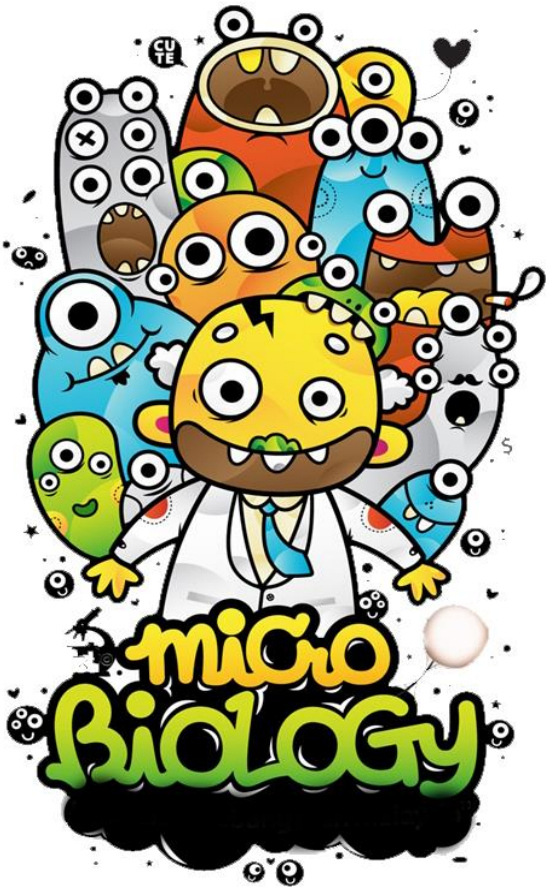

Subject: Sterilization
Lecture No.: 8
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In the **previous lecture** these types of media were mentioned (*routine – previously explained-, selective, differential and enrichment media*).

These media are very important in diagnostic medicine

Selective medium: it encourages the growth of some m.o. but suppresses the growth of others. (selection of a general or specific type of bacteria)

Similar to routine lab media but it includes the addition of a toxic material for everything except the wanted bacteria.

- e.g. an antibiotic can be added to the growth medium so as only m.o. that are resistant to this antibiotic can grow.

Differential medium (indicator media): has an indicator constituent that causes an observable change (color change or pH change) in the medium when a biochemical reaction, (that is characteristic to a certain m.o.) occurs.

- This will allow to distinguish a certain type of m.o. (colony) from others growing on the same plate without the inhibition of the growth of other m.o.

- e.g. blood agar can be used to distinguish hemolytic bacteria (the ability to cause lysis of blood cells).

Enrichment medium: it contains special nutrients that allow the growth of particular m.o. that might not otherwise be present in sufficient numbers, to allow it to be isolated and identified.

- Unlike selective medium, it doesn't suppress the growth of other m.o.
- Blood agar and chocolate (heat-treated blood) agar are also considered as enrichment media and are frequently used to grow fastidious m.o.
- e.g. Salmonella typhi may be in very small numbers in fecal samples, so it is cultured on a medium containing the <u>trace element selenium</u> which supports the growth of this m.o.

It should be also noted that the growth of bacteria does not only depend on the nutrients present in the growth media but also on certain physical factors, such as:

PH:

Each type of bacteria has its own optimum PH, when the PH of the medium diverges from the optimum the growth rate decreases. Most pathogenic bacteria have an optimum PH around PH **7** (*physiologic PH)* and in order to maintain this optimum PH we usually add a buffer system into the growth media.

Temperature:

Most pathogenic bacteria have an optimum temperature around 37°C. So therefore in the incubation of the bacteria we use an incubator, through which we can control the temperature of the environment.

Oxygen:

In terms of oxygen requirements and the extent of its toxicity on bacteria, we divide the bacteria into five different categories:

- ✓ Obligate aerobes:

They usually obtain O_2 from nutrient broth or on the surface of solidified agar, but some may need more. As a result, O_2 can be bubbled through the medium (*with filters to prevent contamination).* It uses oxygen as the final electron acceptor in aerobic respiration.

