Microbiology practicals

Basic principles of microbiological diagnosis

Practical 2: Staphylococci

1. **Introduction to Staphylococci**
    - General characteristics
    - Normal human flora
    - Clinical importance
2. **Diseases Caused by Staphylococci**
    - Skin and soft tissue infections
    - Wound infections and abscesses
    - Osteomyelitis
    - Endocarditis
    - Meningitis
    - Respiratory infections
    - Enterotoxicosis and food poisoning
3. **Specimen Collection**
    - Swabs from skin, wounds, throat, and nose
    - Blood cultures
    - Urine samples
    - Pus and other sterile specimens
4. **Microscopy and Gram Staining**
    - Gram-positive cocci in clusters
    - Differentiation from streptococci and pneumococci
5. **Cultivation of Staphylococci**
    - Growth on blood agar
    - Colony morphology of *Staphylococcus aureus* and *Staphylococcus epidermidis*

- Hemolytic properties

6. **Mannitol Salt Agar**
    - Selective and differential medium
    - Mannitol fermentation by *S. aureus*
    - Differentiation from *S. epidermidis*
7. **Catalase Test**
    - Principle of the test
    - Identification of catalase-positive staphylococci
    - Differentiation from streptococci
8. **Coagulase Testing**
    - Bound coagulase (clumping factor)
    - Free coagulase
    - Slide and tube methods
    - Identification of *S. aureus*
9. **Antimicrobial Susceptibility Testing**
    - Disc diffusion method
    - E-test
    - Interpretation of susceptibility results
10. **Treatment of Staphylococcal Infections**
    - Antistaphylococcal penicillins
    - Methicillin-sensitive and methicillin-resistant strains
    - Common therapeutic options
11. **Summary**
    - Major staphylococcal diseases
    - Laboratory diagnosis
    - Identification methods and antibiotic susceptibility testing.

Practical 3: Streptococci

1. **Introduction to Streptococci**
    - General characteristics of streptococci
    - Morphology and Gram staining
2. **Taxonomy and Classification**
    - Lancefield groups
    - Classification according to hemolysis
    - Important streptococcal species
3. **Major Streptococcal Pathogens**
    - *Streptococcus pyogenes*
    - *Streptococcus agalactiae*
    - Viridans streptococci
    - *Streptococcus pneumoniae*
4. **Clinical Manifestations**
    - Pharyngitis and scarlet fever
    - Neonatal infections
    - Pneumonia and meningitis
    - Endocarditis, septicemia, and dental caries
5. **Diagnostic Approach**
    - Specimen collection
    - Microscopy and Gram staining
    - Cultivation on blood agar
6. **Identification Tests**
    - Bacitracin test
    - CAMP test
    - Optochin test
    - Bile solubility test

7. **Post-Streptococcal Complications**
    - Rheumatic fever
    - Acute post-streptococcal glomerulonephritis
8. **Laboratory Markers**
    - ASLO (ASO) test
    - C-reactive protein (CRP)
9. **Antimicrobial Susceptibility**
    - Penicillin sensitivity
    - Alternative antibiotics
    - Emerging resistance in *S. pneumoniae*
10. **Summary**
    - Medically important streptococci
    - Diagnosis, complications, and treatment of streptococcal infections.

Practical: Neiseria, Haemophilus

Neisseria and Haemophilus Infections – Presentation Outline

1. **Introduction to Neisseria Infections**
    - Main pathogenic Neisseria species
    - Epidemiology and transmission
2. **Neisseria gonorrhoeae**
    - Biological characteristics
    - Virulence factors
    - Clinical significance
3. **Diagnosis of Gonococcal Infections**
    - Specimen collection
    - Microscopy and Gram staining
    - Culture methods
4. **Cultivation and Identification of Neisseria**
    - Chocolate agar
    - Thayer-Martin selective medium
    - Oxidase test
    - Biochemical identification
5. **Neisseria meningitidis**
    - Serogroups
    - Epidemiology
    - Laboratory diagnosis
6. **Antimicrobial Susceptibility Testing**
    - Disc diffusion method
    - Antibiotic resistance
    - Therapeutic options
7. **Introduction to Haemophilus Infections**

