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Standard Operating Procedures (SOPs) and Good laboratory Practices (GLPs) for Cell Culture Facilities

*M. Ravi, Ganesh Venkatraman and Solomon FD Paul.

Department of Human Genetics, Faculty of Biomedical Sciences, Technology and Research, Sri Ramachandra University, Porur, Chennai – 600116. India.

Introduction

A cell culture lab is a place where a considerable amount of cost is incurred; a certain amount of expertise is required along with the inevitable frustrations that are a part of any scientific learning and discovery process. Considering the output potentials, cell culture techniques remain and will continue to be in the forefront of many a research process. In these are the labs, a unique combination of sensitive techniques, delicate but maneuverable sterile *in vitro* environment and precision equipment exists. Therefore, apart from an in depth theoretical understanding of essential concepts and the creative thought process for designing experiments, it is imperative to follow certain precautions and way of working with cell culture techniques. This ensures maximizing the reliability of results obtained combined with minimizing losses. Standard Operating Protocols (SOPs) and Good laboratory Practices (GLPs) are very important for protecting ones work and also others work who share the common facilities. These guidelines would prevent loss of material, critical cell lines, economic and manpower resources.

Dress code

The first attention is sure the dress code for any one who works with cell cultures. The dress code has a dual purpose of protecting the person as well as the work. A dedicated lab coat is a must and no one must enter a sensitive cell culture laboratory without one. The coat should be intended to be used only during cell culture work in a particular laboratory. Gloves and face masks are important accessories, especially while working in bio-hazardous situations, be it chemicals or cell lines/supplements. The footwear used for general purposes are not to be allowed into the culture labs at any cost and it should be a practice to wear shoe-covers or to take suitable adequate precautions before entering a cell culture lab. While within the lab, people with long hair should necessarily tie it up in as short manner as possible and should never let the hair untied.

Preparations before entering the lab

All precautions as above should be ensured. Hands should be scrubbing properly with soap before entering the lab. While inside, the first thing to do is to wipe with a suitable disinfectant or 70% ethanol, if gloved. To make sure the UV lamps are switched off and to check for any other notices/indications which might prevent ones entry into the lab or which would require additional precautions (such as when the labs are fumigated, etc). To make sure that the entry would not affect another persons' work if already in progress

inside the lab. Unless unavoidable, it is advisable to restrict the lab utilization to one particular work at any given time. One should handle the door, knobs etc gently while opening and should not open the door when the incubator door is open by someone else inside the lab.

Inside the lab

Many people can avoid working simultaneously and to avoid discussions while within the culture lab. All work that need to be done need to be planned before entering the lab including the organization of materials required for the methods. Last minute modifications should be restricted to circumvent situations where cell cultures are deviating from the expected conditions, for the further experiments. Once inside the lab, it is to be ensured that all equipment are in optimal working conditions; to look for untoward/unexpected signs such as condensations, change in temperature, CO₂ levels, alarm signals, etc on all equipment.

Laminar flow cabinet

Laminar air flow and UV lamp should be switched on minimum 15 minutes before the actual work. Always switch on the air flow and ensure that the UV lamp is switched off before opening the door. The gauge that gives the air pressure level should be checked to ensure proper functioning and never to obstruct the air vents/intake. The entire working surface should be wiped with 70% ethanol working from back, towards the front. Cluttering should be avoided and to have a clear working space by positioning things to the periphery or sides. Disinfectant dispensers, discard beakers/containers, pipette racks, pipette tip boxes etc are to be positioned in suitable positions for easy access.

The actual work can be towards the center and avoid working very close to the outer edge, towards the worker. While working, the hands should be almost in a stretched position are inside the laminar flow chamber. Working or movement above open culture vessels, containers, etc should be avoided at all costs. Talking or conversing while using the laminar flow cabinet should also should be avoided. Ensure judicious wiping of the work surface during work and to attend immediately and thoroughly to any medium/serum, etc spills on the laminar air flow work surface.

