Culture enrichment and isolation (contd)

- Many microbes have **special growth conditions** or **require precautions** to grow in a laboratory setting, leading to special culture techniques.

- Microbes, often those that we **know little about**, have to be cultured with **undefined** media or growth conditions to provide them maximum nutrients and a broad range of conditions.

- The **use of animals is sometimes necessary** as no simple media can be used.

- However, this presents **technical** and **ethical** issues.

- As **human pathogens** are often studied by microbiologists, **special safety conditions** known as **biosafety levels** are used to keep researches free of infection from the pathogens they study.

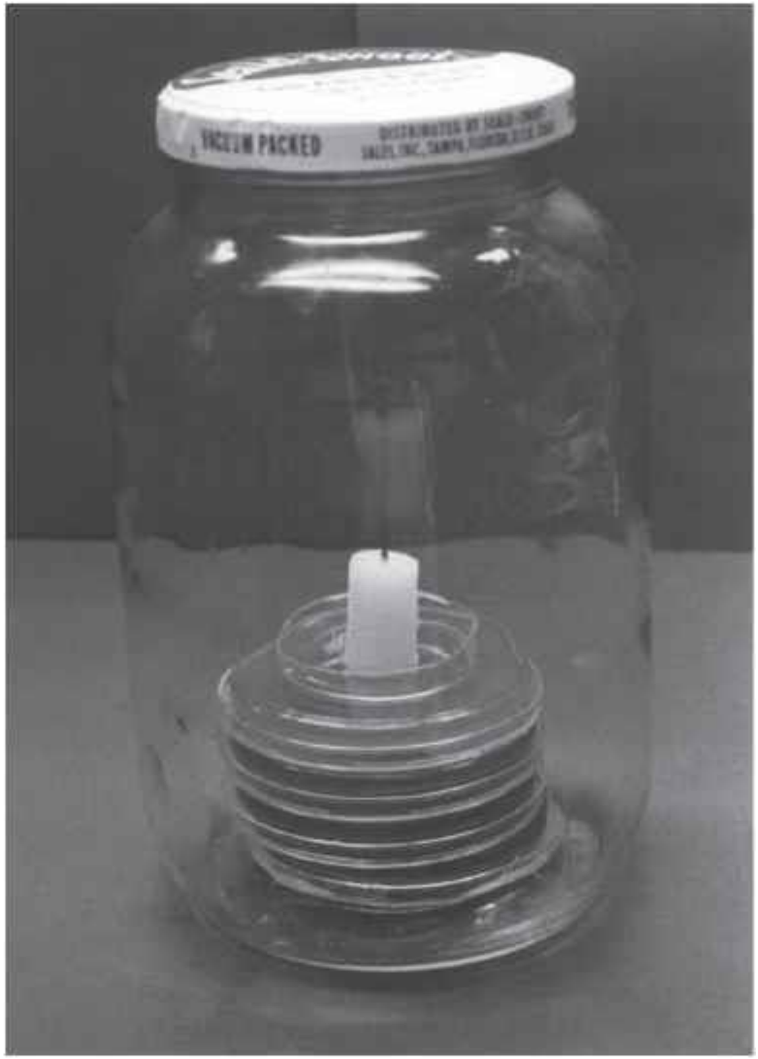
Candle jars and Carbon dioxide incubators

- Some special culture conditions are **relatively simple** as demonstrated by **microaerophiles w**ho require a small amount of oxygen
- Many microphiles are also **capnophiles**, as they require an elevated concentration of carbon dioxide.
- In the laboratory they can be easily cultivated in a **candle jar**.

