PROCEDUR MANUAL LABORATORY
NEUROBEHAVIOUR SYSTEM

SECTION:MICROBIOLOGY

IDENTIFICATION OF BACTERIAL AGENTS AT CENTRAL NERVOUS SYSTEM DISEASE

I. INTRODUCTION

The cerebrum, cerebellum, brainstem, spinal cord, and their covering membranes (meninges) constitute the central nervous system (CNS). Because of the unique anatomic and physiologic features of the CNS, infections of this site can represent special challenges to the microbiologist and clinician. Most CNS infections appear to result from blood-borne spread; for example, bacteremia or viremia resulting from infection of tissue at a site remote from the CNS may result in penetration of the blood–brain barrier. Examples of infectious agents that commonly infect the CNS by this route are *Haemophilus influenzae, Neisseria meningitidis, Streptococcus pneumoniae, Mycobacterium tuberculosis,* and viruses such as enteroviruses and mumps. The initial source of infection leading to bloodstream invasion may be occult (eg, infection of reticuloendothelial tissues) or overt (eg, pneumonia, pharyngitis, skin abscess or cellulitis, or bacterial endocarditis).

Occasionally, the route of infection is from a focus close to or contiguous with the CNS. These possible sources include middle ear infection (otitis media), mastoiditis, sinusitis, or pyogenic infections of the skin or bone. Infection may extend directly into the CNS, indirectly via venous pathways, or in the sheaths of cranial and spinal nerves.

Common Causes of Purulent Central Nervous System Infections

AGE GROUP	AGENT
Newborns (<1 mo old)	Group B streptococci (most common), *Escherichia coli, Listeria monocytogenes, Klebsiella* species, other enteric Gram-negative bacteria
Infants and children	*Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenzae*
Adults	*S. pneumoniae, Neisseria meningitidis*
SPECIAL CIRCUMSTANCES	
Meningitis or intracranial abscesses associated with trauma, neurosurgery, or intracranial foreign bodies	*Staphylococcus aureus, Staphylococcus epidermidis, S. pneumoniae;* anaerobic Gram-negative and Gram-positive bacteria; *Pseudomonas* species
Intracranial abscesses not associated with trauma or surgery	Microaerophilic or anaerobic streptococci, anaerobic Gram-negative bacteria (often mixed aerobic and anaerobic flora of upper respiratory tract origin)

Findings of Cerebrospinal Fluid Analysis: Normal Versus Infection

Clinical Situation	Leukocytes/mm^3	% Polymorphouclears	Glucose (% of blood)	Protein (mg/dL)
Children and Adults				
Normal	0–5	0	≥60	≤30
Viral infection	2–2000 (80)[a]	≤50	≥60	30–80
Pyogenic bacterial infection	5–5000 (800)	≥60	≤45[b]	>60
Tuberculosis and mycoses	5–2000 (100)	≤50	≤45	>60
Neonates				
Normal (term)	0–32 (8)	≤60	≥60	20–170 (90)
Normal (preterm)	0–29 (9)	≤60	≥60	65–150 (115)

The other major procedures that must be performed on all CSF samples in which any infection is suspected include bacterial cultures and Gram staining. If the CSF is grossly purulent and the patient untreated, a Gram stain of the uncentrifuged CSF or of its centrifuged sediment frequently shows the infecting organism and indicates the specific diagnosis. According to the clinical indications and results of CSF cytology and chemistry, other microbiologic tests may be used, including viral cultures, special stains and cultures for fungi and mycobacteria, immunologic methods to detect fungal or bacterial antigens (eg, latex agglutination for *Cryptococcus*), and polymerase chain reactions to detect viral or bacterial nucleic acids.

Tests on specimens other than CSF are selected on the basis of the clinical diagnostic possibilities. If acute bacterial meningitis is suspected, blood cultures should also be used to ensure the diagnosis. Viral cultures of the pharynx, stool, or rectal swabs may provide indirect evidence of CNS infection. In encephalitis, a biopsy specimen of the brain is sometimes obtained for culture, histology, and to demonstrate viral antigen or nucleic acid. Other studies may include acute and convalescent sera for viral serology and serologic tests to detect antibodies to certain fungi, such as *C. immitis*.

Intracranial abscesses can often be detected with radiologic techniques, such as computerized tomography or magnetic resonance imaging. A definitive etiologic diagnosis is established by careful aerobic and anaerobic culture of the contents of the abscess.

A. *Haemophilus influenzae*

The growth of most species of Haemophilus requires supplementation of media with one or both of the following growth-stimulating factors: (1) **hemin** (also called **X factor** for “unknown factor”) and (2) **nicotinamide adenine dinucleotide** (**NAD;** also called **V factor** for “vitamin”). Although both factors are present in blood-enriched media, sheep blood agar must be gently heated to destroy the inhibitors of V factor. For this reason, heated blood (“chocolate”) agar is used for the isolation of Haemophilus in culture.

The cell wall structure of Haemophilus is typical of other gram-negative rods. Lipopolysaccharide with endotoxin activity is present in the cell wall, and strain-specific and species-specific proteins are found in the outer membrane.

Analysis of these strain-specific proteins is valuable in epidemiologic investigations. The surface of many, but not all, strains of H. influenzae is covered with a **polysaccharide capsule,** and six antigenic **serotypes** (**a** through **f**) have been identified. Before the introduction of the H. influenzae type b vaccine, H. influenzae serotype b was responsible for more than 95% of all invasive Haemophilus infections.