**Corresponding Author*

Email: maddalyravi@hotmail.com

All materials that are required inside the laminar flow cabinet should be wiped thoroughly before taking into the laminar flow cabinet. After warming in the water bath, one must make sure the bottles, tubes or other containers are completely wiped with 70% ethanol before placing inside the laminar flow cabinet. It should be ensured that they are dry of the wiping solution before opening. The containers are to be opened while in an angular position, well inside the cabinet edge and not facing the outside. Attention is to be paid to the position of removed caps and if possible, avoid placing them down at all. Placing the caps facing up or down, both have advantages and disadvantages and it is best to retain them in hand while working, especially culture plate lids, etc. Likewise, the positioning of pipettes are important and it should be ensured that they are placed on the pipette tip boxes, etc, facing away from the worker. It is best to discard the tips and place the pipettes onto their racks, but if it is required that the same tip/pipette is to be used frequently within a short span (not advisable though), it should be ensured that the tips do not touch any other surface/material and is placed facing away from the worker. Used pipette tips should be discarded into a suitable discard containing an appropriate disinfectant solution. Pouring/transferring liquids directly should be avoided and suitable liquid handling equipment should always be used within the laminar flow cabinet.

'Sterile' need not necessarily always mean 'clean', where the context pertains to unwanted particulate matter can cause serious disturbances in cell cultures, although they can be rendered sterile; best examples are cotton and paper fibers. It should be ensured that nothing that is used for sterile work inside the laminar flow cabinet goes outside the laminar flow area and everything that goes in should be wiped or the sterility of such material ensured. It is important to clear all material not required from the laminar flow cabinet and wiping of the surfaces after completion of work.

Incubator

The display panel of the incubator settings should be monitored regularly to ensure optimal functioning. Thorough wiping of hands with 70% ethanol if gloved or a suitable disinfectant should be done before touching the incubator. Stacking of culture units should be avoided (flasks, multi well plates and petri plates). The flasks should be positioned with caps facing away from the door; for preventing contaminations and ease of handling too. It is best to position culture flasks, plates etc in a manner that will avoid 'reaching over' and for easy access; to avoid inadvertent disturbances to other culture units. To avoid repeated opening and closure of the doors and to keep the door open for the minimum duration possible. A 'not-to-breath' approach when the incubator door is open and keeping 'at-arms-length' from the inner chamber while taking in or taking out the material from the incubators are to be practiced.

The outside surfaces are to be wiped with a safe disinfectant and all internal surfaces wiped with 70 % ethanol for cleaning purposes. Removal, washing, autoclaving and hot air drying of all trays and other detachable glass and metal parts such as inner doors, hinges, etc will ensure maintenance of sterile conditions and can minimize contaminations considerably. The door rubber linings should be

wiped with 70 % ethanol once a week. The water level in the water tray is monitored to ensure maintenance of desired humidity levels; to clean, wash and autoclave and to replace the water with appropriate microbicidal agents every fortnight. The walls, trays and floor are checked constantly for any signs of unexpected/unwanted color/growth/spills and are to be attended to immediately, if noticed. The HEPA filter for unwanted discoloration/growth and water in the water jacketed incubators should be monitored and replaced based on the manufacturers' instructions.

Water bath

The water should be checked constantly for the level and quality. Containers placed inside the water baths should never be submerged and in fact water should be below the lid level of such containers. The water is replaced every week with distilled or deionized water preferably with addition of a safe microbicidal agent. The thermostat functioning should be monitored and all detachable metal holders, stirrers etc can be autoclaved periodically. Direct contact with water should be avoided when the water baths are switched on.