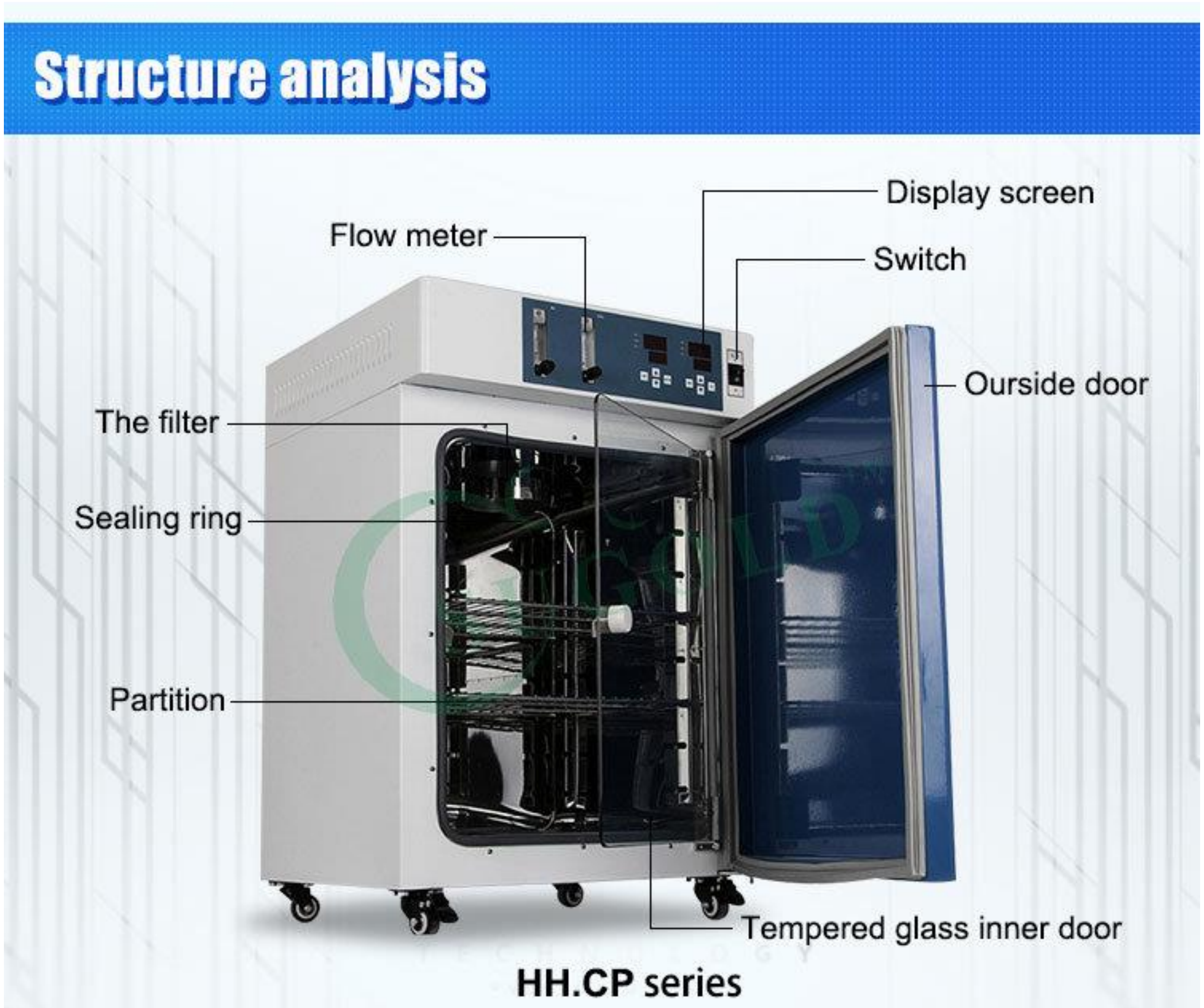
- A candle jar is a container into which a **lit candle** is introduced before sealing the container's airtight lid.

- The candle's flame burns until extinguished by oxygen deprivation, which creates a **carbon dioxide-rich, oxygen-poor atmosphere** in the jar.

- Many labs also have access directly to carbon dioxide and can add the desired carbon dioxide levels **directly to incubators** where they want to grow microaerophiles.



Candle jar



Carbon dioxide incubator

Animals

Animals can often be used **to culture microbes**.

- For example, **armadillos** are often used in the study of **leprosy**.

- The leprosy bacterium is almost impossible to culture using culture media.

- Armadillos have a body temperature of 34°C, **similar to human skin** where leprosy bacteria grow**.**

- The **unusually** low body temperature of armadillos make them susceptible to the leprosy bacterium, *Mycobacterium leprae*.

- **Humans** can acquire a leprosy infection from armadillos by **handling them** or **consuming armadillo meat**.

