Cell Culture Laboratory and Aseptic Technique

Lin Yuan

China Animal Disease Control Center（CADC）
OIE Reference Laboratory for Porcine
Reproductive and Respiratory Syndrome

2015.4.13

Cell Culture Laboratory

- Biosafety Level 2 (BSL-2)
  - BSL-2 is appropriate for moderate-risk agents，cell culture labs should be at least BSL-2.
- Personal Protective Equipment (PPE)
  - Gloves , laboratory coats and gowns, shoe covers, boots, respirators, face shields, safety glasses ,or goggles.

Cell Culture Laboratory

- Cell Culture Equipment
  - Cell culture hood（Class II biosafety cabinet，laminar flow）
  - Incubator (humid 37℃,5% CO_2)
  - Cell counter (Automated Cell Counter or hemacytometer)

Cell Culture Laboratory

- Cell Culture Equipment
  - Water bath（37℃，56℃）
  - Centrifuge，Low speed，with refrigeration
  - Refrigerator and freezer (4℃，–20℃，–70 ℃)

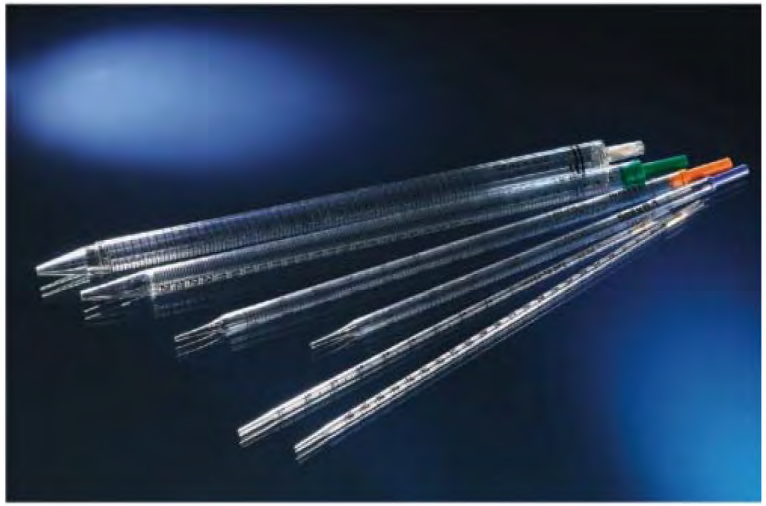

Cell Culture Laboratory

- Cell Culture Equipment
  - Inverted microscope
  - Liquid nitrogen (N_2) freezer or cryostorage container
  - Sterilizer (i.e., autoclave)

Cell Culture Laboratory

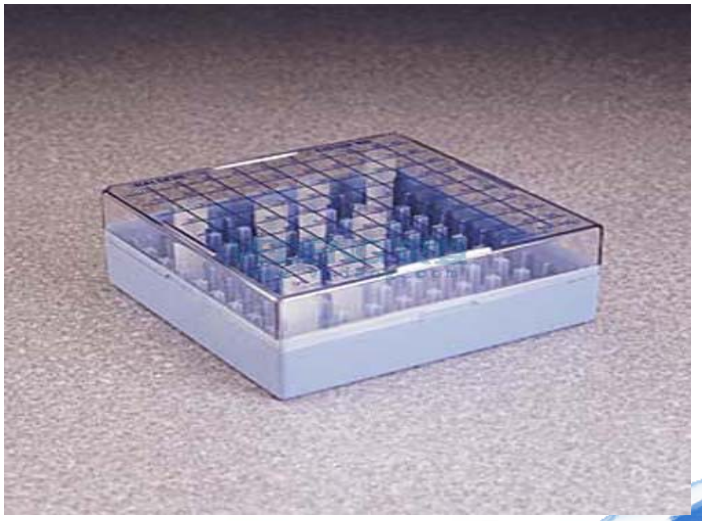
- Supplies
  - Cell culture vessels（flasks，microplates）
  - Assorted sterile pipettes and pipetting device including multi-channel pipette.
  - Sterile capped tubes.
  - Cryogenic vials
  - Centrifuge tubes
  - Liquid waste container

Cell Culture Laboratory



Cell Culture Laboratory

- Supplies
  - Pens/markers
  - Controlled-rate freezing container（-1℃/min）
  - Freezing container



