

Veterinary Microbiology (Unit-1)

Isolation of bacteria in pure culture

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Microbial cultures

- ❖ A population of bacteria grown in the laboratory
- ❖ Foundational and basic diagnostic methods
- ❖ To determine - type of organism, its abundance in sample tested, or both
- ❖ Bacterial contaminants may be present on the plates together with the pathogen of interest
- ❖ Solid media- agar based, semi-solid media, Liquid culture
- ❖ Bacterial cultures periodically transferred, or sub-cultured, to new media to keep bacterial population growing

Pure culture

- Axenic culture
- Single bacterial species
- May originate from a single cell or single organism, cells are genetic clones of one another
- Population (clone, strain) derived from an individual cell and free from other (contaminating) microorganisms
- Aseptic techniques
- Foundation of all research in infectious diseases

Need for pure culture

- ❑ Taxonomic identification
- ❑ Diagnostics of pathogens
- ❑ Virulence and pathogenicity studies
- ❑ Elucidation of metabolic properties
- ❑ Testing sensitivity to antibiotics
- ❑ Genome sequencing
- ❑ Proteomic studies
- ❑ Strain deposition in microbial collections

PURITY OF CULTURE

- ❖ Testing essential for success of microbial identification
- ❖ Very often live but non-growing contaminants may be present in, or near a colony and can be sub-cultured along with chosen organism
- ❖ Non-selective media preferred for final isolation

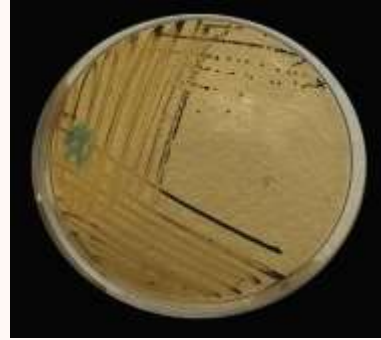
Characteristics:

- ❖ Colonies on solid media alike (same cultural characteristic) on a particular media
- ❖ Cells should have identical form and similar size
- ❖ Same staining property, similar biochemical results

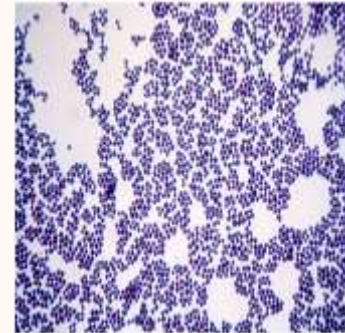
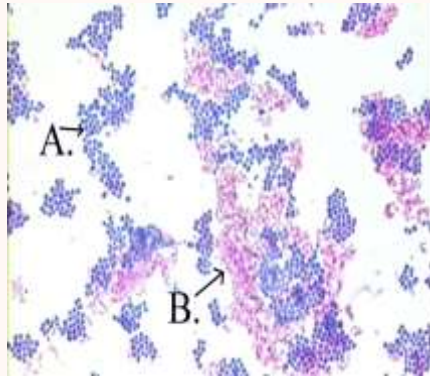




Staining of mixed culture



Staining of pure culture



(Image source-Google)

Obtaining pure culture

- usually derived from a mixed culture
- Diluted, various individual microorganisms become separated far enough apart on an agar surface (isolation plate)
- Isolated colony aseptically "picked off" the isolation plate
- Transferred to new sterile medium
- After incubation, all organisms in new culture descendants of the same organism (pure culture)

