Pure culture technique

a. Clonal populations**:**

i. *Pure culture technique* is a method of culturing microorganisms in which all of the individuals in a culture have descended from a single parent cell.

ii. This is done so as to:

1. Inhibit evolutionary change within cultures
2. Allow the characterization of types of microorganisms without the confounding presence of other.

b. Colony isolation:

i. The basis of *pure culture technique* is the isolation of pure discrete colonies of individual cells, and their descendants, from other colonies of individuals.

ii. This is usually done by culturing methods employing petri dishes such as:

1. streaking
2. pouring
3. spreading

c. Isolation from the wild**:**

i. When isolating microorganisms from complex mixtures it is always a good idea to repeat the isolation procedure at least once (e.g., re-streak an isolated colony) to make sure that an isolated colony is truly derived from only a single cell (i.e., closely overlapping colonies can be indistinguishable from colonies founded from single cells).

ii. Following their isolation from the wild, microorganisms may be characterized by inoculation into differential medium to determine what type of nutrients they require or

can use, and what types of by-products they produce. This aids in identification.

d. Pouring a plate

a. A *pour plate* is a method of melted agar inoculation followed by petri dish incubation.

b. Steps include:

i. cultures are inoculated into melted agar that has been cooled to 45^0C

ii. The liquid medium is well mixed then poured into a petri dish (or *vice versa*)

iii. colonies form within the agar matrix rather than on top as they do when streaking a plate

c. Pour plates are useful for quantifying microorganisms that grow in solid medium.

d. Because the "pour plate" embeds colonies in agar it can supply a sufficiently oxygen deficient environment that it can allow the growth and quantification of microaerophiles.

e. Spreading a plate

a. Quantification technique**:**

-*Spreading a plate* is an additional method of quantifying microorganisms on solid medium.

-Instead of embedding microorganisms into agar, as is done with the pour plate method, liquid cultures are *spread* on the agar surface using a devise that looks more or less like a hockey stick.

b. An advantage of spreading a plate over the pour plate method is that cultures are never exposed to 45^0C melted agar temperatures.

Preservation of cultures

Culture Preservation is important for the following reasons:

i. Scientific reasons

ii. Identification

iii. Vaccine production

iv. Industrial use

Methods of preserving cultures include:

I. Refrigeration

ii. Stabs

iii. Slants

iv. Lyophilisation

v. Freezing

i. Refrigeration (a.k.a., 4^0C)

This is effective short term preservation. All of the following can be refrigerated

- broth cultures
- stabs
- slants
- streaks

ii. Stabbing

In this method, Cultures are usually stabbed deeply into agar using an inoculating needle in bijou bottles, test tubes and other holding wares. The stabs are incubated until visible cultures are formed; they are then sealed and stored at room or lower temperature.

iii. Slant method

Slant method involves streaking the organism of interest onto the surface of a solid medium in a slant tube. The slant is then incubated until visible culture formation. The slant is then sealed and stored at room or lower temperature.

iv. Lyophilisation

Lyophilisation is the freeze-drying of cultures. Cultures are first frozen and then dried under high vacuum. To revive cultures they are rehydrated by broth. Lyophilisation can be effective long term method of storage.

v. Freezing

Broth cultures are mixed with various ingredients (e.g. glycerol) to limit damage upon freezing and then frozen to temperatures ranging from -50^0C to -95^0C. To revive cultures, they are thawed, pelletted, and resuspended into broth. Freezing can be an effective long term method of storage.

Colony morphology

Differentiating colonies

I. Colony morphology gives important clues as to the identity of their constituent microorganisms. Important classes of characteristics include:

1. size
2. type of margin
3. colony elevation
4. colony texture
5. light transmission
6. colony pigmentation

II. Colony size

a. *Colony size* is dependent not just on the type of organism but also on the growth medium and the number of colonies present on a plate (that is, colonies tend to be smaller when greater than ascertain amount are present) and on culture medium characteristics.

b. Usually stabilizes after few days:

- Colony size usually stabilizes after a day or two of incubation.
- Exceptions include:

-slow growing microorganisms

-during growth under conditions that promote slow growth

- With slow growth colonies may continue to experience growth past this time, especially if an effort is made to prevent solid medium from drying out.

III. Type of margin

d. Colonies can vary in the shape of their margins.

e. See illustration below

IV. **Colony elevation**

Colonies can vary in their elevations both between microorganisms and growth conditions, and within individual colonies themselves.

