

Are You Sterile?

Standard Operating Procedure

Effects of Media on Sterility for IVC Cultures

Laboratory: Biotechnology
SLOP prepared by: R. Sanders

Location: Ag Biotech Academy
Last Revision: 1 May 2024

General: The goal of this procedure is to examine the effectiveness of antimicrobials and fungicides in controlling contamination in IVC cultures.

Safety: Safety Glasses

Materials

70% Isopropyl Alcohol
small container
cheesecloth
single hole punch
forceps
plant with young growing leaves
4 – 1 dram vials containing the following media and labels:
Vial #1: Medium only
Vial #2: Medium with fungicide
Vial #3: Medium with antimicrobial agent
Vial #4: Medium with fungicide and antimicrobial agent

Procedure

1. Fill small container with 70% alcohol.
2. Place cheesecloth, single-hole punch and forceps in the container of alcohol.
3. Select a young, growing leaf, which is only about $\frac{1}{4}$ to $\frac{1}{2}$ expanded and remove from the plant.
4. Sterilize the leaf with the cheesecloth soaked in alcohol using procedure from SOP [Surface Sterilization of Leaf Tissues](#).
5. Using the alcohol-sterilized single-hole punch with the hole-side facing up, punch a disc from near the base of the leaf.
6. Then with the alcohol-sterilized forceps, transfer the leaf disc to Vial #1 and cap immediately.
7. Repeat Steps 5 and 6 to ensure vials 2-4 contains a leaf disc.
8. Place all 4 vials on benchtop away from direct sunlight.
9. Observe vials every 24 hours for 5-7 days.
10. Each day observe the vials for contamination and record observations in data table.

*This document may be reproduced for educational purposes, but it may not be reposted or distributed without crediting GrowNextGen and The Ohio Soybean Council and soybean checkoff.