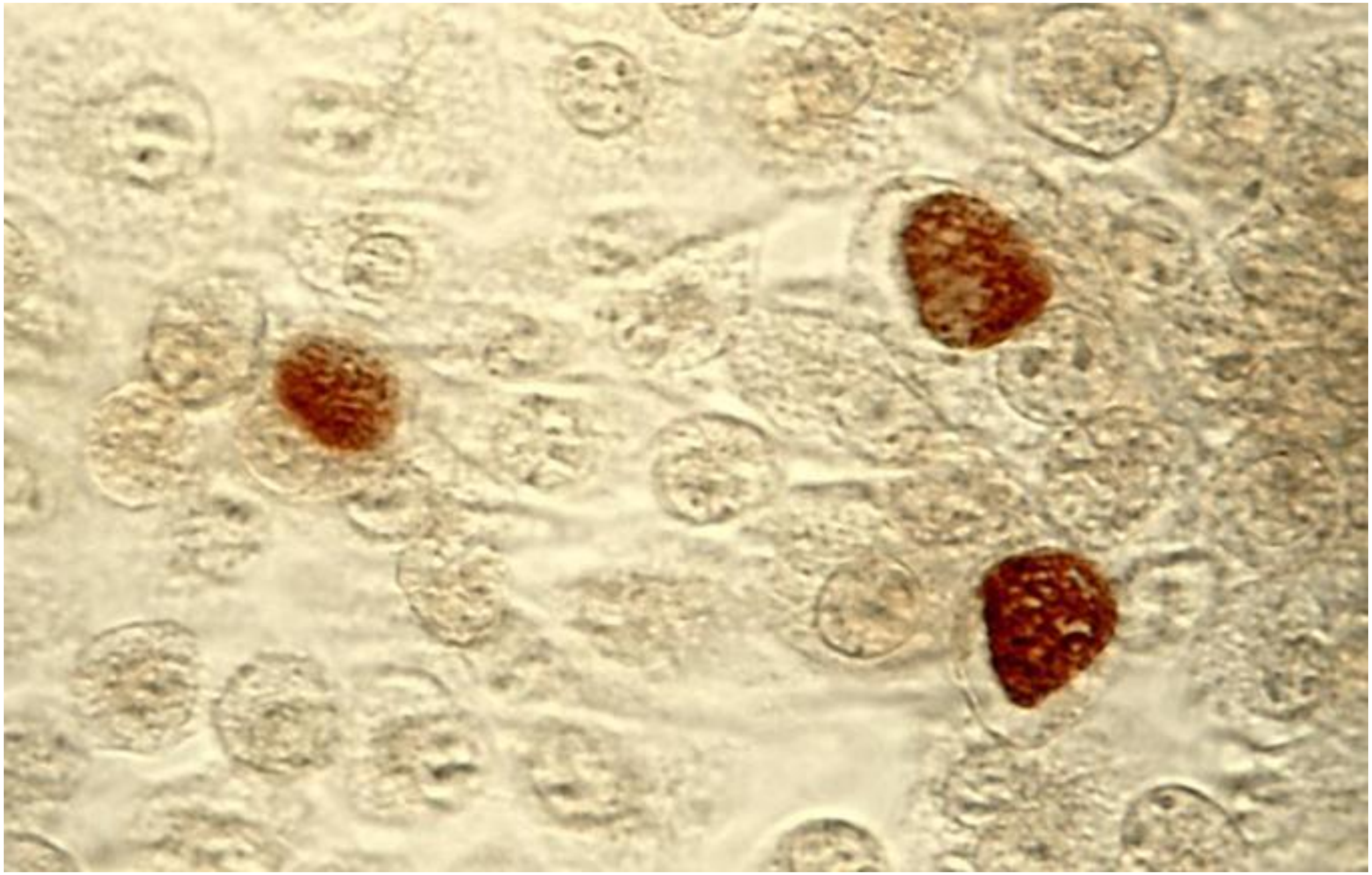
Cell culture

- Some human pathogens are **grown directly on cells** cultured from humans.

- Example is *Chlamydia trachomatis*, the bacteria responsible for the sexually transmitted infection (STI) in humans known as Chlamydia.

- *Chlamydia trachomatis* only grows in humans.

- The human cell culture known as **McCoy cell culture** is used to culture these bacteria.



Brown colored cells are Chlamydia infected cells

Biosafety levels

- As typified by some of the above examples, some microbes have to be **grown in the lab**, and some of them **can infect humans.**
- To deal with this, microbiologists use a **classification of biosafety levels.**
- A **biosafety level** is the **level of the biocontainment precautions** required to isolate dangerous biological agents in an enclosed facility.
- The levels of containment range from the **lowest biosafety level 1 (BSL-1) to the highest at level 4 (BSL-4).**

Biosafety Level 1:

- This level is suitable for work involving **well-characterized agents not known to consistently cause disease** in healthy adult humans.

- These microorganisms have **minimal potential hazard** to laboratory personnel and the environment.