- Importance of *Haemophilus influenzae*
- Clinical relevance

8. **Laboratory Diagnosis of Haemophilus influenzae**
    - Microscopy and Gram staining
    - Culture characteristics
    - Satellite growth phenomenon
9. **Growth Factors and Identification**
    - X factor and V factor requirements
    - Growth factor testing
10. **Capsule and Antigen Detection**
    - Serotyping
    - Quellung reaction
    - Latex agglutination tests
11. **Rapid Diagnosis of Hib Infections**
    - Detection of *H. influenzae* type b antigen
    - Clinical applications in CNS infections
12. **Summary**
    - Key features of *Neisseria* and *Haemophilus*
    - Diagnostic methods and antimicrobial susceptibility
    - Clinical importance of major species.

Practical: Enterobacteriaceae, Campylobacter and Helicobacter

1. **Introduction to Enterobacteriaceae**
    - General characteristics
    - Medically important genera
    - Sources and transmission
2. **Structure and Physiology**
    - Gram-negative rods
    - Biochemical properties
    - O, K and H antigens
3. **Escherichia coli**
    - Clinical significance
    - Laboratory identification
4. **Klebsiella Species**
    - Virulence factors
    - Pneumonia and other infections
    - Capsule detection
5. **Proteus Species**
    - Motility and Rauss phenomenon
    - Urinary tract infections
    - Urease production
6. **Salmonella and Shigella**
    - Clinical importance
    - Laboratory diagnosis
7. **Diagnostic Approach**
    - Sample collection
    - Microscopy
    - Culture methods

- Serological identification

8. **Culture Media**
    - Blood agar
    - Endo agar
    - Deoxycholate-citrate agar (DCA)
9. **Biochemical Identification**
    - TSI (Hajn) agar
    - Urease test
    - Citrate utilization test
    - Indole test
    - Enterotest system
10. **Serotyping**
    - Slide agglutination
    - Polyvalent and monovalent antisera
11. **Campylobacter jejuni**
    - Morphology
    - Culture characteristics
    - Clinical significance
12. **Helicobacter pylori**
    - Biological characteristics
    - Urease activity
    - Association with gastric diseases
13. **Diagnosis of Helicobacter pylori**
    - Urea breath test
    - Principles and interpretation
14. **Summary**
    - Major enteric pathogens

- Laboratory diagnosis
- Key biochemical and serological tests
- Clinical relevance of Enterobacteriaceae, Campylobacter and Helicobacter.

Practical: Bacillus. Pseudomonas, Listeria

Pseudomonas, Bacillus and Listeria – Presentation Outline

1. **Pseudomonas aeruginosa**
    - General characteristics
    - Opportunistic and nosocomial infections
2. **Clinical Manifestations of Pseudomonas Infections**
    - Respiratory tract infections
    - Bacteremia and endocarditis
    - CNS, urinary tract, skin, eye and ear infections
3. **Laboratory Diagnosis of Pseudomonas**
    - Sample collection
    - Microscopy and Gram staining
    - Culture methods
4. **Cultivation and Identification of Pseudomonas**
    - Growth on blood agar
    - Growth on DCA and TSI agar
    - Pigment production
    - Oxidase test
5. **Introduction to Bacillus Species**
    - General characteristics of Bacillaceae
    - Endospore formation
6. **Bacillus anthracis**
    - Morphology and virulence factors
    - Anthrax and routes of transmission
    - Capsule and exotoxins
7. **Laboratory Diagnosis of Anthrax**
    - Microscopy
    - Culture characteristics

