Isolation

1) isolate pathogens from infected plant parts  
we cut off some of the infected tissue a small 1 cm Surface Area to include the injured part and then we wash this parts under running water or sterile dust suspended several times after that we sterilized surface plant parts before the isolation process using a single sterile solutions derived :  
1 - 1% chloride Zibakak  
2 - 5% formalin  
3 - 70% alcohol  
4 - 3% oxygen water  
5 - 2% potassium Bermnkenat  
6 - 1% sodium Azotat  
Overlying the infected parts for a certain plant parts and wash with water after sterilization, sterile distilled several times to completely get rid of the effects of sterile material that has already been used for sterilization. Under the conditions of sterilization movement of the parts which have been prepared on PDA medium and incubator at 28 C.  
2-isolate the fungus from the soil directly:  
Taken a little from agricultural soils at the head of scalpel sterilized is placed in a dish containing the nutrient medium PDA then leave dishes in the incubator, isolated take part on a slide is then put lactophenol blue special dye fungi and the movement of under the microscope.  
3) isolate soil fungi by diluted concentrations:  
1-Taken several replicates of soil samples and random samples are taken from to be studied and then follow the steps below.  
2 - Dilutions and concentrations.  
3 - Added PDA Life antibiotics to prevent bacterial growth.  
4 - Take 1 ml of each conc. is added either above the center or placed in the dish and pour over it and incubate all the dishes in the incubator. At the appropriate time and conditions.

Note: nutrient agar media for isolation bacteria and water agar for isolation actionmycetes

**Isolation of bacteria in pure culture**

To identify a bacterial pathogens, it’s necessary to isolate the bacteria in pure culture, many techniques can be used to isolate different microorganisms.

1. Streak plate technique:

Streak method is routinely employed for isolation of bacteria in pure culture. This technique involves the following steps:

Put a sterile wire loop over the flame of the burner until be red-hot and allow to cool.

Remove very small amount of bacterial culture or clinical materials by sterile wire loop.

Hold the late of medium near to the burner by free hand and put the inoculums on peripheral of the plate and spread over a small area.

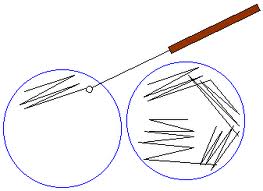
Rotate the plate to 90° and streak over agar surface by making a parallel line vertical to the first one.

Sterile the loop again and rotate the plate toward right angle and making another lines of streak vertical toward quarter by touching the previous lines.

By the same process, repeat the streaking for 3 to 4 times until the whole surface of the agar plate is inoculated.

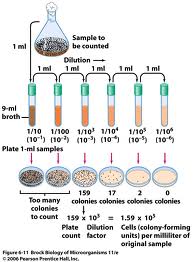
Streak the center of plate in a zigzag motion.

Incubate the plate in an inverted position at 37°C for 18-24hr



2. Spread plate technique:

In this method take 0.1ml of sample of bacterial culture by pipette place over the surface of agar medium. Spread the bacteria culture over the surface by glass spreader then incubate the plate at 37C for 18-24hr. This technique is used for counting bacteria



3. Pouring plate technique:

Add 0.1ml of bacterial culture by using sterile pipette to Petri dish.

Pour the media into the Petri dish and allow to solidify.

Incubate the plate at 37C for 18-24hr.

To differentiate between aerobic and anaerobic bacteria, aerobic bacteria grow and reach to the surface of the agar medium.

**MEDIA CLASSIFIED ACCORDING TO COMPOSITION**

1-Synthetic Media .

2-Non-Synthetic Media: These are complex media prepared from raw materials such as meat

extracts, peptones, yeast extract, and various sugars. They support the growth of a wide variety

of microorganisms.

**INGREDIENTS COMMONLY USED IN MEDIA**

Familiar compounds such as sodium chloride and hydrochloric acid are used both in the

chemistry lab and in media making, but some less familiar ingredients are commonly used in

microbiological media preparation. Some of these are:

1-Beef Extract,

2-Peptone,

3-Yeast Extract,

4-Agar.