- These microorganisms can be handled under **normal laboratory conditions.**

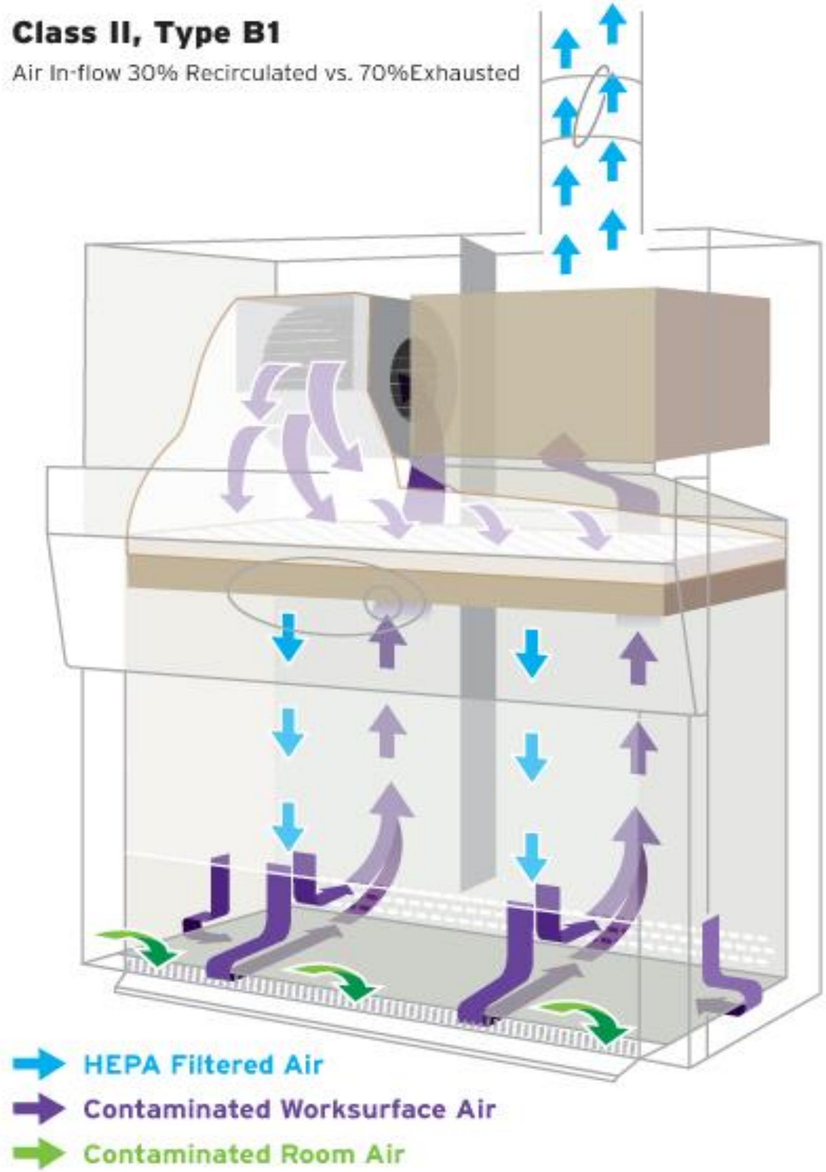
Biosafety Level 2:

- This level is suitable for work involving **agents of moderate potential hazard** to personnel and the environment.

- It includes various bacteria and viruses that cause **mild to severe disease** to humans.

  Example is *Salmonella typhi* that causes typhoid.

- **Special biosafety cabinets** are used which have **vertical and horizontal air shields.**