- ✓ Obligate anaerobes:

In this type of bacteria, the final electron acceptor is not oxygen, it uses nitride or sulfur containing compound, in fact oxygen is toxic to it therefore:

- All molecular O_2 must be removed
- Addition of oxygen-binding agents like thioglycolate, cysteine (a.a) or sodium
- sulfide prevents O_2 from exerting its toxic effects on anaerobes
- If the culture is in plates, special jars are used where special bags containing a Chemical substance are placed to remove O_2 & generate CO_2

- ✓ Facultative anaerobic bacteria:

Can use both oxygen and other electron acceptors in respiration, in other words it can undergo aerobic and anaerobic respiration and clearly oxygen is not toxic to this group of bacteria.

- ✓ Aero-tolerant anaerobic bacteria:

Similar to the facultative anaerobic bacteria, oxygen is not toxic to these species however they do not use oxygen in respiration as they do not have the appropriate metabolic pathways that make use of oxygen as the final electron acceptor.

- Both the Facultative anaerobic bacteria and Aero-tolerant anaerobic bacteria do not have special oxygen requirements for culturing.

- ✓ Microaerophiles:

(The hardest to handle in the laboratory of all the bacterial types)

This group of bacteria require oxygen for respiration, on the other hand high amounts of oxygen can exert toxic effects. They usually require 5% concentration of oxygen, the atmospheric concentration of oxygen in the air is about 21% which is considered toxic, thus this type of bacteria is killed in open air.

A broth tube or agar plate can be incubated in a jar in which a candle is lit before the jar is sealed. The Burning candle uses O2 & adds CO2, when the candle extinguishes = suitable Conditions. (oxygen is 5%).

We can also use an Incubator that controls the oxygen content however this requires more expensive equipments.

Commercial identification techniques:

Are selective, differential and enrichment media mutually exclusive?

No, some types of media can be both selective and differential. For example: **MacConkey agar**, are both selective and differential. MacConkey agar contains crystal violet and bile salts, which inhibit growth of Gram-positive bacteria while allowing growth of Gram-negative bacteria. MacConkey agar also contains the sugar lactose plus a pH indicator that turns colonies of lactose fermenters red (acidic) and leaves colonies of nonfermenters colorless and translucent. Although there are some exceptions, most organisms that are normally found in the human intestines ferment lactose, whereas most pathogens (disease-causing microorganisms) do not.

- ✓ Sulfite Polymyxin Sulfadiazine (SPS) Agar:

It is Used for the detection of Clostridium botulinum, two antibiotics that inhibit the growth of most microorganisms other than Clostridium are added. (**Selective**) Sulfite (present in the medium) is reduced by Clostridium botulinum to sulfide which forms a black iron sulfide precipitate (**Differential**).

How can we identify the specific type of bacteria?

We use a series of differential and selective media for substantial short listing thus reducing the number of possible bacteria.

e.g.

The API (analytical profile index) consists of a plastic tray with 20 micro tubes called cupules, each containing a different kind of dehydrated medium. Each cupule medium is rehydrated and inoculated with a suspension of bacteria from an isolated colony. The tray is incubated; test results determine a seven digits' profile number which identifies the microorganism.

END OF CHAPTER

The control of microorganisms:

The word control means either the killing or the inhibition of microorganisms.

We must be able to distinguish between environmental control and the control of microorganisms in a human body. In the human body we use antibiotics (a subject which will be discussed later in another chapter) while environmental control uses decontamination processes (Sterilization, Disinfection, antisepsis and preservation):

Sterilization:

Complete destruction or removal of all forms of life (including viruses). After treatment the number of surviving microorganisms is zero. Sterilization does not necessary have to kill prions as they are not microorganisms. We perform it with nonliving surfaces to tolerate the very harsh conditions.

