

Plant Tissue Culture



Media ingredients

Tissue Culture

All media contains:

- Deionized and distilled water
- Macro and micro plant nutrients
- Inositol
- Vitamins
- Hormones
- Sugar
- Agar

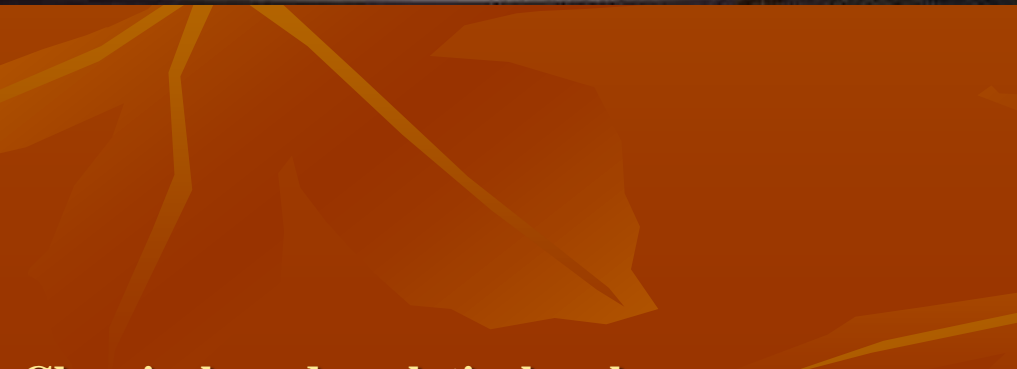
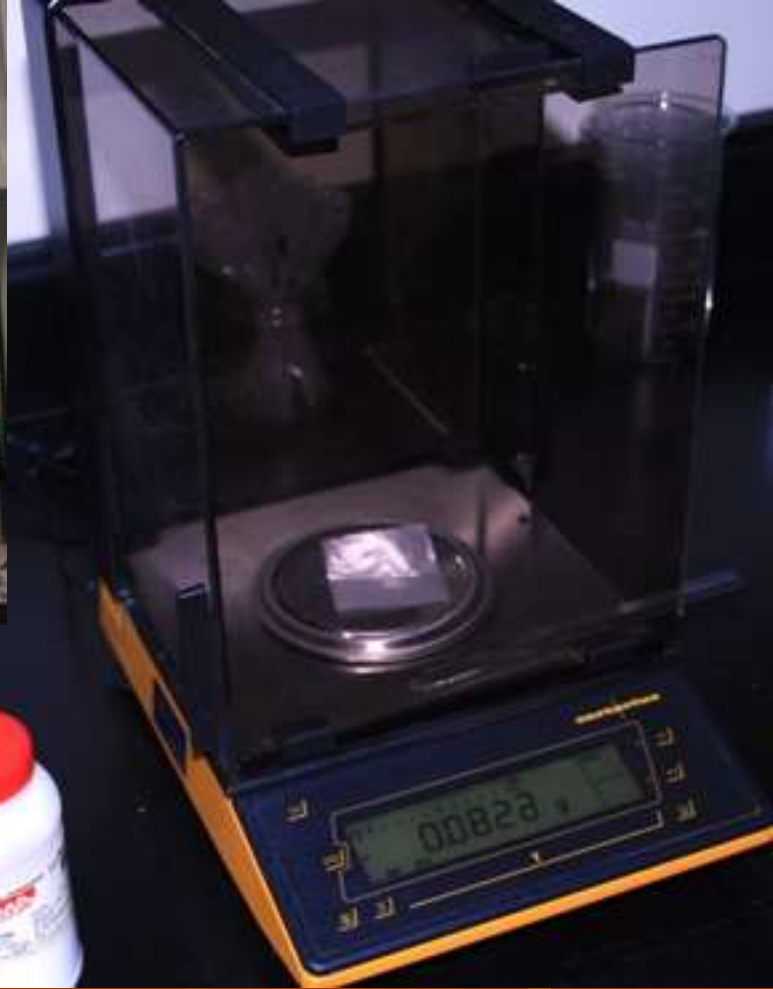
Then:

