150e Laboratory Diagnosis of Infectious Diseases

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The laboratory diagnosis of infection requires the demonstration—either direct or indirect—of viral, bacterial, fungal, or parasitic agents in tissues, fluids, or excreta of the host. Clinical microbiology laboratories are responsible for processing these specimens and also for determining the antibiotic susceptibility of bacterial and fungal pathogens. Traditionally, detection of pathogenic agents has relied largely on either the microscopic visualization of pathogens in clinical material or the growth of microorganisms in the laboratory. Identification generally is based on phenotypic characteristics such as fermentation profiles for bacteria, cytopathic effects in tissue culture for viral agents, and microscopic morphology for fungi and parasites. These techniques are reliable but are often time-consuming. Increasingly, the use of nucleic acid probes is becoming a standard method for detection, quantitation, and/or identification in the clinical microbiology laboratory, gradually replacing phenotypic characterization and microscopic visualization methods. This chapter discusses general concepts of diagnostic testing, with an emphasis on detection of bacteria. Detection of viral, fungal, and parasitic pathogens is discussed in greater detail in separate chapters (see Chaps. 214e, 235, and 245e, respectively).

DETECTION METHODS

Reappraisal of the methods employed in the clinical microbiology laboratory has led to the development of strategies for detection of pathogenic agents through nonvisual biologic signal detection systems. A *biologic signal* is generated by detection of a material that can be reproducibly differentiated from other substances present in the sample. Key issues in the use of a biologic signal are distinguishing it from background noise and translating it into meaningful information. Examples of useful materials for detection of biologic signals applicable to clinical microbiology include structural components of bacteria, fungi, and viruses; specific antigens; metabolic end products; unique DNA or RNA base sequences; enzymes; toxins or other proteins; and surface polysaccharides.

A detector is used to sense a signal and discriminate between that signal and background noise. Detection systems range from the trained eyes of a technologist assessing morphologic variations to electronic instruments such as gas-liquid chromatographs or mass spectrometers. The sensitivity with which signals can be detected varies widely. It is essential to use a detection system that discerns small amounts of signal even when biologic background noise is present—i.e., that is both sensitive and specific. Common detection systems include immunofluorescence; chemiluminescence for DNA/RNA probes; flame ionization detection of short- or long-chain fatty acids; and detection of substrate utilization or end-product formation as color changes, of enzyme activity as a change in light absorbance, of turbidity changes as a measure of growth, of cytopathic effects in cell lines, and of particle agglutination as a measure of antigen presence.

Amplification enhances the sensitivity with which weak signals can be detected. The most common microbiologic amplification technique is growth of a single bacterium into a discrete, visible colony on an agar plate or into a suspension containing many identical organisms. The advantage of growth as an amplification method is that it requires only an appropriate growth medium; the disadvantage is the amount of time required. More rapid amplification of biologic signals can be achieved with techniques such as polymerase chain reaction (PCR), ligase chain reaction (LCR), and transcription-mediated amplification (TMA), all of which target the pathogen's DNA/RNA; enzyme immunoassays (EIAs) for antigens and antibodies; electronic amplification (for gas-liquid chromatography assays); antibody capture methods (for concentration and/or separation); and selective filtration or centrifugation.

DIRECT DETECTION

Direct detection refers to detection of pathogens without the use of culture. Molecular methods of direct detection are discussed below.

MICROSCOPY AND STAINING

The field of microbiology was defined largely by the development and use of the microscope. The examination of specimens by microscopic methods rapidly provides useful diagnostic information. Staining techniques permit organisms to be seen more clearly.

The simplest method for microscopic evaluation is the *wet mount*, which is used, for example, to examine cerebrospinal fluid (CSF) for the presence of *Cryptococcus neoformans*, with India ink as a background against which to visualize large-capsuled yeast cells. Wet mounts with dark-field illumination also are used to detect spirochetes in genital lesions and *Borrelia* or *Leptospira* in blood. Skin scrapings and hair samples can be examined with the use of either 10% KOH wet-mount preparations or the *calcofluor white method* and ultraviolet illumination to detect fungal elements as fluorescing structures. Staining of wet mounts—e.g., with lactophenol cotton blue stain for fungal elements—often is used for morphologic identification.

Bacteria are difficult to see by light microscopy unless they are *stained*. Although simple one-step stains can be used, differential stains are more common.

Gram's Stain Gram's stain differentiates between organisms with thick peptidoglycan cell walls (gram-positive) and those with thin peptidoglycan cell walls and outer membranes that can be dissolved with alcohol or acetone (gram-negative). Morphology and Gram's stain characteristics often can be used to categorize stained organisms into groups such as streptococci, staphylococci, and clostridia (Table 150e-1). Gram's stain is particularly useful for examining sputum for polymorphonuclear leukocytes (PMNs) and bacteria. Sputum specimens from immunocompetent patients with ≥25 PMNs and <10 epithelial cells per low-power field often provide clinically useful information. However, the presence in "sputum" samples of >10 epithelial cells per low-power field and of multiple bacterial types suggests contamination with oral microflora. Despite the difficulty of discriminating between normal microflora and pathogens, Gram's stain may prove useful for specimens from areas with a large resident microflora if a useful biologic marker (signal) is available. Gram's staining of vaginal swab specimens can be used to detect epithelial cells covered with gram-positive bacteria in the absence of lactobacilli and the presence of gram-negative rods—a scenario regarded as a sign of bacterial vaginosis. Similarly, examination of stained stool specimens for leukocytes is useful as a screening procedure before testing for *Clostridium difficile* toxin or other enteric pathogens.

The examination of samples from normally sterile body sites (e.g., CSF or joint, pleural, or peritoneal fluid) with Gram's stain is useful for determining whether bacteria and/or PMNs are present. The sensitivity is such that $>10^4$ bacteria/mL should be detected. Centrifugation often is performed before staining to concentrate specimens thought to contain low numbers of organisms. This simple method is particularly useful for examination of CSF for bacteria and white blood cells or examination of sputum for mycobacteria.

Acid-Fast Stain The acid-fast stain identifies acid-fast bacteria (AFB; mycobacteria) by their retention of carbol fuchsin dye after acid/organic solvent disruption. Modifications of this procedure allow the differentiation of *Actinomyces* from *Nocardia* or other weakly (or partially) acid-fast organisms. The acid-fast stain is applied to sputum, other fluids, and tissue samples when *Mycobacterium* species are suspected. Because few AFB may be present in an entire smear, even when the specimen has been concentrated by centrifugation, identification of the pink/red AFB against the blue background of the counterstain requires a trained eye. An alternative method for detection of AFB is the auramine-rhodamine fluorescent dye stain.