Obtaining pure cultures: routine practice

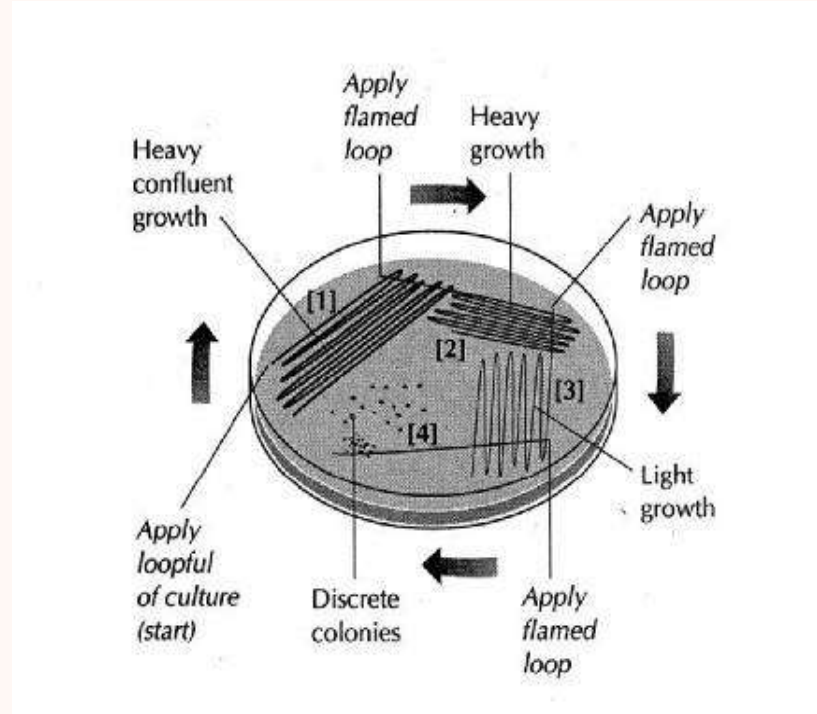
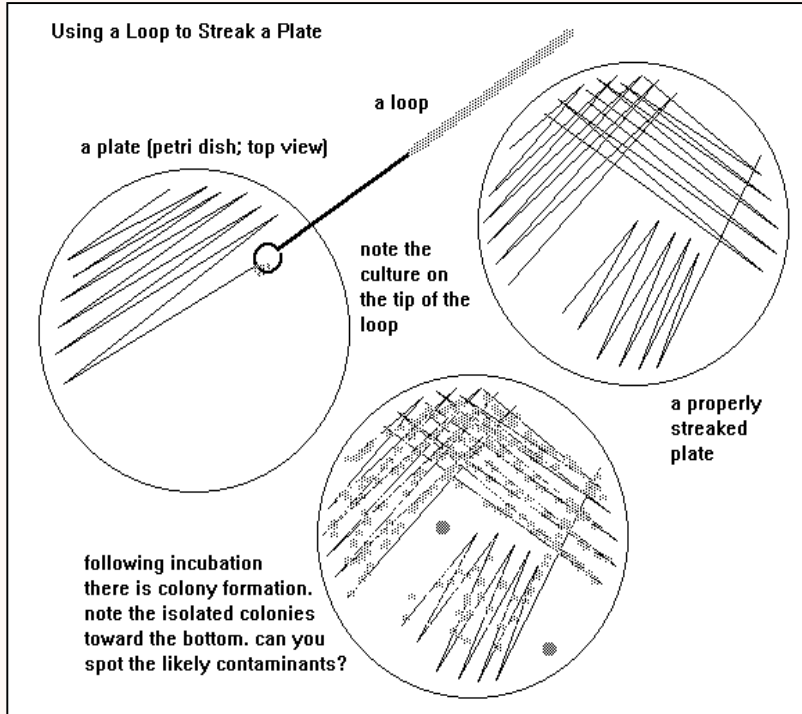
Various physical and chemical treatments as well as technical manipulations are employed

- ❖ Plating (streak plate method, pour plate method, spread plate method)
- ❖ Serial dilution
- ❖ Single cell isolation
- ❖ Use of treatments relatively harmless to the target strain but toxic to contaminants (e.g., phenolics, detergents, elevated temperature, UV or gamma irradiation, antibiotics) and enrichment method

STREAK PLATE METHOD OF ISOLATION

- ❖ Most common way of separating bacterial cells on the agar surface to obtain isolated colonies
- ❖ After incubation
 - area at the beginning of the streak pattern -confluent growth
 - area near the end of the pattern should show discrete colonies

Pure culture – Streak Plates






Spread Plate method

- ❖ Serially diluted specimen spread over the solidified agar media plates as a thin layer with the help of a sterile L-shape glass rod (Spreader)

Pour Plate Method

- ❖ Inoculum from a broth/sample placed in centre of sterile Petri dish using a sterile pipette
 - ❖ Molten cooled agar then poured into the Petri dish containing the inoculum, mixed well and allowed to solidify
 - ❖ After incubation, discrete bacterial colonies found growing both on the agar and in the agar
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Spread-plate method