Disinfection:

It also deals with nonliving surfaces however it is concerned with the ***reduction*** of the level of microorganisms to a safe level. (Depends on application, the level of disinfection of a table is lower than that of an endoscope).

Antisepsis:

Is the killing or inhibition of microorganisms on living tissues; having the effect of preventing or limiting the harmful effects of infection, however here we are dealing with living surfaces. (skin and mucus membrane)

- Both cidal (killing) and static (growth inhibiting) agents (called antiseptics) can be used in antisepsis.

Preservation:

The addition of antimicrobial chemicals to a product (food or drugs) to inhibit the growth or kill microorganisms and to reduce the risk of microbial spoilage and infection of the consumer.

The main principle of preservation is similar to that of disinfection, so why are they considered as separate processes?

Since in preservation the products will be consumed by humans.

Toxicity concerns:

- **Disinfection:**

Little or no toxicity concerns as we are dealing with nonliving surfaces, therefore we would aim to achieve the maximum number of killings possible.

- **Antisepsis:**

We are dealing with living tissues here like intact skin and mucus membrane thus toxicity concerns will be much higher than that of disinfection as a result we may need to compromise efficacy in treatment to prevent toxicity. static agents may also be accepted in this case since living tissues can induce an immunity response that may aid in the killing of the microorganisms.

- **Preservation:**

Much higher toxicity concerns than all the above processes since the products that we are dealing with will eventually enter the systemic circulation. Opposed to the intact skin since it has the stratum cornea that reduces the absorption of the chemical into the systemic circulation.

In medical settings these processes are very important, however what is the criteria that dictates what process of the four is the most suitable for a particular item and what chemical should we use in order to perform such process?

Every hospital has its own infection control committee, and its main function is to set a disinfection policy, which divides all the items in the hospital into three distinct categories according to the risk imposed on the patient upon their contamination.

High risk items:

They have close contact with broken skin or mucous membrane or are those introduced into a sterile area of the body. These should be sterile. e.g. surgical equipment, gloves, catheters, syringes and needles. Some are reusable equipment therefore they must be sterile before each use.

Intermediate-risk items:

They are in close contact with intact skin or mucous membranes and disinfection will normally be applied. e.g. respiratory and anesthetic equipment and bed-pans. We use proper cleaning and disinfection before each use if they are reusable.

Low-risk items or areas:

Which are not in close contact with the patient.e.g. walls, floors, ceiling, etc. require non frequent disinfection.

- In general sterilization is done using physical measures (heat, radiation, filtration) while the other three methods require chemical agents. (with some exceptions)

Chemical biocides:

Chemicals used in decontamination processes are known as chemical biocides: a chemical agent that has the ability to kill or inhibit microorganisms. (*Same definition as that of antibiotic*)

What is the difference between a biocide and an antibiotic?

In order for a biocide to be an antibiotic it Must be proven to be **safe to be taken systemically** (antibiotic) through clinical trial phase. Antibiotics are also highly specific in terms of action. Biocides affect all cells even human cells (not selective).

Biocides are classified according to their efficacy:

High level disinfection:

They are involved in the Destruction of all microorganisms, but not necessarily bacterial spores. Some have good sporoicidal activity and hence they are called 'liquid chemical sterilants' to indicate that they completely kill all microorganisms as in sterilization.

Intermediate level disinfection:

They are involved in the Destruction of all vegetative bacteria (including Mycobacterium tuberculosis), most viruses (except for small naked viruses) most fungi (not fungal spores). They have little or no sporicidal activity (**Mostly used** biocides as they have reasonable efficacy and are relatively safe).

Low level disinfection:

They can destroy most vegetative bacteria (excluding Mycobacterium tuberculosis), some fungi and some viruses (large enveloped viruses only) they are also the **least used** biocides as they have the lowest efficacy.

Please refer to the slides for further information that wasn't mentioned in the record.

GOOD LUCK