

## Part 4: Laboratory Procedures

### Chapter 24

#### Collection, Handling and Processing of Specimens

The correct specimen (as specified in earlier chapters) must be collected.

Expectorated **sputum** is an unreliable source of specimens; the frequency of confusing Gram negative bacteria has been reported at 31%. Sterile saline washing of expectorates has been found tedious and hazardous. To be of value, sputum must have been derived from deep in the respiratory tract. It should be screened for evidence of contamination with oral secretions. Specimens with < 10 squamous epithelial cells/100X field may be of satisfactory quality, especially if containing > 25 polymorphonuclears/100X field. Gram stain should always be done; 97% of *Streptococcus pneumoniae* and *Haemophilus influenzae* infections are detected by Gram stain-directed culture versus 51% by routine culture; overall specificity is 90% but sensitivity is 60-85%. *Streptococcus pneumoniae* isolation may improve if plating occurs within 1 h of collection. > 10 bacteria of the same kind per 1000X field should be reported and a definite identification in cultures attempted. A combination of coagglutination and semiquantitative, microscopy-directed culture of homogenised sputum is optimal. A culture of > 100 000-1M/mL of bacteria preliminarily identified in the Gram should be regarded as significant. Pneumococcal antigen detection, direct fluorescent antigen and culture for *Legionella*, DNA probe for *Mycoplasma pneumoniae*, smear for *Mycobacterium tuberculosis*, monoclonal antibody fluorescence for *Pneumocystis jiroveci* and viral antigen detection tests can also be performed on sputum.

If an adequate specimen of sputum cannot be obtained, if there is no clear diagnosis from expectorated sputum, or if there is poor response to antibiotics chosen on the basis of an expectorated sample, bronchoalveolar lavage is the safest, most reliable method of obtaining authentic specimens. Gram stain and culture should be performed, with > 10 000-100 000 bacteria/mL of fluid being regarded as significant. Pneumococcal antigen detection, direct fluorescent antigen and culture for *Legionella*, DNA probe and culture for *Mycoplasma pneumoniae*, monoclonal antibody fluorescence for *Pneumocystis jiroveci*, smear and culture for *Mycobacterium tuberculosis* and virus isolation can also be performed. For paediatric patients, a specimen may be collected by a respiratory therapist via suction. Gram stain and culture of a bronchoscopy protected specimen brush (with 10 000 bacteria/mL significant) and viral antigen detection in cells may be useful, as may Gram stain, viral culture, pneumococcal antigen detection on pleural fluid. Urine may be used for *Legionella* antigen detection.

**Urinary tract specimens** for anaerobes should be suprapubic percutaneous bladder aspirates. In other cases, contamination with urethral and perineal flora should be avoided. Mid-stream clean catch specimens are suitable for most purposes. Catheter specimens do not distinguish between colonisation and infection and procedure may introduce urethral flora into bladder. Suprapubic aspiration is the best way to obtain an uncontaminated specimen when this is not possible by normal means. Urine from urinary tract diversion specimens has a rich mixed aerobic and anaerobic flora (uterusigmoidostomy > ileal conduit > colon loop). Culture of such specimens is irrelevant and antimicrobial treatment useless. Ascorbate may lessen development of malignancy. Foley catheters are not acceptable for culture, since growth represents distal urethral flora.

Urine specimens should be refrigerated immediately upon receipt in the laboratory unless they are processed at once. Most uropathogens grow well in urine held at room temperature. However, bacterial counts should remain stable for at least 24 h at 4°C. The use of a dip slide at the time of collection to establish the true count is good practice. As the time a specimen is left unrefrigerated increases, so does the percentage of mixed cultures. Less than 5% of urines which are properly collected and transported contain multiple organisms. Exceptions to this rule are urine specimens from patients with neurogenic bladders or chronic indwelling catheters, in which polymicrobial bacteriuria may be detected in 30-80% of cultures. The Becton-Dickinson Urine Culture Tube is useful where there is considerable delay between collection and processing (up to 24 h), though a possibly better system is provided by using a

lyophilised preservative containing boric acid, sorbitol and sodium formate; this provides 94% agreement with fresh specimens in microscopy and 96% agreement in culture after 48 h.

