infections include antigen detection, virus isolation, serology, and molecular techniques. While antigen detection is rapid, there may be many false positives and negatives. The primary method to diagnose many viruses (except EBV, for example) is with viral isolation in cell culture, but this can take weeks. Specimens submitted to culture viral agents may require special transport medium, although media designed for transporting bacteria are often acceptable. Viral detection in culture is carried out by visual examination of culture cells for viral cytopathic effect (CPE)

TABLE 2.1. Characteristic viral inclusions observed in culture.

Virus	Nuclear inclusions	Cytoplasmic inclusions	Syncytia
Herpes simplex virus	Yes	No	Yes
Cytomegalovirus	Yes	Yes	No
Influenza	No	No	No
Respiratory syncytial virus	No	No	Yes
Adenovirus	Yes	No	No
Measles	Yes	Yes	Yes

(Table 2.1). Serology (detection of circulating antibodies) relies on demonstrating a rise in antibody titers in sequential samples.

- *Bacteriology.* Bacteria are diagnosed by direct examination (Gram and acid-fast staining) and culture (aerobic and anerobic) using specific culture media (Fig. 2.2). Specimens for anerobic culture should ideally be submitted in anerobic containers and immediately transported to the laboratory. A specimen submitted in an anerobic container can also be used for aerobic, mycobacterial, and fungal cultures. Mycobacteria are cultured on both solid (e.g., Lowenstein-Jensen and Middlebrook agars) and broth (e.g., BACTEC system, Mycobacterium Growth Indicator Tube or MGIT) media.
- *Mycology.* Fungi can be identified using direct examination (e.g., calcofluor white fluorescent stain, India ink), antigen detection (e.g., cryptococcal antigen), and fungal culture (e.g., Sabouraud agar). Yeasts (unicellular with budding) form small bacterial-like creamy colonies in culture, whereas molds (multicellular with hyphae) make large fuzzy colonies.
- *Parasitology.* Parasites are usually not cultured. They are usually diagnosed on direct examination using both wet mounts and stained slides (e.g., modified acid-fast stains for *Cryptosporidium*, *Cyclospora*, and *Isospora*). Wet mounts are useful to identify motile trophozoites and cysts (often with iodide added). Serology is of limited use in parasitology.

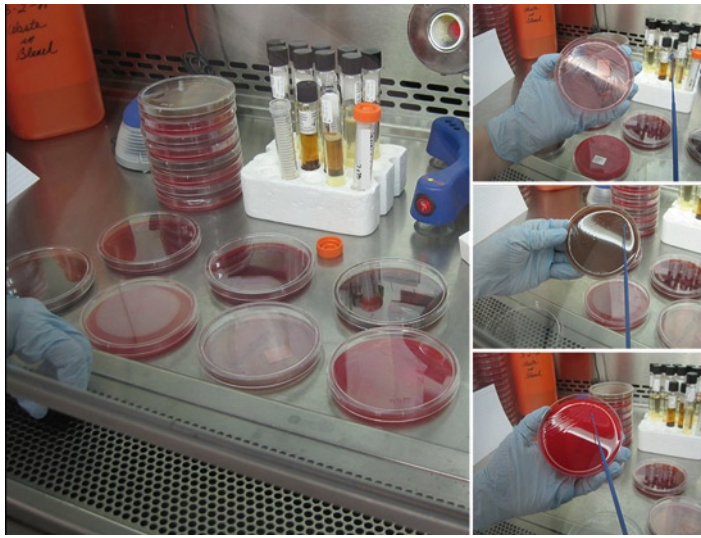

FIG. 2.2. Cytologic specimens received in the microbiology laboratory get plated under a culture hood (*left*) onto various media such as (*top right*) MacConkey agar, (*middle right*) chocolate agar, and (*bottom right*) blood agar. Most pathogens are able to grow on blood agar, which is used to initially culture most specimens. Other culture media like MacConkey agar provide a selective and differential medium for specific bacteria (e.g., Gram-negative bacilli).

Specimen Type

- *Pap test* (*smear*) involves scraping of the cervix with a cervical brush, broom, or wooden/plastic spatula. For anal Pap tests a small brush or cotton-tipped rod is inserted into the anus. Rinsing the collection device or detaching and placing it in a vial containing proprietary preservative fluid (liquid-based cytology) permits material to be processed for ancillary studies (e.g., DNA testing for HPV, gonorrhea, and Chlamydia) and for infections that cannot be reliably identified morphologically. Conventional smears can also be used for ancillary studies (e.g., HPV tests), by scraping material off slides.

