

Simple orchid flasking

The idea of this article is to put to rest the thought that you must have a complete Laboratory setup to germinate Orchid Seed.

Orchid Seed can be germinated with a few simple items, most found in any ones kitchen.

You will need the following items :-

2 linen Tea Towels - White King Bleach - 1lt size Orchid Medium (advertised in all Orchid Magazines) - small 10ml glass bottles/jars - Calcium hypochlorite (CaHc) -

1 litre Demineralised water - Bucket - 6 x 250ml jam jars - disposable gloves - Pressure Cooker - Coffee filter paper - small container egg cup size -

To start with you need to do the following two things -

1. Make up your medium with the one you can purchase through the magazines, you only need to add distilled water to a pot, add the powder slowly until mixed, bring almost to the boil then turn the flame off (with most dehydrated media Im pretty sure it is already at the correct PH, but if you do have access to a ph metre or strips then check it for 5.4) Pour the medium while it is in liquid form into your 6 jars , screw the lids (not too tight) on the jars, then place them in your pressure cooker (which is set for 15psi) and bring up till the steam makes the weight jiggle around (its up to pressure) then turn it down too low and time it for 20 mins. I recommend that you leave the pressure cooker until completely cold before opening it up, then screw the lids tight. Leave this for at least 5 days to make sure you have no fungal flasks.

2. Next you must make up the Calcium hypochlorite 650g/kg (available from Pool Shops). You make up a Stock Solution first - too 276mls of distilled water you add 1/4 teaspoonful (0.85gm) of Calcium hypochlorite, shake well until dissolved. Fold a coffee filter into a 1/4 then shape it like a funnel; place this

funnel in a Amber bottle and slowly pour the 276mls of mixture through the filter. Wrap some Aluminium Foil around the amber bottle to keep out any light; this is stored in your fridge and will last for about 6 months.

Seed Sterilising - in a small container add 5ml (1 teaspoon) of the Stock Solution to 40ml (8 teaspoons) of Distilled Water add 1 drop of detergent and 5 drops of vinegar to bring it down to between ph5 and ph6. (this mixture should be used within 1 hour, if you are interrupted make up a new lot). Place enough seed into the small bottle (enough about the size of a grain of rice; remember this amount of Orchid seed represents 100's of seeds more than enough to germinate) using the above mixture that you have made up from the Stock, pour about 5ml into each of the seed bottles; this needs to be shaken occasional for the next 10-15 mins. One bottle of seed per flask.

You are now ready to start the job, firstly you need to place 100ml of White king in your bucket, add 900mls of tap water. Now put on the disposable Gloves which must be dipped into the White King solution before and after every thing you now do. Place a pile of 5 sheets of newspaper on your table/bench, place the 2 Linen Tea towels into the bucket until totally wet, screw out as much liquid as you can and lay the two on the newspaper ; all the work is down between the two tea towels.

Next take one Flask, seed bottle and empty small container and wash it well in the W/King solution and place in-between the two tea towels, (remember to mark/number the flasks to the seed bottles; so you will know when you have finished what each flask has sown in it.) while keeping the tea towels together as much as possible, unscrew the flask and also the top off the seed bottle and pour contents into the flask, replace the lid and take you hands out and wait for 5 mins. Re-enter under the towel and without lifting up the towel very slowly tip the flask so the contents tip into the empty container. During the 5 mins some of the seed would have dropped to the bottom of the solution and attached itself to the medium; sure, you will have poured out some of the seed, but you will still have plenty of seed still left on top of the medium. Take the flask out and screw it up tightly. Continue this process until you have finished the flasking you have to do.

The idea of this process is you don't do any replating of the protocorms, as you are only sowing a small amount of seed in each flask.

The flasks need to be in a well lit place (not outside in the sun as they will cook) the idea situation is to set them under a Fluro Tube (placed about 30cm above) and lit up for a period of 12 hours each day. Germination time varies according to genera sown, but some can germinate in 10 days.

I think the hardest part of Flasking, is the waiting.

The procedure described above came from an article written by Fred J. Bergman.

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ADVANCED ORCHID FLASKING

After using the "Simple Flasking" for awhile, I had reached the problem of "replating" (seperating the protocorms into other flasks to enable them to grow on) and while the process for seeding was found to be very good, you will remember it entailed carrying out the process under a Tea Towel and without being able to see what you are doing, would make it impossible to place the protocorms in the flask.

