

The establishment of an animal cell culture

[Science](#), [Biology](#)



Introduction

Animal Cell Culture

Animal Cell culture is a technique for understanding the structure and function of cells, nowadays it has very good implications in biotechnology. Cultured animal cells are commercially used for the production of interferon, vaccines and clinical materials like growth hormones and urokinase.

Planning of Animal Cell Culture Laboratory

The major requirement that makes different tissue culture from most other laboratory techniques is the need to maintain aseptic condition. It is important that the tissue culture laboratory should be dust free and have less people. The introduction of laminar-flow hoods has greatly simplified the problem and allows the utilization of unspecialized laboratory. For designing the animal tissue culture laboratory following items should be considered:

- Pressure balance: a tissue culture laboratory should be at positive pressure relative to surrounding work areas.
- Laminar flow hoods. Consider where air inlets and extracts must be placed. It is preferable to duct laminar-flow hoods to the exterior to improve air circulation and remove excess heat (300–500 W per hood) from the room.

Construction and Services

The rooms should be supplied with filtered air. If Category I containment is required for the tissue culture laboratory it will be required to be at negative pressure relative to other areas. In this case adjacent rooms, which lead to

the aseptic area, should be regarded as buffer zones and should also receive filtered air but at positive pressure

Aseptic Room

Six main functions need to be accommodated in the laboratory sterile handling, incubation, preparation, washup, sterilization and storage.

Sterile Handling Area

Sterile work should be located in a quiet part of the tissue culture laboratory and should be restricted to tissue culture.

Laminar Flow

The introduction of laminar-flow hoods with sterile air blown onto the work surface affords greater control of sterility at a lower cost than providing a separate sterile room.

Preparation Area

Media Preparation

The need for extensive preparation of media in small laboratories can be avoided if there is a proven source of reliable commercial culture media. Although a large enterprise may still find it more economical to prepare its own media, smaller laboratories may prefer to purchase readymade media. These laboratories would then need only to prepare reagents, such as salt solutions (EDTA), bottle these and water, and package screw caps and other small items for sterilization. In that case, although the preparation area should still be clean and quiet, sterile handling is not necessary, as all the items will be sterilized.

Washup

Washup and sterilization facilities are best situated outside the tissue culture lab, as the humidity and heat that they produce may be difficult to dissipate without increasing the airflow above desirable limits. Autoclaves, ovens, and distillation apparatus should be located in a separate room if possible with an efficient extraction fan. The washup area should have plenty of space for soaking glassware and space for an automatic washing machine, should you require one.

Storage

Storage must be provided for the following items ensuring sterile and nonsterile are kept separate and clearly labelled Sterile liquids, at room temperature (salt solutions, water, etc.), at 4°C (media), and at –20°C or –70°C (serum, trypsin, glutamine, etc.) Sterile and nonsterile glassware, including media bottles and pipettes Sterile disposable plastics Screw caps, stoppers, etc., Gloves, disposal bags, etc.

Laboratory Facilities for Tissue Culture

The laboratory facilities for animal tissue culture consist of:

- Sterile area
- Tissue culture equipment's

Sterile Area

For processing the animal tissues for culture purpose a sterile or aseptic area is needed. This working place must be free from any kind of contamination.

Two types of sterile work areas are generally recommended. They are:

1. (a) Laminar flow cabinet
2. (b) Bio-safety cabinet.

Laminar Flow Cabinet

It is a specially designed chamber inside which animal tissue for culture purpose is being handled in an aseptic condition. It is completely open in front to allow the researcher to work comfortably and handle the equipment's present inside the laminar flow cabinet.

Layout of Animal Cell Culture Laboratory

Motor blows air into the laminar flow cabinet through a coarse filter, where large dust particles are separated. This air then passes through a 0.3 µm HEPA (High Efficiency Particulate Air). This keeps all contaminants away from the work surface. Such arrangement does not give protection to researcher against pathogenic organisms. Hence, laminar flow cabinet cannot be used in any cell or tissue culture which may contain a human pathogen (disease causing organism). There are two types of laminar used in the animal cell culture lab are Vertical and the horizontal laminar.

Bio-Safety Cabinet

Bio-safety cabinet provides a sterile environment for tissue culture in addition to making provision for the safety of researcher against human pathogens. Class I Biological Safety Cabinets The Class I cabinet has the most basic and rudimentary design of all biological safety cabinetry available today. A stream of inward air moving into the cabinet contains aerosols generated during microbiological manipulations. It then passes through a filtration system that traps all airborne particles and contaminants. Finally,

clean, decontaminated air is exhausted from the cabinet. The filtration system usually consists of a pre-filter and a HEPA (High Efficiency Particulate Air) filter. Class II Biological Safety Cabinets Like Class I safety cabinets, Class II cabinets have a stream of inward air moving into the cabinet. This is known as the inflow and it prevents the aerosol generated during microbiological manipulations to escape through the front opening. However, unlike Class I cabinets, the inflow on Class II cabinets flows through the front inlet grille, near the operator. None of the unfiltered inflow air enters the work zone of the cabinet, so the product inside the work zone is not contaminated by the outside air. Class II Type A (A1/A2) Biological Safety Cabinets The Class II Type A biological safety cabinet is the most common Class II cabinet. It is also the most common safety cabinet of all the different types available. It has a common plenum from which 30% of air is exhausted, and 70% re-circulated to the work area as the downflow.

In the A2 cabinet, about 70% of air from the positive plenum is recirculated as downflow, and the remaining 30% is discharged to the lab through the exhaust filter. Class II Type B1 Biological Safety Cabinets The Class II Type B1 biological safety cabinet was originally specified by the American National Cancer Institute. It has a common plenum from which 70% of air is exhausted, and 30% re-circulated to the work area as the downflow. Class II Type B2 Biological Safety Cabinets In the Class II Type B2 cabinet all inflow and downflow air is exhausted after HEPA filtration to the external environment without recirculation within the cabinet. Type B2 cabinets are suitable for work with toxic chemicals employed as an adjunct to microbiological processes under all circumstances since no re-circulation

occurs. In theory, Type B2 cabinets may be considered to be the safest of all Class II biological safety cabinets since the total exhaust feature acts as a fail-safe in the event that the downflow and / or exhaust HEPA filtration systems cease to function normally.

Tissue Culture Lab Equipments

The equipment's required for the animal tissue culture are the followings:

- Autoclave
- Centrifuge
- Microscope
- Incubator (capable of regulating the percentage of CO₂)
- Water bath
- Refrigerator
- Freezer (for -20°C)
- pH meter
- Chemical balance
- Stirrer
- Bunsen burner/spirit lamp
- Culture vessels with screw cap
- Pasteur pipettes
- Inverted microscope
- Liquid Nitrogen freezer
- Liquid Nitrogen storage flask
- Bench centrifuge
- Soaking bath

- Pipette cylinder and Water purifier.

Applications of Tissue Culture

1. Animal cell culture was primarily aimed to study infection of animal viruses.
2. Later on it was used to produce a wide range of biological products of commercial importance such as antibodies, enzymes, hormones, immuno-regulators.
3. Recently tissue culture technique has been used in the manufacture of viral vaccines, tissue plasminogen activator, interferon-a, monoclonal antibodies and tumour specific antigens.
4. Production of Foot and Mouth disease vaccines (FMD vaccines) is the most important example of the use of large scale cell culture. There are several other vaccines including polio vaccine, bovine leukaemia virus (BLV) vaccines, rabies vaccines etc. which are produced on commercial basis using cell cultures
5. Impact of new drugs can be evaluated using cell and tissue culture techniques.

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