

LABORATORY MANUAL

Microbiology, Parasitology and Immunology

Chief Editor Liping Zhang







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Foreword

Global developments in medicine and health shape trends in medical education. And in China education reform has become an important focus as the country strives to meet the basic requirements for developing a medical education system that meets international standards. Significant medical developments abroad are now being incorporated into the education of both domestic and international medical students in China, which includes students from Hong Kong, Macao and Taiwan that are taught through mandarin Chinese as well as students from a variety of other regions that are taught through the English language. This latter group creates higher demands for both schools and teachers.

Unfortunately there is no consensus as to how to improve the level and quality of education for these students or even as to which English language materials should be used. Some teachers prefer to directly use original English language materials, while others make use of Chinese medical textbooks with the help of English language medical notes. The lack of consensus has emerged from the lack of English language medical textbooks based on the characteristics of modern medical education in China.

In fact, most Chinese teachers involved in medical education have already attained an adequate level of English language usage. However, English language medical textbooks that reflect the culture of the teachers would in fact make it easier for these teachers to complete the task at hand and would improve the level and quality of medical education for international students. In addition, these texts could be used to improve the English language level of the medical students taught in Chinese. This is the purpose behind the compilation and publishing of this set of English language medical education textbooks.

The editors in chief are mainly experts in medicine from Capital Medical University (CCMU). The editorial board members are mainly teachers of a variety of subjects

from CCMU. In addition, teachers with rich teaching experience in other medical schools are also called upon to help create this set of textbooks. And finally some excellent scholars are invited to participate as final arbiters for some of the materials.

The total package of English medical education textbooks includes 63 books. Each textbook conforms to five standards according to their grounding in science; adherence to a system; basic theory, concepts and skills elucidated; simplicity and practicality. This has enabled the creation of a series of English language textbooks that adheres to the characteristics and customs of Chinese medical education. The complete set of textbooks conforms to an overall design and uniform style in regards to covers, colors, and graphics. Each chapter contains learning objectives, core concepts, an introduction, a body, a summary, questions and references that together serve as a scaffold for both teachers and students.

The complete set of English language medical education textbooks is designed for teaching overseas undergraduate clinical medicine students (six years), and can also serve as reference textbooks for bilingual teaching and learning for 5-year, 7-year and 8-year programs in clinical medicine.

We would like to thank the chief arbiters, chief editors and general editors for their arduous labor in the writing of each chapter. We would also like to acknowledge all the contributors. Finally, we would like to acknowledge Higher Education Press. They have all provided valuable support during the many weekends and evening hours of work that were necessary for completing this endeavor.

President of Capital Medical University

Director of English Textbook Compiling Commission

Zhaofeng Lu

August 1st, 2011

Preface

This laboratory text is used to guide the students' learning and training of microbiology, parasitology and immunology lab techniques, procedures and experiments. The outcome expected is students' capacity of doing research in laboratory of microbiology, parasitology and immunology.

The manual consists of two parts: Part I —outlines the 8 basic experiments. Part II —outlines the 21 comprehensive experiments of microbiology, parasitology and immunology. The involved experiments are designed to help enhance and deepen the students' understanding of microbiology, parasitology and immunology.

This laboratory manual is designed to meet the needs of foreign students at Capital Medical University(CMU) in Beijing. LABORATORY MANUAL Microbiology, Parasitology and Immunology is also a reference for all the students taking the pathogenic biology & immunology experiments.

Liping Zhang May, 2012

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Laboratory Safety and Laboratory Regulations

In the medical microbiological and parasitological laboratory, you will deal with potentially dangerous pathogens. For the safety and convenience of everyone working in the laboratory, the followed laboratory rules are important:

- 1. A lab coat should be worn and buttoned while in the laboratory. You must wear shoes that cover the tops of your feet to prevent injury from broken glass, spilled chemicals, and dropped objects. Sandals are not permitted in the lab! Long hair should be tied back.
- 2. No food, drinks are permitted to be carried into the laboratory at any time. Don't touch your mouth and nose with any object in laboratory. Mouth pipetting is strictly forbidden in the laboratory. Keep your hands away from your mouth and eyes.
- 3. Since some of the microorganisms used in this class are pathogenic or potentially pathogenic (opportunistic), it is essential to follow proper aseptic technique in handling and transferring all organisms.
- 4. Take only a laboratory manual, notebook and pen to the workbench. All other items should be safely stored in authorized clean areas in order to avoid damage or contamination.
- 5. Be very carefully when doing experiments. Avoid spilling material and contaminating your skin, eyes or clothing. Dispose of all used hazardous biological materials in the appropriate waste containers.
- 6. Return all chemicals, reagents, cultures, and glassware to their appropriate places. Slides, cultures or instruments are not allowed to carry out of the teaching room without authorization.
- 7. Clean the laboratory bench space and turn off all equipment, heat sources, light, water and close windows and doors before leaving the laboratory. Wash your hands thoroughly before leaving the laboratory.
- 8. In case of accident, report immediately to your lab instructor so that appropriate measures may be taken. If you are injured in the lab, immediately contact your course instructor.

