

# Bacteria Classification

*Pseudomonas* spp

Lab 7

# Practical Taxonomy

## Scientific Classification of *Pseudomonas* Spp.

Domain: Bacteria

Phylum: Proteobacteria

Class: Gammaproteobacteria

Order: Pseudomonadales

Family: Pseudomonadaceae

Genus: *Pseudomonas*

Species: *P. aeruginosa*

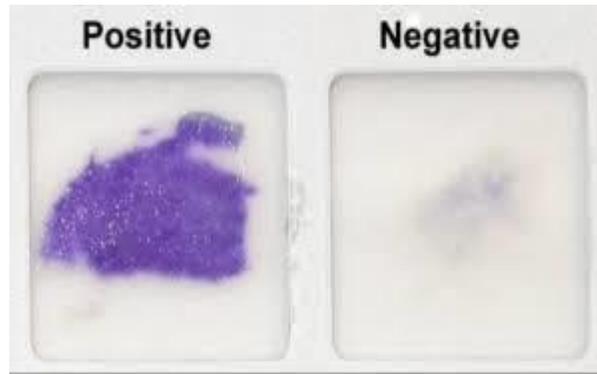
*Pseudomonas* genus include 191 species

**Identification of *P. aeruginosa***

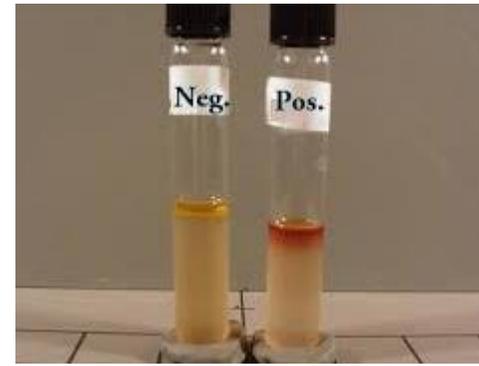
<b>Test</b>	<b>Results</b>
<b>Gram Stain</b>	-
<b>Oxidase</b>	+
<b>Indole Production</b>	-
<b>Methyl Red</b>	-
<b>Voges-Proskauer</b>	-
<b>Citrate</b>	+
<b>Hydrogen Sulfide Production</b>	-
<b>Urea Hydrolysis</b>	+
<b>Phenylalanine Deaminase</b>	-
<b>Lysine Decarboxylase</b>	-
<b>Motility</b>	+
<b>Gelatin Hydrolysis</b>	+
<b>Acid from lactose</b>	-
<b>acid from glucose</b>	-
<b>acid from maltose</b>	-
<b>acid from mannitol</b>	+
<b>acid from sucrose</b>	-
<b>nitrate reduction</b>	+
<b>DNase</b>	-
<b>Lipase</b>	+
<b>Pigment</b>	<b>+ (bluish green pigmentation)</b>
<b>Catalase</b>	+



