

Basic Sterile Cell Culture

Work Area: Cell culture cabinet. Do not use to store items, and position reagents so that they do not block vents or laminar flow around individual reagents.

Supplies are nearby, so that person doing culturing does not need to move. Lighting kept to minimum as to avoid free radical oxidation of reagents (unless sterilizing with UV prior to passing cells.)

Passing suspension cells: Sterilizing done with Et-OH and iodine solution. Scrubbed with aseptic foam onto glove (optional)

Sterile techniques: **Sources of contamination:** Technician, water bath, excessive supplies in cabinet, length of time of procedure.

Passing Cells: Cloudy sample implies need to transfer.
Note technician's pipetting technique.

All reagents and supplies are in line, care is taken to avoid crossing over, and cap is closely monitored.

Pipette don'ts: do not touch shaft of pipette, open only in hood, close when not in use, and use cotton plugs when possible.

Cell Culture Plastic: Quite variable: FYI...Culture wells, culture bottles and culture flasks.

Equipment: Keep sterile, especially water baths, cell culture hood and incubators.

Microbes Types: Virus, mycoplasma, bacteria (first three, not easily visible, but can often see effects.) Fungal and molds often grossly visible.

Brownian movement: Random oscillation due to molecular bombardment. Can make inert particles move and thus appear living.

Chief sources of microbial contamination: poor sterilization, room air, dust, aerosols (from talking and sneezing), malfunctioning hood, chalk dust.