I have read and understood the above rules and agree to follow them.

Signed	Date
Name (Please print)	

CONTENTS

Part 1 Basic Experiments	• 1
Experiment 1 Observation of Bacterial Morphology	. 3
I. Using Oil Immersion Lens of the Microscope	• 3
[]. Basic Morphology and Special Structure of Bacteria	
Experiment 2 Bacterial Staining and Microscopy	
[. Gram Stain ·····	
[]. Acid-fast Stain (Ziehl-Neelsen Method)	
Experiment 3 Preparation of the Basic Medium	
Experiment 4 Examination Methods of Parasitic Infections	
I. Direct Fecal Smear Method for Detecting Ascaris Eggs	
[]. Examination for Ascaris Larvae in Lung Tissue of Mice	
Ⅲ. Saturated Brine Flotation Method for Detecting Hookworm Eggs ·····	
IV. Fecal Culture Method for Hookworm Larvae	
V. Cellophane Tape Method for Detecting Pinworm Eggs	
VI. Direct Sputum Smear or Centrifugal Sedimentation Method for Detecting Paragonimus Eggs	
■ Squash Method for Detecting Striated Muscle Larvae of Trichinella	
₩. Test of Concentration of Eggs by Sedimentation for Detecting S. Japonicum Eggs	19
[X]. The Miracidium Hatching Method for Detecting S. Japonicum	
X. Circumoval Precipitation Test (COPT) for Diagnosing Schistosomiasis	20
XI. Direct Stool Smear with Iodine-stain for Detecting Amoeba Cysts	21
Experiment 5 Agglutination Reaction	
I. Direct Agglutination Reaction—Identification of the ABO Blood Group System	24
II. Indirect Agglutination Reaction—Anti-streptolysin O Test	
III. Indirect Agglutination Inhibition Reaction—Latex Pregnancy Test	27
Experiment 6 Precipitation Reaction	
Experiment 7 Enzyme-linked Immunosorbent Assay (Sandwich ELISA Assay) ·······	34
Part 2 Comprehensive Experiments	37
Unit 1 Isolation of Microorganisms from the Environment and Human Body	38
Experiment 8 Bacteria in the Air and Ultraviolet Radiation	
Experiment 9 Bacteria on the Finger Tip and Solid Surface	41
Unit 2 Detection of Skin Infections	43
Experiment 10 Cultural Characteristics of Bacteria and Fungi (Teaching Demonstration) · · · · · · · · · · · · · · · · · · ·	44

I. Clostridiun	n Perfringens in the Milk Medium · · · · · · · · · · · · · · · · · · ·	44
	abouraud Dextrose Medium	45
Experiment 11	Observation of Plasmodium, Toxoplasma Gondii, Trichinella Spiralis,	
	Ethiococcus Orannosus and Insecta	46
I. Plasmodiu	m ······	46
[]. Toxoplasm	na Gondii	48
∏. Trichinella	a Spiralis	49
IV. Echinococc	us Granulosus	51
	ion of Respiratory Infections	
Experiment 12	Isolation & Identification of Pyogenic Cocci	59
I. Isolation a	nd Culture of Mimic Pus Swab and Throat Swab	59
∏. Coagulase	Test ·····	61
Experiment 13	Cultural Characteristics of Respiratory Infected Bacteria	63
I. The Colon	ies of Pathogenic Cocci on Blood Agar Plates(Teaching Demonstration)	63
	rium Tuberculosis in Lowenstein-Jensen Solid Medium(Teaching Demonstration)	64
	Observation of Paragonimus Westermani	
	tion of Enteric Infections	
Experiment 15	Isolation and Identification of Pathogenic Enterobacteria	68
I. Isolation o	f Artificial Fecal Specimens	68
II. Biochemica	al Activities of Enteric Pathogens—Kligler Iron Agar Test · · · · · · · · · · · · · · · · · · ·	69
■. Serologic I	dentification of Enteric Pathogens	70
	Widal Test ····	72
Experiment 17	$Observation \ of \ Ascaris \ lumbricoides \ , Hookworms \ , Enterobius \ Vermicular is \ ,$	-
	Taenia, Entamoeba and Cryptos poridium	76
I . Ascaris Lu	umbricoides ····	76
]]. Hookworn	ns (Ancylostoma Duodenale and Necator Americanus) ·····	79
	Vermicularis ·····	82
IV. Intestinal	Cestodes (Taenia Solium and Taenia Saginata)	83
	Protozoa (Entamoeba Histolytica and Entamoeba Coli) ·····	
VI. Cryptospo		
Unit 5 Detect	tion of Hepatic Infections ·····	
Experiment 18	Observation of Clonorchis Sinensis	
Experiment 19	Observation of Schistosoma Japonicum	
Unit 6 Detect	tion of Genitourinary Tract Infections	
Experiment 20	Observation of Chlamydia Trachomatis Inclusion	
Unit 7 Antimi	crobial Resistance	99
Experiment 21	Bacterial Conjugation: the Transfer of Plasmids Coding for Antibiotic	
	Resistance	100