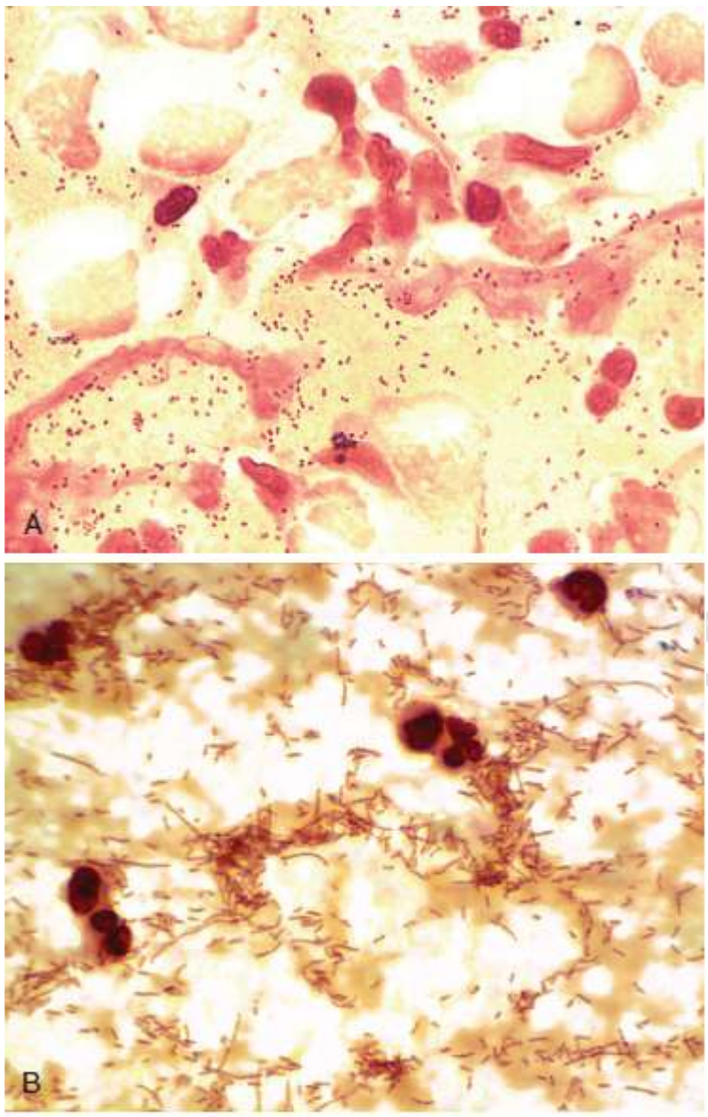


FIGURE 24-1 Gram stains of *Haemophilus influenzae.* A, Small coccobacilli forms seen in sputum from patient with pneumonia. B, Thin pleomorphic forms seen in a 1-year-old unvaccinated child in Africa with overwhelming meningitis.

These organisms can spread locally and cause disease in the ears (otitis media), sinuses (sinusitis), and lower respiratory tract (bronchitis, pneumonia). Disseminated disease, however, is relatively uncommon. In contrast, encapsulated *H. influenzae* (particularly serotype b [biotype I]) is uncommon in the upper respiratory tract or is present in only very small numbers but is a common cause of **disease in unvaccinated children** (i.e., meningitis, epiglottitis [obstructive laryngitis], cellulitis). Pili and nonpilus adhesins mediate colonization of the oropharynx with *H. influenzae*. Cell wall components of the bacteria (e.g., lipopolysaccharide and a low-molecular-weight glycopeptide) impair ciliary function, leading to damage of the respiratory epithelium. The bacteria

can then be translocated across both epithelial and endothelial cells and can enter the blood. In the absence of specific opsonic antibodies directed against the polysaccharide capsule, high-grade bacteremia can develop, with dissemination to the meninges or other distal foci.

The major virulence factor in *H. influenzae* type b is the antiphagocytic polysaccharide capsule, which contains ribose, ribitol, and phosphate (commonly referred to as **polyribitol phosphate [PRP]**). Antibodies directed against the capsule greatly stimulate bacterial phagocytosis and complement-mediated bactericidal activity.

H. influenzae type b was the most common cause of pediatric meningitis, but this situation changed rapidly when the conjugated vaccines became widely used. Disease in nonimmune patients results from bacteremic spread of the organisms from the nasopharynx and cannot be differentiated clinically from other causes of bacterial meningitis. The initial presentation is a 1- to 3-day history of mild upper respiratory disease, after which the typical signs and symptoms of meningitis appear.

Person-to-person spread in a nonimmune population is well documented, so appropriate epidemiologic precautions must be used.

