Logbook & Laboratory
Work Guide of Medical Microbiology and Immunology
For third year medical students

2013-2014
Student name: ..............................................

Student number: ...........................................

Number of practical section:.............................

Group number: ..............................................
Preface

The purpose of this study guide and workbook is to introduce the essentials of clinical and practical microbiology as well as to provide the student with an organized, user-friendly tool to better enable him or her to understand laboratory aspects of microbiology using an interactive format of lab quizzes. This book is composed of three sections. In the first section, the students studies the basic methods for microbial isolation and identification and how to examen their characteristic morphologies, cultural characters and biochemical behavior, all help the student to establish a laboratory identification of medically important microbes. Also in the first section the students study different methods of sterilization and disinfection and laboratory biosafety. The second section of this book comprises clinical microbiology cases of medically important microorganisms, and how to proceed in laboratory diagnosis of infections caused by these microorganisms. In the end of each session, the student is asked to observe the spots demonstrated in the laboratory and to draw some of these demonstrations in the workbook as a part of student's activities. The end of each session includes as well some highlight points that help the students to self assess the knowledge that they acquired from the session. The third section of the book includes the skill lab, which improve infection control practice and heighten awareness of risks associated with poor infection control practice in health care setting, laboratory and community. We hope you enjoy this workbook and we also hope it makes your study of microbiology a bit easier. We thank all those who supported and helped in the preparation of this work.

Prof. Mohammad Abo Elela

Professor and Head of Medical Microbiology & immunology Department
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Course specification

Overall Aims of Course:

• To educate students about the basic features of general bacteriology, virology and mycology.

• To provide students with an understanding of the immune system, its protective functions and its role in the patho-physiology of infectious and non-infectious diseases.

• To learn about microbial genetics and advanced biotechnology techniques.

• To define the following terms: antimicrobial agent, antibiotic, chemotherapeutic agent and to describe the mechanisms of action of antimicrobial chemotherapeutic agent with the origin and the different mechanisms of drug resistance.

• To familiarize students with the common infections and diseases of medical importance, their microbial causes, as well as laboratory diagnosis, treatment, prevention and control of such diseases.

• To enable the students to practice the principles of sterilization and infection control.

Intended Learning Outcomes of Course (ILOs):

a-Knowledge and Understanding:

By the end of this course the student should be able to:

a1- Illustrate general bacterial morphology, physiology and genetics.

a2- Recognize the host parasite relationship and microbial pathogenesis.

a3- Explain the physiology of the immune system, its beneficial role, as well as its detrimental role in immunopathology (hypersensitivity, autoimmunity and transplant rejection).

a4- Recognize the most important infectious clinical conditions and outline the diagnosis, treatment, prevention and control of the most likely organisms causing such diseases.

a5- Describe the morphology, culture character, antigenic structure and virulence factors of microorganisms of medical importance.

a6- Describe the most important methods of decontamination and principles of infection control.
a 7- Describe the basics of antimicrobial uses and resistance.
A8- Understand the impact of molecular technology in microbiology and immunology.

**b- Intellectual Skills:**

By the end of this course the student should be able to:

b1- Interpret results of microbiological, serological and molecular tests

b2- Formulate a systematic approach for laboratory diagnosis of common infectious clinical conditions and select the most appropriate tool to the identification of the causative organism.

b3- Evaluate according to evidence the causal relationship of microbes and diseases.

b4- Categorize a microorganism as a bacterium, virus or fungus according to standard taxonomy.

**c-Professional and Practical Skills:**

By the end of this course the student should be able to:

c1-Elicit medically important bacteria based on microscopic examination of stained preparations.

c2-Perform a Gram stain and a Ziehl-Neelsen stain and identify, according to morphology and characteristics, stained preparations.

c3- Elicit culture media, biochemical tests and serological tests commonly used for bacterial identification and distinguish positive and negative results.

C4- Perform hand wash and identify different methods of physical and chemical methods of sterilization.

C5- Use the virtual lab to learn standard practices and procedures, get familiarity with the equipments, apply the scientific method and develop the online critical thinking skills.

C6- Improve infection control practice by skill lab and heighten awareness of risks associated with poor IC practice in health care setting, laboratory and community.

**d-General and Transferable Skills:**

By the end of this course the student should be able to:

d1- Establish a concise scientific activity according to standard scientific thinking and integrity.
d2- Explain the danger of handling and use of infectious agents on community and environment as a part of their ethical heritage.

d3- Interact and communicate effectively with other health care professionals during infection control practice.
STUDENT ASSESSMENT

Attendance Criteria:
Students should attend no less than 70% of practical classes and/or small group sessions as an essential prerequisite to be legible for the final exams.