So the next step was to make up a "FLASKING CHAMBER". I have seen a number of different ideas as to what different people have made up. I chose to buy a 3ft Glass Aquarium Tank which I had two round holes cut in the side; to this I glued a very long glove to the hole so I could place my hands through. I turned the tank upside down so the opening was downward, placing this on a sheet of white malamite.

Sterilising Here lies the success or failure in flasking! The inside of the chamber must be sterile at all times and this is obtained by spraying it well before you commence; I started using bleach (20% bleach /80% water) but found after a number of months the fumes were making me feel sick, so I changed to using Methylated Spirits (ethanol) (70% ethanol /30% water) and this also dries quick where the bleach left the chamber wet all the time. For the flasks I found after using a number of chemicals I came back to dipping them into a bleach Solution of (10% bleach /90% water) these are placed wet inside the chamber. Tools - forceps, etc are also dipped into the bleach solution before placing in the chamber; many people place them into a container of the solution inside the chamber to sterilise in-between flasks, I had a small spirit lamp which I flamed them by dipping into neat Alcohol. I found this a better way to cut out any cross infection from flask to flask. This action made the inside of the chamber Sterile and I had very little fungal problems.

Green Pod Flasking you need a container which contains neat Methylated Spirits or Bleach, a tooth brush to dip and scrub, a scalpel to cut the pod, a small square

of glass to cut on(remember all of this is done inside the chamber; and also everything that goes in must be dipped in the solution first) I have seen mention of the need to scrub for 30 mins, I have always found about 5 mins is ample (while the inside of the pod is sterile, the outside is covered in dirt and fungus, this must be removed), you then cut the pod in half with the scalpel; unscrew the lid of the flask and with the blade scrape a small amount of the seed over the top of the medium (try not to over do it) if you have dropped it in piles then gently with the blade (or a piece of stainless steel stiff wire that has a bend on the end) move the seed over as much of the medium as you can. Place the lid back in the Flask; job done.

Dry Seed Flasking I still used the same process as I mention in the "[Simple Orchid Flasking](#)"; the only change is I don't have to use the tea towels.

Replating Protocorms observing all the protocol for sterilising as explained in "Green Pod Flasking", we will assume you have the flasks in the chamber (both the "Mother Flasks" and the flasks you will replate into).

The idea is to allow the plants to grow quick and strongly and if left in the "Mother Flask" (a flask that you sow the seed into is called a "Mother Flask") they will not make much progress, so you do one of two things, 1. Spread; by taking an amount of the protocorms and just spread them on top of the medium. 2. Place a set number into a flask by actually planting them into the medium. I prefer the 2nd way as I like to place about 15 to 20 protocorms (the number depends on the size of the flask you use) in a flask, I just push the end of the protocorm a little way into the medium to hold it upwards (the top of the protocorm usually has a point) this allows the protocorms to grow into plants and progress. Im often asked at what size do you replate; I recommend that you start when the protocorms are large enough to pickup, about the size of a match head. Of course you can replate at any size you want too.

Flasks & Mediums

Flasks -I use two sorts of flasks, an Unvented one for Mother Flasks and a Vented one for all Replating , a vent is necessary to allow the gas and air to transfer from the flask. There are a number of ways to vent a flask I use 250ml glass jars with Plastic lids (auto-clavable) I drill a 1/4" hole in the lid to the side; you can buy small round Teflon patches or place some plastic tubing in the hole and fill it with non-absorbent cotton wool or just stuff cotton wool in the hole. I have used all

three mentioned, but have now come back to the tube with the cotton wool plug, I'm finding it is 100% fool proof.

Mediums - For seeding I have found Sigma's "Phytamax" Maintenance Medium (P6688) used at either 1/2 or full strength the best, others are Vacin & Went and Murashige & Skoog either full, 1/2 or 1/4 strength, use these for "seeding". For replating Sigma's "Phytamax" Medium (P1056) has been found to grow most genera very well, and other companies also make up proprietary mediums to much the same formulas all over the world. I also make up a few mediums using general fertiliser which work quite well also.

Sterilising Medium - Most proprietary mediums you purchase are in powder form and have everything included, apart from Agar (I use 7gms/litre, this gives a firm medium) you just add the powder to distilled water and follow the directions in "[Simple Orchid Flasking](#)"

Laminar Flow Cabinet

Because I started to do flasking for my local Species Society (to provide "plant of the Month") I have moved up from the chamber which restricted the number of flasks I could have in it at one time, so I bought a "[Laminar Flow Cabinet](#)", this enables me to work without restriction regarding flasks and also with the front being open I can move around better. I have a Bunsen Burner inside and now flame all the tools; any fungal problems I did have (and believe me if you think you can run a lab 100% clean, you are kidding yourself) with the use of the B/burner and 70% Ethanol spraying of the cabinet, I have most of the problems that I have experienced to a minimum.