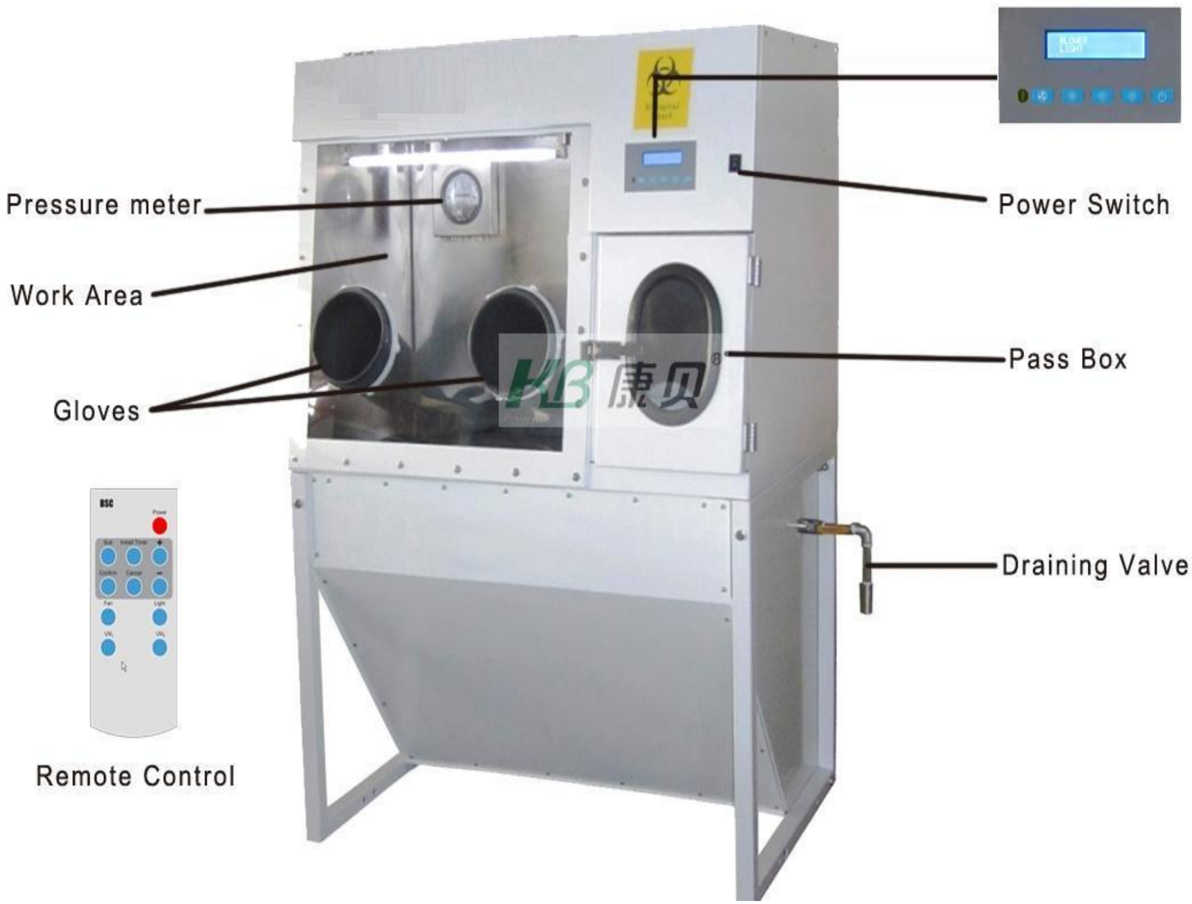

Class II, Type B1
Air In-flow 30% Recirculated vs. 70%Exhausted
HEPA Filtered Air
Contaminated Worksurface Air
Contaminated Room Air

Biosafety Level 3:

- This level is applicable to microorganisms that may cause **serious or potentially lethal disease after inhalation**.

- It includes various bacteria, parasites, and viruses that can cause **severe to fatal disease** in humans, but for which **treatments exist** (e.g. *Mycobacterium tuberculosis*, yellow fever).

Class III Biological safety Cabinet



Biosafety Level 4:

- This level is reserved for work with **dangerous and exotic agents that pose a high individual risk** of aerosol-transmitted laboratory infections.

- These agents cause **severe to fatal disease** in humans for which vaccines or other **treatments are not available**.

- These include **Marburg virus**, and the **Ebola virus**.

- **Very few** laboratories are biosafety level 4.

Positive pressure suit: A scientist puts on a positive pressure suit, something needed to work with the most dangerous human pathogens in a biosafety level 4 laboratory.

Isolation of Microorganisms

- Microorganisms occur in natural environment like soil.
- They are **mixed** with several other forms of life.
- Many microbes are **pathogenic**.
- They cause a **number of diseases** with a variety of symptoms, depending on how they interact with the patient.

- The **primary culture** from natural source will normally be a **mixed culture** containing microbes of different kinds.

- But in laboratory, the various species may be **isolated from one another.**

- A culture which contains **just one species** of microorganism is called a **pure culture**.

- The **process of obtaining a pure culture** by separating one species of microbe from a mixture of other species, is known as **isolation** of the organisms.

Methods of Isolation:

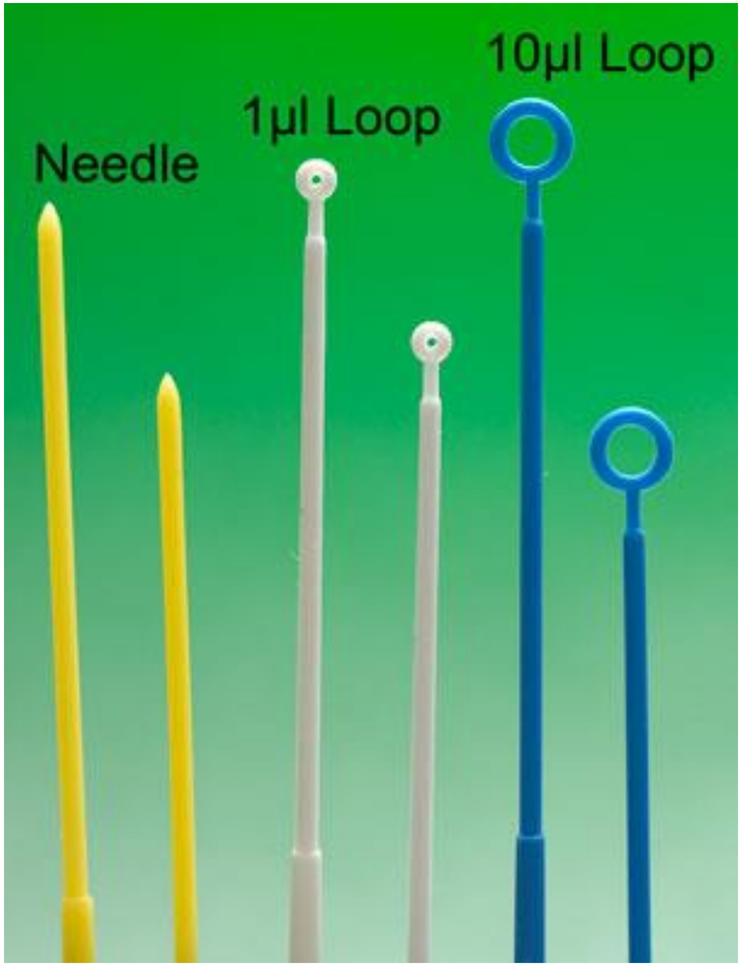
- There are **special techniques** employed to obtain pure cultures of microorganisms.
- In few cases it is possible to secure pure culture by **direct isolation or direct transfer.**
- This can be done only in those situations in which **pure culture occurs naturally.**
- Type of specimens taken for culturing will depend on the **nature and habitat** of microbes.