B. *Neisseria meningitidis*

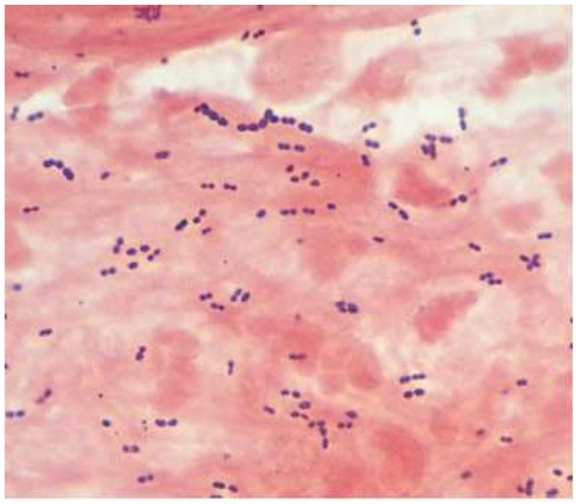
Strains of N. meningitidis can colonize the nasopharynx of healthy people without producing disease or can cause community-acquired meningitis, overwhelming and rapidly fatal sepsis, or bronchopneumonia Neisseria species are aerobic **gram-negative** bacteria, typically coccoid shaped (0.6 to 1.0 μm in diameter) and arranged in pairs **(diplococci)** with adjacent sides flattened together (resembling coffee beans [Figure 23-1]). All species are oxidase positive and most produce catalase, properties that combined with the Gram stain morphology allow a rapid, presumptive identification of a clinical isolate. Acid is produced by oxidation of carbohydrates (not by fermentation), a property that was historically used to differentiate Neisseria species. More rapid methods such as mass spectrometry are now used to identify these bacteria. *N. meningitidis* grows on blood agar and has variable growth on nutrient agar. The cell wall structure of *N. gonorrhoeae* and *N. Meningitidis* is typical of gram-negative bacteria, with the thin peptidoglycan layer sandwiched between the inner cytoplasmic membrane and the outer membrane. The major virulence factor for *N. meningitidis* is the polysaccharide capsule. Antigenic differences in the **polysaccharide capsule** of *N. meningitidis* are the basis for serogrouping these bacteria in vitro and play a prominent role in determining if an individual strain will cause disease. Thirteen serogroups are currently recognized, with 6 serogroups (A, B, C, W135, X, and Y) associated with endemic and epidemic disease.

Pathogenic and nonpathogenic strains of *Neisseria* have **pili** that extend from the cytoplasmic membrane through the outer membrane. Pili mediate a number of functions, including attachment to host cells, transfer of genetic material, and motility, and the presence of pili in *N. gonorrhoeae* and *N.meningitidis* appears to be important for pathogenesis, in part because the pili mediate attachment to nonciliated epithelial cells and provide resistance to killing by neutrophils. The pili are composed of repeating protein subunits **(pilins)** that have a conserved region at one end and a highly variable

region at the exposed carboxyl terminus. Other prominent families of proteins are present in the outer membrane. The **porin proteins** are integral outer membrane proteins that form pores or channels for nutrients to pass into the cell and waste products to exit. *N. Gonorrhoeae* and *N. meningitidis* have two porin genes, *porA* and *porB.* The gene products, **PorA and PorB proteins,** are both expressed in *N. meningitidis,* but the *porA* gene is silent in *N. Gonorrhoeae*. Iron is essential for the growth and metabolism of *N.gonorrhoeae* and *N. meningitidis*. These pathogenic neisseriae are able to compete with their human hosts for iron by **binding host cell transferrin** to specific bacterial surface receptors. The specificity of this binding for human transferrin is likely the reason these bacteria are strict human pathogens. The presence of this receptor is fundamentally different from most bacteria that synthesize siderophores to scavenge iron. Another major antigen in the cell wall is **lipooligosaccharide (LOS).** This antigen is composed of lipid A and a core oligosaccharide but lacks the O-antigen polysaccharide found in lipopolysaccharide (LPS) in most gram-negative rods. The lipid A moiety possesses endotoxin activity. Both N. gonorrhoeae and N. meningitidis spontaneously release **outer membrane blebs** during rapid cell growth. These blebs contain LOS and surface proteins and may act to both enhance endotoxin-mediated toxicity and protect replicating bacteria by binding protein-directed antibodies. N. gonorrhoeae and N. meningitidis produce **immunoglobulin (Ig)A1 protease,** which cleaves the hinge region in IgA1. This action creates immunologically inactive Fc and Fab fragments.

C. *Streptococcus pneumoniae*

The pneumococcus is an **encapsulated** gram-positive coccus. The cells are 0.5 to 1.2 μm in diameter, oval, and arranged in pairs (commonly referred to as **diplococci**) or short chains. Colonial morphology varies, with colonies of encapsulated strains generally large (1 to 3 mm in diameter on blood agar; smaller on chocolatized or heated blood agar), round, and mucoid, and colonies of nonencapsulated strains smaller and flat. All colonies undergo autolysis with aging—that is, the central portion of the colony dissolves, leaving a dimpled appearance. Colonies appear α-hemolytic on blood agar if incubated aerobically and may be β-hemolytic if grown anaerobically. The α-hemolytic appearance results from production of **pneumolysin,** an enzyme that degrades hemoglobin, producing a green product.



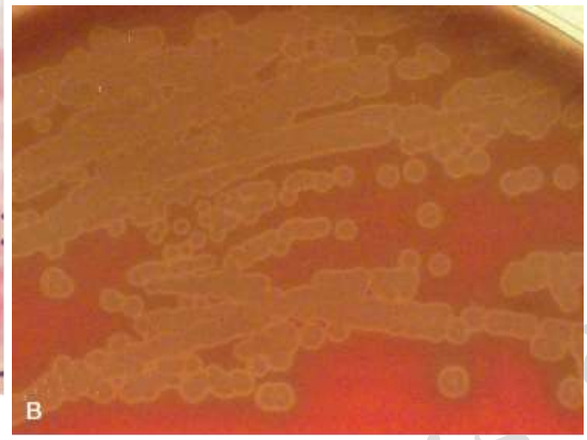


FIGURE 19-7 Gram stain of *Streptococcus pneumoniae.*

The organism has fastidious nutritional requirements and can grow only on enriched media supplemented with blood products. *S. pneumoniae* can ferment carbohydrates, producing lactic acid as the primary metabolic byproduct. *S. pneumoniae* grows poorly in media with high glucose concentrations because lactic acid rapidly reaches toxic levels in such preparations. Similar to all streptococci, the organism lacks catalase. Unless an exogenous source of catalase is provided (e.g., from blood), the accumulation of hydrogen peroxide inhibits the growth of *S. pneumoniae*, as observed on chocolatized blood agar.