- Colony morphology

8. **Bacillus cereus**
    - Food poisoning syndromes
    - Clinical manifestations
    - Laboratory diagnosis
9. **Endospores**
    - Structure and properties
    - Sporulation and germination
    - Endospore staining methods
10. **Cultivation of Bacillus Species**
    - Blood agar characteristics
    - Differentiation between *B. anthracis* and *B. cereus*
11. **Listeria monocytogenes**
    - General characteristics
    - Sources and transmission
12. **Listeriosis**
    - Risk groups
    - Clinical manifestations
    - Neonatal and pregnancy-associated infections
13. **Laboratory Diagnosis of Listeria**
    - Gram staining
    - Wet mount microscopy
    - Tumbling motility
    - Culture on blood agar
14. **Treatment of Listeriosis**
    - Antibiotic therapy
    - Treatment recommendations

15. **Summary**

- Major characteristics of *Pseudomonas aeruginosa*, *Bacillus* spp., and *Listeria monocytogenes*
- Important diagnostic methods
- Clinical significance and treatment.

Practical: Corynebacterium diphtheriae, Bordetella pertussis

1. **Introduction to Bordetella**
    - *Bordetella pertussis* and whooping cough
    - Epidemiology and prevention
2. **Virulence Factors of Bordetella pertussis**
    - Pertussis toxin
    - Adenylate cyclase toxin
    - Tracheal cytotoxin
    - Dermonecrotic toxin
    - Filamentous hemagglutinin
3. **Pertussis (Whooping Cough)**
    - Transmission and incubation period
    - Catarrhal stage
    - Paroxysmal stage
    - Convalescent stage
4. **Laboratory Diagnosis of Bordetella pertussis**
    - Sample collection
    - Microscopy and Gram staining
    - Culture methods
5. **Cultivation and Identification of Bordetella**
    - Bordet-Gengou agar
    - Charcoal blood agar
    - Colony morphology
6. **Additional Diagnostic Methods**
    - Direct fluorescent antibody test
    - Enzyme immunoassay (EIA)
    - PCR
    - Western blot

7. **Introduction to Corynebacteria**
    - Clinically important species
    - Human and zoonotic infections
8. **Corynebacterium diphtheriae and Diphtheria**
    - Clinical manifestations
    - Pseudomembrane formation
    - Systemic complications
9. **Microscopy and Morphology of Corynebacteria**
    - Gram-positive pleomorphic rods
    - Metachromatic granules
    - Albert staining
10. **Cultivation of Corynebacteria**
    - Blood agar
    - Tellurite blood agar
    - Tinsdale agar
11. **Pathogenicity of Corynebacterium diphtheriae**
    - Tissue invasion
    - Diphtheria toxin production
12. **Biochemical Identification**
    - Fermentation reactions
    - Differentiation from other corynebacteria
13. **Detection of Toxigenicity**
    - Elek test
    - Animal model
14. **Antimicrobial Susceptibility and Treatment**
    - Antitoxin therapy
    - Antibiotic treatment

- Management of carriage state

15. **Summary**
    - Major characteristics of *Bordetella pertussis* and *Corynebacterium diphtheriae*
    - Diagnosis, pathogenicity and treatment of pertussis and diphtheria.

Practical: Mycobacterium

1. Introduction to the Genus *Mycobacterium*
    - General characteristics
    - Acid-fast bacteria
    - Cell wall structure and slow growth
2. Medically Important Mycobacteria
    - *Mycobacterium tuberculosis*
    - *Mycobacterium leprae*
    - Nontuberculous mycobacteria (NTM)
3. Physiology and Structure of Mycobacteria
    - Mycolic acids
    - Lipid-rich cell wall
    - Acid-fastness and resistance mechanisms
4. Classification of Mycobacteria
    - *M. tuberculosis* complex
    - Runyon classification of NTM
    - Growth characteristics and pigmentation
5. Epidemiology of Tuberculosis
    - Reservoir and transmission
    - Risk factors
    - Drug-resistant tuberculosis
6. Pathogenesis and Immunity
    - Intracellular survival in macrophages
    - Cell-mediated immune response
    - Formation of granulomas
7. Clinical Manifestations of Tuberculosis
    - Pulmonary tuberculosis
    - Extrapulmonary tuberculosis