V. Colony texture

Surface appearance:

i. Colonies can vary in their texture.

ii. Possible textures include:

1. shiny to dull
2. smooth to wrinkled

3. rough
4. granular
5. mucoid

NOTE: A shiny, smooth, and/or mucoid appearance tends to be associated with the presence of capsular material.

VI. Colony light transmission

The *light transmission* through colonies can range from:

- complete (transparent)
- through intermediate (translucent)
- through completely lacking (opaque)

VII. Colony pigmentation

Colonies can come in a rainbow of colors.

SOME DEFINITIONS

- **Petri dish** -*Petri dishes are circular*, vertical sided plates used to contain agar and with tops for aseptic purposes.
- **Loop** -A platinum wire formed into a *loop* is heated to an orange glow to sterilize it then is used to transfer a culture from one physical location to another. Platinum wire is used as inoculation needle because when heated, it cools fast.
- **Streaking** -*Streaking* is a method of applying cultures to solid medium:

i. a sterile loop is cooled and brought into contact with a culture

ii. the loop is then brought into contact with the surface of solid medium whereupon it is streaked (i.e., dragged) along the surface of the solid medium

iii. colonies grow along the points of the streak

Streaking a plate

Colony isolation: A petri dish is streaked in manner such that individual colonies may be isolated.

Control of Microbes by Physical and Chemical Agents

I. Definitions

A. Antimicrobial agent = general terms for an agent that kills microbes or inhibits their growth

1. prefix designates organism type (bacteri-, fungi-)

2. suffix designates whether it kills (-cidal) or inhibits growth (-static)

B. Sterilization = destruction or removal of ALL living cells, spores, or viruses

C. Disinfection = killing, inhibition, or removal of all microbes that may cause disease by disinfectant; usually on inanimate objects.

D. Sanitation = reduction of microbial populations to levels that are considered safe for public health by cleaning (partial disinfection) by sanitizer; usually on inanimate objects.

E. Antisepsis = prevention of infection of living tissue by application of antiseptic.

F. Antibiotic = a microbial product or derivative that kills or inhibits growth of a susceptible microbe.

II. Conditions influencing effectiveness of antimicrobial agent

A. Population size: larger population size = longer time to kill all organisms because death is logarithmic.

B. Population composition

1. Some organisms are more resistant to killing than others

2. Some growth stages are more resistant than other (i.e. endospores, stationary phase cells)

C. Concentration/intensity of agent

Generally and up to a point, the more concentrated the agent, the more effective it is at killing. There are exceptions to this. 70% ethanol is more effective than 100%.

D. Duration of exposure (contact time): longer exposure = more killing

E. Temperature: many times higher temperature = greater killing

F. Local environment

1. lower pH enhances killing

2. Organic material protects antimicrobial agents

Use on inanimate objects	Use on living tissue		
sterilant	disinfectant	sanitizer	antiseptic antibiotic

III. Use of physical methods in control

A. Heat

1. Mechanism of action: denaturation of proteins and DNA, disruption of

membranes, oxidation of proteins (for dry).

2. Quantitative descriptions

a) thermal death point = lowest temperature in which a microbial population is killed in 10 minutes;

b) thermal death time = shortest time needed to kill all organisms in a microbial suspension at a particular temperature; depends on original number of microbes.

c) decimal reduction time (D) = time required to kill 90% of microbes in a sample at a particular temperature

d) z value = increase in temperature required to drop D 10-fold (larger Z value = more heat resistant)

3. Types of heat

(a) Moist heat

(i) Boiling

Ten minutes of heating destroys most vegetative cells but not bacterial endospores. Hence, we can regard this as a form of disinfection since not all population are completely destroyed.

(ii) Autoclaving

This involves the use of autoclave @ a pressure of 15 psi , high temperature of 121°C for 15 minutes. Bacterial endospores and vegetative forms are destroyed. It is a form of sterilization. Examples include bact. Media, rubber

appliances, surgical instruments, cotton wool and discarded cultures.

(iii) Pasteurizing

This involves heating (usually below boiling) to kill all pathogens and to reduce the number of microbes that may cause spoilage(disinfection).e.g milk is pasteurized at 63°C for 30 mins.

(iv) Tyndallization (fractional stream sterilization)

This involves alternating treatments (x3) of heat at 100° for 30 min followed by incubation at 37°C for 1 day which allows germination of spores and then subsequent killing (sterilization).