Oct. 99 up date - I have now purchased a "[Glass Bead Steriliser](#)" from India and this takes the place of the "Bunsen Burner"; while the b/burner did a great job, there was always the chance of either a fire or the flame going out, so for safety sake I felt it was important to change over. While using 3 sets of tweezers I am able to have one set in the steriliser, one set cooling off while I use the 3rd set. Since using the steriliser I now note that fungal is almost a thing of the past. Click onto Lamina Flow Cabinet above to see how it is setup.

Dec 2000 update - Dry Seed - I now have found an excellent result for sterilizing Dry Seed. I make a solution of 100ml of Distilled Water and I add 5ml of 'White King' (contains 42g/l of Sodium Hypochlorite) or (Clorox) to a total of 105ml, I

don't add any wetting agent as 'White King' already contains it. Using very small bottles (5ml) with screw top lids, number them for your records. Add the dry seed first, when all the bottles have been filled with the seed, I then add the liquid (screw the lids tight) so I can then have them all about the same time limit (5min) remember to shake the bottles a number of times and then place all the bottles in the bleach solution before placing in the cabinet. I let the liquid/seed settle and using a pipet (a tapped glass tube which attach either a rubber squeeze ball or a retracting pump) and draw most of the solution out, adding Sterile Water (distilled water auto-claved for 20 mins.) too wash the seed: this is then tipped into the flask and left for up to 20 mins. To settle on top of the medium. I tip the flask to the side and using the pump I take the excess water out. Re the Pipets- I have a numbered rack (numbered 1 to 20) and place the pipets in each slot, this way I use one pipet per flask, you don't end up with a mixture of seed germinating in a flask. Remember the glass Pipets must be auto-claved to make them sterile; I place them in a oven bag, sealed with tape and usally place them on top of the flasks in the Pressure Cooker. The bag must also be washed as all other tools and flasks are before placed in the cabinet.

[Want lots more information on flasking?????](#)

I would suggest you obtain a very up to date book titled "Asymbiotic Technique of Orchid Seed Germination" by Aaron Hicks from The Orchid Seedbank Project in the USA. This book covers from A to Z on flasking and covers lots of old and new ideas , mediums, sterilising etc. etc. Available from the Author Aaron Hicks direct (go to our 'Links' page and click onto The Orchidseed Bank for details and prices)

[Flasking Cool Growing Orchid Seed](#) by Bob Hamilton. A very good article on flasking, I feel is applicable to most types of Orchids.

[Lotte&Thomas's Flasking Site](#) - Another excellent site describing lots of Orchid Propagating Techniques, including 'Flasking under Steam'.

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Lamina Flow Cabinet

& Glass Bead Steriliser



Flasking Room

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Flasking Cool Growing Orchid Seed by Bob Hamilton

Recently, there have been questions about sterilizing media for growing orchids in vitro -- flasking. Some discussion of microwave ovens came up. My answer is rather oblique to the question of micro waving and definitely not terse!.

I have been doing my own "bottles" for about 6 years. Most of what I have learned has been through empirical methods. Using the algorithm, ready, fire, aim, I've learned to reliably flask Odonts and Masdevallias. Basically, I had to learn to flask cool growing orchids because there were no cool commercial laboratories available to me. I had disastrous results with the labs I tried. I also like to doing things for myself.

First off, let me say no one will put more care and effort into your flasks than you will. In addition, techniques such a ploidy conversion or selecting plants for replant based on vigor are not available from a commercial lab. You will be amazed at how many of your crosses germinate when you are in control. When I sent pods to outside labs, I got low germination reports. When I switched to doing my own flask work the percentage went well over 90%.

I try and sow all my orchid seed "dry pod". This means the pod is mature on the plant, the seed was ready to dehisce. For reliable sowing of dry seed it is important not to get the seed pod wet after it begins to split or pathogenic fungus can grow into the embryos making sterilization impossible. There are times when a seed pods must be taken "green". In the oncidinae, some intergeneric crosses never mature. I understand fertility in Phals can be much higher when pods are harvested immature -- green.

Should the maternal parent in green pod culture be virused, (sadly, I heard Gary Gallup of Gallup & Stribling say that almost 50% of Phal meristems bought from wholesale growers are virused; hence, Gallup now propagates only from seed) cutting the seed case can spread virus to the uninfected embryos. To prevent this, I take efforts to sanitize the surface of the seed pod and use a red hot scalpel when I cut it into two halves.