Of the many rapid methods for detecting bacteriuria available, acridine orange staining is the most sensitive (98% at 10 000 cfu/mL, 99% at 100 000 cfu/mL), requires only 2 minutes and costs only 50 cents per test. However, it does require fluorescent equipment. Gram stain for the presence of bacteria in an uncentrifuged urine specimen has a sensitivity of 93% and false positive rate of 5%. The urine dipstick leucocyte esterase and nitrite tests have sensitivity of 79-88% and specificity of 80-96%. These results are superior to microscopic analysis for pyuria. Chemstrip LN and Bac-T-Screen both detect bacteriuria and pyuria. Both take 2 minutes per test and have 93-94% sensitivity at detecting pyuria (lower in leucopenic patients). The Bac-T-Screen is more sensitive at detecting bacteriuria (93% at 10,000 cfu/mL and 97% at 100 000 cfu/mL versus 79% and 92%) but costs nearly 3 times as much per test. The Lumac system has the highest sensitivity (98%) and predictive value of negative (99.5%), takes 35 minutes and is 50% more expensive again. The most effective method appears to be screening of urines at the point of collection with Boehringer Mannheim Combur-9 dipsticks, eliminating all urines which do not show any abnormality; this gives a virtually 100% correlation with full laboratory testing. The IRIS automated urine microscopy detects more than twice as many abnormalities as are found by manual microscopy, but it remains to be proved that the significance of this is such as to justify its cost. The presence of leucocytes and/or haematuria and/or bacteria on microscopy suggests, but does not prove, urinary tract infection. Many patients with increased numbers of white cells in the urine do not have urinary tract infection. The most common cause is probably vaginal contamination, but inflammatory processes anywhere in the body may result in the presence of increased numbers of leucocytes in the urine. Again, many patients with urinary tract infection do not have increased numbers of white cells in the urine. Dysmorphic red cells are seen in patients with glomerular disease. Such patients should be investigated with renal function assessments and possibly a renal biopsy.

If standard culture methods are used, results can be speeded up by realising that 85% of urines with 100 000 organisms/mL will, after 4-6 h incubation, produce recognisable colonies which can be presumptively identified by a combination of colonial morphology and spot tests with an accuracy of > 90%. Specimens from patients with urinary tract infection usually produce counts of > 100 000. Lower counts are related to contamination from external urethra and vagina. Specimens from patients without infection will generally cluster < 1000. At the 10 000-100 000 level, there is a 5% chance of infection, and cultures should be repeated. Two specimens yielding a titre of 100 000 or more make the probability of infection about 95%. False positives are found mainly in women and are related to contamination. False negatives are related to patients taking antimicrobials, diuresis or rare tuberculous or anaerobic infections. Enterococci, staphylococci and diphtheroids do not grow well in urine and will sometimes show up as borderline cultures (10 000-100 000). A titre of 10,000 of Gram positive organisms is cause for suspicion warranting a repeat culture. *Haemophilus* infections constitute 0.3% of urine cultures; this does not warrant routine screening but, in the case of abnormalities of the urinary tract with recurrent infections, especially in young girls, and when results suggest infection in the presence of negative cultures, the possibility of *Haemophilus* infection should be considered and appropriate media employed. Cultures for other fastidious organisms (*Mycobacterium*, *Ureaplasma urealyticum*, *Gardnerella vaginalis*, anaerobes) may also be warranted. The adoption of a criterion of infection of a colony count of 10 000/mL in urines with 10 000 leucocytes/mL of uncentrifuged urine and of 100 000/mL in those with < 10 000/mL gives a sensitivity and specificity of 99%.

**Blood cultures:** Isolates from blood cultures should always be fully identified and no isolate should be discarded as a contaminant without proper investigation. A single draw of 35-42 mL maximises sensitivity and minimises risk of contamination.

The use of a biphasic bottle frequently allows earlier isolation, in most cases enabling colonies to be picked and used in identification and susceptibility tests as soon as growth is evident. Caution should, however, be used in interpreting rapid staphylococcal fibrinogen/protein A tests and oxidase tests on colonies taken from the slopes of these bottles; they frequently give misleading results. This system also allows greater recovery of *Streptococcus pneumoniae* and simplifies the subculture process, resulting in decreased labour, contamination and cost. *Pseudomonas*, coagulase negative *Staphylococcus*, *Staphylococcus aureus*, *Bacillus*, *Escherichia coli*, *Klebsiella*, *Serratia*, *Acinetobacter*, *Alcaligenes*, *Neisseria* and *Candida* show diminished growth in unvented vacuum-exhausted bottles, while significantly more isolates of *Corynebacterium*, *Haemophilus*, *Flavobacterium*, *Moraxella*, *Bacteroides* and *Peptostreptococcus* are