Virulent strains of *S. pneumoniae* are covered with a complex **polysaccharide capsule.** The capsular polysaccharides have been used for the serologic classification of strains; currently, 94 serotypes are recognized. Purified capsular polysaccharides from the most commonly isolated serotypes are used in a **polyvalent vaccine.**

S. pneumoniae can spread into the central nervous system after bacteremia, infections of the ear or sinuses, or head trauma that causes a communication between the subarachnoid space and the nasopharynx. Although **pneumococcal meningitis** is relatively uncommon in neonates, *S. pneumoniae* is now a leading cause of disease in children and adults. Mortality and severe neurologic deficits are 4 to 20 times more common in patients with meningitis caused by *S. pneumoniae* than in those with meningitis resulting from other organisms.

D. *Mycobacterium tuberculosis*

They are nonmotile, non–spore-forming, aerobic gram-positive rods that stain **acid-fast** (i.e., resist decolorization with weak to strong acid solutions) due to the presence of medium to long chains of mycolic acids in their cell wall. This staining property is important because only five genera of acid-fast bacteria are medically important. All acid-fast organisms are relatively slow-growing bacteria, requiring incubation for 2 to 7 days *(Nocardia, Rhodococcus, Gordonia, Tsukamurella)* to as long as 1 month or more *(Mycobacteria).* Currently, more than 350 species of acid-fast bacteria have been described; however, the number associated commonly with human disease is relatively limited. The spectrum of the infections associated with the acid-fast genera is

extensive and includes insignificant colonization, cutaneous infections, pulmonary disease, systemic infections, and opportunistic infections

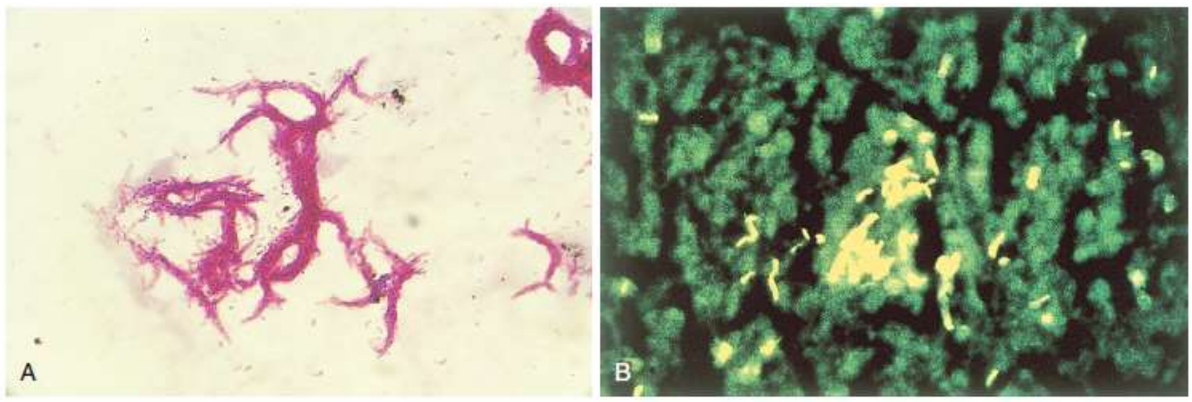


FIGURE 22-5 Acid-fast stains of *Mycobacterium tuberculosis.* **A**, Stained with carbolfuchsin using the Kinyoun method. **B**, Stained with the fluorescent dyes auramine and rhodamine using the Truant fluorochrome method.

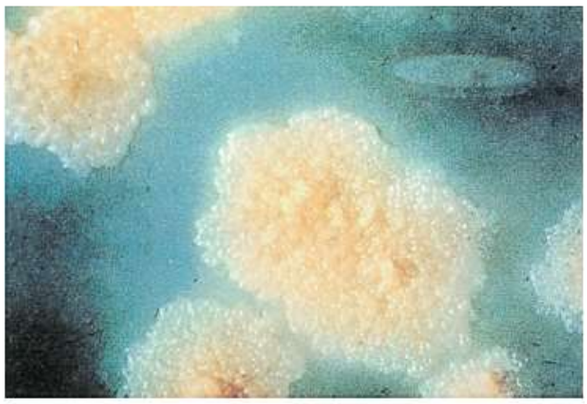


FIGURE 22-1 *Mycobacterium tuberculosis* colonies on Löwenstein-Jensen agar after 8 weeks of incubation. (From Baron EJ, Peterson LR, Finegold SM: *Bailey and Scott's diagnostic microbiology,* ed 9, St Louis, 1994, Mosby.)

Pathogenesis and Immunity

M. tuberculosis enters the respiratory airways, and infectious particles penetrate to the alveoli where they are phagocytized by alveolar macrophages. In contrast with most phagocytized bacteria, *M.tuberculosis* **prevents fusion of the phagosome with lysosomes** (by blocking the specific bridging molecule, early endosomal autoantigen 1 [EEA1]). At the same time, the phagosome is able to fuse with other intracellular vesicles, permitting access to nutrients and facilitating intravacuole replication. Phagocytized bacteria are also able to evade macrophage killing mediated by reactive nitrogen intermediates formed between nitric oxide and superoxide anions by catalytically catabolizing the oxidants that are formed. So in this state, the bacteria are able to evade the immune system and replicate. However, in response to infection with *M.tuberculosis,* macrophages secrete **interleukin (IL)-12** and **tumor necrosis factor (TNF)-α.** These cytokines increase localized inflammation with the recruitment of T cells and natural killer (NK) cells into the area of the infected macrophages, inducing T-cell differentiation into **TH1 cells (T-helper cells),** with subsequent secretion of **interferon (IFN)-γ.** In the presence of IFN-γ, the infected macrophages are activated, leading to increased phagosome-lysosome fusion and intracellular killing. In addition, TNF-α stimulates production of nitric oxide and related reactive nitrogen

intermediates, leading to enhanced intracellular killing. Patients with decreased production of IFN-γ or TNF-α, or who have defects in the receptors for these cytokines, are at increased risk for severe progressive mycobacterial infections.