Dry seed is sterilized with 20% by volume, Clorox bleach in tap water. Dry seed is placed in a 16 mm X 150 mm glass test tube. A drop of wetting agent and the Clorox/water mixture is added. About 1 inch is left empty at the top of the test tube and a cotton ball is placed in the top. This is shaken for 5 minutes and then the cotton is pushed to the bottom. The cotton compresses the seed at the bottom while the Clorox/water is poured off. Sterile water is added and the cotton plug is pulled back up toward the top. This action is repeated several times rinses away the Clorox/water. The final time the cotton is pushed down to the bottom it is not pushed all the way. A little water is left so that after the cotton is removed the seed can be poured into a mother bottle.

(Be sure and bring water reservoirs to a boil before placing in an autoclave. A standard 15 minute autoclave cycle will not heat a large volume of water sufficiently to sterilize it. In addition, replace Clorox bottle monthly after opening. This material is volatile and subject to reduction in strength through dissipation of the chlorine)

I sow my seed in 1/2 pint wide mouth canning jars. These have a low profile, about 3" so I can stack the several high in my pressure cooker. I sow 2 -3 mother bottled of each cross to reduce the chance of loosing a cross through contamination. If the contamination rate from sowing is 5% per bottle, 2 bottles mothers reduces the chance of loosing a cross from 5% to .25% and 3 mothers even less. For mother media, I use Sigma Vacin & Went media with 20 grams of sugar, 2-3 grams of charcoal and 7 grams of Sigma type E agar, pH adjusted is adjusted with nitric acid to 5.4. (I understand from my Paph friends (people who grow Paphs) that a higher pH, 5.8-6.2 plus in the dark is more effective for Paphs. Cost is around \$1.50 per liter. I use about 3/4" of media per mother bottle.

I sterilize in a pressure cooker. I have two large ones to save time. After flasks are filled with media I wipe the inside top walls near the lids with a towel. I believe removing any splashed agar during filling from these areas lessens the chance of later contamination. Flasks are placed in the pressure cooker and the lid secured. With the pressure cookers vent open, bring it to a boil and allow the media to come up to temperature (5-10 minutes). It is very important to replace all the air in the pressure cooker with steam. Hot air is not effective for sterilization at the normal time/temperature specified. I sterilize with 15 psi of steam for 20 minutes. I have also seen 15 psi used for 15 minutes (15 psi of steam is the equilibrium pressure for water at 121C).

To prevent the lids of the canning jars from forming a vacuum when cooling, I give each lid a slight twist before capping so they do not lay flat. I don't tighten down the screw bands, I only give them about 1/3 of a turn so they are in place. After autoclaving, I place the pressure cooker under my laminar flow bench to cool. I open the pressure cooker vent when the pressure is about 0-1 psi. After unloading and cooling I give each lid a final twist tight. By waiting for these jars to cool before tightening I prevent a vacuum from forming.

In answer to questions about using a microwave oven to sterilize I offer a this guess why it doesn't work well. Microwaves heat water by rotating the molecules 180 degrees rapidly. Water molecules are dipoles (opposite charge on each end of the molecule) and this causes them to align to the electromagnetic field. The field reverses 2.3×10^8 times per second causing the water to heat by friction. Materials such as plastic and glass are not directly heated by microwaves.

I suspect microwaves will effectively kill micro-organisms if they are uniformly in the field; however, the distribution of microwaves in a typical oven is poor and there are substantial area where nodes occur with effectively no energy. We have all had to rotate our food to get it to evenly heat. Perhaps someone with more experience can elaborate. Bottom line, microwave ovens don't do a good job at sterilizing flasks. I do use mine to boil water and media prior to autoclaving).

I store "mothers bottles" on a shelf, ready for use. Because they are unvented, they do not dry out or contaminate with time. When I do open them, they are not under vacuum which keeps them from "sucking" in a volume of air at high velocity upon prying off the lid, lessening the chance of

contamination. I also autoclave erlenmeyer flasks the same way, i.e. the stoppers canted and laid on the top, not sealed. In this position there is no danger of them "popping off" during sterilization. I push the stoppers in place when I unload the pressure cooker and let them cool in the flow bench.

After sowing mother bottles I place the solid lid back on and screw down the band. I don't vent these containers until after contamination free germination has occurred, 4-6 weeks typically.

