FACULTY OF ENGINEERING AND TECHNOLOGY

TOPIC-Instruments Used in Animal Biotechnology

History of Animal Cell Culture

There are various equipments used in animal cell culture and the basic equipments required to carry out the animal cell culture are enlisted as follows.

Incubator	Laminar flow hood	Low-temperature freezer
Microscope	Cell counter	Glassware washing machine
Sterilizer	Vacuum Pump	Colony counter
Washing up instrument	CO2 incubator	Closed-circuit machine
Sterilizing and drying oven	Preparation and quality control	Cell sizing
Centrifuge	Temperature recording	Time-lapse
Water purification	Bulk culture	Controlled-Rate cooler
Cell freezing	Pipette aids and automatic pipetting	Cinemicrography
		Centrifugal elutriator
		Fluorescence activated Cell sorter

Sterile work area required for cell culture:

Room should be devoid of traffic, and if possible it should be equipped with an air flow cabinet that provides filtered air surrounding to the work surface.

A HEPA (High Efficiency Particle Air Filter) filtered air is appropriate but is not economical.

The laboratory must be especially designated for clean culture work and it should be strictly restricted to culture the primary animal tissue and micro-organisms in or near the cell culture laboratory.

The laboratory coats should be placed at the entry gate of the laboratory and should not be used outside the lab. A laminar flow hood (i.e. biosafety cabinet) is supposed to the simplest and the most cost effective way to supply aseptic conditions.

While permitting the containment of infectious splashes or aerosols produced by many microbiological processes, the laminar flow hood provides an aseptic work area.

In order to meet the diversified research and clinical needs, Three kinds of laminar flow hoods, have been designated as Class I, II and III.

Incubator:

An incubator will be needed in order to supply the suitable temperature environment for cell growth at 30-40^0 C. Depending on the type of cells being cultured, the incubation temperature will vary.

An incubator that has been designated to permit CO2 to be supplied from a main supply or gas cylinder is needed in order to maintain an atmosphere of between 2-5% CO2 is maintained in the incubator.

In the medium, the concentration of CO2 is kept in the equilibrium with sodium bicarbonate.

In general, several cell lines can be retained in an atmosphere of 5% CO2: 95% air at 99% relative humidity.
Dry incubators are relatively cost-effective, but the cell cultures are needed to be incubated in sealed flasks to avoid evaporation.

In a dry incubator, if the water dish is placed, it can supply some humidity however, they do not provide appropriate control of atmospheric conditions in the incubator.

Humid CO_2 incubators are relatively expensive, however it allows superior control of culture conditions.

They can be used to incubate cells that are cultured in petri-dishes or multiwell plates that needs a regulated atmosphere of high humidity and increased CO2 tension.

Refrigerators and freezer (-20 °C) for specimen storage:

Both refrigerators and freezer are very essential for storage of liquid media at 2–8°C and for enzymes (e.g. trypsin) and some media components (e.g., glutamine and serum) at –5°C to –20°C.

To store medium and buffers, a refrigerator or cold room is needed.

A freezer is required for keeping pre-aliquoted stocks of serum, nutrients and antibiotics.

Cryogenic Storage

There is high possibility for genetic instability in cell lines of continuous culture as their passage number increases, hence, it is necessary to prepare working stocks of the cells and preserve in cryogenic storage.

It is to be noted that the cells should not be stored in 20^{o}C or -80^{o}C freezers as their viability reduces when they are not stored at these temperatures.

Liquid nitrogen freezers permit storage in the vapor phase just above the liquid at temperature between -140^{o}C and -180^{o}C, or submerged in the liquid at a temperature below -196^{o}C.

The possibility of leaky vials or ampules exploding during removal is highly reduced by use of vapor phase storage, however, the liquid phase systems generally have longer static holding times, and are thus, more cost-effective.

Microscopes:

In order to examine the cultures in flasks and dishes, a simple inverted microscope is needed.
The morphological changes in cultures should be recognized as they are the first indicators for the identification of deterioration of a culture.

Although, a microscope of very high quality will be needed for chromosome analysis or autoradiography work, a very simple light microscope with X100 magnification will suffice for routine cell counts in a hemocytometer.

Tissue culture ware:

A diverse tissue culture plasticware is found, the most general being specially treated polystyrene.

Even if all tissue culture plasticware should support cell growth maximally, it is necessary to make sure that the new supplier facilitates the growth of cultures.

Cells can be kept in petri dishes or flasks (25 cm2 or 75 cm2) , that have added the benefit that the flasks can be gassed and then sealed so that a CO2 incubator should not be used.

This is especially useful in case if incubators fail.

Filter sterilization

Media which can not be autoclaved must be sterilized through a membrane filter of 0.22 µm pore size.
These can be obtained in different designs to filter a wide range of volumes.

They can be bought as sterile disposable filters, or they can be sterilized in appropriate filter holders by autoclaving.

Centrifuge

Periodically, to increase the concentration of cells or to wash off a reagent, cell suspensions require centrifugation.
For most purposes, a small bench-top centrifuge, preferably with proportionally controlled braking, is enough.

Refrigeration is not necessary, although, set at room temperature, it can be used to prevent overheating of cell samples.

At 80 to 100 g, cells sediment satisfactorily; higher g may cause damage and encourage pellet agglutination.