Pipettes

Pipettes should not be shared between laboratories with each laminar flow cabinet equipped with a dedicated set which is not taken out of the cabinet. More pressure than required should not be applied on the plunger neither while aspirating nor during dispensing as this can induce volume errors and improper functioning of the pipettes. Similarly, there is an optimal time duration which is required to aspirate and dispense a certain volume of liquid and while slower operation is not required, faster operation will sure cause improper results. Gentle handling of pipettes is essential. Pipettes should never be positioned with the tip adaptor side facing above the horizontal angle; should be facing lower than parallel to the floor level. Tips should not be retained in the pipettes after use and pipettes should always be placed on suitable stands or for brief periods on the tip boxes, but never on the laminar cabinet floors. Always position the tip adapter side facing away from the user; towards the back of the laminar flow cabinet and never touch the pipette tips to any surface during liquid dispensing including the container rims, edges, walls etc. Minimize multiple uses of a single tip. While aspirating liquids, the pipette entry into a container should be restricted to a minimum. This is achieved by tilting the container instead of taking the pipette too much into the container. All direct contact with both sterile unused and used pipette tips should be avoided and used tips should be discarded into a discard container half filled with a suitable disinfectant solution. The used tips should be processed immediately for re-sterilization tips and to avoid wastage of time for washing, etc. Tips used for potential hazardous/infectious substances should be discarded as per governing regulations. Periodic calibration and autoclaving should be done based on the manufacturers' recommendations.

Medium/serum/buffer bottles, etc

Ensure proper cleaning and sterility, devoid of moisture and store in clean place. Wash, autoclave and dry immediately prior to use. The

containers should not be submerged in water below the lid level while warming in a water bath; water should be below the cap level. The containers should be wiped thoroughly with 70 % ethanol before taking into the laminar flow cabinet and the caps should never be opened or loosened outside the laminar flow hoods. It is best to tilt the containers while pipetting solutions/ liquids and liquids should never be poured straight into other containers. Appropriate liquid handling systems should be used for the transfer of liquids into and out of the containers. It is best to cover lids and a portion of the container just below the lids with Para film or similar wraps during storage. As only the inside of the containers and the material contained are sterile, the outside need to be wiped with 70% ethanol every time they are taken into the laminar flow cabinets. When dispensing liquids, when the caps are removed from the containers, the placements of the caps inside the laminar flow cabinets can be very important. Care should be taken to minimize contact area with surfaces, although the surfaces are considered sterile. Caps placed facing down or up might have advantages and disadvantages and it is best to avoid keeping them down at all and to acquire the skills to hold them while dispensing liquids as much as possible. Placing the caps facing down is better provided that the surfaces are sure to be sterile. Any spills or drips on the rim, outer surface should be wiped carefully before re capping, sealed properly and stored appropriately.

Culture flasks

Individual flasks should not be taken out of their packing outside the laminar flow cabinets and while taking them out of their packing, it should be ensured the caps are closed tight within the pack prior to removal. Flasks should be held by their sides and not the cap or the flat culture surface sides. The cap side should be facing away from the worker, towards the back of the laminar flow cabinets while working and should not be handled with wet gloves or hands. Flasks should be tilted while dispensing cell suspensions, media, etc into the flasks. Direct contact to any surfaces and the flask opening with dispensing pipette tips, etc should be avoided and liquids should not be directly poured into the flasks from the containers. Always, the use of appropriate liquid handling equipment is advisable. Scratches and smudges should be avoided on the inside and the outer surfaces to for optimal observations. Spills and drips on any outer surfaces of the flasks should be avoided. It should be ensured that the caps are appropriately closed during incubation based on the type of flasks (vented, filter caped, etc).

Only appropriate volumes of media and other culture supplements should be added to the flasks and flasks are not to be used for storage or any other purposes not intended. All flasks should be labeled appropriately before placing into the incubators and stacking should be avoided within laminar flow cabinets and also inside incubators. Used flasks should be discarded appropriately and reuse or other usage of disposable culture flasks after the intended cell culture use should not be attempted.

Culture Plates & Petri plates

Most of the precautions are as applicable for usage of the culture flasks. In addition, it should be made sure not to leave the plates open even while within the laminar flow cabinets when not really

essential. Sufficient care is necessary while handling the plates during transit between laminar flow cabinets, microscopes and the incubators. It is necessary to avoid touching the top and bottom surfaces and tilting. Plates should always be handled by the sides through the grooved provisions. It is advisable to avoid complete removal of the lids while dispensing liquids into and out of the plates and the sides can be sealed with parafilm or other appropriate tapes if the procedure warrants. Can avoid complete covering of the plates, except for light sensitive procedures where the plates need to be covered completely with aluminum foil, etc.

