

# Tissue Culture for Beginners: What It Takes to Setup a Lab

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## INTRODUCTION

Tissue culture is a valuable tool for plant propagators. In tissue culture bud or other plant tissue is used to produce a large number of genetically identical uniform plants. This procedure is often used when other methods of propagation are unsuccessful. Setting up a plant tissue culture lab requires a basic knowledge of plant culture procedures. There are a number of reference books useful for plant culture procedure and lab design. *Plants From Test Tubes* by Lydiane Kyte and John Kleyn is very helpful and gives a great deal of information for setting up a successful lab.

A laboratory generally consists of three components; media preparation, culture transfer, and culture growth. Lab design is as unique as the individual setting up the lab. A lab can be a number of rooms or one large room. It is important that the lab is isolated from other operations and traffic to keep contamination at a minimum. The lab needs adequate heating and cooling so windows and doors can be kept closed. At Evergreen Nursery a mobile home was purchased and placed at the nursery. The kitchen is used for media preparation, the living room for culture growth, a bedroom for culture transfer and the remaining areas are for storage.

## MEDIA PREPARATION

A media preparation area can be a simple kitchen. Culture media are prepared by mixing together stock solutions or adding water to powdered pre-measured media formulas. Water must be purified using a still, deionizer, or reverse osmosis. For small labs bottled distilled water is inexpensive and readily available. A refrigerator to store stock solutions, a sink to wash glassware, a stove to heat media, pressure cookers, and cupboards to store supplies are necessary. Specialized equipment needed for media preparation includes lab glassware and a hotplate stirrer or other automatic stirring device for mixing media. A balance is necessary for weighing stock solution and media components. A pH meter is used for adjusting medium pH to its proper level and dissolving growth regulators. Individual culture vessels can be test tubes, baby food jars, or other commercially available culture vessels. Media sterilization can be accomplished using an autoclave or stove top pressure cooker. The media preparation area is for producing sterile media, it is important that it is kept clean.

## TRANSFER ROOM

The transfer room is where cultures are transferred from old to new media. Cultures must be sterile so transfer work is done in a sterile environment. A transfer hood consists of an enclosed HEPA filter with a blower forcing air through the filter into the transfer area. Transfer hoods can be purchased complete or in kit form. Prices vary widely depending on the type of construction. Transfer hoods are often left on continuously to purify the transfer room air.

Cultures cannot be manipulated by hand. Sterile instruments such as tweezers and scissors are used to cut and pull plants apart. Instruments must be continually sterilized during transfers and may be sterilized several different ways. Alcohol lamps are inexpensive but dangerous. Devices such as a Bacti-Cinerator can be purchased, they rapidly heat the instruments without a flame. Other supplies utilized in the transfer of cultures are tube or jar covers, marking pens to label cultures, and alcohol and paper towels to wipe down the transfer hood before and after transfers. Often prepared media are stored ready for use in the transfer room.

### **GROWTH ROOM**

Plant cultures require light and moderate temperatures for proper growth. A culture growth room can be a room with wood or metal shelves. Fluorescent fixtures are suspended approximately 18 to 24 inches above the cultures on each shelf. The growth room should be kept closed from outside air if possible. If the room has windows they should be sealed. Employee traffic should be kept to a minimum. It is important to have adequate heating and cooling.

### **SHOOT HARVEST**

Shoots may be cut in the lab and sent to the rooting area or the culture itself is opened and shoots harvested in the greenhouse. Shoots produced in the lab are often treated as softwood cuttings and stuck in greenhouse media. Shoots are tender and root rapidly. Pots or trays filled with media are stuck and placed in a high humidity environment. Flats covered with clear domes can be placed under lights or a mist tent system can be constructed.

### **CONCLUSION**

An efficient small tissue culture lab can be set up for under \$10,000. Once the lab is set up it must be staffed. Personnel must be trained in media preparation and sterile techniques. Labor is an ongoing cost and must be considered in the lab cost. A lab can be very valuable for propagating specific plants needed in your operation. Before setting up a lab estimate lab set up and labor costs to determine if it is more economical to produce the shoots yourself or contract a commercial lab to propagate the shoots for you.

### **LITERATURE CITED**

- Lydiane, K. and J. Kleyn.** 1996. Plant from test tubes. Timber Press, Portland, Oregon.  
**Torres, K.** 1989. Tissue culture techniques for horticultural crops. Van Nostrand Reinhold, New York, New York.