- Media pH is adjusted
- Tubes are filled with 12 mL media and capped
- Tubes are sterilized in autoclave at 121°C for 20 minutes (at 15 psi)



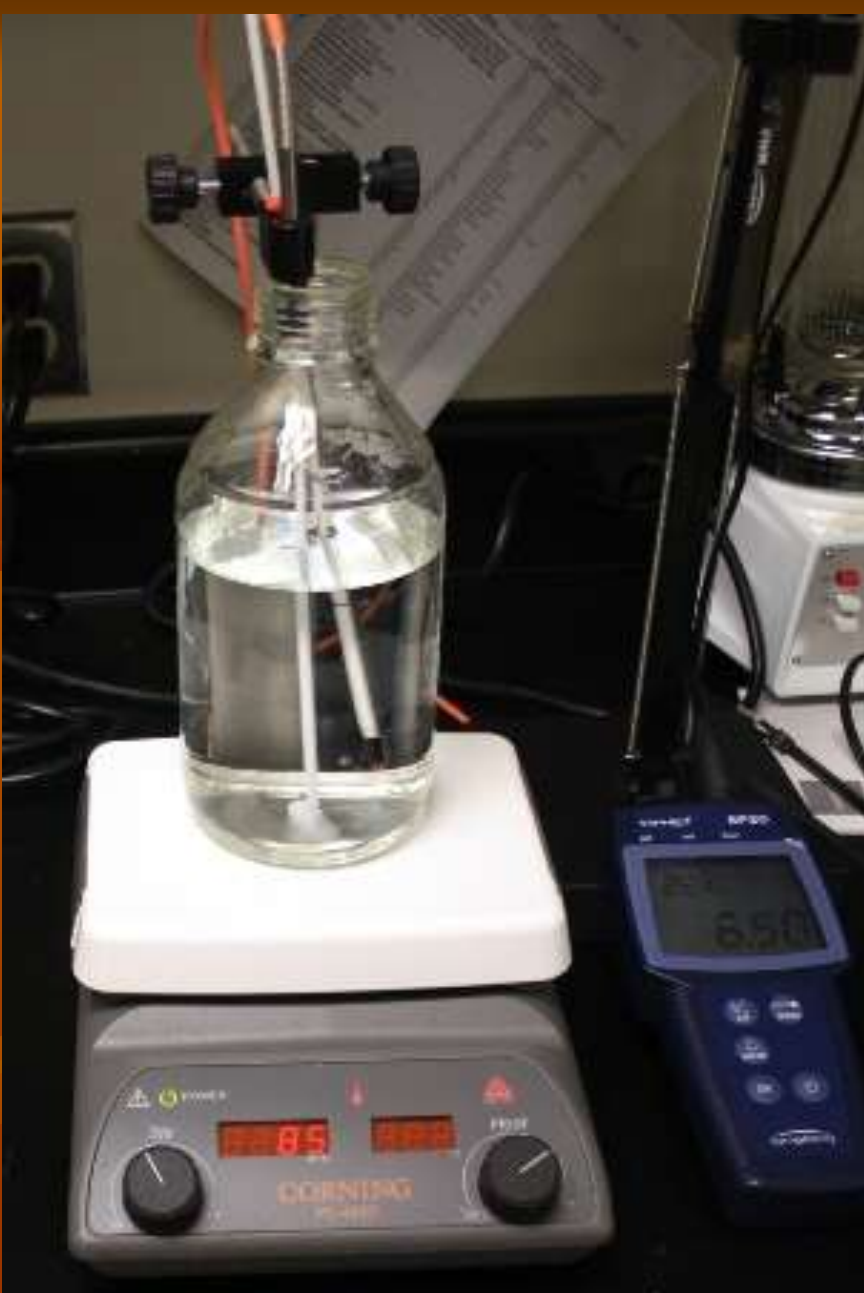
Water deionizer and distiller

Tissue Culture



Chemicals and analytical scale

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Hotplate/stirrer with media bottle, pH meter, and autoclave

Tissue Culture

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Woody Plant Medium (WPM) for Rhododendrons