Experiment 22	Antibiotic Sensitivity Test	102
Unit 8 Imm	ne Cells Function Assay	105
Experiment 23	Macrophage Phagocytosis Assay ·····	106
Experiment 24	Neutrophil Phagocytosis Assay ·····	109
Experiment 25	Plaque Forming Cells Assay (PFC Assay)	111
Experiment 26	Lymphocyte Transformation Assay ·····	114
Experiment 27	Classification of T Cell Subset and FACS Analysis	116
Experiment 28	Cytokine Measurement by ELISPOT ·····	121
Appendix ·		125
Appendix 1	Commonly Used Dye and Staining	125
Appendix 2	Media ·····	130
Appendix 3	Commonly Used Reagents and Buffers	132
Index ······		133

Part 1

Basic Experiments

Observation of Bacterial Morphology

Objectives

- (1) To learn how to use oil immersion objective lens to observe the morphology of bacteria.
 - (2) To understand the basic shapes and special structures of bacteria.

I. Using Oil Immersion Lens of the Microscope

Principle

Bacteria are relatively small in size, usually on the order of 1 μ m in diameter. Bacteria can be visible only under light microscope employed with a 100-power objective lens with 10-power ocular lens.

Normally, when light waves travel from one medium into another, they bend. Therefore, as the light travels from the glass slide to the air, the light waves bend and are scattered similar to the "bent pencil" effect when a pencil is placed in a glass of water. The microscope magnifies this distortive effect. Also, if high magnification is to be used, more light is needed.

Immersion oil has the same refractive index (1.515) as glass (1.52), therefore, provides an optically homogeneous path between the slide and the lens of the objective. Light waves thus travel from the glass slide, into glass-like oil, into the glass lens without being scattered or distorting the image (Fig. 1-1). In other words, the immersion oil "traps" the light and prevents the distortion effect that is seen as a result of the bending of the light waves.

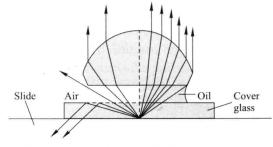


Fig. 1-1 The working principle of oil immersion objective lens

Procedures

1. The identification of oil immersion objective lens(Fig. 1-2)

- (1) The oil immersion objective lens is the longest.
- (2) There are some marks on oil lens, such as objective magnification "100×", the numeric aperture "NA 1. 25" and "Oil".
 - (3) Oil immersion objective lens has a black and white band.

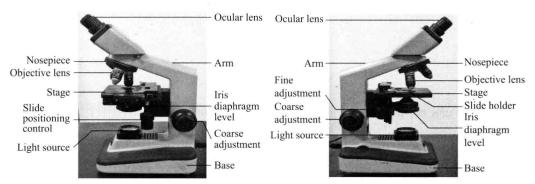


Fig. 1-2 An Olympus microscope

2. The procedure for using oil immersion objective lens

(1) Setting the focus

To switch on the power and open the aperture fully. Raise condenser to the same high level as the stage. Rotating the revolving nosepiece to make sure that the low power objective lens is clicked into place. Adjustment of light intensity may be necessary. The light should be strong when stained samples are observed, whereas the light should be weak when the unstained samples are observed.

(2) Observation of specimens using low-power objective lens

Lower the stage to its lowest position using the coarse focusing knob. Place the slide on the stage and hold it with a pair of stage clips. Locate desired portion of specimen in the center of the field. Rotate the coarse adjustment knob to raise the stage to the highest. While looking through the eyepiece, use the coarse-adjustment knob to lower the stage slowly to focus on the slide. After the outline of the specimens appear, turn the fine-adjustment knob until the object is in sharp focus. If there is no specimen visible, it is probably not in the field of view. Move the slide so that the specimen is directly over the condenser lens. Center the specimen in the field of view.

(3) Observation of specimens using high-power objective lens

Rotate the high-power objective into the position over the slide. While looking through the eyepieces, use only the fine adjustment knob to obtain a sharp image. Moving the stage clips to make the slide around until the area you wish to examine to be located at the central stage.

(4) Observation of slide using oil immersion objective lens Rotate the objectives so that there is not a lens directly over the slide.