recovered from unvented bottles. Therefore, one type only should never be used. Routine subculturing of biphasic bottles is unnecessary, but unvented bottles should routinely be subcultured at 6-17 h and again at 48 h. If a biphasic bottle is not used, the vented bottle should be treated similarly. In this case, also, agitation of the vented bottle significantly decreases the detection time and increases the number of positive blood cultures detected. Contamination rates and costs with these systems are about equal to the biphasic. Repeat subculture of known positive blood cultures is costly and ineffective in detecting polymicrobial bacteremias. Isolation rates can be significantly increased by use of lysis-centrifugation, eg, DuPont Isolator. This gives > 10% higher isolation rates than conventional 2 bottle systems (especially *Staphylococcus aureus*, fungi and mycobacteria, although the additional of oleic acid to conventional systems increases the yield of the latter to an equivalent extent), but recovery of *Streptococcus pneumoniae* is less good than with conventional systems and the contamination rate is 12% higher. The method involves direct plating of the system, eliminating subculture. This method is also useful for viral isolation and should always be used in investigating fungemia. With patients receiving i.v. lipids, media containing 2.5% olive oil should be used. The method's main advantage is in decreasing detection time. Isolation rates also depend on the volume of blood cultured, average yields from 30 mL of blood being 61% greater than that from 10 mL of blood.

The Bactec automated system provides similar isolation rates to conventional methods (except for *Streptococcus pneumoniae*; also, *Coccidioides immitis* produces visible growth but a negative growth index) and is cost effective for volumes in excess of about 6000 specimens per year. Cost per bottle is only about 40% of that for Isolator and biphasic systems, while labour involved is about equal to the biphasic. The radiometric system can also be used for rapid identification of mycobacteria.

Antimicrobials present in blood can frequently be removed by use of Bactec 16B medium (which does not always work for ticarcillin or moxalactam) or Marion's antimicrobial removal device (which may not work for moxalactam, cefotaxime or cefoperazone). However, studies have not convincingly shown that this translates into a higher yield of positives. On the other hand, the membrane filtration technique of Sullivan, Sutter and Finegold yields twice as many positives as the best conventional system from patients on antimicrobial therapy.

Gram staining should be the first step in investigating any positive blood culture. Gram positive cocci will almost always be staphylococci, streptococci or anaerobic cocci. 99% of staphylococci and streptococci can be correctly identified by microscopy. *Neisseria*, *Haemophilus*, *Bacteroides* and Gram positive bacilli can also usually be identified from microscopy. If diphtheroids are seen, it may be worth while doing a hanging drop preparation to look for the distinctive tumbling motility of *Listeria monocytogenes*.

It is frequently possible to obtain a quick identification of *Escherichia coli* by spinning down a portion of the culture fluid and performing an indole test on the supernate. Direct inoculation of MICROID or API20E from the culture fluid is also frequently possible, while the AMS (Vitek) will often give a direct identification in 3-8 h and direct susceptibility in 5-8 h. Blood for viral culture (cytomegalovirus and herpes simplex virus routinely isolated; arboviruses, arenaviruses, Epstein-Barr virus, HIV-1 and enterovirus in newborns not routinely isolated) should be collected during the acute phase of the infection and not refrigerated.

Blood cultures should also be obtained when a **CSF** specimen is taken. If only one tube of CSF is collected, it should be submitted to microbiology first; otherwise, the second tube collected is usually used. Coxsackievirus, echovirus, enterovirus and mumps virus are routinely cultured from CSF. Arboviruses, herpes simplex virus, lymphocytic choriomeningitis virus and rabies virus may also be cultured, but this is not routinely done.

Contamination and drying of **routine smears and cultures for bacteria (including mycobacteria) and fungi** must be avoided.

**Wound swabs** should be taken from the advancing margin of the lesion. For abscesses, tissue or fluid is always superior to a swab. If swabs must be used, 2 should be collected: 1 for culture and 1 for Gram stain. Agents are usually not recovered from animal bite wounds < 12 h old. The yield of potential pathogens from cellulitis aspirates is only 25-35%. A decubitus swab provides little clinical information and a tissue biopsy or needle aspirate is always to be preferred. The same applies to superficial tissue samples of gangrenous tissue. For otitis externa, vigorous swabbing is required, because surface swabbing may miss streptococcal cellulitis.

