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MEDICAL MICROBIOLOGY
LABORATORY MANUAL

# 《医学微生物学实验技术指导》编委名单


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# PREFACE

This laboratory manual has evolved over the years to meet the needs of students taking microbiology. The intention is to provide the student with an organized, user-friendly tool to better enable him or her to understand laboratory aspects of microbiology as well as to hopefully make learning laboratory material a bit easier.

Each lab experiment is set up as a complete module that demonstrates some microbiological principle or technique. Each experiment begins with a detailed discussion that provides all the information needed to understand that lab. The discussion section is followed by a detailed step-by-step procedure. The results section of each experiment is where the student records important results and conclusions that will enable him or her to prepare for the practical portion of each lab quiz.

As proficiency in microbiological techniques is considered an essential component of the course, students are expected to work independently on some technical aspect of microbiology and will be graded based on their techniques and their results. The lab notebook will be collected and graded in the end of each lab period. If your lab notebook is missing for any reason, a grade of 0 will be assigned. Unannounced skills tests will be given in the end of the semester.

We hope you enjoy this laboratory manual and also hope it makes your study of microbiology a bit easier. Keep in mind that the labs are meant to be informal and your instructor is more than willing to answer any questions on either lab or lecture topics.

Bin-Bo Liu
Dong-Ying Liu
2006.1

# 前　言

医学微生物学是一门基础医学课程，《医学微生物学实验技术指导》是与之配套的书籍，旨在突出病原微生物的生物学性状及致病性的基础实验技术，为诊断和防治由病原微生物感染所致疾病奠定必要的实验基础。本书根据医药院校本、专科生医学微生物学的教学要求，遵循实用性、科学性、先进性和启发性的原则，从实验目的、材料、方法、结果分析及思考题等方面入手，较为系统和全面地介绍医学微生物学的基本实验技术，主要包括各类病原微生物的形态与结构检查法，常见病原微生物的分离培养与鉴定，细菌代谢产物和致病性的检测，消毒与灭菌，变异现象等。随着分子生物学技术在微生物学领域的广泛应用，本书也介绍了噬菌体、质粒、核酸分子杂交、PCR 等技术。为了方便读者，本书在附录中列举了常见培养基、试剂、染色液和缓冲液的配制，以及菌种保藏方法等，在每一个实验的最后列出了思考题，其目的是增强读者的思维想象力。

为了适应新形势下的医学教学改革和双语教学的需要，本书以中英文的形式编写，以利于提高学生的本专业科技英文水平。本书可作为医学专业七年制、五年制本科、专科生的医学微生物学实验教材，也可作为医院、卫生防疫部门及从事微生物学工作的技术人员的参考书。

限于编者的学识水平，书中不足之处，诚望读者批评指正。

刘斌波　刘东瀛

2006.1

# CONTENTS

# MICROBIOLOGY LABORATORY SAFETY RULES

A primary feature of the microbiology laboratory is that living organisms are employed as part of the experiment. Most of the microorganisms are harmless; however, whether they are non-pathogenic or pathogenic, the microorganisms are treated with the same respect to assure that personal safety in the laboratory is maintained. Careful attention to technique is essential at all times. Care must always be taken to prevent the contamination of the environment from the cultures used in the experiments and to prevent the possibility of the people working in the laboratory from becoming contaminated.

It is essential that students follow all rules established by the lab instructor, lab manager, or lab assistant to ensure the safety of all individuals in the class. Failure to follow established rules may result in dismissal of the individual from the class. For the safety and convenience of everyone working in the laboratory, it is important that the following laboratory rules be observed at all times:

1. Place only those materials needed for the day's laboratory experiment on the bench tops. Purses, coats, extra books, etc., should be placed in the lab bench storage areas or under the lab benches in order to avoid damage or contamination.

2. Since some of the microorganisms used in this class are pathogenic or potentially pathogenic (opportunistic), it is essential to always follow proper aseptic technique in handling and transferring all organisms.

3. No smoking, eating, drinking, or any other hand-to-mouth activity while in the lab. If you need a short break, wash your hands with disinfectant soap and leave the room.

4. If you should spill a culture, observe the following procedures:

a. Immediately place the culture tube in the plastic baskets found in the hood in the back of the room so no one else touches the contaminated tube.

b. Have your partner spray isopropyl alcohol liberally over the spill. Be sure your Bunsen burner is turned off before you spray any alcohol ! After a few minutes, use paper towels to dry the area.

c. Both you and your partner wash your hands with disinfectant soap.

d. Notify your instructor.

