Introduction to aseptic techniques in microbiology

Microorganisms are abundant in the environment, living in soil, in water, on the surfaces of plants and animals and inside many types of multicellular organisms, including humans. Fortunately, most microorganisms live freely in the environment without causing harm to humans or other organisms; in fact, most of them are beneficial in a variety of ways. For these reasons, humans seek to control only certain types of microorganisms and under relatively few specific circumstances. We exert control over microorganisms associated with food materials, so that foods can be stored and made available for human consumption without being consumed by microorganisms first.

Sterilization

The process of sterilization eliminates or eliminates all living microorganisms, including viruses. After undergoing this procedure, any material is considered sterile. Sterilizing control techniques are typically only used on inanimate objects.

The majority of sterilization is carried out using a physical agent, such as heat, A few chemicals can be categorized as sterilizing agents due to their capacity to eliminate spores. Remember that the destruction of spores is not always a necessity, because most of the infectious diseases of humans and animals are caused by non-spore-forming microbes. sterilization achieved by many method:

Physical Methods

1. Heat: denatures proteins

A. Moist heat:

1. Boiling: 100°C, 10 min , lead to Kills: vegetative cells, most viruses

In boiling, objects (Glass-wares instruments) are subjected to boiling water (at temperature 100C) for 10 minutes.

Boiling kills vegetative forms of bacteria & many viruses within about 10 minutes. Endospores and some viruses are not destroyed so quickly (Within 10 minutes) e.g. Hepatitis virus can survive up to 30 minutes of boiling and some bacterial spores can survive for more than 20 hours.

Boiling is therefore not a reliable sterilization procedure.

This method is not recommended for the sterilization of instruments used for surgical procedures and should be regarded only as a mean of disinfection

Applications: sanitization of water, dishes, cookware, etc.

2. Steam:

Autoclaving by 121°C, 15 min and 15psi,

This sterilization technique uses moist heat, or steam under pressure, that is hotter than boiling water. Compared to boiling water, saturated steam under pressure has a higher temperature and greater penetration power. The most popular tool for autoclaving is the autoclave.

Pasteurization

Pasteurization is developed by Louis Pasteur to prevent the spoilage of beverages, milk, juices, etc.

In pasteurization, the product is subjected to controlled heat treatment where specific types of microorganisms are killed but does not kill all organisms.

Temperature used for the pasteurization depends upon the product to be pasteurized and method of pasteurization.

In the case of pasteurization of milk, it is heated to 71.6°C for at least 15 seconds in the high temperature short time method or 62.9°C for 30 minutes in the low temperature holding method.

2) Fractional sterilization (Tyndallization)

In tyndallization, objects, media which decompose at high temperature are exposed to free flowing steam at a temperature of 1000C for 20 minutes for three consecutive days with an incubation period of 24 hours at Room Temperature.

John Tyndall has first proposed this method of sterilization.

This method is also called Fractional sterilization.

Arnold steamer is used for this type of sterilization.

Arnold steamer consists of tinned copper cabinet. The lid is conical, enabling drainage of condensed steam and a perforated tray filled above the water level ensures that the material placed on it is surrounded by steam.

B. Dry heat:

1. Incineration (Burning): turn into ash, Complete destruction of everything

Applications: sterilization of inoculating instruments, waste disposal.

2. Sterilization oven: 170°C, 2 hrs.

Kills: vegetative cells, viruses, endospores (sterilization)

Applications: sterilization of instruments that can tolerate heat

3. Low Temperature: decrease chemical reaction rates → slow or stop cell division.

A. Refrigeration: 4°C

Applications: short term food preservation

B. Freeze: –20°C or lower (liquid nitrogen -196°C)

Freezing is used extensively to control microorganisms associated with food materials, drugs, research chemicals, etc. Freezing effectively inhibits the growth of most microorganisms by stopping metabolic processes, but is rarely cidal to bacteria