- A Stock: 4 ml (into 150 ml ddH₂O in 250 ml media bottle)
- B Stock: 4 ml
- CaCl₂ x 2 H₂O: 0.0254 g
- D Stock: 2 ml
- E Stock: 2 ml
- F Stock: 2 ml
- G Stock: 2 ml
- Inositol: 0.02 g
- Low Hormone Stock: 12 ml
- Sucrose: 4 g, dissolve, and then adjust volume to 200 ml using ddH₂O
- pH: Adjust to 5.2 (1 to 4 drops 0.5 N NaOH)
- Agar: 1.5 g (heat until clear on hot plate)
- Dispense: put 20 ml per test tube and cap
- Autoclave: 115 psi for 20 min. (then remove racks after 1 hour and allow to cool on a sharp angle for several hours)

Media example: stock solutions reduce the time required to make commonly used media

Preparation

- Brush off dirty clothes and hair outdoors.
- Tie back long hair and remove dirty footwear before entering Rm. 1662.
- Have laminar flow hood on for 5 to 10 minutes before opening a tube.
- Wash hands with antibacterial soap and water and dry thoroughly.
- Put on a clean lab coat and apply small amount of hand cream.
- Remove all non-essential items from laminar flow hood.
- Drip 70% ethanol to work surface and wipe dry with paper towel.
- Do NOT get alcohol into the back of hood (it harms the HEPA filter).
- Have forceps and scalpel soaking in 70% ethanol for at least 2 minutes initially and then for at least 10 seconds between transfers.
- Keep alcohol deep in hood so it does not get spilled. Lite alcohol burner away from alcohol.

Sterile transfer technique

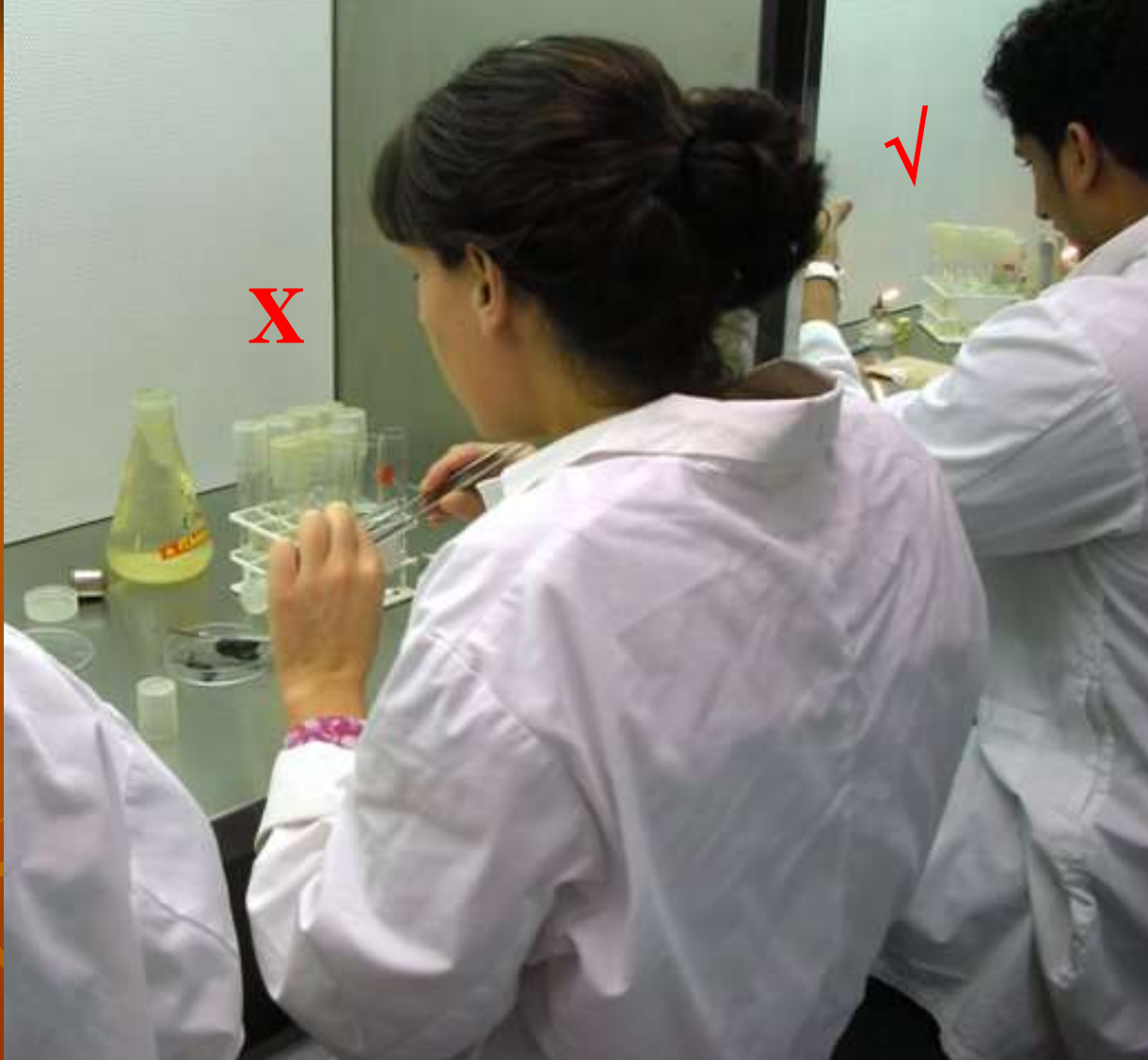
Transferring Process (reverse if left handed)