- Common signs and symptoms

8. Laboratory Diagnosis of Mycobacterial Diseases
    - Immunological tests
    - Microscopy
    - Nucleic acid-based methods
    - Culture and identification
9. Sample Collection for Tuberculosis Diagnosis
    - Sputum
    - Respiratory samples
    - Gastric lavage
    - Extrapulmonary specimens
10. Microscopy and Ziehl-Neelsen Staining
    - Principle of acid-fast staining
    - Diagnostic significance
11. Culture of *Mycobacterium tuberculosis*
    - Growth characteristics
    - Culture methods
12. Immunodiagnosis of Tuberculosis
    - Mantoux tuberculin skin test
    - Interferon-gamma release assay (IGRA)
13. Treatment of Tuberculosis
    - First-line antituberculous drugs
    - Multidrug therapy
    - Management of drug-resistant infections
14. Antituberculous Drug Susceptibility Testing
15. Prevention and Control of Tuberculosis
    - BCG vaccination

- Surveillance and prophylaxis

16. *Mycobacterium leprae*
    - Biological characteristics
    - Epidemiology and transmission
17. Leprosy (Hansen Disease)
    - Tuberculoid form
    - Lepromatous form
    - Clinical features
18. Diagnosis of Leprosy
    - Microscopy
    - Skin testing
    - Limitations of culture
19. Treatment and Control of Leprosy
    - Drug regimens
    - Prevention and case management
20. Summary
    - Major mycobacterial pathogens
    - Diagnosis, treatment and prevention of tuberculosis and leprosy.

Practical: Anaerobes, Treponema, Leptospira

1. **Introduction to Anaerobic Bacteria**
    - Classification according to oxygen tolerance
    - General characteristics
2. **Clinically Important Anaerobes**
    - Gram-negative rods
    - Gram-positive cocci
    - Clostridia and other anaerobic bacilli
    - Gram-negative cocci
3. **Microscopy of Anaerobic Bacteria**
    - Gram staining
4. **Cultivation of Anaerobes**
    - Non-selective media
    - Selective and differential media
5. **Anaerobic Culture Conditions**
    - Anaerobic jars
    - Atmosphere generation systems
    - Biological methods
6. **Clostridia**
    - Major pathogenic species
    - Clinical importance
7. **Clostridium difficile**
    - Pseudomembranous colitis
    - Antibiotic-associated diarrhea
    - Toxins A and B
8. **Clostridium perfringens**
    - Gas gangrene
    - Tissue-destroying enzymes

9. **Clostridium tetani**
    - Morphology
    - Tetanospasmin
    - Pathogenesis of tetanus
10. **Tetanus**
    - Clinical effects of toxin
    - Treatment and prevention
11. **Clostridium botulinum**
    - Characteristics
    - Sources of infection
12. **Botulism**
    - Food-borne botulism
    - Wound botulism
    - Infant botulism
    - Treatment
13. **Spirochetes**
    - General characteristics
    - Medically important genera
14. **Treponema pallidum**
    - Taxonomy
    - Diseases caused by treponemes
15. **Syphilis**
    - Primary syphilis
    - Secondary syphilis
    - Latent syphilis
    - Tertiary syphilis
16. **Laboratory Diagnosis of Syphilis**

- Direct detection methods
- Serological testing

17. **Nontreponemal Tests**
    - VDRL
    - RPR
18. **Treponemal Tests**
    - FTA-ABS
    - TPHA
    - MHA-TP
    - TP-PA
19. **Interpretation of Syphilis Serology**
    - Screening tests
    - Confirmatory tests
20. **Summary**
    - Clinically important anaerobic bacteria
    - Clostridial diseases
    - Syphilis diagnosis and laboratory testing.