Cryopreservation is an important need and care should be taken while handling all aspects to ensure safety of both material and of the personnel. Liquid nitrogen levels in the cryocans should be monitored regularly and should be transferred with appropriate equipment and protective accessories. New or dry cryocans should not be filled with liquid nitrogen in one step, but should be added gradually over a period of time. A small quantity is added initially and rest of the can is filled after about two hours. The storage straws/canisters should be placed in their proper niches and the closing caps/stoppers should be able to glide in without obstructions. The levels of liquid nitrogen should be maintained so that all cryovials and canisters should be submerged in the liquid nitrogen. A register containing the details of all cell lines preserved the number of such vials with individual label details and a register for recording the liquid nitrogen level monitoring and replenishment should be maintained. Evaporative and spillage waste of liquid nitrogen should be minimized during storage, transport and transfer. Cryoprotective gloves should be worn while working with cryocans, liquid nitrogen, during introduction and retrieval of cryovials, etc. Only approved material should be used for all activities related to liquid nitrogen and to direct contact with liquid nitrogen or the surfaces of containers/vials that are frozen during thawing and revival procedures should be avoided.

Refrigerator

Overloading should be avoided and storage should be restricted to essential material. Storage should be done in a way that does not block either illumination or vents. Everything stored in the refrigerators should be labeled. Doors of any compartments should not be left open for longer duration than required to place or retrieve material. Regular maintenance such as defrosting should be carried out and the door rubber linings should be wiped with 70 % ethanol every week. Spills or drips should be avoided inside the refrigerators and material should be stored in appropriate containers and compartments based on the storage requirements/recommendations. The outer surfaces should be wiped clean every week and unnecessary stickers/notes, or other materials either on the outer walls or on the top should be avoided.

Other considerations

Air conditioning vents and units must be in regular maintenance, especially for cleaning of the air filters. Periodic observations for unwanted growth, discoloration and condensations should be made. All bench surfaces within a cell culture facility should be regularly wiped with appropriate disinfectants. The areas around microscopes, centrifuges, incubators or any other equipment placed

on the surfaces should be given special attention and should be wiped before and after use of the equipment. Clutter should be avoided and restriction of the placement of most important and regularly used material only should be followed within the cell culture facility. The floor should be mopped on a daily basis with appropriate disinfectant solution and scratches, breaks etc that can harbor potential contaminating material should be avoided/rectified immediately.

All autoclaved/sterile materials are to be handled to ensure the maintenance of the sterile conditions. The containers should be opened only inside the laminar air flow cabinets after appropriate precautions such as wiping the outer surfaces with 70 % ethanol. All autoclaved material should be stored appropriately covered / capped, within appropriate storage devices/containers, neatly labeled. The tubing/plumbing of all liquid handling systems should be cleaned/flushed with appropriate disinfectant solutions and contaminations inside the tubing should be avoided.

Disposing of any material that was used within a cell culture facility should be done with necessary precautions and should adhere to norms as prescribed by National Accreditation Board for Testing

Laboratories (NABL). Care should be taken to avoid cross contaminations and for the protection of health safety issues during disposing material. Biodegradable, biohazardous substances and non biodegradable substances should be appropriately disposed off utilizing appropriate processing of the disposable material prior to disposals.

Everything that needs to be retained inside a cell culture lab and within storage areas such as refrigerators, incubators, etc should be appropriately labeled giving sufficient details to avoid inadvertent loss/discard of material. Bottles and other containers that are stored in lower temperatures and that need to be warmed prior to use, it is advisable to use moisture proof labels or to simply cover the labeled area with a transparent cellophane tape to avoid erasing the marks during wiping or by condensation.

The entire lab should be fumigated once a month or as appropriate. During the fumigation process, a prominently display should be placed on the door indicating that fumigation is in progress. All material used for fumigation should be removed from the lab after the fumigation process. Formaldehyde along with potassium permanganate overnight is an efficient method of fumigation.

This article is adapted from the book 'Animal Cell Cultures, M. Ravi et. al., Samanthi Publications, India. ISBN: 978-81-906565-1-1.