Student Assessment Methods:
5.1 - Written Exam to assess ILOs: a1-8
5.2 – MCQs to assess ILOs:a1-8, b1-4
5.3 - Practical examination to assess ILOs: a1-8, b1-4, c1-4
5.4 - Oral exam to assess ILOs:a1-8, b1-4, c1-4, d1-3.

Assessment Schedule:

Mid year exam 35 marks
Oral Examination 20 marks
Practical Examination 40 marks divided as
  • Practical exam. 37 marks
  • Skill lab 3 marks
Log book and practical book 5 marks
Final Written Exam 100 marks
Total 200 marks
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Title: MICROSCOPY

Objectives:
By the end of this session, students should acquire the following knowledge:
1- Types of microscopes.
2- Structure of ordinary light microscope.
3- Precautions for the use and care of microscopes.
4- Magnification power of the microscope.
5- Uses & principle of Dark ground microscope.
6- Uses & principle of Phase contrast microscope.
7- Uses & principle of Fluorescence microscope.
8- Uses, advantages, disadvantages& principle of Electron microscope.

Assessment questions

Complete the following sentences:
The magnification power of the ordinary light microscope is .........................

The resolution of electron microscope is .....................................................

The oil used during microscopic examination is ...........................................

The microscope used for examination of refractile organisms is ..................

The microscope used for examination of viruses is .................................
Section no: ............................................  Tutor: ..........................................................
Date: ..................................................

Title: Staining Methods

Objectives:
By the end of this session, students should acquire the following knowledge and skills:
1- How to prepare a dried fixed film.
2- Types of stains.
3- Procedure and principle of Gram stain.
4- Procedure and principle of Ziehl-Neelsen stain.
5- The use and procedure of Hanging drop preparation.

Student activities:

1. Gram positive cocci
2. Gram positive bacilli
3. Gram negative cocci
4. Gram negative bacilli
5. Hanging Drop

Assessment questions
Organisms which resist decolourization & retain the colour of the basic dye are called:
a) Gram +ve.  b) Gram -ve.  c) Alcohol fast.

Mark

Supervisor
**Title: Sterilization**

**Objectives:**
By the end of this session, students should acquire the following knowledge:

1. Definition of sterilization and disinfection.
2. The physical methods of sterilization and their uses.
3. The chemical methods of sterilization and their uses.
4. The difference between disinfection and antisepsis.

**Student activities:**

Define sterilization:

------------------------------------------------------------------------------------------------------------------------

Define disinfection:

------------------------------------------------------------------------------------------------------------------------

Metal instruments can be sterilized by the following methods except:

a. Boiling.
b. Autoclave.
c. Koch’s steamer.
d. Formaldehyde vapour.
Title: Culture Media

Objectives:
By the end of this section, students will acquire the following knowledge:
1. Importance of culture media.
2. Different types of culture media.
3. Methods of sterilization of different types of culture media.
4. Suitable media for culture of different groups of organisms and characteristics of growth.

Student activities

Nutrient agar plate  Nutrient agar slope  Deep agar

Blood agar plate  Chocolate agar plate  Löffler’s serum slope
Assessment questions

Match the following organisms with their respective culture media:

1. Neisseria gonorrhoea a. Blood agar
2. Tubercle bacilli b. MacConkey’s medium
3. Diphtheria bacilli c. Dorset egg medium
4. Salmonella d. Löffler serum
   e. Chocolate agar

Mark

Supervisor
Title: Collection of samples for bacteriological examination

Objectives:

By the end of this session, the student should be able to know the following:

1- The general rules that should be applied during collection of samples for laboratory diagnosis.
2- The appropriate samples required to diagnose a case of respiratory tract infection.
3- How to transport a respiratory specimen to the lab after collection.
4- The proper samples needed to diagnose a case of intestinal infection.
5- The appropriate methods of collection and transport of urinary specimens.
6- How to collect a CSF sample from a case of meningitis.
7- The appropriate samples required for diagnosis of wound sepsis.
8- The blood culture technique for diagnosis of septicemia.

Assessment questions

What is the proper bacteriological sample to diagnose a meningococcal carrier?