Following **isolation methods** are usually employed to isolate microbes from mixed cultures:

1. **Streaking**
2. **Plating**
3. **Dilution**

1. Streaking:

- This is **most widely used** method of isolation.
- The technique consists of pouring a suitable **sterile medium** into **sterile petri plate** and allowing the medium to **solidify**.
- By means of a **sterile loop** or **straight needle** or a **sterile bent glass-rod**, a small amount of growth preferably from a broth culture or bacterial suspension is taken.



Needles and loops

Glass spreader

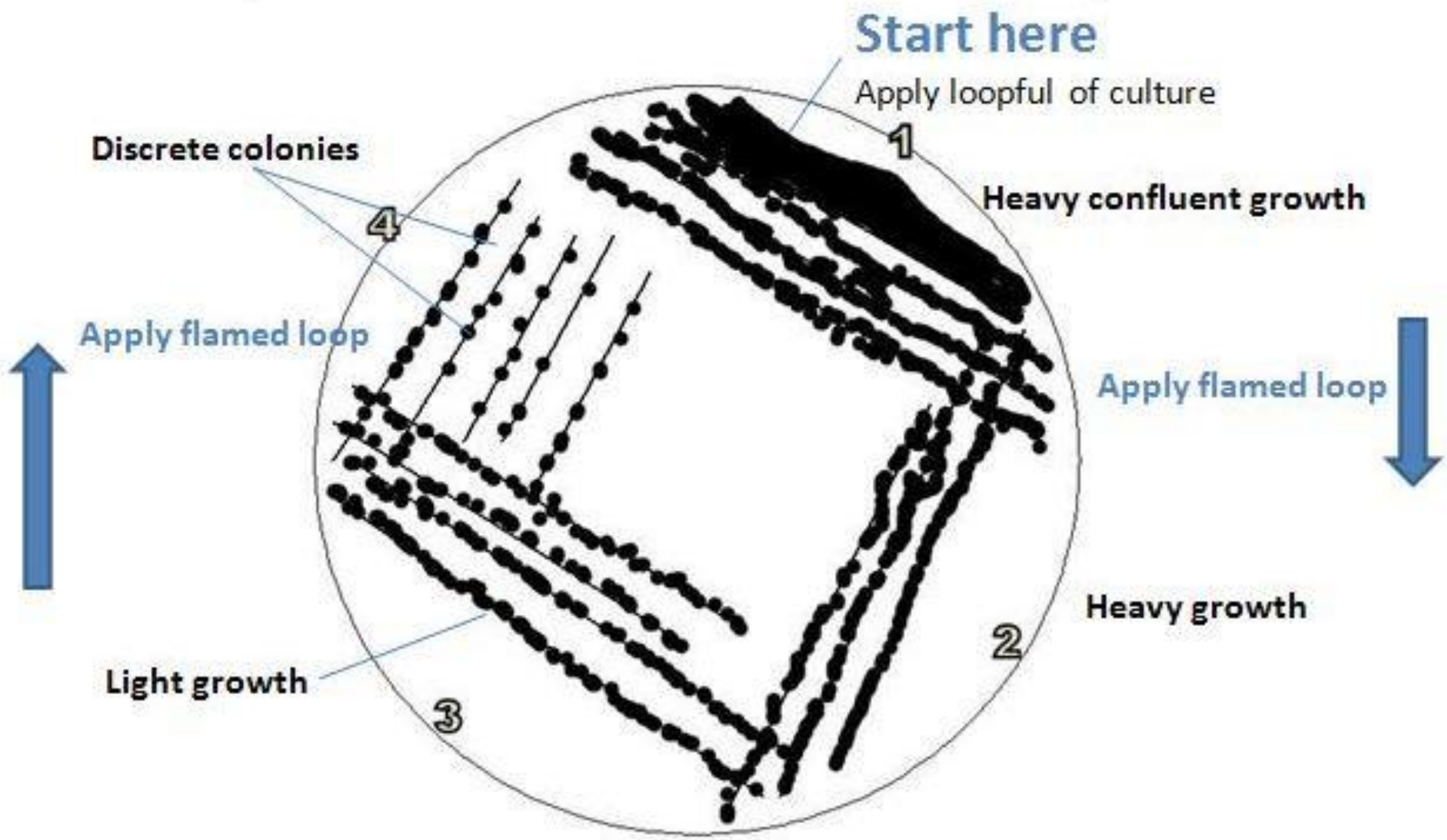
- The bacterial suspension is **streaked back and forth** across the surface of agar until about **one third** of the diameter of the plate has been covered.