The effectiveness of bacterial elimination is in part related to the size of the focus of infection. Alveolar macrophages, epithelioid cells, and **Langhans giant cells** (fused epithelioid cells) with intracellular mycobacteria form the central core of a necrotic mass that is surrounded by a dense wall of macrophages and CD4, CD8, and NK T cells. This structure, a **granuloma,** prevents further spread of the bacteria. If a small antigenic burden is present at the time the macrophages are stimulated, the granuloma is small and the bacteria are destroyed with minimal tissue damage. However, if many bacteria are present, the large necrotic or caseous granulomas become encapsulated with fibrin that effectively protects the bacteria from macrophage killing. The bacteria can remain dormant in this stage or can be reactivated years later when the patient's immunologic responsiveness wanes as the result of old age or immunosuppressive disease or therapy. This process is the reason that disease may not develop until late in life in patients exposed to *M.tuberculosis.*

Specimens inoculated onto egg-based (e.g., **Löwenstein-Jensen**) and agar-based (e.g., **Middlebrook**) media generally take 4 or more weeks for *M. tuberculosis* to be detected. However, this time has been shortened approximately 2 weeks through the use of specially formulated **broth cultures** that support the rapid growth of most mycobacteria. The ability of *M. tuberculosis* to grow rapidly in broth cultures has been used for performing rapid susceptibility tests. The technique, **MODS** or ***m*icroscopic *o*bservation *d*rug susceptibility assay,** uses an inverted light microscope to examine 24-well plates inoculated with Middlebrook broth and decontaminated sputum. *M. tuberculosis* growth can be detected as tangles or cords of growth in the broth, generally after 1 week of incubation. Incorporation of antimycobacterial drugs in the broth enables rapid, direct susceptibility testing with clinical specimens. This technique is widely available in laboratories servicing resource-limited countries where drug-resistant strains of *M. tuberculosis* are widespread.

E. Enterovirus

the enteroviruses are extremely small (22 to 30 nm in diameter), naked virions with icosahedral symmetry. They possess single-stranded, positive-sense RNA and a capsid formed from 60 copies of four nonglycosylated proteins (VP1, VP2, VP3, VP4). Replication and assembly occurs exclusively in the cellular cytoplasm; one infectious cycle can occur within 6 to 7 hours. Most enteroviruses can be isolated in primate (human or simian) cell cultures and show characteristic cytopathic effects. Some strains, particularly several coxsackievirus A serotypes, are more readily detected by inoculation of newborn mice. In fact, the newborn mouse is one basis for originally classifying group A and B coxsackieviruses. Group A coxsackieviruses cause primarily a widespread, inflammatory, necrotic effect on skeletal muscle, leading to flaccid paralysis and death. Similar inoculation of group B coxsackieviruses causes encephalitis, resulting in spasticity and

occasionally convulsions. Echoviruses and polioviruses rarely have an adverse effect on mice, unless special adaptation procedures are first employed.

In acute enterovirus-caused syndromes, diagnosis is most readily established by virus isolation from throat swabs, stool or rectal swabs, body fluids, and occasionally tissues. Viremia is usually undetectable by the time symptoms appear. When there is CNS involvement, cerebrospinal fluid (CSF) cultures taken during the acute phase of the disease may be positive in 10 to 85% of cases (except in poliovirus infections, in which virus recovery from this site is rare), depending on the stage of illness and the viral serotype involved.

Direct isolation of virus from affected tissues or body fluids in enclosed spaces (eg, pleural, joint, pericardial, or CSF) usually confirms the diagnosis. Isolation of an enterovirus from the throat is highly suggestive of an etiologic association; the virus is usually detectable at this site for only 2 days to 2 weeks after infection. Isolation of virus from fecal specimens only must be interpreted more cautiously; asymptomatic shedding from the bowel may persist for as long as 4 months (see Fig 36–1). The polymerase chain reaction with reverse transcription and complementary DNA amplification (RT-PCR) can also be used to detect enteroviral RNA sequences in tissues and body fluids, thus greatly enhancing diagnostic sensitivity and speed.

F. Mumps virus

Mumps virus is a paramyxovirus, and only one antigenic type is known. Like fellow members of its genus, it contains single-stranded, negative-sense RNA surrounded by an envelope. There are two glycoproteins on the surface of the envelope; one mediates neuraminidase and hemagglutination activity, and the other is responsible for lipid membrane fusion to the host cell.

After initial entry into the respiratory tract, the virus replicates locally. Replication is followed by viremic dissemination to target tissues such as the salivary glands and central nervous system (CNS). It is also possible that before development of immune responses, a secondary phase of viremia may result from virus replication in target tissues (eg, initial parotid involvement with later spread to other organs). After an incubation period of 12 to 29 days (average, 16 to 18 days), the typical case is characterized by fever and swelling with tenderness of the salivary glands, especially the parotid glands. Swelling may be unilateral or bilateral and persists for 7 to 10 days. Several complications can occur, usually within 1 to 3 weeks of onset of illness. All appear to be a direct result of virus spread to other sites and illustrate the extensive tissue tropism of mumps.