Sample is pipetted onto surface of agar plate (0.1 ml or less)



Sample is spread evenly over surface of agar using sterile glass spreader

Incubation

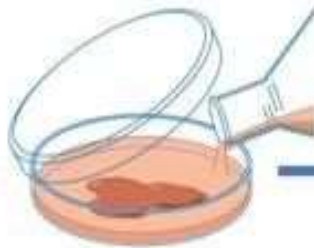


Typical spread-plate results

Pour-plate method

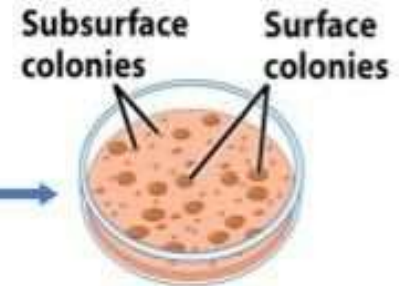


Sample is pipetted into sterile plate



Sterile medium is added and mixed well with inoculum

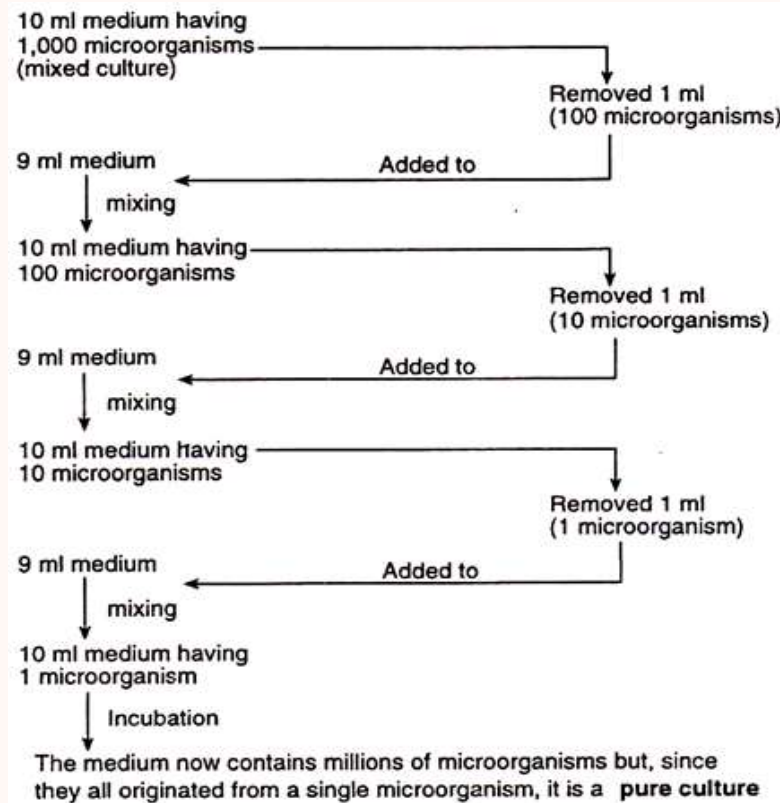
Incubation



Typical pour-plate results

Serial dilution

- ❑ Microorganisms not successfully cultivated on solid media and grow only in liquid media
- ❑ Inoculum subjected to serial dilution in a sterile liquid medium
- ❑ Large number of tubes of sterile liquid medium inoculated with aliquots of each successive dilution



Single Cell Isolation Methods

- ❑ An individual cell of the required kind is picked out by this method from the mixed culture and is permitted to grow

Capillary pipette method:

Several small drops of a suitably diluted culture medium are put on a sterile glass-coverslip by a sterile pipette drawn to a capillary

One then examines each drop under the microscope until one finds such a drop, which contains only one microorganism

This drop is removed with a sterile capillars pipette to fresh medium

The individual microorganism present in the drop starts multiplying to yield a pure culture

Micromanipulator method

Pick out a single cell from a mixed culture

Used with a microscope to pick a single cell (particularly bacterial cell) from a hanging drop preparation