- It is called **well culture.**

- The needle is then **flamed** and streaking in done at **right angles** to and across the **first streak**.

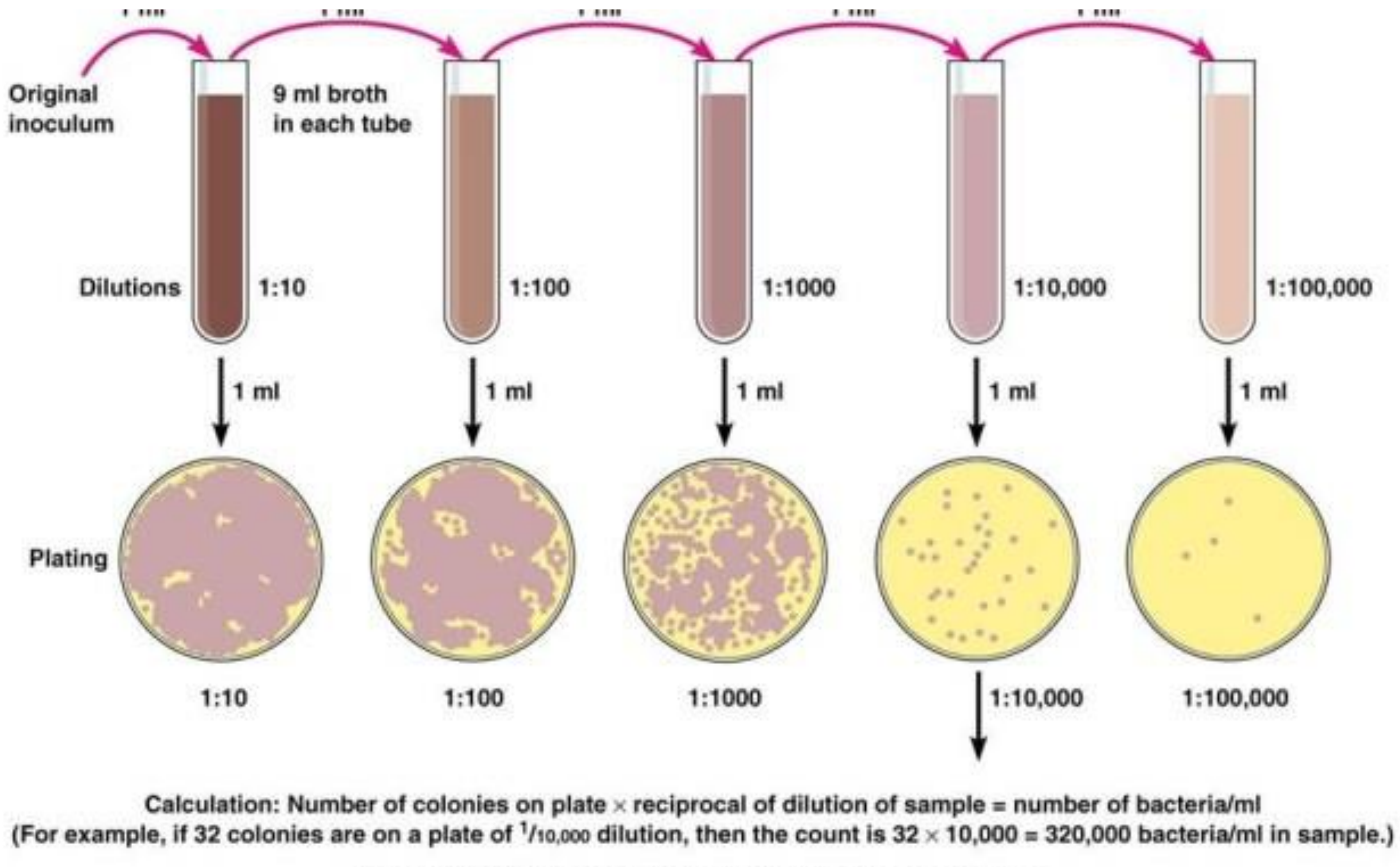
- This serves to **drag bacteria out in a long line** from the initial streak.

- When this streaking is completed the needle is **again flamed** and streaking is done at **right angles** to the second streak and parallel to the first.