- Remove the cap from the tube and hold between two fingers.
- Move tube from left to right hand, holding between thumb & index finger.
- Flame the tool over the alcohol burner to remove the alcohol. There's no need to heat the tool.
- Pick up the surface sterilized plant tissue or seed and place it into the agar inside the tube.
- Quickly recap the tube and place in rack. Important: Seal tube with parafilm wrap.

Minimizing contamination & health risks

- Personal hygiene is important. Work at least half way into hood.
- If surface sterilizing plant material then follow instructions carefully.
- Do not talk or breathe heavily during the transferring process.
- There should be nothing between an open tube and the back of the hood to block airflow.
- Instruments need to be in 70% ethanol for at least 10 seconds.
- Keep the tip of tools pointing downwards so fingers stay dry and do not get burned. Be careful not to dip a lit tool into the alcohol flask!
- Do NOT open any tube with fungal contamination. Staff will check for and discard contaminated cultures regularly.

Sterile transfer technique



Work with hands at least half way inside the hood
Ensure nothing is blocking the air flow between back of hood and the specimen!

Sterile transfer technique

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African Violet Explant Establishment (SI)

- Soak two young leaves in 10% bleach with 1% tween added for 10 minutes maximum. Vortex.
- Rinse in three changes of sterile deionized water (5 minutes in each rinse).
- Cut center portion of two leaves out into four 1 cm X 1 cm in size in sterile petri dish (avoid midrib).
- Aseptically transfer the pieces into the agar and submerge about half-way into it.



Methods and some materials (tubes with media, 10% bleach, cultures, vortex, Parafilm)



Bleach (10%) the African violets leaf piece for no more than 10 minutes!

Sterile transfer technique

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Parafilm tubes 2 to 3 times around to keep spores of bacteria and fungi out.

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New SII cultures



- Old culture: transplanting should be done at least every two months
- Fungal contamination.

Culture maintenance

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Effects of Varying Cytokinin and Auxin Levels on African Violet Differentiation in Tissue Culture

↓ Cytokinin (mg/l BA)					
↓	Auxin:	Auxin:	Auxin:	Auxin:	Auxin:
↓	0 mg/l NAA	0.1 mg/l NAA	0.5 mg/l NAA	1.0 mg/l NAA	2.0 mg/l IAA
0					
.01					
0.1					
1.0					

Hormonal effects

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- Roots in culture are only useful for certain plants that are very difficult to root on a mist bench or under a humidity dome.
- Even then it's only the SIII culture where we want to see roots.
- Media must be well washed off or a disease will likely kill the plant.

Hormonal effects: too high auxin to cytokinin ratio may result in only roots being formed!