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Dept. Medical Lab. Techniques
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Lecture-1: Specimen Management

General Methods to the Diagnosis of a Bacterial Infection

1. Obtain a specimen from the infected site precisely.
2. Stain the specimen using the appropriate procedure, e.g., Gram stain or acid-fast stain.
3. Culture the specimen on the appropriate media, e.g., blood agar plates, nutrient plates, ...etc. The plates should be streaked in such a manner as to obtain isolated colonies (pure culture). The plates should be incubated in the presence or absence of **oxygen** as appropriate.
4. Identify the organism using the appropriate tests, e.g., sugar fermentation, DNA probes, antibody-based tests such as agglutination, or immunofluorescence. Note special features such as hemolysis and pigment formation.
5. Perform antibiotic susceptibility tests.

How do you diagnose a Bacterial Infection when the culture is negative?

1. Detect antibody in the patient's serum.
2. Detect antigen in the patient's specimen.
3. Detect nucleic acids in the patient's specimen.

Bacteriologic Methods:

- **Blood Cultures:** Blood cultures are performed most often when sepsis, endocarditis, osteomyelitis, meningitis, or pneumonia is suspected. The organisms most frequently isolated from blood cultures are **two** gram-positive cocci, *Staphylococcus aureus* and *Streptococcus pneumoniae*, and **three** gram-negative rods, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*.

Blood cultures are checked for **turbidity or for CO₂ production daily for 7 days or longer**. If growth occurs, Gram stain, subculture, and antibiotic sensitivity tests are performed. If no growth is observed after 1 or 2 days, blind sub-culturing onto other media may reveal organisms. Cultures should be held for 14 days when infective endocarditis, fungemia, or infection by slow-growing bacteria, e.g., *Brucella*, is suspected.

What is a Blood Culture?

- A blood culture is a laboratory test in which blood is injected into bottles with culture media to determine whether microorganisms have invaded the patient's bloodstream.



- **Throat Cultures:** Throat cultures are used primarily to detect the presence of group A β -hemolytic streptococci (*Streptococcus pyogenes*), an important and treatable cause of tonsillitis and pharyngitis. They are also used when diphtheria, gonococcal pharyngitis, or thrush (*Candida*) is suspected.

*Note that a Gram stain is typically not done on a throat swab because it is impossible to distinguish between the appearance of the normal flora streptococci and *S. pyogenes*.*

- **Sputum Cultures:** Sputum cultures are performed primarily when pneumonia or tuberculosis is suspected. The most frequent cause of community-acquired pneumonia is *S. pneumoniae*, whereas *S. aureus* and gram-negative rods, such as *K. pneumoniae* and *P. aeruginosa*, are common causes of hospital-acquired pneumonias. **If tuberculosis is suspected, an acid-fast stain should be done immediately and the sputum cultured on special media, which are incubated for at least 6 weeks.** In diagnosing aspiration pneumonia and lung abscesses, anaerobic cultures are important.
- **Spinal Fluid Cultures:** Spinal fluid cultures are performed primarily when meningitis is suspected. Spinal fluid specimens from cases of encephalitis, brain abscess, and subdural empyema usually show negative cultures. The most important causes of acute bacterial meningitis are **three encapsulated** organisms: *Neisseria meningitidis*, *S. pneumoniae*, and *Haemophilus influenzae*.
- **Stool Cultures**

Stool cultures are performed primarily for cases of enterocolitis. The most common bacterial pathogens causing diarrhea are *Shigella*, *Salmonella*, and *Campylobacter*. *E. coli* O157 strains are also an important cause of diarrhea.

A direct microscopic examination of the stool can be informative from two points of view:

1- A methylene blue stain that reveals many leukocytes indicates that an invasive organism rather than a toxigenic one is involved.

2- A Gram stain may reveal large numbers of certain organisms, such as staphylococci, clostridia, or campylobacters. Gram stain of the stool is not usually done because the large numbers of bacteria in the normal flora of the colon make the interpretation difficult.

For culture of *Salmonella* and *Shigella*, a selective, differential medium such as **MacConkey** or Eosin methylene blue (**EMB**) agar is used. These media are selective because they allow gram-negative rods to grow but inhibit many gram-positive organisms.

- **Urine Cultures**

Urine cultures are performed primarily when pyelonephritis or cystitis is suspected. By far the most frequent cause of urinary tract infections is *E. coli*. Other common agents are *Enterobacter*, *Proteus*, and *Enterococcus faecalis*.

Urine in the bladder of a healthy person is sterile, but it acquires organisms of the normal flora as it passes through the distal portion of the urethra. To avoid these organisms, a **midstream specimen**, voided after washing the external orifice, is used for urine cultures.

- **Genital Tract Cultures**

Genital tract cultures are performed primarily on specimens from individuals with an abnormal discharge or on specimens from asymptomatic contacts of a person with a sexually transmitted disease. One of the most important pathogens in the genital tract is *Neisseria gonorrhoeae*. The laboratory diagnosis of gonorrhea is made by microscopic examination of a gram-stained smear and by culture of the organism.

Specimens are obtained by swabbing the urethral canal (for men), the cervix (for women). A urethral discharge from the penis is frequently used. Because *N. gonorrhoeae* is very delicate, the specimen should be inoculated directly onto a Thayer-Martin chocolate agar plate or onto a special transport medium (e.g., Trans-grow).

Gram-negative diplococci found *intracellularly* within neutrophils on a smear of a urethral discharge from a man have over 90% probability of being *N. gonorrhoeae*.



Non-gonococcal urethritis and cervicitis are also common infections. The most frequent cause is *Chlamydia trachomatis*, which cannot grow on artificial medium but must be grown in living cells. Because *Treponema pallidum*, the agent of syphilis, cannot be cultured, diagnosis is made by microscopy and serology. **The presence of motile spirochetes with typical morphologic features seen by darkfield microscopy of the fluid from a painless genital lesion is sufficient for the diagnosis.** The serologic tests fall into two groups: 1- the **non-treponemal antibody** tests such as the Venereal Disease Research Laboratory (VDRL) or rapid plasma reagin (RPR) test and 2- the **treponemal antibody** tests such as the fluorescent treponemal antibody-absorption (FTA-ABS) test.

- **Wound & Abscess Cultures**

The bacteria most frequently isolated differ according to anatomic site and predisposing factors. Abscesses of the brain, lungs, and abdomen are frequently caused by anaerobes such as *Bacteroides fragilis* and gram-negative cocci such as *Staph. aureus* and *Strep. pyogenes*. Traumatic open-wound infections are caused primarily by members of the soil flora such as *Clostridium perfringens*; surgical-wound infections are usually due to *Staph. aureus*. Infections of dog or cat bites are commonly due to *Pasteurella multocida*, whereas human bites primarily involve the mouth anaerobes.