For each of the following gastrointestinal infections, mention the most suitable sample for diagnosis:

a- Cholycystitis: ............................................................

b- Food poisoning: ..........................................................

c- Cholera chronic diarrhea: ..............................................
Title: **Laboratory Diagnosis of Infection**

**Objectives:**

By the end of this session the student should be able to know and gain skills about the following:

1. The value of direct microscopic examination of specimens.
2. The plating out technique for culture of specimens.
3. The various methods for anaerobiosis.
4. The schematic identification of bacteria isolated from clinical specimens.
5. How to describe the colonial characters of isolated bacteria.
6. The scheme for description of microorganisms under a microscope.
7. The principles of some biochemical tests used for identification of isolated bacteria.
8. The value of animal inoculation in the identification of an unknown organism.
9. The different methods of bacterial typing.

**Student activities:**

- Plating out technique
- Antibiotic sensitivity plate

**Assessment questions**

What is the value of direct microscopic examination of specimens?

---

Mark

Supervisor
Title: **Serological Tests**

**Objectives:**

At the end of this session, the student should know:

1. Nature of antigen-antibody reactions.
2. Specificity and cross reactivity.
3. Factors affecting measurement of antigen-antibody reactions.
4. Types of serological tests (agglutination, precipitation, complement fixation).
5. The principle of each test.
6. Applications of serological tests.
7. Commonly used serological tests e.g. blood typing.

**Student activities**

A. **The students should perform the following tests:**

1. Blood grouping of various blood samples.
2. Latex agglutination tests: ASO and CRP

B. The students will observe the demonstrated Widal tube agglutination tests and interpret the positive results.

C. The students will observe the demonstrated Wasserman test and interpret its results.

- Slide agglutination
- Widal test
- Widal test
- Salmonella typhi
- Salmonella paratyphi A
**Assessment questions**

**Mark the correct answer (✓)**

Direct coomb’s test is used for the diagnosis of

- Hemolytic disease of the newborn
- Pregnancy
- Rh typing

Elek’s test used to detect the toxigenic Diphtheria bacilli is an example of

- Agglutination test
- Complement fixation test
- Precipitation test

Mark

Supervisor
Title: Staphylococcal infections

Objectives:
By the end of this session, the student should gain the following knowledge:
1- The different species of staphylococci.
2- The different diseases caused by Staph.aureus.
3- The appropriate samples to diagnose each of the staphylococcal diseases.
4- The morphological characters of staphylococci.
5- The cultural characters of staphylococci.
6- The principle of each of the biochemical tests used to identify Staph.aureus as well as the methods and interpretation of these tests.
7- How to differentiate the species of staphylococci.
8- How to diagnose staphylococcal food poisoning.
9- Phage typing of Staph.aureus.

Student activities:

- Gram stained film of Staphylococci in culture
- Coagulase Test
- B- haemolytic Colonies of Staph.aureus on blood agar
- Catalase Test
**Assessment questions**

Staph. aureus shows the following type of arrangement:

a- Clusters.  
b- Chains.  
c- Chinese letter.  
d- Pairs.

Which of the following media is selective for the isolation of Staph. aureus?

a- Nutrient agar  
b- Blood agar  
c- Mannitol salt agar  
d- DNAase agar

List the cultural characters of Staphylococcal isolates

1- .................................................................
2- .................................................................
3- .................................................................
4- .................................................................
Title: Streptococci

Objectives:
By the end of this session the student should be able to:

1. Identify Streptococci.
2. Know the basis of classification, grouping and typing of Streptococci.
3. Diseases caused by and remote complications of streptococcal infections.
4. Samples collected to diagnose each of streptococcal infections.
5. How samples are processed until an accurate diagnosis is obtained.

Student activities:

- Gram stained film of *Strept. pyogenes* in sputum
- Gram stained film of *Strept. pneumoniae* in sputum
- α-Haemolytic Colonies of *Strept. pneumoniae*
- β-Haemolytic Colonies of *Strept. pyogenes*
- Optochin sensitivity Test
- Bile solubility Test

Assessment questions
List some specific tests that help in the diagnosis of scarlet fever

a. .......................................................... b. ..........................................................

Mark

Supervisor

22
Title: Neisseria

Objectives:

By the end of this session the student should be able to:

6. Identify Neisseria
7. Know the basis of classification and typing of Neisseria
8. Diseases caused Neisseria infections.
9. Samples collected to diagnose each disease.
5. How samples are processed until an accurate diagnosis is obtained.

Student activities:

The student should be able to:

1. Examine Gram-stained film from samples possibly containing Neisseria.
2. Perform and read the related biochemical activities and reactions.