5. Report any cuts, burns, or other injuries to your instructor.

6. Using a wax marker, properly label all inoculated culture tubes or petri dishes with the name or the initials of the microorganism you are growing, your initials or a group symbol, and any other pertinent information. Place all inoculated material only on your assigned

incubator shelf, the shelf corresponding to your lab section. Culture tubes should be stored upright in plastic beakers, while petri dishes should be stacked and incubated upside-down.

7. After completing an experiment, dispose of all material properly:

a. Remove all wax pencil markings from culture tubes and place the tubes upright in the plastic baskets found in the disposal hood at the back of the room.

b. Put all used pipettes and swabs in the biowaste disposal containers found in the back of the room.

8. Use caution around the Bunsen burners. In a crowded lab it is easy to lean over a burner and ignite your hair or clothing.

9. Always clean the oil off of the oil immersion lens of the microscope with a piece of lens paper at the completion of each microscopy lab.

10. Return all Equipment, reagents, and other supplies to their proper places at the end of each lab period.

11. Disinfect the bench top with isopropyl alcohol before and after each lab period. Be sure your Bunsen burner is turned off before you spray any alcohol !

12. Always wash your hands with disinfectant soap before leaving the laboratory.

# 微生物学实验室规则

微生物学是一门实验性很强的学科，在做实验时，经常用到病原微生物，因此，在进入实验室期间，必须严格遵守下列规则。

一、进实验室必须穿好工作服，离室时脱下。非必需物品不要带入实验室。

二、禁止在实验室内抽烟与饮食，不准随地吐痰，注意保持室内整洁。

三、要严格遵守操作规程。实验时保持安静，勿高声谈笑或随便走动，以免影响他人实验。

四、吸过菌液的吸管、毛细吸管，要投入含有2%3%来苏或5%石碳酸液的玻璃筒中，不得放在桌上，亦不可冲洗于水槽内。用过的玻片也应放于含消毒液的器皿内。

五、实验过程中如发生意外，应进行紧急处理。

1. 皮肤破伤：先除尽异物，用蒸馏水或生理盐水冲洗后，涂以2%碘酒。

2. 灼伤：涂以凡士林油、5%鞣酸。

3. 菌液进入口腔：应立即吐出，以大量清水漱口，必要时，根据菌类不同服用相关药药进行预防。

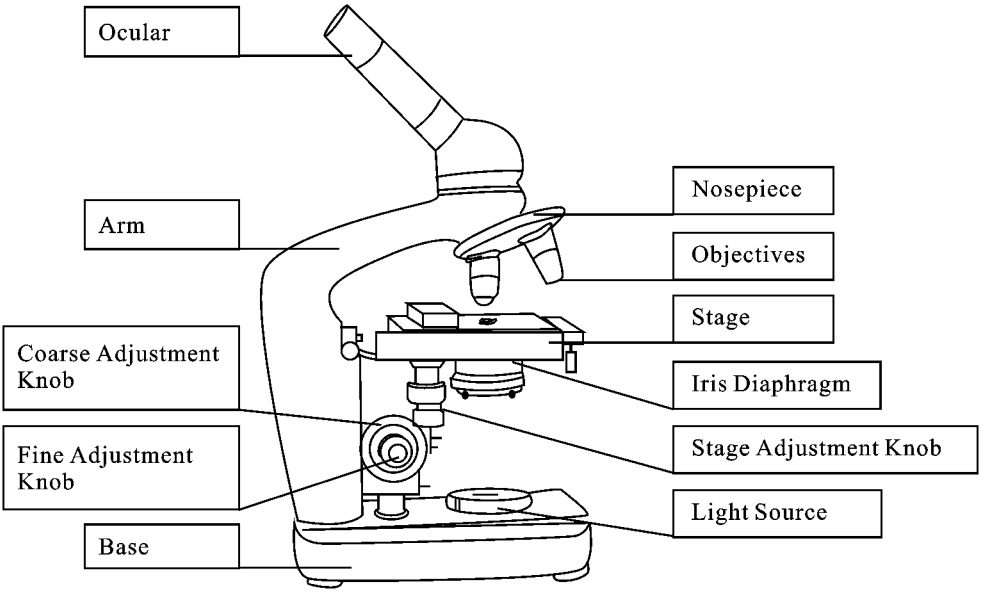
4. 菌液流洒桌面：立即以抹布浸沾2%3%来苏或5%石炭酸液后，盖在污染部位半小时方可抹去。若手上沾有活菌，亦应浸泡于上述消毒液1020分钟后，再以肥皂水洗。

六、爱护公物，节约使用实验材料。实验完毕后，整理桌面，需培养的物品要放入培养箱。做好实验室清洁，关好水电等，洗手后离室。

# Experiment 1
# INTRODUCTION TO MICROSCOPY

Microorganisms are too small to be seen with the naked eye so a microscope must be used to visualize these organisms. While a microscope is not difficult to use it does require some practice to develop the skills necessary to use the microscope to its maximum capabilities.