Complications, which can occur without parotitis, include infection of the following:

1. Meninges: Approximately 10% of all infected patients develop meningitis. It is usually mild, but can be confused with bacterial meningitis. In about one third of these cases, associated or preceding evidence of parotitis is absent.

2. Brain: Encephalitis is occasionally severe.

3. Spinal cord and peripheral nerves: Transverse myelitis and polyneuritis are rare.

4. Pancreas: Pancreatitis is suggested by abdominal pain and vomiting.

5. Testes: Orchitis is estimated to occur in 10 to 20% of infected men. Although subsequent sterility is a concern, it appears that this outcome is quite rare.

6. Ovaries: Oophoritis is an unusual, usually benign inflammation of the ovarian glands.

Other rare and transient complications include myocarditis, nephritis, arthritis, thyroiditis, thrombocytopenic purpura, mastitis, and pneumonia. Most complications usually resolve without sequelae within 2 to 3 weeks. However, occasional permanent effects have been noted, particularly in cases of severe CNS infection, in which sensorineural hearing loss and other impairment can occur.

Mumps virus can be readily isolated early in the illness from the saliva, pharynx, and other affected sites, such as the cerebrospinal fluid (CSF). The urine is also an excellent source for virus isolation. Mumps virus grows well in primary monolayer cell cultures derived from monkey kidney, producing syncytial giant cells and viral hemagglutinin. Rapid diagnosis can be made by direct detection of viral antigen in pharyngeal cells or urine sediment.

The usual serologic tests are enzyme immunoassay (EIA) and indirect immunofluorescence to detect IgM- and IgG-specific antibody responses. Other serologic tests are also available, such as complement fixation, hemagglutination inhibition, and neutralization. Of these, the neutralization test is the most sensitive for detection of immunity to infection.

II. HOMEWORK ASSINGMENT:

to be collected to your tutor at the day of lab activity

1. What are bacterial agent in common to cause central nervous system infection?
2. How many routes the bacterial agent can trough the central nervous system?
3. Explain procedure about taking sample from meningitis patient!
4. Explain procedure about taking sample from abscess cerebral patient!
5. Explain the procedure diagnosis laboratory for bacterial agent from meningitis patient!
6. What are principle and procedure of simple staining?

III. OBJECTIVE

At the end of the activity the students will understand and can describe about:

1. The bacterial agent who makes central nervous system infection
2. The procedure of taking sample from meningitis and cerebral abscess patient
3. Identification bacterial agent cause central nervous system infection by biochemical test
4. Perform the procedure of simple staining

IV. MEASUREMENT/EXAMINATION

There are microbial examination in the cerebrospinal fluid simple staining procedure:

A. Smear preparation

B. Simple staining

C. Microscopical observation

V. EQUIPMENTS

1. Bunsen burner
2. Inoculating loop
3. Glass slide
4. Pencil glass
5. Filter paper
6. Squirt bottle
7. Light bright microscope

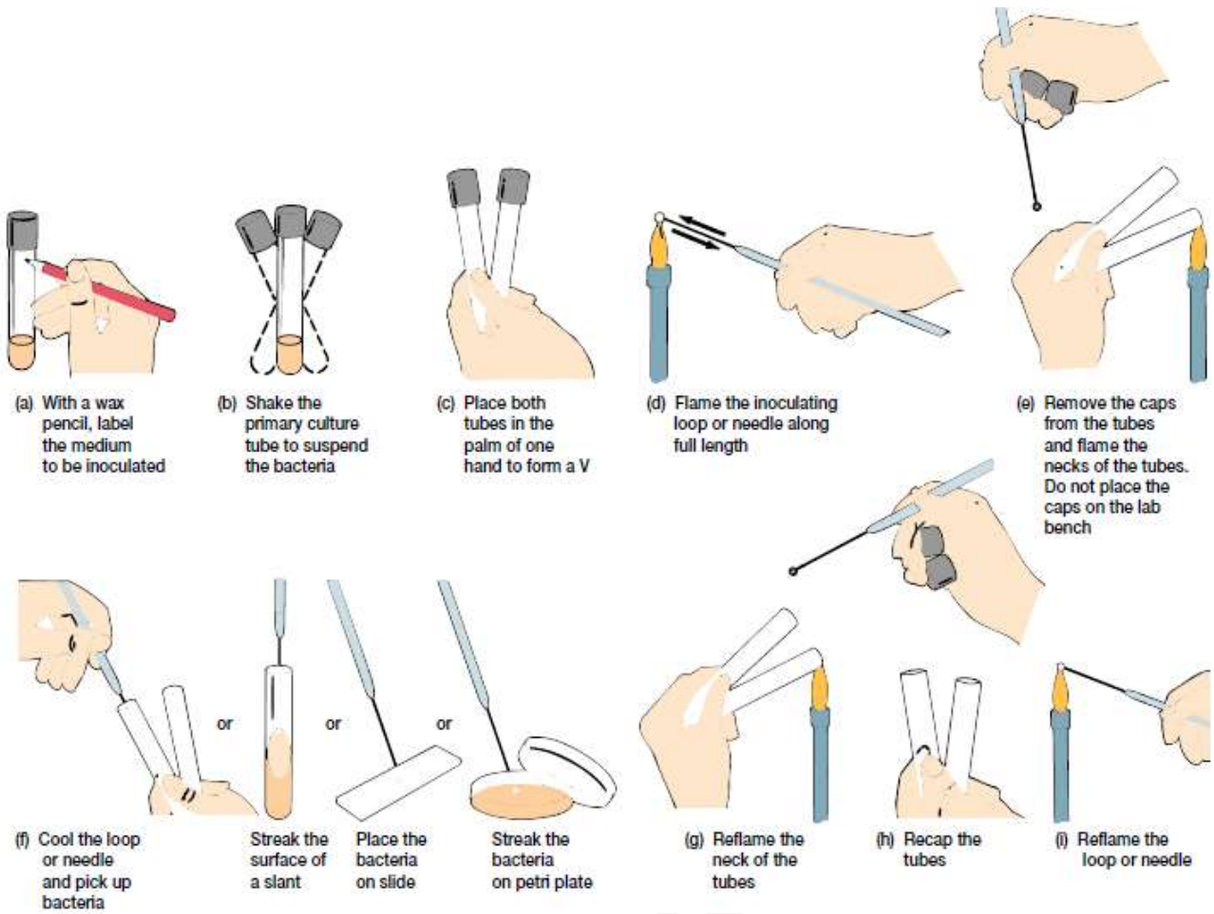
VI. REAGENTS:

