## **Sterile cabinet DIY**

Sterile orchid seeds and protocorms can be handled over a boiling water bath in the kitchen, but everything is easier and the risk of contamination much lower if you have a sterile cabinet. Professionals use sophisticated laminarair-flow work stations, which are not available for most amateurs, but you can easily build a sterile cabinet that is almost as efficient as the professional LAF-version, and it can be done for less than 100 euro. All you need is a kitchen cupboard with one shelf, an air purifier with a high efficiency pure air (HEPA) filter with 99.9 percent removal of particles down to 2 µm, a piece of polycarbonate glass, and a roll of gaffer tape. The cupboard that I used was a second-hand Ikea cupboard that I got for free, the air purifier was the cheapest Bionaire model from the local electronics store, and the polycarbonate glass was from an old poster frame. The dimensions should be approximately width: 60 cm, depth: 60 cm, height 70-90 cm, and a 15-cm opening.

The principle is very simple: First, make a hole at the top of the cupboard that fits the air purifier. The inlet of the air-purifier should be on the outside and the outlet on the inside of the cabinet. If it is impossible to have the on/off switch on the outside, simply have the switch permanently turned on, and use the outlet switch on the wall as the main switch, or unplug the whole thing when not using it. Second, drill a lot of small holes in the shelf in a regular pattern, and place the shelf about one-fifth from the top of the cupboard. The purpose of the shelf is to prevent turbulence. Without the shelf, the air will whirl around inside the cabinet and drag spores from bacteria and fungi into the cabinet. With a perforated shelf, a small over-pressure will build up in the upper room and press the air evenly through the holes which minimizes turbulence. Also, the air-purifier may conveniently be placed on the shelf.

Third, attach the polycarbonate glass plate to the front of the cupboard, there should be a 15-cm open slit at the bottom. Finally, seal everything with gaffer tape. A small lamp inside the sterile cabinet helps, but is not absolutely necessary.

A few routines have to be learned when using the sterile cabinet. First, turn on the air purifier to get air flow. Then wipe the inside with a tissue soaked in ethanol to sterilize the surfaces. It is important not to do this in the opposite order because then the cabinet will fill up with ethanol fumes that may explode when you turn on the air purifier. The cabinet is ready for use after 5 to 10 minutes when all the air in the cabinet has been exchanged with sterile air.



My home-made sterile cabinet, cheap, ugly, and very efficient. The dimensions are  $60 \times 60 \times 80$  cm. The opening is 15 cm.



Topview.

I keep a pocket lighter and a beaker with ethanol inside the cabinet for flaming the tools. Remember to keep a lid on the ethanol beaker which may otherwise catch fire. Forceps, inoculation loops etc. should be flamed every time you handle them. Right before use, dip the tool in ethanol and set the tool on fire with the lighter. Hold it a while so that it cools, and then use it to sow the sterilized seeds or move protocorms to fresh medium. The tools should be flamed again every time they have touched any surface inside the cabinet (except the inside of sterile flasks or sterile seeds in hypochlorite solution) or when starting on a new flask.



Cheap air purifiers with 99.9% removal of particles down to 2  $\mu m$  are available at amazon.co.uk for instance this Prem-I-Air HEPA Air Purifier (£53). Remember to buy extra filters as it is often difficult to get new filters after some years when new models are introduced.



Small professional LAF workstations (laminar air flow cabinets) can be purchased online for less than 1000 Euro for instance this <u>CJ600-P</u>, which may be even cheaper (400 Euro) if you order directly from the Chinese producer <u>Huanghua Faithful Instruments</u>.