There are several types of microscopes but the only one used in this laboratory is the compound light or bright-field microscope. Individual microscopes will vary depending on the manufacturer but all microscopes have the same basic features as follow.




These microscopes are known as compound microscopes because there are two magnifying lenses in the microscope. One magnifying lens is in the ocular and one is in the objective. Each contributes to the magnification of the object on the stage. The total magnification of any set of lenses is determined by multiplying the magnification of the objective by the magnification of the ocular. The nosepiece rotates allowing the objectives to change and thus change the magnification of the microscope. The stage is where the slide is placed. The stage adjustment knobs allow the slide to be moved easily. Light provides the illumination for the specimen. To control the amount of light reaching the eye the iris diaphragm may be opened

or closed using the lever just under the stage. On low magnifications less light is need than on higher magnifications. Too much light on low magnification may mask the specimen, particularly something as small as a bacterial cell. The coarse and fine adjustment knobs are used to focus on the specimen. When a slide is on the stage there is a space between the objective and the slide. This space is known as the working distance. The coarse adjustment knob will cause the working distance to visibly change while the fine adjustment knob is for final, fine focusing. The ability to see things using a microscope is limited by the resolving power of the microscope. The resolving power of a microscope is the distance two objects must be apart and still be seen as separate and distinct. For the light microscope this is 0.25 μm. Objects closer together than 0.25 μm will not be distinctly seen. Increasing the magnification will not make the objects more distinct, just bigger. Each objective has the magnification of the objective written on the objective. The magnification of the ocular is also inscribed on the ocular. Low magnifications are used for quickly examining the slide to find a appropriate area to examine. Higher magnifications allow the examination of a particular object on the slide.

When you look through the ocular you will see a lighted circle. This is known as the field of view or the field. When you look through the microscope you will move the iris diaphragm lever and notice how the brightness of the light changes. As you move the objectives to provide increased magnification you will look at a smaller section of the slide. Be sure you move the object you want to view into the center of the field before moving to the next objective. These microscopes are parfocal. Once you have focused on an object using one objective the object will be approximately in focus on the next objective. Use of the fine focus knob will sharpen the focus.

# I. Use of the microscope

## 1. Materials/Equipment (for each student)

- Microscope complete with low power, high power and oil immersion objectives.
- Various prepared slides of bacteria.
- Immersion oil and xylol.
- Rubbing lens paper.

## 2. Methods

In this first experiment, you will calibrate the 40× and 100× objectives of your compound microscope. Then you will use the compound light microscope to assess the shape and associations of bacteria that have already been fixed to slides and stained.

**A. Procedure for Focusing**

a) Obtain a slide.

b) Use the coarse adjustment knob to obtain maximum working distance.

c) Place the slide on the stage. The slide should fit into the slide holder but is not placed under the slide holder. Use the stage adjustment knob to move the slide over the hole in the

stage.

d) Rotate the low power objective in place.

e) Use the coarse adjustment knob to obtain the minimum working distance. Develop the habit of watching this process to be sure the objective does not crash into the slide.

f) Look through the ocular. Adjust the light with the iris diaphragm lever if necessary. Slowly turn the coarse adjustment knob until something comes into focus. Use the fine adjustment knob to sharpen the focus.

g) Using the stage adjustment knob move the slide around until you find an area you wish to examine more closely. Move the slide until the object you wish to examine is in the center of the field.

h) Rotate the high power objective into place. Use the fine adjustment knob to sharpen the focus. Do not use the coarse adjustment knob. Adjust the light using the iris diaphragm lever if necessary.

i) Rotate the high power object halfway to the next position. Place a drop of immersion oil on the slide, and then rotate the oil immersion objective into place. The objective should be immersed in the oil on the slide. Use the fine adjustment knob to sharpen the focus. Adjust the light using the iris diaphragm lever if necessary.

j) When you finish viewing the slide use the coarse adjustment knob to maximize the working distance and remove the slide from the stage. If you want to look at another slide, begin the process over. If you are finished with the microscope clean the microscope and return it to storage.

