



## Standard Laboratory Operating Procedure #101 The Effects of Media on Sterility for IVC Cultures

**Laboratory:** Biotechnology

**Location:** Lab Station

**SOP prepared by:** R. Sanders and B. Wolf

**Last Revision:** 6 January 2014

**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 #100.
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.