(b) Dry heat

(i) Hot air

This is the use of hot air through hot air oven (160-170°C for 2-3 hours) to sterilize items that cannot be in contact with water and that can withstand very high temperature. Examples include powders, glass wares, liquid paraffin etc. This method cannot be used for items that cannot stand exposure to heat for long times and a lot of time is needed in this method.

(ii) Direct flaming

This involves exposing the appliances to direct flames(sterilization).Examples include inoculation loop, spatula, mouth of test tubes and flasks.

B. Filtration

This is the process of separating microbial contaminants from liquids using filters. Sterilization here depends on pore size of filters used.

Types of filters

a) Depth filters

b) Membrane filters

c) HEPA filters – High efficiency particulate air filter

Advantages: easy, can filter heat sensitive materials

Disadvantages: filters are expensive, some things do not filter well

C. Radiation

1. 260 nm ultraviolet (UV) light

(a) Mode of action: Inhibits DNA replication by causing formation of thymine dimers in DNA

(b) Advantage: Very effective sterilant

(c) Disadvantage: Cannot penetrate solid or opaque objects

1. Ionizing radiation –

(a)Mode of action: very short wavelength radiation that cause atoms to loose electrons or ionize thereby causing;

(1) breakage of hydrogen bonds

(2) oxidization of double bonds

(3) polymerization of some molecules

(4) production of free radicals

(b) Types of radiation

(1) X rays – artificially produced

(2) Gamma rays – emitted during radioisotope decay

(c) Advantage: Can penetrate better than UV

(d) Disadvantage: Concern about radioactive contamination, production of toxic or carcinogenic byproducts, alteration of nutritional value; takes a long time

D. Cold

1. Mode of action: prevents growth by decreasing rate of chemical and biochemical reactions

2. Microbiostatic for most organisms - does not disinfect

3. Important in food preservation

E. Desiccation

1. Mode of action: disruption of metabolism

2. Microbiostatic for most organisms

3. Important in food preservation

F. Osmotic pressure

1. Mode of action: plasmolysis

2. Important in food preservation

IV. Use of chemical agents in control

A. Ideal properties

1. Effective against broad range of microbes

2. Effective at high dilutions and in the presence of organic material

3. Nontoxic to people and objects

4. Stable

5. Non-offensive odour

6. Soluble

7. Inexpensive

8. Sporocidal

B. Types of chemical agents

1. phenolics

a) phenols and related compounds such as Lysol

b) mode of action: denature proteins; disrupt membranes

c) advantages: 1, 2, 4

d) disadvantages: 3, 5

2. alcohol

a) 70% ethanol; isopropanol

b) mode of action: denature proteins; dissolve lipids

c) advantages: 1 ,3 (antiseptic), 7

d) disadvantages: 8; 4 (contact time)

3. halogens – iodine based

a) iodine (I_2) or more commonly organic carrier: iodine complex (iodophor) – Betadine

b) mode of action: oxidized cell constituents, especially proteins at –SH groups; iodinates proteins and inactivates them frequently at tyrosine residues

c) advantages: 1, 3, 4, 6, 8 (at high concentrations)

d) disadvantages: some pseudomonads can survive in iodophors

4. halogens – chlorine based

a) chlorine gas; sodium hypochlorite (bleach); calcium hypochloride

b) mode of action: conversion to hypochlorous acid (HOCl) and then atomic oxygen which oxidizes cell components

c) advantages: 1, 3, 6

d) disadvantages: 2, 4, 8

5. heavy metals

a) Hg; Ag; arsinic; Zn; Cu; silver nitrate; silver sulfadiazine; copper sulfate

b) mode of action: combine with proteins (usually via sulfhydryl groups) to inactive them; can precipitate proteins

c) advantages:

d) disadvantages: for many (3), bacteriostatic,

6. Surface acting agents

a) Soaps and detergents

(1) mode of action: help to remove organisms from surfaces

(2) not very effective disinfectants

b) quaternary ammonium compounds

(1) positively charged quaternary nitrogen and long hydrophobic chain

(2) mode of action: disrupt membranes, may inactivate some proteins

(3) advantagess: 3, 4

(4) disadvantage: 8

7. aldehydes

a) formaldehyde; glutaraldehyde

b) mechanism of action: inactivates proteins by crosslinking to numerous functional groups on proteins including (-NH2, -OH, -COOH, -SH)

c) Advantages: 1, 8 (sterilant)

d) Disadvantages: 3, 5

8. sterilizing gases

a) ethylene oxide

b) mode of action: inactivates proteins by alkylating them

c) advantages: penetrates plastics, 1, 8 (sterilant)

d) disadvantage: 3