An alternative focusing technique is to firstly focus on the slide with the yellow-striped 10× objective by using only the coarse focus control and then without moving the stage, add immersion oil, rotate the white-striped 100× oil immersion objective into place, and adjust the fine focus and the light as needed.

**B. Procedure for Cleaning a Microscope**

a) Turn off the light and unplug the cord. Store the cord appropriately.

b) Using the coarse adjustment knob to obtain maximum working distance and remove the slide from the stage.

c) Using lens paper clean all the lenses starting with the cleanest first-ocular, low power, high power and oil immersion. Use lens cleaner if necessary.

d) Clean any oil off of the stage using paper towels.

e) Rotate the scanning objective into place. Use the coarse adjustment knob to obtain minimum working distance.

f) Return the microscope to the appropriate storage area.

## II. Reason for using immersion oil

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 mi-

croscope magnifies this distortion effect. Also, if high magnification is to be used, more light is needed.

Immersion oil has the same refractive index as glass and, 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. 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.

## Review Questions

1. How to correctly clean the eyepiece and the objective lenses before and after each lab?
2. Define ocular lens and objective lens.
3. How to place a slide in the slide holder of a mechanical stage correctly?
4. Focus on a specimen using 10X, 40X, and 100X objectives.
5. Adjust the light using the iris diaphragm lever for optimum contrast after focusing.
6. State the reason for using immersion oil at 1000X.

(Dong-Ying Liu, Xian-Zhou Liu)

# 实验一　显微镜的构造与使用

显微镜是一种放大仪器。由于微生物的体积微小，因此，显微镜是微生物学实验中的重要工具之一。根据不同的研究目的和要求，可分别选用光学显微镜、暗视野显微镜、相差显微镜、荧光显微镜和电子显微镜等。其中以普通光学显微镜（通常称显微镜）最为常用，尤其是油浸镜（简称油镜）。

## 实 验 目 的

一、学习并掌握油镜的使用技术。

二、熟悉显微镜的保养技术。

三、了解油镜的基本原理。

## 实 验 项 目

### 一、普通光学显微镜的构造

显微镜的构造大致如下：

1. 光学部分：接物镜（包括高倍镜、低倍镜和油镜）、接目镜、集光器、反光镜等。

2. 机械部分：镜筒、镜臂、镜座、回转板、倾斜关节、调节器（包括粗调节器和细调节器）、载物台、光圈、次台等。

## 二、显微镜的使用方法

**材料**

普通光学显微镜、香柏油、擦镜纸、细菌染色标本片等。

**方法**

1. 油镜的识别　显微镜油镜头上常有下列几种标记：

(1)放大倍数是 90×或 100×。

(2)镜头前端常有一黑圈。

(3)油镜前面的透镜最小。

(4)刻有“油”或外文“HI”(homogene immersions)、“oil oel”等字样。

2. 油镜的使用程序

(1)对光：显微镜直立在桌上，将集光器升起与载物台孔平行，并将虹彩光圈轻轻开至最大，再转动反光镜，使光线集中于集光器。光强时用平面，光弱时用其凹面。

(2)放置标本片：将标本片放置在载物台上，用弹簧夹或标本推进器固定，将待检部分移置接物镜下。

(3)观察：先用低倍镜找到标本所在处，再换油镜观察。使用油镜时，须在载玻片上先滴加香柏油一滴，并置台孔中央，眼睛从镜筒侧面观察，并顺时针方向扭动粗螺旋，使镜筒徐徐下降，让油镜头浸入油内接近标本表面，并轻轻接触玻片，然后将眼睛移至目镜，边观察边向上(逆时针方向)徐徐扭动粗螺旋，至视野中看到模糊物像时，再换用细螺旋调至物像清晰为止。调节时千万不可使用强力将物镜任意下降，以免压碎标本片，甚至将贵重油镜头损坏。检查完毕，向上扭动粗螺旋，将镜筒提起，取下标本片，用擦镜纸将油镜头上的油擦净。

## 三、显微镜的保养

1. 显微镜是贵重的精密光学仪器，使用时应爱惜，避免碰撞，更不能随意拆散玩弄。拿显微镜时，必须用双手，一手持镜臂，一手托镜座。

2. 每次实验前用纱布擦去显微镜外面的灰尘，接物镜、接目镜须用软绸或擦镜纸拭擦，切不可用粗布、粗纸，以免损害透镜。不要随意抽出接目镜，以免灰尘落入镜筒，影响清晰度。

