# WORKING STANDARDS FOR CELL CULTURES

## 1.- OBJECT AND SCOPE

- The purpose of this document is to provide users with the most appropriate procedure for understanding the basic rules of handling sterile crops. Its application will allow the good use of the equipment by the users of the Service.

## 2.- USE OF THE CULTURE LABORATORY

- Users should be aware of the basic rules for handling sterile crops. The cell culture laboratory is suitable for working with primary cultures of human, mouse and insect cells and for the culture of established lines.  The use of viruses and vectors is restricted. In principle, vectors that can be used in a P1 room and that have passed the approval of the Service's managers will have permission to enter, giving their consent to work in a P1 room.

- No user of the Cell Culture Service will be able to use the facilities of the culture laboratory without having registered with the CABD reservation service, for which they will have to be authorised by the responsible technician Corín Díaz Ramos.

- All users will be obliged to carry out an exhaustive cleaning of the room following a cleaning shift established among the users of the room.

- All users undertake to comply with the rules of use of the service, respecting the booking hours of the room and the hygiene and biosafety rules established for its correct use. These rules are posted at the entrance to the room, however the following rules are highlighted:

    * Do not enter the cell culture room if the UV light is on in the cabin or if there is a person working inside.

    * No one may use the vertical flow cabin in the cell culture room for anything other than sterile product handling.

    * All users will be obliged to reserve the room in advance for the total estimated working time. Always try to start and finish in the time reserved.

*All users will be obliged to use their own culture media, material, etc. They may never use another person's material without consulting them.

- Before starting work in the room:

   - Use a clean gown that will be hung at the entrance of the room and that will be used exclusively for the cultivation room. Each user must have their own duly identified gown

   - Wash your hands well with soap and EtOH 70%

   - Clean the cabin with EtOH 70% and irradiate with UV inside the cabin the instruments and plastics to be used 15 minutes before starting to work.

- While working in the room:

   - When using the booth, always work keeping the booth environment sterile.

   - If gloves are used inside the booth, they should never touch handles, microscopes, refrigerator doors or incubators. Before the hands leave the flow booth, they should be removed and thrown away. The use of gloves is imperative when working with human material.

   - If medium or any other liquid falls into the cabinet it is MANDATORY to clean it immediately with 70% EtOH. When the work is finished, a more exhaustive cleaning must be done.

- At the end of the work in the room:

   - Collect and store all the material in the place that your group will be assigned within the room.

- If the flow cabin is used, it must be cleaned afterwards with disinfectant. Never use bleach or pure ethanol to clean the metal base or the glass of the booth as they are corrosive and fix the salts in the medium. The UV of the booth must be switched on and left to work for at least 30 minutes

- If the vacuum pump is used, the 50% diluted lye suction paste must then be passed through to clean and disinfect the duct for 5 minutes. Never let the tank remain more than ¾ full.

- It is strictly forbidden to leave any biological waste bottles inside or outside the booth, or any rest of the material that has been used.

- Make sure that the waste bags are not too full, before that happens you must close it and put a new autoclave bag.

- Every time the grow room is used, it should be clean and free of any trace of someone having passed through it.

- When you are finished, make sure that the cabin, microscope, vacuum pump and centrifuge are turned off if there is no user reserved after you.