Practical: Virological Tests and Diagnostic Methods

1. **Introduction to Virological Diagnosis**
    - Principles of laboratory diagnosis of viral diseases
2. **Laboratory Diagnosis of Viral Infections**
    - Cytopathic effects
    - Detection of viral particles
    - Virus isolation
    - Detection of viral components
    - Serological methods
3. **Specimen Collection for Virological Examination**
    - Types of clinical specimens
    - Transport and storage requirements
4. **Common Specimens Used in Virology**
    - Respiratory specimens
    - Blood, CSF, urine and stool
    - Tissue and lesion samples
5. **Viral Transport Medium**
    - Composition and purpose
6. **Diagnostic Methods in Virology**
    - Direct examination
    - Virus isolation
    - Serological diagnosis
7. **Direct Detection of Viruses**
    - Antigen detection
    - Electron microscopy
    - Light microscopy
    - Viral genome detection
8. **Virus Isolation Methods**

- Cell culture
- Embryonated eggs
- Experimental animals

9. **Serological Diagnosis**
    - Antibody detection
    - Classical and modern serological methods
10. **Electron Microscopy**
    - Principles
    - Applications in viral diagnosis
    - TEM and SEM
11. **Virus Cultivation Systems**
    - Biological systems
    - Embryonated eggs
    - Tissue culture systems
12. **Cultivation in Embryonated Eggs**
    - Advantages
    - Routes of inoculation
13. **Cell Culture Techniques**
    - Primary cell cultures
    - Semi-continuous cell cultures
    - Continuous cell cultures
14. **Cytopathic Effects in Cell Culture**
    - Cell rounding
    - Plaque formation
    - Syncytium formation
    - Inclusion bodies
15. **Limitations of Cell Culture**

- Sensitivity
- Contamination
- Viruses that cannot be cultured

16. **Serological Interpretation**
    - Primary infection
    - Reinfection
    - Seroconversion
17. **Major Serological Tests**
    - Virus neutralization test
    - Complement fixation test
    - Hemagglutination inhibition assay
    - ELISA and ELISPOT
    - Immunoblotting and immunofluorescence
18. **Molecular Methods in Virology**
    - Genome detection
    - PCR-based methods
    - Future role in viral diagnostics
19. **Summary**
    - Diagnostic approaches in virology
    - Virus isolation and cultivation
    - Serological and molecular methods
    - Interpretation of laboratory results

Practical: Fungi and Mycoses

1. **Introduction to Fungi**
    - General characteristics
    - Fungal cell structure
    - Ergosterol and fungal cell wall
2. **Fungal Morphology and Replication**
    - Yeasts
    - Molds
    - Hyphae and mycelium
    - Dimorphic fungi
3. **Fungal Spores**
    - Sporangiospores
    - Conidia
4. **Classification of Mycoses**
    - Superficial mycoses
    - Cutaneous mycoses
    - Subcutaneous mycoses
    - Systemic mycoses
    - Opportunistic mycoses
5. **Superficial Mycoses**
    - Clinical characteristics
    - Common causative agents
6. **Cutaneous Mycoses**
    - Dermatophytes
    - *Epidermophyton*
    - *Microsporum*
    - *Trichophyton*
    - Dermatomycoses caused by *Candida*

7. **Laboratory Diagnosis of Cutaneous Mycoses**
    - KOH preparation
    - Specimen collection
    - Culture methods
8. **Subcutaneous Mycoses**
    - Sporotrichosis
    - Chromoblastomycosis
    - Zygomycosis
    - Phaeohyphomycosis
    - Mycetoma
9. **Systemic Mycoses**
    - Host defense mechanisms
    - Risk factors for infection
10. **Major Endemic Systemic Mycoses**
    - Coccidioidomycosis
    - Histoplasmosis
    - Blastomycosis
    - Paracoccidioidomycosis
11. **Coccidioidomycosis**
    - Transmission
    - Clinical manifestations
    - Diagnosis and treatment
12. **Histoplasmosis**
    - Pathogenesis
    - Clinical forms
    - Laboratory diagnosis
13. **Blastomycosis**