3. 用油镜时，不可使镜臂弯曲，致使载物台倾斜，香柏油外溢。非油镜头不可与香柏油接触。

4. 显微镜的光学部分，如镜头、反光镜等，应避免日光照射；强酸、强碱、氯仿、酒精、乙醚等都能去漆或损坏机件，不可使用。油镜每次用毕，须立即以擦镜纸或软绸布擦净油渍。如香柏油已干或镜头模糊不清，可用擦镜纸沾少许二甲苯擦净，并随即用干擦镜纸擦干。因二甲苯能溶解粘固透镜的胶质，日久会使镜片移位或脱落，故尽量少用。

5. 显微镜用毕，将接物镜转成“八”字形，集光器稍下降，然后转动粗调节器，使镜筒下移，以避免接物镜与集光器相碰受损，然后送入镜箱。

## 附：油镜的原理

使用油镜要加香柏油的原理：因为油镜的透镜很小，从载玻片透过的光线通过空气($n=1.0$)，因介质密度不同，发生折射现象，使射入镜筒的光线很少，物像不清。若在油镜与载玻片中间加入和玻片折射率($n=1.52$)相近的香柏油($n=1.515$)，则使通过的光线不致产生折射而损失，因此能清楚地看到物像，如图 1－1 所示。

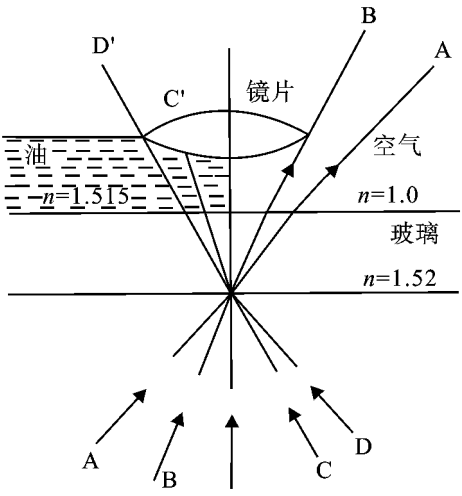



图 1－1　显微镜油镜的原理

## 注 意 事 项

1. 油镜头的识别。
2. 显微镜的使用方法步骤及保养。

## 思　考　题

1. 在使用油镜时，能否用其他种类的油剂或水来代替香柏油，为什么？
2. 在使用高倍镜或低倍镜观察标本时，能否像油镜那样使用香柏油，为什么？

（饶邦忠　向　宇）

# Experiment 2
# SIZES, SHAPES, AND ARRANGEMENTS OF BACTERIA

When studying bacteria or other microorganisms, it is often essential to evaluate the size of the organism. By tradition, the longest dimension (length) is generally stressed, although width is sometimes useful for identification or other study. In microbiology, we deal with extremely small units of metric length (micrometer, nanometer). The main unit of length is the micrometer ($\mu$m) which is $10^{-6}$ of a meter or approximately 1/25,400 of an inch. The average size of a rod-shaped (cylindrical) bacterium is 0.5-1.0 $\mu$m wide by 1.0-4.0 $\mu$m long. An average coccus-shaped (spherical) bacterium is about 0.5-1.0 $\mu$m in diameter. A volume of one cubic inch is sufficient to contain approximately nine trillion average-sized bacteria. It would take over 18,000,000 average-sized cocci lined up edge-to-edge to span the diameter of a dime !

## I. The basic shapes of bacteria

Bacteria are unicellular prokaryotic microorganisms. Most bacteria come in one of three basic shapes: coccus, rod or bacillus, and spiral. Bacteria divide by binary fission, a process by which one bacterium splits into two. In this lab, you will compare the relative size and shape of some microorganisms.

### 1. Coccus

A coccus-shaped bacterium is usually spherical, although some appear oval, elongated, or flattened on one side. Cocci are approximately 0.81.2 $\mu$m in diameter and may be seen, based on their planes of division and tendency to remain attached after replication, in one of the following arrangements.

a) Division in one plane produces either a diplococcus arrangement (cocci in pairs) or a streptococcus arrangement (cocci in chains).

b) Division in two planes produces a tetrad arrangement (cocci forming a square of four).

c) Division in three planes produces a sarcina arrangement (cocci forming a cube of eight).

d) Division in random planes produces a staphylococcus arrangement (cocci in irregular, often grape-like clusters).