- *Wet prep* can be performed at the patient bedside to provide immediate microscopic examination of vaginal specimens (swab or secretions) for the presence of *Trichomonas vaginalis*, clue cells, and yeast in a saline suspension (e.g., 2 mL 0.9% NaCl) with/without potassium hydroxide (20% KOH with dimethylsulfoxide).
- *Tzanck test* is a scraping of an ulcer base to look for infected cells with herpetic changes due to herpes simplex virus (HSV) or varicella-zoster virus (VSV). As these are often prepared by clinicians and received on prepared slides, they are subject to air-drying artifact without residual material to perform immunocytochemistry. Due largely to sampling issues, there is a high rate of false-negative results even when the virus is present.
- *Scrapings*, *swabs, or impressions* may be performed to diagnose oropharyngeal (e.g., *Candida*) as well as conjunctival and corneal (e.g., Herpes simplex keratitis and trachoma) infections. The technique for performing impression cytology of the ocular surface is described in Chap. 12.
- *Washings*, *brushings, and lavage* typically involve an invasive procedure (e.g., bronchoscopy) with infusion and reaspiration of sterile saline solution. Samples can be submitted for cytological evaluation and culture.
- *Fluids* from effusions and other anatomic sites (e.g., CSF, urine, joints) can be shared for cytological evaluation and a variety of microbiology tests (e.g., serology, culture. PCR). Cytologic preparation techniques include cytocentrifuge (larger volumes), cytospins (smaller volumes <5 mL), membrane filtration, liquid-based preparations, and cell blocks. The sensitivity of microscopy can be improved by increasing the concentration of the specimen. Specimens should be prepared soon after collection to prevent degeneration. If this is not possible, refrigeration and/or addition of equal volume of 50% alcohol can be used. At least 2–5 mL is required for bacterial culture and >10 mL for fungi and/or mycobacteria because these latter organisms are generally present in low numbers.
- *Fine needle aspiration* (*FNA*) permits procured material to be immediately evaluated on site for appropriate triage. Aspirated material can be used to make smears and liquid based preparations and cell blocks, or sent for culture, flow cytometry and

molecular studies. Material submitted for culture requires the use of sterile containers into which patient's material is added along with minimal sterile saline. Isolation of pathogens in fine needle aspirates is almost always considered significant and not due to contamination.

Specimen Sites

- *Genital tract.* Collection of genital specimens includes Pap smears, swabs, Tzanck preparations of ulcers, and infrequently FNA. Typically, specimens in female patients to detect pathogens are acquired at the time of performing a Pap test. Many common and uncommon pathogens can be identified on a Pap smear (see Chap. 5). A wet preparation can be used to make a rapid diagnosis of vaginitis. A definitive diagnosis of certain pathogens may require culture, especially in cases of suspected sexual abuse.
- *Urinary tract.* The normal urinary tract is usually devoid of bacteria, except for microflora of the urethral mucosa. Nevertheless, urine can become contaminated with bacteria of the vaginal canal or perineum. For urinary tract infections, a midstream "clean-catch" urine specimen is preferable. A 24-h urine sample is recommended for suspected schistosomiasis. Catheterized urine can be collected, but not urine from catheter bags. Other specimens from the urinary tract that may be required to diagnose infection include suprapubic aspirates (used mainly in neonates and small children), upper tract brushings and washings, urinary diversions (e.g., ileal conduit), and kidney FNA.
- *Respiratory tract.* Sputum is often submitted for the diagnosis of infection. Multiple, early morning specimens improve sensitivity because they harbor pooled overnight secretions, and hence they are more likely to contain concentrated bacteria. All cytopreparatory techniques (pick and smear, Saccomanno, cytocentrifugation, liquid based) are suitable. Sputa should be processed as soon as possible, because after 20 h of refrigeration there is a significant decrease in recoverable organisms. For pneumocystis, the yield from sputum is generally low. Various grading schemes (e.g., Bartlett grading system, Murray and Washington

grading system) have been used to assess the quality of sputum samples for Gram staining. These schemes use the number of squamous epithelial cells and leukocytes as well as the presence of mucus in sputum samples. For example, greater numbers of epithelial cells indicates oropharyngeal contamination. These grading schemes do not apply for all infections (e.g., Legionella spp., mycobacteria, fungi, and viruses). Bronchial washings, brushings, and bronchoalveolar lavage specimens are recommended for the optimal recovery of microorganisms. Organisms seen in sputum and specimens collected via bronchoscopy may be present due to contamination from the oral cavity rather than true infection of lungs. Transbronchial and percutaneous FNA may be required in some patients.

- *Central nervous system.* Specimens that can be submitted for microbiology studies include CSF obtained by lumbar puncture or other means (subdural tap, ventricular aspiration, or collected from a shunt), stereotactic FNA (e.g., abscess), and tissue biopsy. Specimens should be prepared as soon as possible after collection. If this is not possible, addition of an equal amount of 50% alcohol may help preserve the specimen. Refrigeration may adversely affect the recovery of certain microorganisms. Rapid diagnostic tests are available including India ink preparation for *Cryptococcus neoformans* and wet preparation for free-living amebae. FNA may identify toxoplasmosis, mycobacteria, cryptococcus, ameba, and cysticercosis.
- *Gastrointestinal system*. A variety of cytologic specimens can be obtained from the gastrointestinal tract including stool samples, ano-rectal swabs, secretions, and FNA. The development of fiber-optic endoscopy has greatly expanded the ability to obtain specimens for cytological evaluation (e.g., esophageal and gastric brushings and washings), including endoscopic ultrasound FNA of the liver and pancreas, as well as bile duct brushings.
- *Musculoskeletal system.* Usually such specimens are obtained by FNA, which includes aspiration of joint fluid. Joint fluid clots quickly, and if not submitted into appropriate containers that contain anticoagulant cell counts cannot be performed.
- *Skin.* Cytologic specimens include superficial scrapings, swabs and for vesicles, bullae, pustules, and deep palpable subcutaneous lesions FNA.

- *Fluids.* Effusions due to bacterial infection (e.g., *Staphylococcus* spp., *Streptococcal* spp.) appear turbid and purulent macroscopically while mycobacterial-infected effusions often have a shiny green appearance. Fungi (e.g., *Candida*, *Cryptococcus*) and parasites (e.g., *Echinococcus*, *ameba*, *Strongyloides*) may produce a purulent or serous effusion, whereas viral infected-fluids (e.g., coxsackie, herpes) are usually serous.

Suggested Reading

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