**Gram Negative**



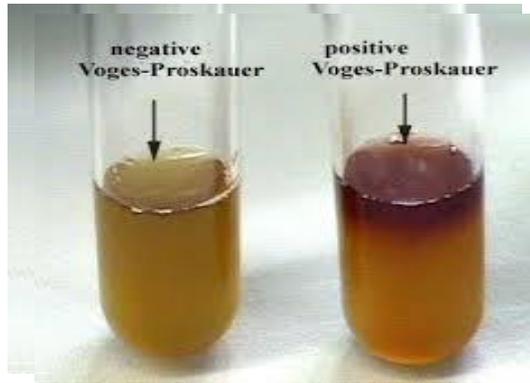
**Oxidase test**



**Indole Production**



**Methyl Red Test**



**Voges-Proskauer test**



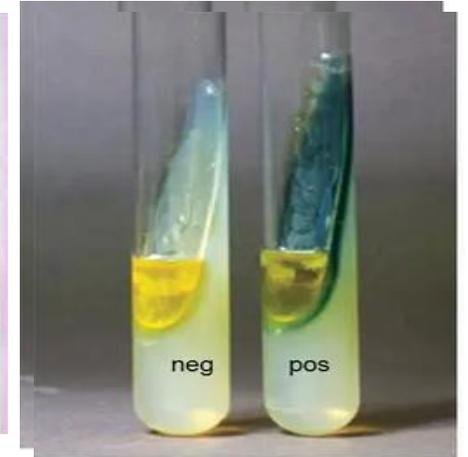
**Citrate test**



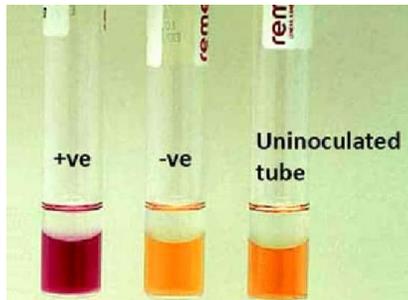
**Hydrogen Sulfide (H<sub>2</sub>S)**



**Urea Hydrolysis test**



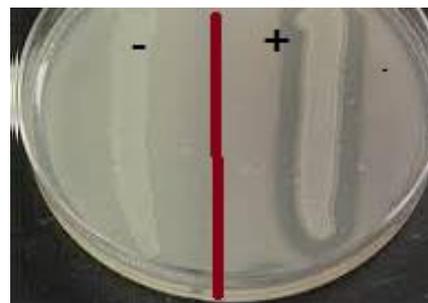
**Phenylalanine Deaminase**



**Lysine Decarboxylase**



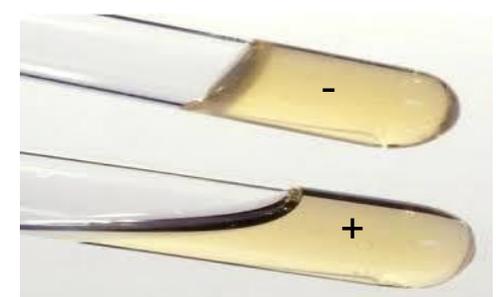
**Motility test**



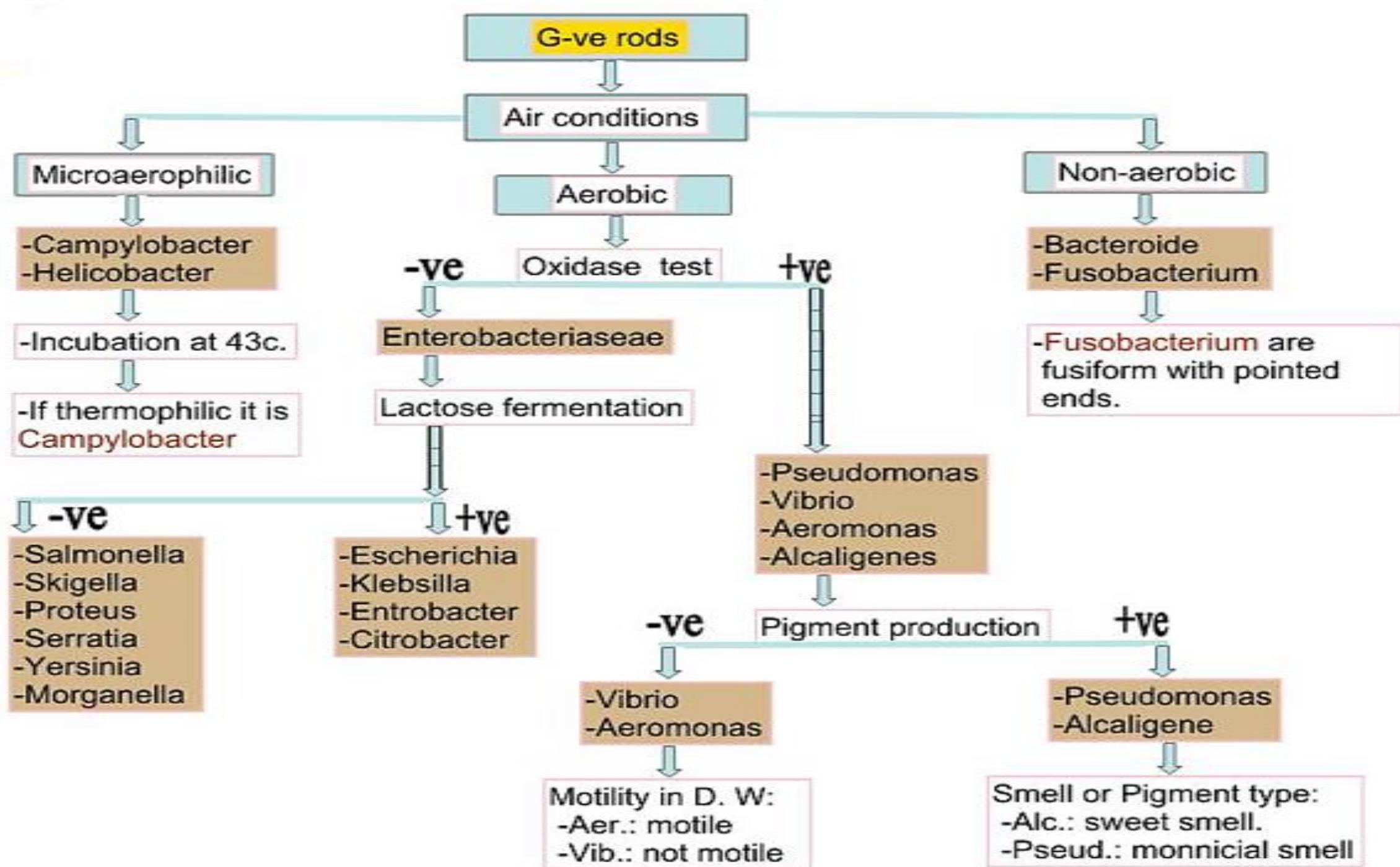
**Lipase test**

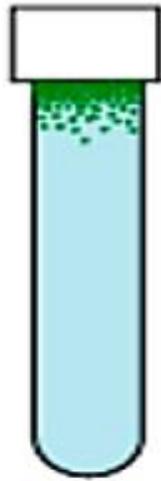


**pigment production**

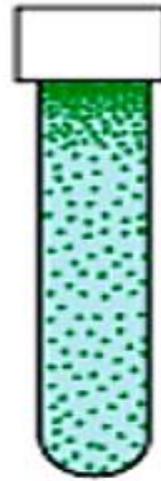


**Gelatin Hydrolysis**

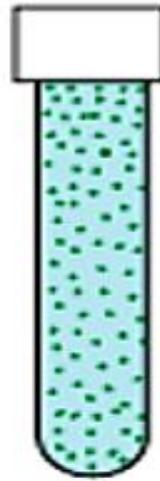




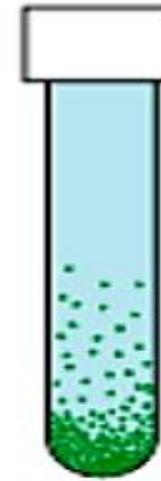
Obligate  
aerobe



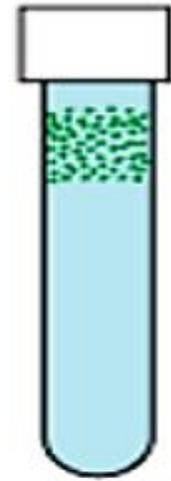
Facultative  
anaerobe



Aerotolerant  
anaerobe



Strict  
anaerobe



Microaerophile

**Enzyme content**

+ SOD  
+ Catalase

+ SOD  
+ Catalase

+ SOD  
- Catalase

- SOD  
- Catalase

+ SOD  
+/- Catalase  
(low levels)

Superoxide dismutase (SOD)

# Pigments

***P. aeruginosa***, is capable of producing a wide variety of pigments. The blue pigment, pyocyanin, is produced only by *P. aeruginosa*. Fluorescein (**pyoverdine**), a yellow pigment that fluoresces under ultraviolet light, is produced by *P. aeruginosa* and other free-living less pathogenic Pseudomonas species. Pyocyanin and fluorescein combined produce a bright green color that diffuses throughout the medium.