As you observe these different cocci, keep in mind that the procedures used in slide

preparation may cause some arrangements to break apart or clump together. The correct form, however, should predominate. Also remember that each coccus in an arrangement represents a complete, single, one celled organism.

### 2. Bacillus (rod)

A bacillus or rod is a hotdog-shaped bacterium having one of the following arrangements.

a) Bacillus: a single bacillus.

b) Streptobacillus: bacilli in chains.

c) Coccobacillus: oval and similar to a coccus.

A single bacillus is typically 0.5-1.0 μm wide and from 1-4 μm long. Small bacilli or bacilli that have just divided by binary fission may at first glance be confused for cocci so they must be observed carefully. You will, however, be able to see bacilli that have not divided and are definitely rod-shaped as well as bacilli in the process of dividing.

### 3. Spiral

Spiral-shaped bacteria occur in one of three forms.

a) Vibrio: an incomplete spiral or comma-shaped.

b) Spirillum: a thick, rigid spiral.

c) Spirochete: a thin, flexible spiral. Some spirochetes are over 100 μm in length.

## II. The special structure of bacteria

A typical bacterium usually consists of four basic structures: a cell wall, a cytoplasmic membrane, a fluid cytoplasm and a nuclear region. Some bacteria have specific structure: endospore, flagella, capsule and pili. In this lab, you will observe the three of special structure bacteria, but the pili are too thin to be observed by light microscope.

### 1. Endospore (spore)

Certain bacteria may produce endospores under unfavourable environmental conditions. Endospores are mainly found in Gram-positive organisms, including the Gram-positive *Clostridium* and *Bacillus*. Endospores may be formed in a central, terminal, or sub-terminal position in the cell and their shape varies from ellipsoidal to spherical. The location of the endospore in the cell is usually characteristic of the species. For example, the location and shape of the *Bacillus subtilis* endospore is different from the location and shape of the *Clostridium* endospore. Therefore, the presence or absence of endospores and the description of the endospore is useful to a microbiologist as an aid in identification.

### 2. Glycocalyx (capsule and slime layer)

All bacteria secrete some sort of glycocalyx, an outer viscous covering of fibers extending from the bacterium. If it appears as an extensive, tightly bound accumulation of gelatinous material adhering to the cell wall, it is called a capsule. If the glycocalyx appears unorganized and more loosely attached, it is referred to as a slime layer.

### 3. Flagella

Motile bacteria possess filamentous appendages known as flagella, which act as organs of locomotion. The filament is the rigid, helical structure that extends from the cell surface. It is composed of the protein flagellin arranged in helical chains so as to form a hollow core. Bacteria flagella are 10-20 μm long and between 0.01 and 0.02 μm in diameter and come in a number of distinct arrangements:

a) Monotrichous: a single flagellum, usually at one pole.

b) Amphitrichous: a single flagellum at both ends of the organism.

c) Lophotrichous: two or more flagella at one or both poles.

d) Peritrichous: flagella over the entire surface.

### 4. Pili

Pili are thin, protein tubes originating from the cytoplasmic membrane and are found in virtually all gram-negative bacteria but not in many gram-positive bacteria. The pilus has a shaft composed of a protein called pilin. At the end of the shaft is the adhesive tip structure having a shape corresponding to that of specific glycoprotein or glycolipid receptors on a host cell. There are two basic types of pili: 1) short attachment pili, also known as fimbriae that are usually quite numerous, and 2) long conjugation pili, also called "F" or sex pili, that are very few in number.

## III. View of the basic shapes of bacteria

### 1. Materials/Equipment (for each student)

- Light microscope.
- Various prepared slides of bacteria.
- Immersion oil and xylol.
- Lens papers.

### 2. Methods

Take a prepared slide of the following bacteria and observe the shapes of the bacterium with a light microscope. Note the appearance, size, arrangement and the colour of each species bacteria.

A. ***Staphylococcus aureus***: Gram-positive; as the genus name implies, cells mainly adherent in irregular clusters (cocci in irregular, often grape-like clusters).

B. ***Escherichia coli***: a small rod-shaped, Gram-negative bacillus.

**C. Vibrio**: vibrios are recognized as short, non-flexuous, comma-shaped Gram-negative bacilli.

Remember that in the process of making the slide, some of the coccal arrangements will clump together and others will break apart. Move the slide around until you see an area representing the true arrangement of each organism. Also remember that small bacilli (such as *Escherichia coli*) that have just divided by binary fission will look similar to cocci. Look