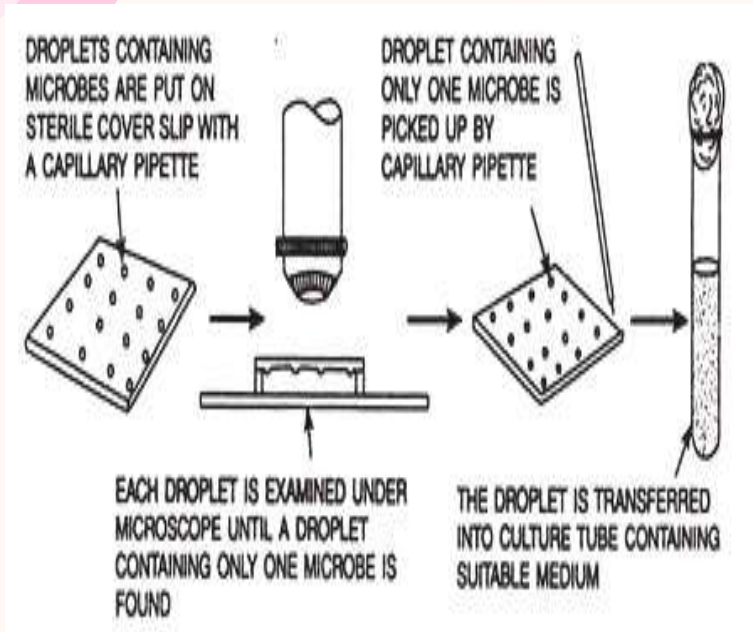
Hanging drops of a diluted culture are placed on a special sterile coverslip by a micropipette

Now a hanging drop is searched, which contains only a single microorganism cell

This cell is drawn into the micropipette by gentle suction and then transferred to a large drop of sterile medium on another sterile coverslip

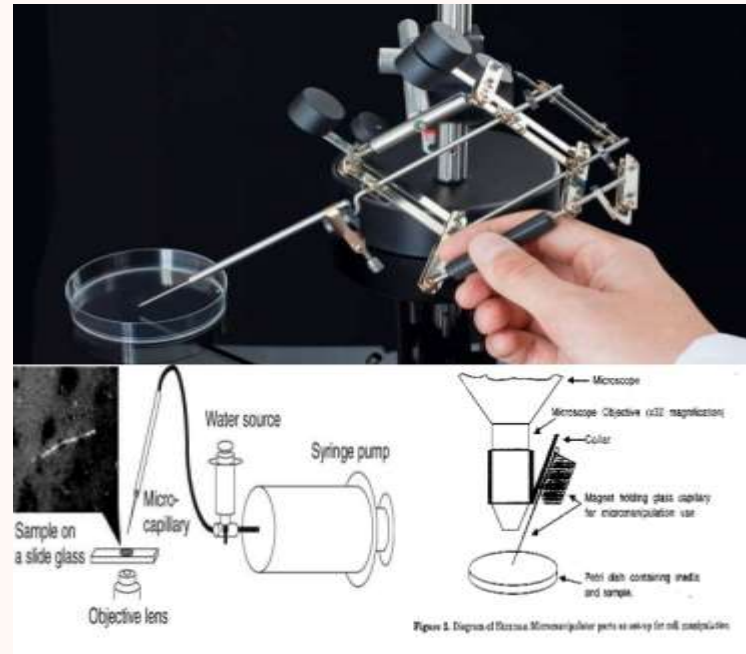
When the number of cells increases in that drop, the drop is transferred to a culture tube having suitable medium

This yields a pure culture of the required microorganism



Capillary pipette method

Source- <https://www.biologydiscussion.com/organism/culture-organism>



Micromanipulator method

Source- <https://www.slideshare.net>

Enrichment Culture Method

- Microorganisms present in relatively small numbers or have slow growth rates
- Provides a specially designed cultural environment
- Make conditions of growth very favourable for an organism of interest, unfavourable for competing organisms
- Mixed microbial population inoculated in a medium with a defined (but limited) chemical composition and allowed to grow under controlled conditions (temperature, air supply, light, pH, etc.)
- Only suits the growth of a particular type of microorganisms with specific characteristics

MAINTENANCE OF PURE CULTURE

- Necessary to maintain the viability and purity of the microorganism by keeping the pure culture free from contamination
- Transferred periodically onto or into a fresh medium (subculturing) to allow continuous growth and viability of microorganisms
- Subject to aseptic conditions to avoid contamination
- Microbial culture preservation
aims at maintaining a microbial strain alive, uncontaminated, and without variation or mutation, as like original isolate