**In fact, pigmentation is one of the most erratic of all phenotypic traits. Usually, pigment production can be induced or enhanced in special culture media, but repeated transfer of strains in the laboratory sometimes results in total loss of pigment production. The production of pyocyanin is unique to the species, and this is enhanced by culture on **King's A medium**, which contains potassium and magnesium salts in sufficient concentration to suppress pyoverdine production. Pyoverdine, is optimally produced on **King's B medium**, which contains less of these**

## Oxidase test:

- ❑ The oxidase test is used to identify bacteria that produce Cytochrome C oxidase, an enzyme of the bacterial electron transport chain. When present, the cytochrome C oxidase oxidizes the reagent **(tetramethyl-p-phenylenediamine)** to **(indophenols)** purple color end product. When the enzyme is not present, the reagent remains reduced and is colorless.
- ❑ All bacteria that are oxidase positive are aerobic, and can use oxygen as a terminal electron acceptor in respiration. This does NOT mean that they are strict aerobes.
- ❑ Bacteria that are oxidase-negative may be anaerobic, aerobic, or facultative; the oxidase negative result just means that these organisms do not have the cytochrome C oxidase that oxidizes the test reagent. They may respire using other oxidases in electron transport.

### Expected results of Oxidase test

1. **Positive:** Development of dark purple color (indophenols) within 10 seconds.
2. **Negative:** Absence of color.

## Procedure of Oxidase test:

- ✓ Take a filter paper soaked with the substrate tetramethyl-p- phenylenediamine dihydrochloride.
- ✓ Pick the colony to be tested with wooden or platinum loop and smear in the filter paper.
- ✓ Observe inoculated area of paper for a color change to deep blue or purple within 10 seconds

### Note:

The oxidase test must be performed from 5% Sheep blood agar or another medium without a fermentable sugar. Fermentation of a carbohydrate results in acidification of the medium (e.g., lactose in MacConkey Agar or), and a false negative oxidase test may result if the surrounding pH is below 5.1.

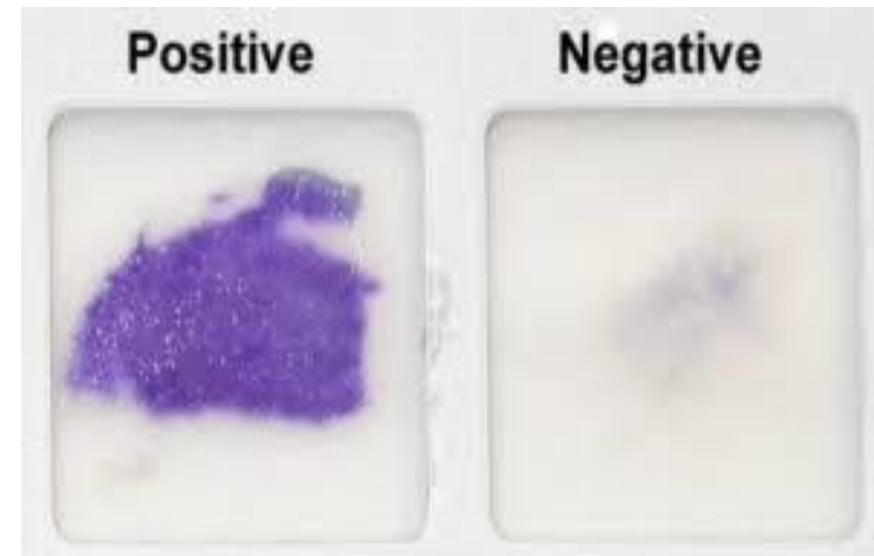
### Oxidase test results

Bacterial genera characterized as oxidase positive include

**Neisseria** and **Pseudomonas**.

Genera of the **Enterobacteriaceae** family

are characterized as oxidase negative.