When it is time to vent these I remove the band and metal lid and replace it with a "Suncap". Suncaps are sold by Sigma and are clear, mylar 5" x 5" squares with a 4 mm teflon sub micron filter in the center. There is a technique for doing this. Suncaps are sterilized, about 30 at a time between the pages of 5" x 7" telephone notepads. These are wrapped in foil. After autoclaving, I store these under the laminar flow hood and just tear off a page and use the Suncap - voila, they stay sterile this way until use. It is hard to sterilize more than 30 Suncaps per notepad as the paper is a poor thermal conductor and if it is too thick, 15-20 minutes in a pressure cooker won't drive the heat into the center of this mass. I also use fresh steel bands on the suncap. I have two sets of bands. Those that I use for sterilizing and those I use for "Suncapping". The ones that go through the pressure cooker get rusty and the high friction from the rust tends to warp and twist the Suncap. A steel band that isn't rusty works much better. Jars and the bands are recycled.

I add these insights about filters. Suncaps are made of a stretched teflon membrane similar to Gore-Tex. The pore size is sub micron, hence bacteria and pathogenic fungus cannot enter. This prevents contamination. The Suncap filter membrane is very thin and has a very high pore density thus suncaps tend to allow a lot of evaporation. It is almost like having a 4 mm hole in the top of a flask; hence, one has to watch flasks for excessive dehydration. I have begun blocking off the open area with a small round paper label to minimize this problem. An alternative is micro porous tape used for bandaging wounds.

I use cotton in rubber stoppers on erlenmeyer's and here the pore size is actually rather large; however, the path length is very long and evaporation is much lower than with Suncaps. One can refer to the suncap as a membrane filter while cotton is a "tortuous path" filter. Both work well. Diffusion, temperature fluctuations and changes in barometric pressure assure 1 or 2 percent of the flask volume is exchanged daily. I might add a caveat, if you use rubber stoppers and cotton, unwashed cotton (available from an upholstery supply) reduces the chance of contamination. Unwashed cotton contains oils that repel water. Wet cotton can grow pathogens through it. You may also want to put a drop of CuSO_4 in water (saturated) or some picric acid in water (a few percent) on the cotton. This acts as a biocide and prevents pathogens from growing through the cotton. (Yes, I know picric acid is used in making hand grenades; however, I have never had a rubber stopper explode and picric acid a great biocide.)

When germinated embryos develop into small balls 2-3 mm in diameter and begin to show the emergence of a tip (called the leaf primordium) it is time to spread them onto replate media. I use several replate medias. Gallup & Stribling distributes Hill's Replate Media with Banana:

Gallup & Stribling

645 Stoddard Ln.

Santa Barbara, CA 93108

1-805-969-5991

It is popular around the world because it is great media. It is also fairly expensive. A good alternative is a media worked out by Terry Root of the Orchid Zone, my variation follows:

Sigma Phytomax Maintenance Media, M6668 (contains sugar and charcoal)

7 grams of type E agar

1 jar of banana baby food (113 grams or 4 oz.)

pH adjusted to 5.4 (higher for Paphs?)

For salt sensitive plants such as Masdevallia:

Vacin and Went

7 grams of type E agar

1 jar of banana baby food (113 grams or 4 oz.)

pH adjusted to 5.4

I move to 1 pint narrow mouth mason jars for spreads and final replates. I recommend the protocorms be spread sparsely for the 1st spread. It is likely if this is done correctly final replates can be done from the 1st. spread. A spread will take several months before it is ready for replating. This final replate will take a few additional months before plants are ready to come out of the bottle. Protocorms can be kept in the mothers for a fairly long period of time so one can time the replate process so flasks are ready to plant out in the spring (avoid taking flasks out in the winter).

The procedure for spreads and replates is fairly simple. I use a very small replate hook, a 1/8" stainless rod, about 12" long, with a small bent hook on one end. I fashioned this with jewelers saw and some files and heat. I do not use tweezers; however, some flaskers do (they cause cramps in my hands). The "hook" is easy to sterilize in a flame and cools quickly. I use Suncaps on spreads and replates.

Growing takes place on Metro wire shelves under fluorescent lights. The shelves are 2'x 4' with two single lamps fixtures per shelf. The ballast are electronic to prevent local heating and the

cheapest, cool-white bulbs (cheap ones) are used. Light levels are ~ 300 ft. candles. The lights are run for 8 hours per day so as not to disturb my neighbors or arouse the nepharious instincts of kids in the neighborhood. I try and keep the flask room under 75F during the day and over 60F at night. Excessive day temperatures causes problems with proliferation in the flask. (I grow cool orchids so I do not have experience with warmer growers.) I do know that the flask rooms I have visited at commercial growers are always comfortable.

Hope the above information proves useful.

Bob Hamilton - Dec 94

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