Assessment questions

Mention one method for rapid diagnosis of meningococcal meningitis other than the direct smear

Mark

Supervisor
Title: Bacillus and Corynebacterium group

Objectives:

At the end of this session, the student should know and gain the following skills:

1. The ability to identify *Bacillus anthracis*.
2. The ability to differentiate between *Bacillus anthracis* and other bacillus species.
3. The ability to make laboratory diagnosis of Diphtheria cases or carriers.

Student activities:

- Beta haemolytic colonies of *Bacillus cereus*
- Colonies of Anthracoids on blood agar plate
- Gelatin liquefaction test
- Gram stained film of *Bacillus anthracis*
- Gram stained film of *C. diphtheriae* in culture
- Elek immunoprecipitation test

Assessment questions

Mc Fadyean stain is used to demonstrate .........................

Mark

Supervisor
Title: Genus *Clostridium*

**Objectives:**
By the end of this session, the student should gain the following knowledge:
10- The important members of genus *Clostridium*.
11- The different diseases caused by *Clostridium*.
12- The appropriate samples to diagnose each of the Clostridium diseases.
13- The morphological characters of *Clostridium*.
14- The cultural characters of *Clostridium*.
15- The principle of each of the biochemical tests used to identify *C. perfringens* as well as the methods and interpretation of these tests.
16- How to diagnose Tetanus.
17- How to diagnose Gas gangrene.
18- How to diagnose botulism.
10- Methods of detection of toxins produced by *Clostridium species*.

**Student activities:**

- [ ] Gram stained film of *Clostridium tetani*
- [ ] Acidic litmus milk

**Assessment questions**

Clostridial toxins can be detected by:
1- ........................................
2- ........................................
3- ..............................................................

Mark

Supervisor
Title: Mycobacteria

Objectives:

By the end of this session, the student should gain the following knowledge and skills:

1. The diseases caused by the genus Mycobacteria.
2. The diagnosis of infections caused by Mycobacteria.
3. The stains used for diagnosis of M. tuberculosis.
4. Identification of acid fast bacilli.
5. The media used for isolation of M. tuberculosis.
6. Skin testing for TB.

Student activities:

- Sputum smear stained by ZN stain showing Acid fast bacilli
- Lowenstein Jensen media
- Colonies of M. tuberculosis on LJ media

Assessment questions

Mention the principle of tuberculin test

Describe the CSF picture of TB meningitis
Title: ENTEROBACTERIACEAE

Objectives:

By the end of this section, students should acquire the following knowledge and skills:

1. Examination of Gram stained smear of Gram-negative bacilli from culture.
2. Basis of classification and special features of different members of Enterobacteriaceae family.
3. Laboratory diagnosis of diseases caused by different members of Enterobacteriaceae family.

Student activities:

- Gram stained film of E. coli
- L.F. Colonies of E. coli
- Mucoid colonies of Klebsiella
- B.R. of E.coli
- B.R. of Klebsiella
- B.R. of Citrobacter
Assessment questions

True (T) or False (F)

1. Some strains of E.coli are capsulated  (   )
2. Citrobacter is indole positive.   (   )

Mark

Supervisor
Title: Vibrio, Pseudomonas, Hemophilus, Bordettela, Yersinia, Mycoplasma, rickettsia & chlamydia

Objectives:
At the end of this session, students will be able to learn and gain the following skills:

1. Learn the main diseases caused by these organisms
2. Mode of transmission of these organisms
3. The difference between these organisms in their growth conditions and biochemical reactions.

Student activities:

Assessment questions

Choose the single best answer:

**Urea breath test is diagnostic to:**
- a. V cholera
- b. Campylobacter
- c. Helicobacter
- d. Spirochetes

- **Haemophilus influenza** type b can be detected in the cerebrospinal fluid by Quelling reaction or latex agglutination.
  - True
  - False

Mark

Supervisor
**Title: Spirochetes**

**Objectives:**

By the end of this session the student should be able to know and gain skills about the following:

10- The special characters of spirochetes family.
11- The pathogenic species in spirochetes.
12- The disease caused by Terponema pallidum.
13- Methods of diagnosis of syphilis.
14- Methods of diagnosis of relapsing fever.
15- The pathogenesis of leptospirosis.

**Student activities: Wasserman test**

- Strong positive
- Moderate positive
- Weak positive
- Negative test
- Antigen anticomplementry
- Antibody anticomplementry

**Assessment questions**

What are the samples for diagnosis of leptospirosis?