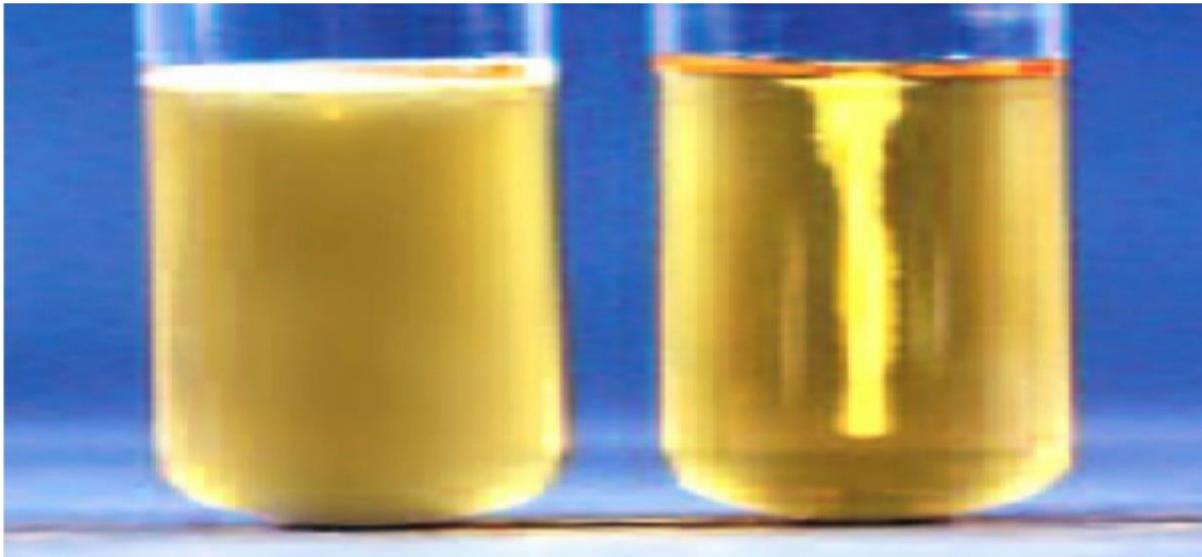


# Detection of Motility:

## 1- Semi- Solid Agar

In semi-solid agar media, motile bacteria "swarm" and give a diffuse spreading growth that is easily recognized by naked eye. Motility may thus be detected more easily than by the microscopical "hanging drop" method. The exact optimal concentration of agar depends on the particular brand used and must be determined by trial, usually it is about 0.4% of Japanese agar or 0.2% of New Zealand agar. This is dissolved in nutrient broth or peptone water. It is important that the final medium should be quite clear and transparent. It is inoculated by stabbing with a straight transfer needle. Motility is detectable as diffuse growth radiating from the central stab line.

The incorporation of tetrazolium chloride (TTC) at a final concentration of 0.005% in the medium is helpful. TTC is used by the bacteria as an electron acceptor. In its oxidized form, TTC is colorless and soluble; when reduced it is red and insoluble. A positive result for motility is indicated when the red (reduced) TTC is seen radiating outward from the central stab. A negative result shows red only along the stab line.



**Figure .2 Motility test in simmedium without TTC** : On the left is *Proteus vulgaris* (motile); *Shigella sonnei* (nonmotile) is on the right. Notice that motility of *P. vulgaris* is seen only as haziness in the medium. Tubes must be compared to uninoculated controls to discriminate between faint haziness and motility.

## **2- The Hanging Drop Slide**

**Purpose :** To observe bacteria in a hanging drop, study their morphology, and determine their motility

### **Materials**

- **24-hour broth culture of *Proteus vulgaris*.**
- **24-hour broth culture of *Staphylococcus epidermidis*.**
- **2 hollow-ground slides.**
- **Several cover glasses.**
- **Bunsen burner or bacterial incinerator.**
- **China-marking pencil or permanent marking pen.**
- **Petroleum jelly (Vaseline) .**

## **Procedure**

- **With a toothpick, spread a small ring of Vaseline around the concavity of a depression slide (figure - 3- a). Do not use too much Vaseline.**
- **After thoroughly mixing one of the cultures, use the inoculating loop to aseptically place a small drop of one of the bacterial suspensions in the center of a coverslip (figure - 3- b).**
- **Lower the depression slide, with the concavity facing down, onto the coverslip so that the drop protrudes into the center of the concavity of the slide (figure - 3- c). Press gently to form a seal.**
- **Turn the hanging drop slide over (figure - 1-d) and place on the stage of the microscope so that the drop is over the light hole.**
- **Examine the drop by first locating its edge under low power and focusing on the drop. Switch to the high-dry objective and then, using immersion oil, to the 90 to 100× objective. In order to see the bacteria clearly, close the diaphragm as much as possible for increased contrast. Note bacterial shape, size, arrangement, and motility. Be careful to distinguish between motility and Brownian movement.**

