CELL CULTURE ROOM PROCEDURES

1- Follow the procedures shown in the electrical panel for the UV lamp lighting, aspiration and air exchange of the cell culture room (1, 2, 3 and 4).

In the pre-room:

2- Wear coat, cap and mask. Wear gloves and spray them with ethanol.
3- Before entering the cell culture room, make sure that pipettes, tips and various tubes are there.

In the cell culture room:

4- Turn on the UV of the hoods and switch them off after 15 min.
5- Turn on the water bath and the incubator shaker. Clean both the table surface and the surface of the microscope using ethanol.

Remember that the outside of the packs of pipettes, flasks and anything else that is still packed by plastic, should be sprayed with ethanol. Only after they have been cleaned, can they be opened through the appropriate openings and placed on the opportune shelves.
It is also important that the pipettes are opened properly to avoid contaminations. ***So you never must work quickly****.*

6- Turn off the UV of the hoods and hang the UV lamp in its support.
7- Clean your pipettor and the micropippetes with ethanol, paying particular attention to clean the portion near the tip.

Remember that the micropipettes must not be left inside the hood with the UV on because they alter. Therefore, as soon as you have finished working, they must be stored in the drawers.

8- Employ a waste containing some bleach inside the hood; before entering, clean it externally. Clean with ethanol everything that goes into the hood (e.g. medium, tubes etc).
9- Exit one or more flasks of the same patient from the incubator.

a. If the medium is not heavily metabolized or does not need to be replaced , conserve the flask or, if necessary, mix its contents, using a pipette in which to insert a yellow tip with no filter at the extremity.

b. If the medium is metabolized, transfer all the contents of the flask in a tube and spin-dry at 1000-1200 rpm for 5 min. After aspirating the supernatant, resuspend the pellet in the fresh medium and plate the cells in the respective flask.

10- Once finished working, clean the work plan; keep your cell medium in the refrigerator (sign any aliquot or medium you have so that if it is accidentally moved or forgotten it is easy to go back to the owner).
11- Clean the pipettor and the micropipettes with ethanol and store them in the drawers. Clean the steel top of the hood, first with ethanol and then using Incidin.
12- If the waste container is full, close it and take one empty.
13- If no one else has to work, clean the microscopes and cover them, switch off the water bath and the incubator shaker.
14- Turn off the light and follow the procedures shown in the electrical panel to turn off (steps 1, 2, 3 and 4), taking care not to put out the switch of the incubators or refrigerator.

Once a week, make sure the cell culture room is treated with SPYCLON.

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Therefore:
1. Make sure that air treatment, room aspiration and UV lamps are turned off;
2. Open the hoods, the incubator shaker, the centrifuge and uncover the microscopes;
3. Turn on the Spyclon;
4. The next day before entering the cell culture room make sure the air treatment and aspiration is switched on.

Procedures for the treatment of surgical samples

Any surgical piece that arrives, even if small, should be divided as follows:
1- A small piece to be frozen in nitrogen
2- Small pieces to be frozen at -80° C
3- A small piece to be paraffinized

The pieces should be stored as soon as possible, should not be left at room temperature for a long time because the RNA could degrade and the morphology could impaired.
They must be carefully cataloged.
Digest what remains following precisely the pre-established methodologies, not changing anything of what has already been standardized.
If one of the medium components is missing, it is better not to improvise or add it later, as it is considered as a modification to the method and therefore not comparable.
Every operator who uses surgical instruments must:
- wash them with diluted bleach using the specific brush (do not leave the surgical instruments in bleach as they could be damaged)
- rinse them with water
- then wash them using the specific detergent and carry out the last rinsing with distilled water
- put the surgical instruments in the heater; the technicians will pack and autoclave them as soon as possible

For trypsinization, the procedure is as follows:
- the trypsin should be taken out of the refrigerator at least 10 minutes before use
- the flask must be washed in PBS to remove excess serum that would inactivate the trypsin
- add trypsin to the cell pellet or flask (500 µl or 1.5 - 2 ml should be sufficient) and incubate them at 37° C. For cells, 3-5 minutes are enough to disgregate the spheres, instead the flasks should be checked under a microscope. Wash at least twice with PBS to remove trypsin residues.