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Introduction

Aseptic or sterile technique is the execution of tissue culture procedures without introducing contaminating microorganisms from the environment. In doing tissue culture work, 70% of the problems are due to a lack of good sterile technique. Microorganisms causing the contaminatic problems exist everywhere, on the surface of all objects and in the air. A conscious effort must made to keep them out of a sterile environment. Because many and sometimes awkward manipulations are required for various techniques, tissue culture media used are often supplem with antibiotics. Antibiotics do not eliminate problems of gross contamination which result from sterile technique or antibiotic-resistant mutants. Autoclaving renders pipettes, glassware, and solutions sterile.

Nutrient medium cannot be autoclaved. The compounds in nutrient medium are destroyed by tl heat of autoclaving. Medium must therefore be sterilized by passing it through a sterile filter sm enough in pore size to hold back bacteria and mycoplasmas (Millipore Sterivex - GS 0.22µm disposable filter units).

You may find yourself involved in a procedure which sterile technique "rules-of-thumb" do not c Therefore, you must constantly be aware that microorganisms are everywhere and take proper to keep them out of your cultures. When first developing your aseptic technique you must alwa thinking of sterility. Eventually it will become second nature to you. Mastering good aseptic tech will save you considerable frustration in the future. Furthermore, the same principles for good a technique also minimize biohazard risk to the investigator when infectious organisms or danger chemicals are used.

Associated Procedure

General Guidelines

All users are restricted to working in their assigned BSC and CO_2 incubator space, unless they the explicit consent of the individual PIs sharing the other BSC/incubators.

Lab coats and gloves should be worn when handling any of the biological material in the tissue facilities.

The use of Ethanol to disinfect gloves should not be used due to is tendency to comprimise the integrity of the glove material. Should researchers wish to disinfect their gloves, it is recommen they double glove.

Gloves must be removed before exiting the tissue culture room. If you need to pass between tv adjacent rooms, remove one glove to allow you to open the door.

Absolutely no food or drink is allowed in the tissue culture room.

Users must place their waste in the proper containers (yellow biohazard bag, sharps, regular w

Ensure that the microscope is turned off when not in use.

It is imperative to discard your vacuum waste. This must be done daily in order to prevent fung growth.

Be considerate of those who will be using the facility after you. Disinfect all surfaces (including tops and microscope if necessary) after using the tissue culture room and irradiate the BSC wit while you clean up after yourselves.

If you have encountered a contamination please inform other researchers using the tissue cultu room so that appropriate action can be taken.

Report any equipment problems or incidents of contamination to the Core Coordinator.

Specific Guidelines

Please note that the use of UV light as a disinfectant is now strongly discouraged. Cl disinfection (e.g. Ethanol or Virox) of the hood is now prefered.

Lift the sash of the cabinet to the designated height and ensure it is operating within safe paran

1. Wipe your work area and hands with Virox before starting. Before placing anything in the BS ensure that it has been wiped with Virox. Wipe the aspirating tubing with Virox as well.

2. Never uncover a sterile flask, bottle, petri dish, etc., until the instant you are ready to use it. F the cover as soon as you are finished. Never leave it open to the environment.

3. Sterile pipettes should never be taken from the wrapper until they are to be used. Keep your pipettes at your work area. Sterile pipettes do not have to be flamed. Pipetting your cells with a pipette will kill them.

4. When removing the cap from a bottle, flask, etc., do not place the cap with the open end upr the lab bench. Do not hold the opening straight up into the air. If possible, tilt the container so tl any falling microorganisms fall onto the lip.

5. Do not draw from different bottles with the same pipette. Because such a pipette has been exposed; the chance for contamination is too great. Use a sterile pipette for each bottle, especi when pipetting medium.

6. When aspirating media, use a sterile glass transfer pipette at the end of the aspirating tube. remove it from the container, shake the container gently so that a transfer pipette “sticks out” in of sticking your hand into the container and touching the other pipettes.

7. Techniques should be performed as rapidly as possible to minimize contamination

8. When finished your procedure, aspirate 10% bleach for several seconds through the aspirati tubing. This will kill any microorganisms that have lodged themselves into the tubing and that h the potential to grow and contaminate your cultures

9. Wipe down the tissue culture hood with Virox when you are finished. Never use bleach as th causes pitting and corrosion to the cabinet

10. Empty the Vacusafe container down the drain (with plenty of water), rinse with water and as more bleach into the container as a courtesy to the next user.

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