Start here
Apply loopful of culture
1
Heavy confluent growth
Discrete colonies
4
Apply flamed loop
Apply flamed loop
Heavy growth
2
Light growth
3

2. Plating:

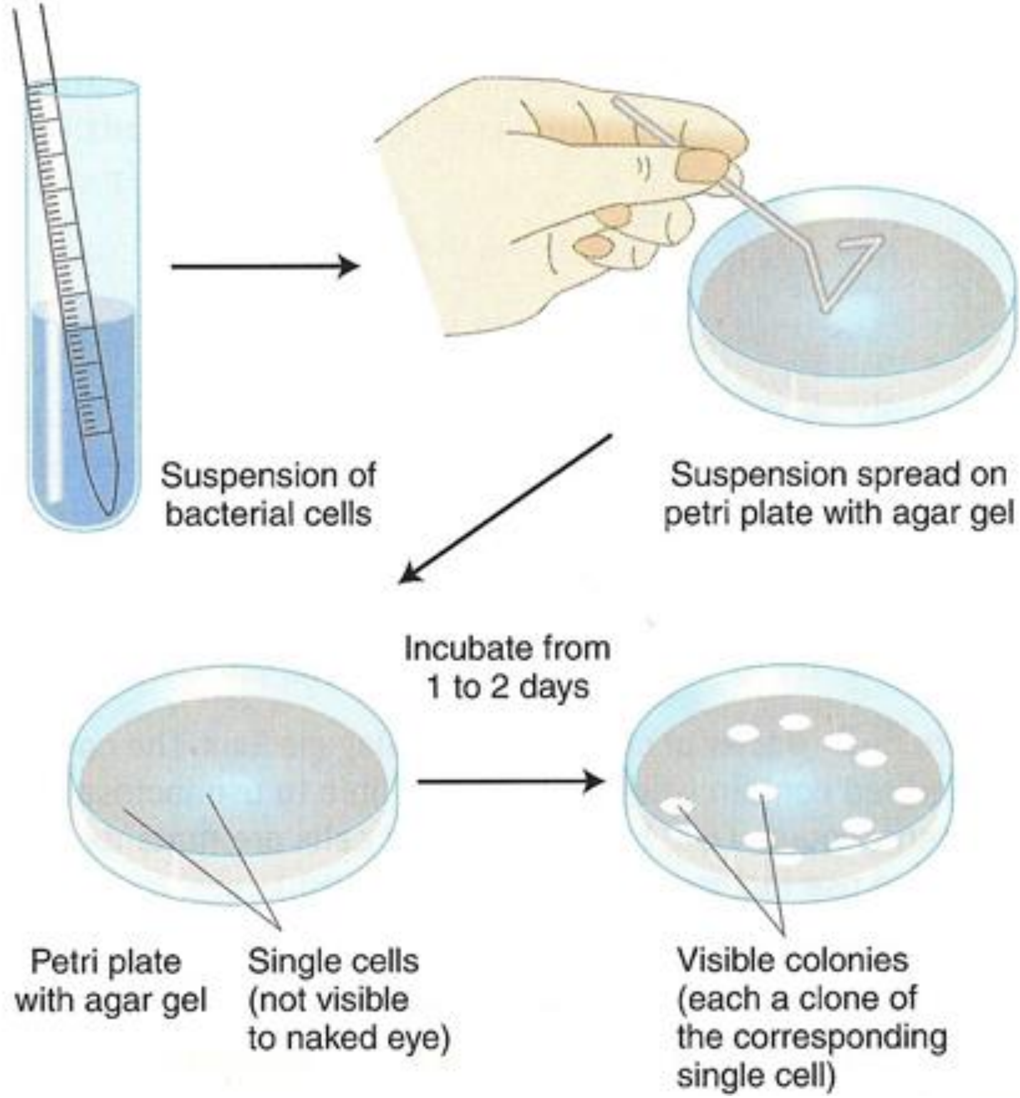
- It includes **diluting of a mixture of microorganisms** until only a few hundred bacteria are left in each ml of the suspension.



- A **very small amount of the dilution** is then placed in a sterile petri plate by means of a sterile loop or pipette.
- The melted agar medium is **cooled to about 45°C** and is poured into plate.
- The microorganisms and agar are **well mixed**.
- When the agar is solidified the individual bacterium will be held in place and will grow to a **visible colony**.
- By this technique, **anaerobic** and **microaerophilic** bacteria can also grow and their colonies can be used for further analysis.

3. Dilution:

- This method is used for the microorganisms which are **small in number dominated by other bacteria.**
- Direct culture yields heavy growth so it is **very difficult** to find bacterial colonies of these **minority bacteria**.
- By **serial dilution**, the colonies will be **separated and a pure culture will be obtained.**


Suspension of
bacterial cells
Suspension spread on
petri plate with agar gel
Incubate from
1 to 2 days
Petri plate
with agar gel
Single cells
(not visible
to naked eye)
Visible colonies
(each a clone of
the corresponding
single cell)


Thank You!