1. ................................................................................................................
2. ................................................................................................................

Mark

Supervisor
**Title: Virology cases**

**Objectives:**

At the end of this session, the student should know:

1- How can viruses be detected in clinical specimens.
2- Tests used for detection of antiviral antibodies.
3- Methods of cultivation and identification of viruses.
4- Methods of quantification of viruses.

**Student activities:**

1- The student should know the instruments demonstrated.
2- The student should try to search on the web and find the following
   a. Pictures of the cytopathic effects of some viruses.
   b. The electron microscopic picture of some viruses.

**Assessment questions**

Viral antigens can be detected by:

a. ELISA
b. Complement fixation test.
c. Immunofluorescence.
d. All of the above.
e. None of the above.
Title: Mycology cases

Objectives

By the end of this session, the student should gain the following knowledge

1. Types of mycosis and proper sample taken in each type
2. Value and methods of direct examination of samples
3. Different media used for isolation of fungi
4. Identification of fungal growth on culture eg C. albicans

Student activities:

- Candida by gram stain
- Candida by LPCB stain
- Septated hyphae by LPCB
- Non septated hyphae by LPCB
- Aspergillus niger head by LPCB
- Aspergillus fumigatus head by LPCB
- Aspergillus niger black hairy colonies on SDA
- Candida white creamy colonies on SDA
- Candida white creamy colonies on SDA
Assessment questions

1. Value of KOH (10-30 %) in direct examination of fungi sample
   a. Inhibit saprophytic fungi
   b. Inhibit bacteria flora
   c. Keratolytic
   d. All of above
   e. Non of above

2. Complete undisrupted morphology of fungi is seen in
   a. SDA
   b. Wood light
   c. EM
   d. Slide culture or microculture
   e. All of above

3. Stains used for fungal diagnosis are
   a. Stained with lactophenol cotton blue
   b. Histopathology by PAS & silver stain
   c. Geimsa stain (histoplasma)
   d. India ink (-ve stain) for Cryptococcus capsule
   e. All of above

4. Media used for culture of fungi are
   a. Blood agar
   b. SDA
   c. Dermatophyte test media
   d. Potato carrot bile salt media
   e. All of above
Title: Standard Infection Control measures for Hospital Acquired Infections

Objectives:

At the end of this section the students should know the following:

1. The importance of hand washing.
2. The proper hand washing technique and indications.
4. Decontamination of Equipment.
5. Dealing with Blood and other Body Fluids.
7. Proper use of Personal Protective Equipment: (Eye / Face Protection, Mask, Aprons, Gloves).

Student activities

**Practice hand washing technique:**

1. Rub hands palm to palm.
2. Right palm over left dorsum with interlaced fingers and vice versa.
3. Palm to palm with fingers interlaced.
4. Backs of fingers to opposing palms with fingers interlocked.
5. Rotational rubbing of left thumb clasped in right palm and vice versa.
6. Rotational rubbing, backwards an forwards with clasped fingers of right hand in left palm and vice versa.
**Practice:**

**Action in the event of a sharps injury or contamination with blood:**

- Encourage bleeding, squeeze the injury, do not suck.
- Wash the skin thoroughly with soap and water, do not scrub.
- Liberal use of antiseptic solution.
- Irrigate contaminated mucous membranes eg mouth and eyes with large quantities of tap water.
- Cover the injury with waterproof dressing and seek medical advice without delay.

**Assessment questions**

The most important for controlling nosocomial infections is:

- a) Safe use of sharps
- b) Hand hygiene
- c) Sterilization of equipment
- d) a+c
- e) None of the above

The concentration of alcohol needed to be effective for hand hygiene is 40%  
☐ True  ☐ False

Burning or incinerating is one way to destroy needle disposable boxes.  
☐ True  ☐ False
Skill lab

Introduction:
Students should be introduced to the principles and practice of infection control in three settings—hospital, clinic, and the community, through a series of skills. The emphasis would be on the development of systematic approach to infection control practice.

Objectives:
Improve infection control practice and heighten awareness of risks associated with poor IC practice in health care setting, laboratory and community.

Structure:
The practical course is structured into a number of learning activities which are supported by graphics, video, animations, a number of challenges and a quiz.

Course layout and contents:
The practical course would be conducted during the academic year for the third year students once/week for 25 student/session. Course contents are delivered in 3 hours/class.

SKILLS ALLOCATED:
1. Principles of hand hygiene.
2. Dressing and removal of PPE.
4. Infectious waste disposal.