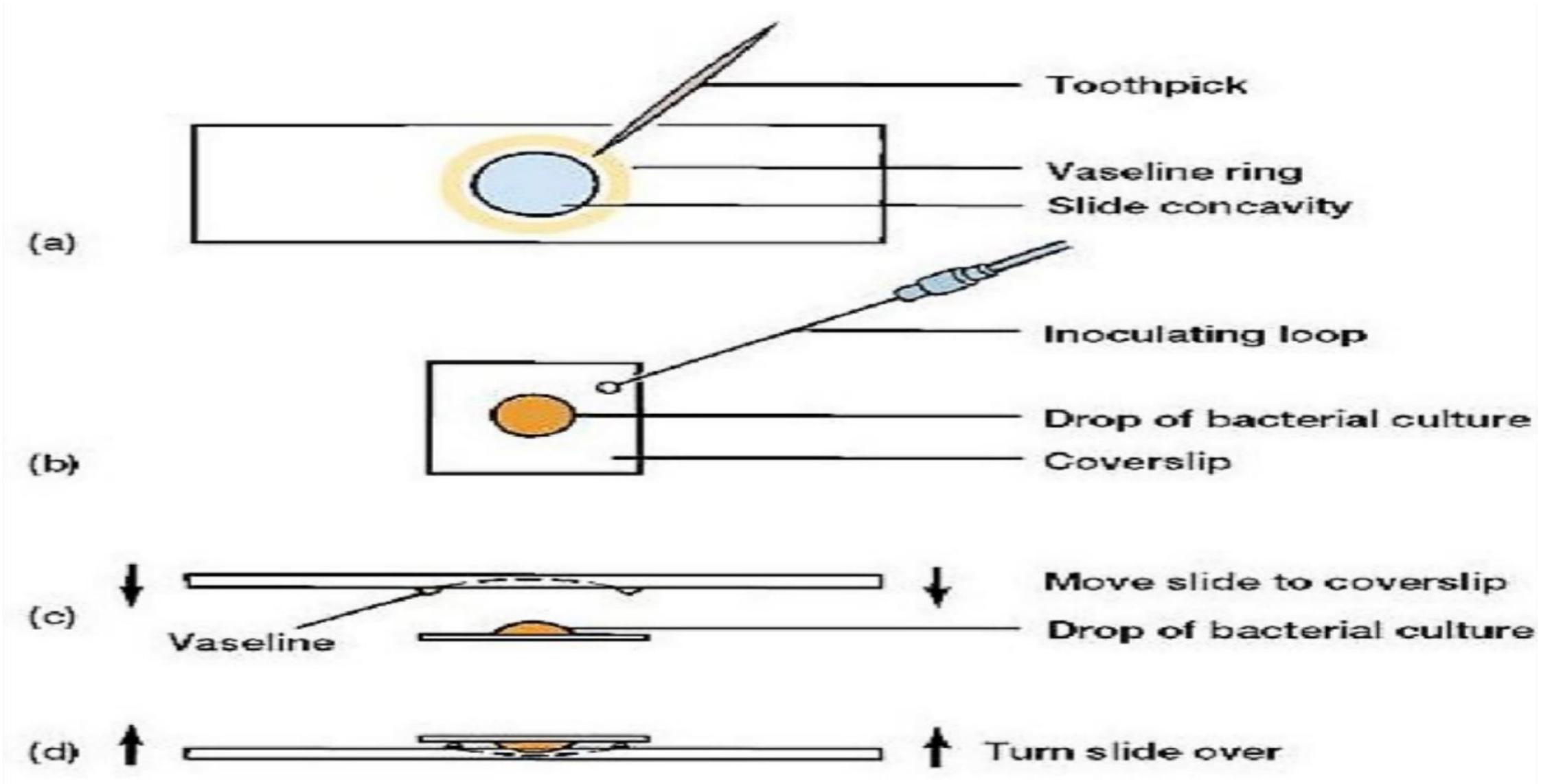


Figure .3 Hanging-drop preparation using petroleum jelly to seal the cover glass to the slide.

