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Aseptic Technique

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INTRODUCTION

Aseptic techniques are deterrent measures that are employed to ensure that laboratory equipment is sterile and pure cultures are not contaminated. All organisms are regarded as potential pathogens. Most of the organisms can be opportunistic and cause infection. Microorganisms are all over the atmosphere, therefore, they rest on the surfaces such as desks, benches, and other laboratory equipment and working surfaces at any time. Organisms in the environment cause contamination which affects the isolation of pure stock cultures.

There is a variety of processes that constitute aseptic techniques. These include carrying out tests in microbiology, culture transfer, pure culture isolation, and media inoculation. Some of the benefits of incorporating aseptic techniques in a laboratory include avoiding contamination by foreign bacteria of cultures that are natural in the environment. Additionally, aseptic techniques assist in conserving a pure culture stock while moving the cultures to new media. It is also essential to maintain high standards of sterile environment to ensure that the single species are well isolated from a mix, thus obtaining a pure culture. This paper discusses various aseptic techniques used to prevent cross contamination, the practicality of which is proven by an experiment performed.

STERILIZATION

Cleaning of surfaces before and after laboratory sessions is termed as sterilization. Sterilization can be accomplished by cleaning all laboratory equipment, machines, and materials with a 10% solution that has a bleaching effect, washing hands before and after handling laboratory procedures, ensuring that the only materials on the working bench are those that one needs on the desk, keeping test tubes and Petri dishes covered, and using a different sterile wire loop for every different sample.

It is also very important to work quickly to avoid exposure to microbes in the atmosphere. Use of the bio-safety cabinet is also essential to prevent aerosols from being released from the laboratory environment. This requires holding tubes and bottles at a certain slant to reduce the expanse of air-borne microbes that can get into them. To effectively sterilize the inoculating loops, the entire piece of the metallic inoculation wire is heated until it is red hot to ensure complete sterilization has been attained. Additionally, before an inoculum (agar or broth) is picked the inoculating loop should have been totally cooled.

Some of the aseptic techniques that are used in laboratories to avoid contamination include flaming and incineration. Flaming is used in wire loops, agar slants, and test tube mouths. When a wire loop is held in the Bunsen burner flame all potential opportunistic microorganisms are killed, hence the loop is sterilized. While the wire loop is cooling caution should be taken to ensure that organisms are not picked from the environment just before the inoculums are picked for culturing since the microorganisms are air-borne.

FLAMING

The effective way of cooling a loop so that organisms are avoided is by touching the very edge of agar then transfer the culture into a plate. In case it is a broth, once the loop is put in the broth a sizzling noise is produced by the contact. This produces some aerosols that would contain microorganisms. The aerosols are airborne and consequently have a probability of entering the human body through the respiratory tract. Therefore, it is recommended that the loop is not removed from the broth immediately to ensure the aerosols are not released to the environment.

The agar slants are contained in a test tube. Often, cultures in addition to broths cultures are transferred to agar slants. When inoculating in an agar slant precautionary measures are taken. The loop should be slightly drawn in a zigzag manner on the surface of the agar carefully ensuring that the media surface is not broken. A sterile needle can also be used in place of the loop to stab the media.

The mouth of a test tube is also passed over a Bunsen burner to kill any contaminants. This creates a current that pushes air out of the tube. Consequently, this deters microbes that can cause contamination to penetrate the test tube. The Bunsen burner produces heat that warms up the air around the working area, thus lowering the chances of culture contamination by airborne microorganisms. A loop can also be sterilized using an incinerator, where the loop is put till it is red hot. To prevent contamination, culturing can be done under a hood or a bio-safety cabinet alongside flaming. This helps achieve pure culture growth.

MATERIALS AND METHODS

Materials

The materials required for the Ubiquity exercise include;

- An unpeeled banana
- A computer keyboard
- Money
- An unopened soda
- Money
- Table
- Agar plates
- Laptop marker for labeling agar plates
- Hands
- Wipes
- Ethanol
- Trash

Method

Expose the sterile agar plates to various environments (an unpeeled banana, an unopened soda, a computer keyboard, and money). Prevent cross contamination. Incubate the plates for 24 hours. Observe the plates for growth and record the findings.

RESULTS

After exposing sterile agar plates to the four different environments: an unopened soda, an unpeeled banana, money, and a computer keyboard, these were the recorded results.

ENVIRONMENT	NO. OF COLONIES
An unpeeled banana	None
An unopened Soda can	None
A computer keyboard	Over 100
money	Over 100

The following results reflect the effects of hand-washing and table disinfection:

	NO. OF COLONIES
Hands before washing	Over 100
Hands after washing	1-50
Dirty table	50-100
Clean table	1-50

SUMMARY OF FINDINGS

The results recorded evidently show that the environments provided were both sterile and pure. Money and a computer keyboard were contaminated environments because of exposure to microorganisms that are found in the air, shown by production of over 100 colonies in the culture. The unpeeled banana and unopened soda can were sterile environments since they were not exposed to the airborne organisms as indicated by no growth in the culture plates.

Additionally, before washing hands and cleaning the table the colonies were over 100 for hands and 1-100 for the dirty table. Consequently, after washing hands and cleaning the table the colonies were reduced to 1-50 colonies. Thus, hands and tables are exposed to microbes, but after washing hands and cleaning the tables the amount of microbes is reduced.

DISCUSSION

The recorded results show the number of colonies that grew after exposure of the sterile agar plates to four environments. For an unpeeled banana there were no colonies; the unopened soda similarly had no colonies. For the computer keyboard there were over 100 colonies, and for money there were also over a 100. The unopened soda and an unpeeled banana had no colonies since the environment was sterile and had not been exposed to the microorganisms in the air. As stated earlier, there are a lot of microbes that are air-bone.

On the other hand, the computer keyboard and money environments had over 100 colonies. This consequently means that the environments were contaminated with microbes and microorganisms that could be pathogens. The contamination was due to the exposure of the computer keyboard and money to the contaminated atmosphere or air. If the keyboard was sterilized then the colony could have been reduced to below 100, since the microbes are killed through aseptic techniques of sterilization using a bleaching solution.

The experiment demonstrated the essence of maintaining clean surfaces after and before laboratory sessions. Before washing hands over 100 colonies were produced and after hand washing the number of colonies was reduced to 1-50. Before the table was cleaned the number of colonies was 50-100 and afterwards there were 1-50 of them. This again displays the importance of applying the aseptic techniques to avoid contamination.

The colony growth was due to contamination from the environment. There are a variety of microbes that grow on the media once an agar plate is exposed to the environment. Hands, tables, a computer keyboard, and money are exposed to dust and other particles in the air that contain microbes or even pathogens. There are also aerosols released from the laboratory to the environment in the event culturing is not performed in a fume chamber.

CONCLUSION

It is evident, that though the microbes are not visible, they are present in various surfaces of the environment. Microbes and pathogens are air-borne and, therefore, it is very important to maintain aseptic techniques such as cleaning in the laboratory. Sterilizing surfaces and washing hands before and after handling laboratory equipment is essential, as well as culturing in a bio-safety cabinet.

A bio-safety cabinet is laboratory equipment that is used to cultivate cultures. The cabinet has sterilization techniques in place such as radiations and free flowing air. The free flowing air allows the aerosols to circulate reducing the contamination probability. Cultures are cultivated in a bio-safety cabinet to ensure that pathogenic microorganisms contained in aerosols are not released into the environment.