For **anaerobes**, the specimen collection method must preclude contamination by anaerobic flora of mucocutaneous surfaces. Specimen transportation must avoid excessive exposure to air. Valid specimens are blood cultures; aspirates of closed spaces such as pleural fluid, peritoneal fluid, ascitic fluid, joint fluid, cerebrospinal fluid;

specialised procedures that bypass mucocutaneous surfaces, such as transtracheal aspiration, direct lung aspirate, protected bronchoscopic brushing, suprapubic bladder aspiration, culdocentesis; deep aspiration of wounds and loculated abscesses; surgical specimens and tissue biopsies from any normally sterile site; Bartholin's gland; bile; bone marrow; Fallopian tube; intrauterine device for *Actinomyces*; ovary; placenta via caesarean section; sinus aspirate; stool for *Clostridium*; swab or tissue from surgical wound; endometrial aspirate from uterus.

The most practical and efficient system of **swab transport** for bacteriological systems is the use of cotton wool swabs pretreated with Sorensen's buffer placed in SIFF medium. Stuart or Amies medium may also be used. Even with transport medium, delays of > 1 h in transit of wound swabs, sputa, tracheal aspirates and urine can cause alterations to the microbial flora and loss of clinically significant species. *Trichomonas vaginalis* will remain viable for ? 24 h on dacron swabs transported in Amies medium.

Pathogenic *Neisseria* are particularly sensitive to cold, heat and lack of CO<sub>2</sub>.

For **genital lesions**, a swab and slide of transudate from the base of the lesion is the preferred specimen.

Great care should be taken to avoid loss of a **minute biopsy or corneal scraping**.

Swabs for **viral culture** must be collected directly into viral transport medium (Virocult (Medical Wire) is the most efficient system). Stuart's transport medium rapidly inactivates most viruses, and calcium alginate swabs should not be used. Swabs for herpes should be refrigerated rather than frozen. Cytomegalovirus and herpes simplex virus are routinely isolated from cervical, urethral and vaginal swabs; molluscum contagiosum virus and papillomavirus are not cultivable. Adenovirus, coxsackievirus A, cytomegalovirus, herpes simplex virus, enterovirus 70 and Newcastle disease virus are routinely isolated from conjunctival swabs. Influenza virus, parainfluenza virus, rhinovirus and respiratory syncytial virus are routinely isolated from nasal and nasopharyngeal swabs, aspirates or washings, though influenza virus and respiratory syncytial virus are usually detected by antigen assay (ELISA or EIA). Adenovirus, cytomegalovirus, enterovirus, herpes simplex virus, influenza virus, measles, mumps and parainfluenza virus are routinely isolated from throat swabs; respiratory syncytial virus may also be isolated by non-routine methods. Adenovirus, enterovirus, measles virus and rubella virus are routinely isolated from swabs taken from the base of maculopapular rash lesions, while coxsackievirus A, echovirus, herpes simplex virus and varicella-zoster virus are similarly isolated from vesicular rashes (vesicle aspirate in viral transport medium preferred for varicella-zoster). Poxviruses may be isolated from similar specimens by non-routine methods. Exudates, cellular scrapings and washings should be collected into buffered tryptose phosphate broth with gelatine or Hank's balanced salt solution with gelatine. Adenovirus, cytomegalovirus (collect 2 or 3 specimens on successive days), herpes simplex virus and mumps are routinely isolated from urine, while polyomavirus (JC virus) and rubella virus may be isolated by non-routine methods. Urine and throat washings for cytomegalovirus should be held in 70% sorbitol. Other specimens should be refrigerated or frozen. All specimens should be processed within 3 hours if possible. Viral and chlamydial tests require separate collection and transport kits.

**Gastric aspirates or washings** for mycobacteria must be processed (neutralised) promptly.

The use of transport media with **faeces** provides little benefit, either for culture or for parasitological examination. Speed of transport and prevention of drying and extremes of temperature are more important factors. If it is desired to use a preservative to transport faeces for parasitology, sodium acetate-acetic acid-formalin is probably the best single agent, though albumen-coated slides are required. Specimens for parasites must not be contaminated with urine or water, dried out, or contain bismuth, barium, magnesium, mineral oil or gallbladder dye (requires 21 d clearance). Adenoviruses and enteroviruses are routinely isolated from faeces; *Rotavirus* is usually detected by enzyme immunoassay.

**Rectal swabs** should be reserved for detecting gonorrhoea (specimen taken from anal crypts, avoiding faeces as much as possible) or for *Shigella* or *Campylobacter* (faeces must be seen on swab) in patients unable to provide faeces or for herpes simplex or rectal carriage of group B streptococci.

The proper timing of **specimens for serology and antibiotic serum assay** should be carefully observed.

In the laboratory, the value of **each specimen** should be carefully evaluated and poor quality or unnecessarily duplicated specimens not processed.