1. Carbol fuchsin
2. Methylene blue
3. Water
4. Immersion oil

VII. PROCEDURES

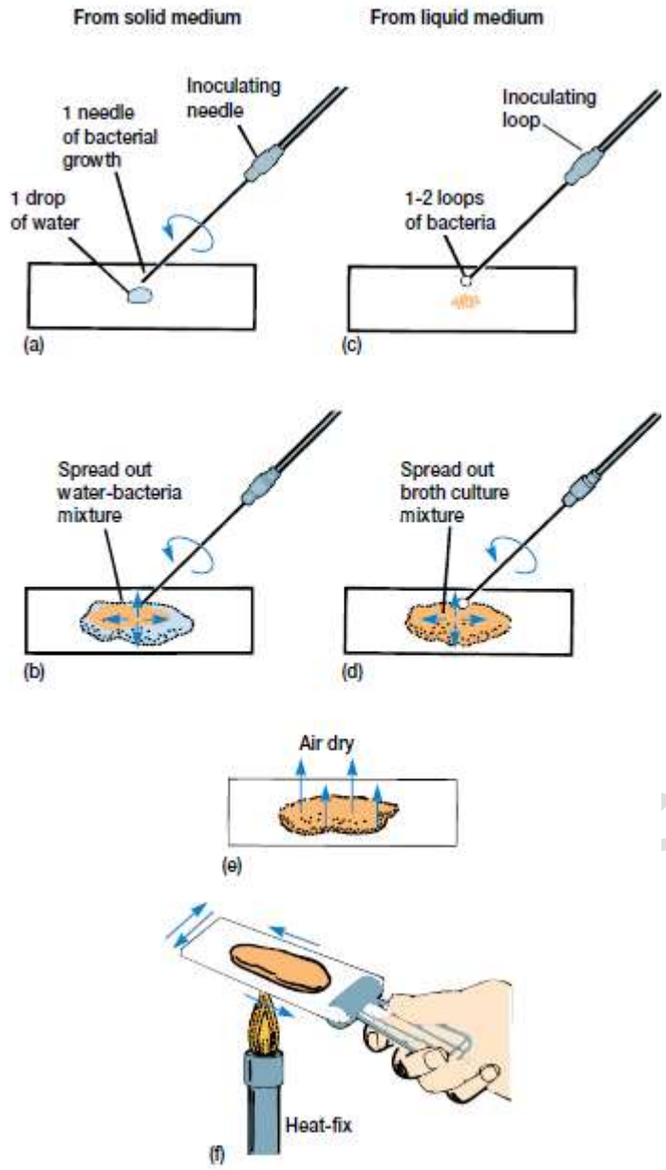
Smear Preparation

1. With the wax pencil, mark the name of the bacterial culture in the far left corner on each of three slides.
2. For the broth culture, shake the culture tube and, with an inoculating loop, aseptically transfer 1 to 2 loopfuls of bacteria to the center of the slide. Spread this out to about a d-inch area. When preparing a smear from a slant or plate, place a loopful of water in the center of the slide. With the inoculating needle, aseptically pick up a very small amount of culture and mix into the drop of water. Spread this out as above. (Three slides should be prepared; one each of *S.pneumoniae, S.aureus,* and *K.pneumoniae*)



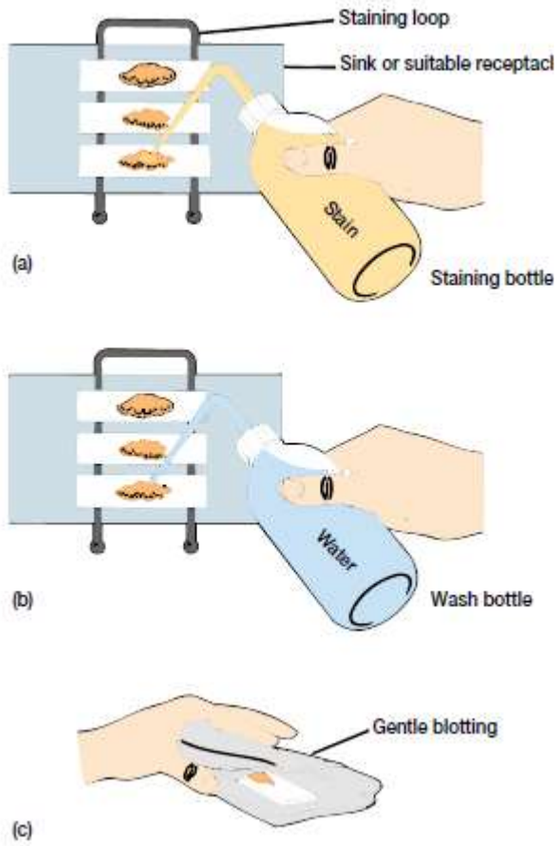
3. Allow the slide to air dry, or place it on a slide warmer.