- Epidemiology
- Clinical manifestations
- Diagnosis

14. **Paracoccidioidomycosis**
    - Clinical presentation
    - Laboratory diagnosis
15. **Opportunistic Mycoses**
    - Risk groups
    - Major opportunistic fungal pathogens
16. **Cryptococcosis**
    - *Cryptococcus neoformans*
    - Meningitis
    - Diagnosis and treatment
17. **Aspergillosis**
    - Clinical forms
    - Risk factors
    - Disseminated disease
18. **Candidiasis**
    - *Candida albicans*
    - Pathogenesis
    - Clinical manifestations
19. **Chronic Mucocutaneous Candidiasis**
    - Clinical features
    - Treatment
20. **Pneumocystis Pneumonia**
    - *Pneumocystis jirovecii*
    - Clinical manifestations

- Diagnosis and treatment

21. **Clinical Specimens in Mycology**
    - Respiratory samples
    - Skin and nail specimens
    - Blood and body fluids
22. **Conventional Laboratory Diagnosis**
    - Microscopy
    - Culture
    - Histopathology
    - Immunological methods
    - Molecular methods
23. **Microscopic Examination**
    - Direct microscopy
    - KOH preparation
    - Staining techniques
24. **Culture and Identification**
    - Sabouraud agar
    - CHROMagar
    - Rice agar
    - Fungal identification
25. **Antifungal Therapy**
    - Polyenes
    - Azoles
    - Allylamines
    - Echinocandins
    - Flucytosine
    - Griseofulvin

26. **Summary**

- Fungal morphology and classification
- Major fungal diseases
- Diagnostic methods
- Principles of antifungal treatment

Practical: Parasitology

1. **Introduction to Parasites**
    - Classification of human parasites
    - Protozoa, helminths and arthropods
2. **Protozoa**
    - General characteristics
    - Life cycle
    - Trophozoite and cyst stages
3. **Flagellates**
    - Intestinal and urogenital flagellates
    - Blood and tissue flagellates
4. **Giardia intestinalis**
    - Transmission and life cycle
    - Clinical significance
5. **Trichomonas vaginalis**
    - Morphology
    - Transmission
    - Clinical features
6. **Trypanosoma Species**
    - African trypanosomiasis
    - Chagas disease
    - Clinical manifestations
7. **Leishmania Species**
    - Cutaneous leishmaniasis
    - Visceral leishmaniasis
    - Clinical significance
8. **Amoebae**
    - General characteristics
    - Reproduction and movement

9. **Entamoeba histolytica**
    - Intestinal and extraintestinal disease
10. **Naegleria**
    - Primary amoebic meningoencephalitis
11. **Sporozoa**
    - Life cycle
    - Sexual and asexual reproduction
12. **Toxoplasma gondii**
    - Sources of infection
    - Life cycle
    - Clinical manifestations
13. **Diagnosis of Toxoplasmosis**
    - Microscopy
    - Serology
    - PCR
14. **Plasmodium Species**
    - Major malaria parasites
    - Life cycle and transmission
15. **Malaria**
    - Clinical manifestations
    - Complications
    - Species-specific features
16. **Helminths**
    - Classification
    - Nematodes, cestodes and trematodes
17. **Nematodes**
    - Intestinal and tissue nematodes
    - Medically important species

18. **Ascaris lumbricoides**
    - Morphology
    - Laboratory diagnosis
19. **Enterobius vermicularis**
    - Enterobiasis
    - Clinical manifestations
    - Scotch tape test
20. **Dracunculus medinensis**
    - Guinea worm disease
21. **Cestodes (Tapeworms)**
    - General characteristics
    - Major genera
22. **Taenia Species**
    - Beef and pork tapeworms
23. **Trematodes (Flukes)**
    - General characteristics
    - Medically important species
24. **Schistosoma Species**
    - Schistosomiasis
25. **Laboratory Diagnosis of Parasitic Infections**
    - Stool sample collection
    - Concentration techniques
    - Microscopic examination
26. **Stool Processing Methods**
    - Flotation techniques
    - Sedimentation techniques
    - Sample fixation
27. **Summary**

- Major protozoan infections
- Helminthic diseases
- Laboratory diagnosis of parasitic infections