TABLE 150e-1 INTERPRETATION OF GRAM'S STAIN[a]

Morphology	Additional Features	Species
Gram-Negative Bacteria		
Bacilli	Regular bacilli	Enterobacteriaceae (*Escherichia coli, Salmonella* spp., *Shigella* spp., others)
		Pseudomonas aeruginosa
		Acinetobacter baumannii
		Stenotrophomonas maltophilia
	Small pleomorphic coccobacilli	*Haemophilus influenzae*, other *Haemophilus* spp.
Cocci	Diplococci	*Neisseria meningitidis*, *Neisseria gonorrhoeae*, *Moraxella catarrhalis*
Curved	Comma-shaped	*Vibrio* spp.
	Comma- or S-shaped	*Campylobacter* spp.
Gram-Positive Bacteria		
Bacilli	Small regular bacilli	*Listeria monocytogenes*
	Small pleomorphic bacilli in irregular palisading (picket-fence) arrangement	*Corynebacterium* spp.
	Large regular bacilli, sometimes with spores	*Bacillus* spp., *Clostridium* spp.
	Bacilli arranged in branching chains	*Nocardia* spp., *Actinomyces* spp.
	Pale purple or unstained "ghost cells" that are acid-fast	*Mycobacterium* spp.
Cocci	Cocci arranged in pairs and short chains	*Streptococcus pneumoniae*, *Enterococcus* spp.
	Cocci arranged in long chains	*Streptococcus pyogenes*, *Streptococcus agalactiae*, other *Streptococcus* spp.
	Cocci arranged in clusters	*Staphylococcus* spp., *Micrococcus* spp.

[a]Some important bacteria cannot be seen with Gram's stain because they are too small or too slender or do not retain the stain. These bacteria include *Treponema pallidum, Borrelia burgdorferi, Chlamydia* spp., *Mycoplasma* spp., and *Ureaplasma* spp.

Fluorochrome Stains Fluorochrome stains such as acridine orange are used to identify white blood cells, yeasts, and bacteria in body fluids. Capsular, flagellar, and spore stains are used for identification or demonstration of characteristic structures.

Immunofluorescent Stains The *direct* immunofluorescent antibody technique uses antibody coupled to a fluorescent compound (e.g., fluorescein) and directed at a specific antigenic target to visualize organisms. When samples are examined under appropriate conditions, the fluorescing compound absorbs ultraviolet light and re-emits light at a higher wavelength that is visible to the human eye. In the *indirect* immunofluorescent antibody technique, an unlabeled (target) antibody binds a specific antigen. The specimen is then stained with a fluorochrome-labeled antibody directed at the target antibody. Because each unlabeled target antibody attached to the appropriate antigen has multiple sites for attachment of the second antibody, the visual signal is amplified. Immunofluorescence is used to detect viral antigens (e.g., cytomegalovirus, herpes simplex virus, and respiratory viruses) within cultured cells or clinical specimens as well as many difficult-to-grow bacterial agents (e.g., *Legionella pneumophila*) in clinical specimens.

MACROSCOPIC ANTIGEN DETECTION

Latex agglutination assays and EIAs are rapid and inexpensive methods for identifying organisms, extracellular toxins, and viral agents by means of protein and polysaccharide antigens. Such assays may be performed directly on clinical samples or after growth of organisms on agar plates or in viral cell cultures. Antibodies coupled to a reporter (such as latex particles or an enzyme) are used for detection of antibody–antigen binding reactions.

Direct agglutination of bacterial cells with specific antibody is simple but relatively insensitive; latex agglutination and EIAs are more sensitive. Some cell-associated antigens, such as capsular polysaccharides and lipopolysaccharides, can be detected by agglutination of a suspension of bacterial cells when antibody is added; this method is useful for typing of the somatic antigens of *Shigella* and *Salmonella*. EIAs employ antibodies coupled to an enzyme, and an antigen–antibody reaction results in the conversion of a colorless substrate to a colored product. Most of these assays provide information about whether antigen is present but do not quantify the antigen. EIAs are also useful for detecting bacterial toxins—e.g., toxins produced by Shiga toxin–producing *Escherichia coli*.

Rapid and simple immunoassays for antigens of group A *Streptococcus*, influenza virus, and respiratory syncytial virus can be used in the clinical setting without a specialized diagnostic laboratory. Such tests usually are reasonably specific but may have only modest sensitivity.

DETECTION OF PATHOGENIC AGENTS BY SEROLOGIC METHODS

Measurement of serum antibody provides an indirect marker for past or current infection with a specific viral agent or other pathogens, including *Brucella*, *Legionella*, *Rickettsia*, and *Helicobacter pylori*. Serologic methods can be used to determine whether an individual has protective antibody levels or is infected by a specific pathogen. Determination of an antibody level as a measure of current immunity is important in the case of viral agents for which there are vaccines, such as rubella virus and varicella-zoster virus; assays for this purpose normally use one or two dilutions of serum for a qualitative determination of protective antibody levels. Quantitative serologic assays to detect increases in antibody titers most often employ paired serum samples obtained at the onset of illness and 10–14 days later (i.e., acute- and convalescent-phase samples). Since the incubation period before symptoms are noted may be long enough for an antibody response to occur, the demonstration of acute-phase antibody alone is often insufficient to establish the diagnosis of active infection as opposed to past exposure. A fourfold increase in total antibody titer between the acute- and convalescent-phase samples is regarded as evidence for active infection. In addition, IgM may be useful as a measure of an early, acute-phase antibody response. For certain viral agents, such as Epstein-Barr virus, the antibodies produced may be directed at different antigens during different phases of the infection. For this reason, most laboratories test for antibody directed at both viral capsid antigens and antigens associated with recently infected host cells to determine the stage of infection.

DETECTION OF PATHOGENIC AGENTS BY CULTURE

SPECIMEN COLLECTION AND TRANSPORT

To culture bacterial, fungal, or viral pathogens, an appropriate sample must be placed into the proper medium for growth. The success of efforts to identify a specific pathogen often depends on the