4. Pass the slide through a Bunsen burner flame three times to heat-fix and kill the bacteria.

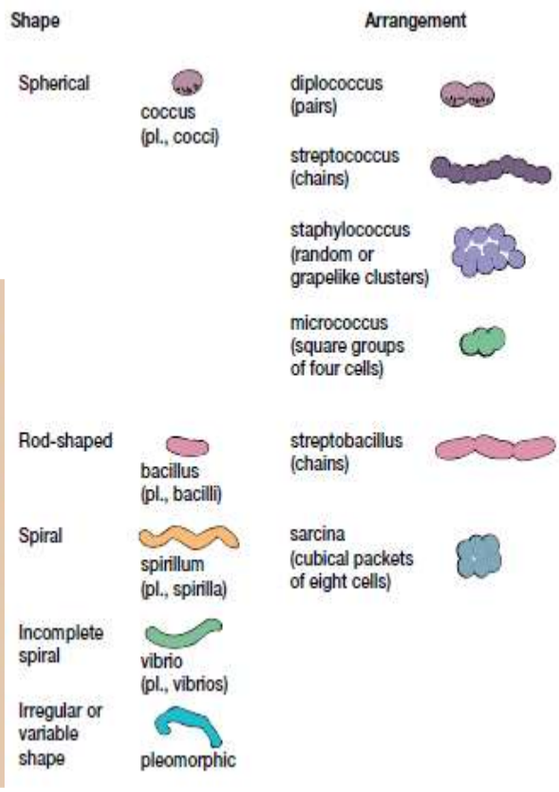


Simple Staining

1. Place the three fixed smears on a staining loop or rack over a sink or other suitable receptacle.
2. Stain one slide with alkaline methylene blue for 1 to 1d minutes; one slide with carbolfuchsin for 5 to 10 seconds; and one slide with crystal violet for 20 to 30 seconds.
3. Wash stain off slide with water for a few seconds.
4. Blot slide dry with bibulous paper. Be careful not to rub the smear when drying the slide because this will remove the stained bacteria.

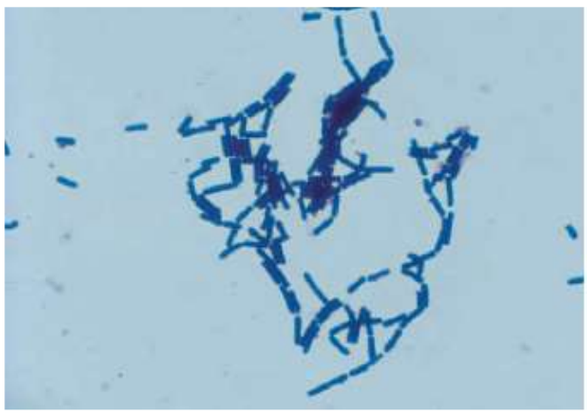


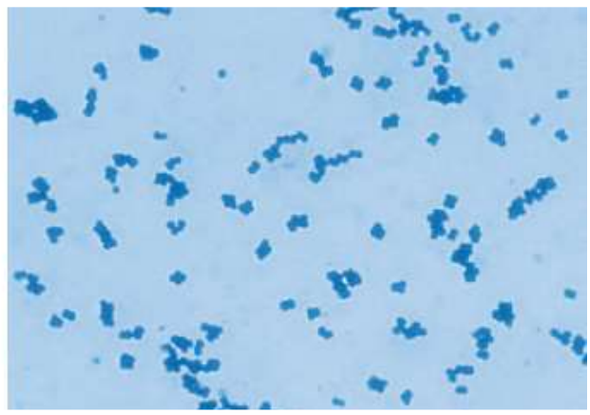
5. Examine under the oil immersion lens and complete the report.
6. You may want to treat smears of the same bacterium with all three stains in order to compare them more directly. It is also instructive to cover bacterial smears for varying lengths of time with a given stain in order to get a feel for how reactive they are and the results of overstaining or understaining a slide preparation.



HINTS AND PRECAUTIONS

(1) When heat-fixing a smear, always make sure that the smear is on the top of the slide as you pass it through the flame. (2) Bacteria growing on solid media tend to cling to each other and must be dispersed sufficiently by diluting with water. If this is not done, the smear will be too thick and uneven. Be careful not to use too much paste in making the smear. It is easy to ruin your results by using too many bacteria. (3) Always wait until the slide is dry before heat-fixing. (4) Fixing smears with an open flame may create artifacts. (5) The inoculating loop must be relatively cool before inserting it into any broth. If the loop is too hot, it will spatter the broth and suspend bacteria into the air. Always flame the inoculating loop after using it and before setting it down. (6) When rinsing with water, direct the stream of water so that it runs gently over the smear.





VIII. REPORTING RESULT:

Smear Preparation and Simple Staining

1. Complete the following drawings and table for the simple staining procedure.

Streptococcus pneumoniae	*Staphylococcus aureus*
Klebsiella pneumoniae	

Drawing of representative field

Bacterium			
Magnification			
Stain			
Cell form (shape)			
Cell color			

Background color			
Cell grouping			

Review Questions

1. What are the two purposes of heat fixation?
2. What is the purpose of simple staining?
3. Why are basic dyes more successful in staining bacteria than acidic dyes?
4. Name three basic stains.
5. Why is time an important factor in simple staining?
6. How would you define a properly prepared bacterial smear?
7. Why should you use an inoculating needle when making smears from solid media? An inoculating loop from liquid media?

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