### **3- Unstained Wet Film (Wet Mount)**

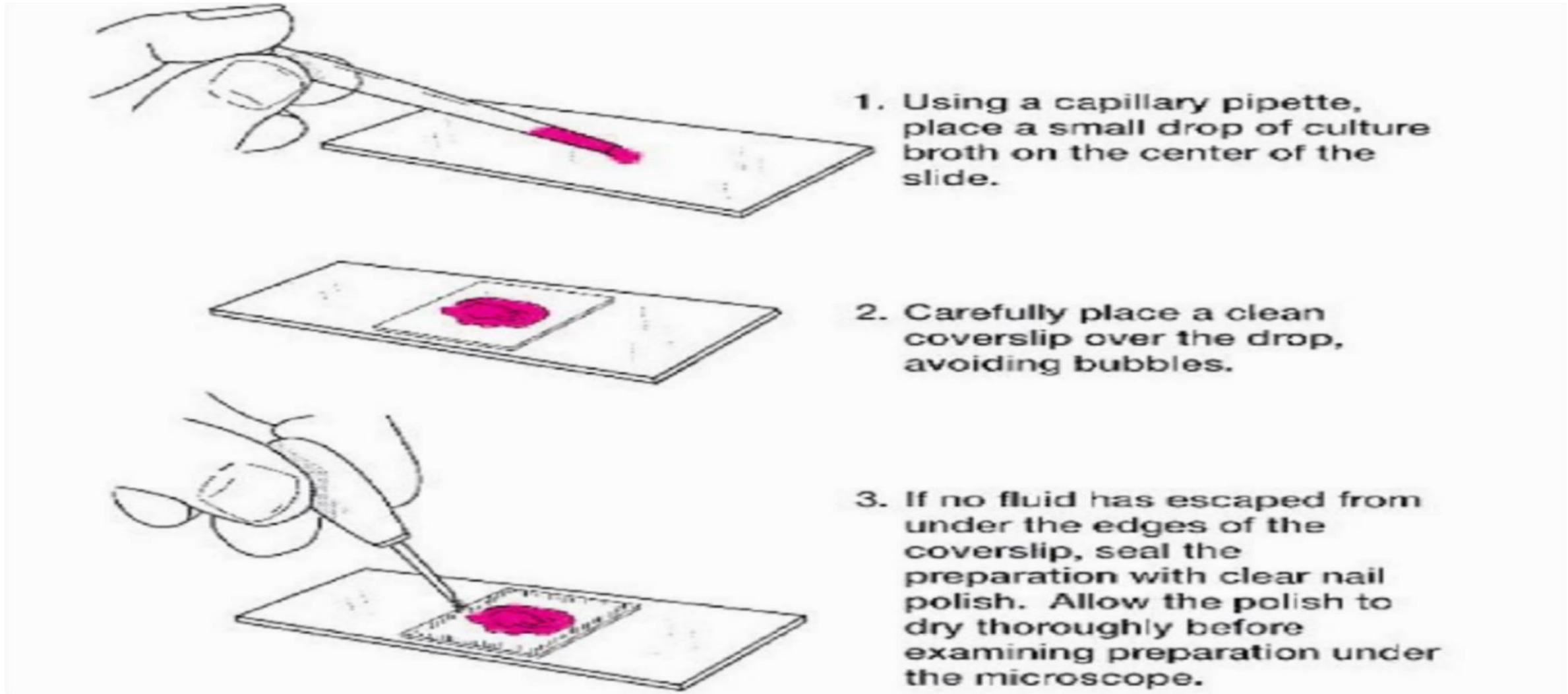
**Purpose:** To observe bacteria in a simple wet mount and determine their motility.

#### **Materials**

- 24-hour broth culture of *Proteus vulgaris*.
- 24-hour broth culture of *Staphylococcus epidermidis*.

#### **Procedures**

- 1. Using a pipette bulb, aspirate a small amount of the *Proteus* culture with a capillary pipette and place a *small* drop on a clean microscope slide (fig. 4, step 1).**
- 2. Carefully place a clean cover glass over the drop, trying to avoid bubble formation (fig. 4, step 2). The fluid should not leak out from under the edges of the cover glass. If it does, wait until it dries before sealing.**
- 3. If you examine the slide immediately, you need not seal the coverslip. Otherwise, seal around the edges of the coverslip with a thin film of clear nail polish (fig. 4, step 3). Be certain the nail polish is completely dry before examining the slide under the microscope.**



**Figure .4.** Wet-mount preparation.