Cell Culture Laboratory

- Cell cultures and reagents
  - 70% alcohol in spray bottle
  - Cell culture medium （DMEM 、MEM、EMEM、PRMI 1640）
  - Serum（FBS（Fetal bovine serum）/NBCS（new born calf ））
  - Antibiotics（Pen / Strep）
  - Trypsin
  - Trypan blue dye
  - DMSO（Dimethyl sulfoxide）

Aseptic Technique

- Contamination sources
  - Non-sterile supplies, media, and reagents
  - Airborne particles laden with microorganisms,
  - Unclean incubators
  - Dirty work surfaces

Aseptic Technique

- Aseptic technique
  - Sterile work area
  - Good personal hygiene
  - Sterile reagents and media
  - Sterile handling.

Aseptic Technique

Sterile Work Area

- The cell culture hood should be properly set up and be located in an area that is restricted to cell culture that is free from drafts from doors, windows, and other equipment, and with no through traffic.
- You may use **ultraviolet light** to sterilize the air and exposed work surfaces in the cell culture hood between uses.

Aseptic Technique

Sterile Work Area

- The work surface should be uncluttered and contain only items required for a particular procedure
- Before and after use, the work surface should be disinfected
- For routine cleaning, wipe the work surface with **70% alcohol** before and during work

Aseptic Technique

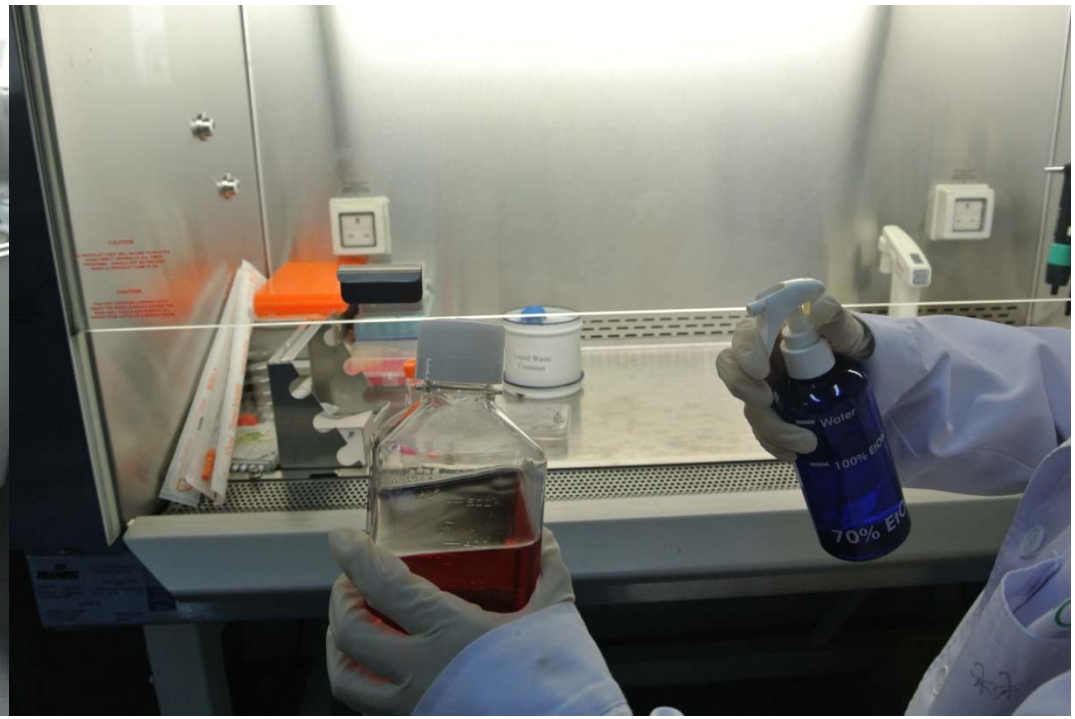
- Good Personal Hygiene
  - Wash your hands before and after working with cell cultures. wearing personal protective equipment also reduces the probability of contamination from shed skin as well as dirt and dust from your clothes.

Aseptic Technique

- Sterile Reagents and Media
  - Always sterilize any reagents, media, or solutions prepared in the laboratory using the appropriate sterilization procedure

Aseptic Technique

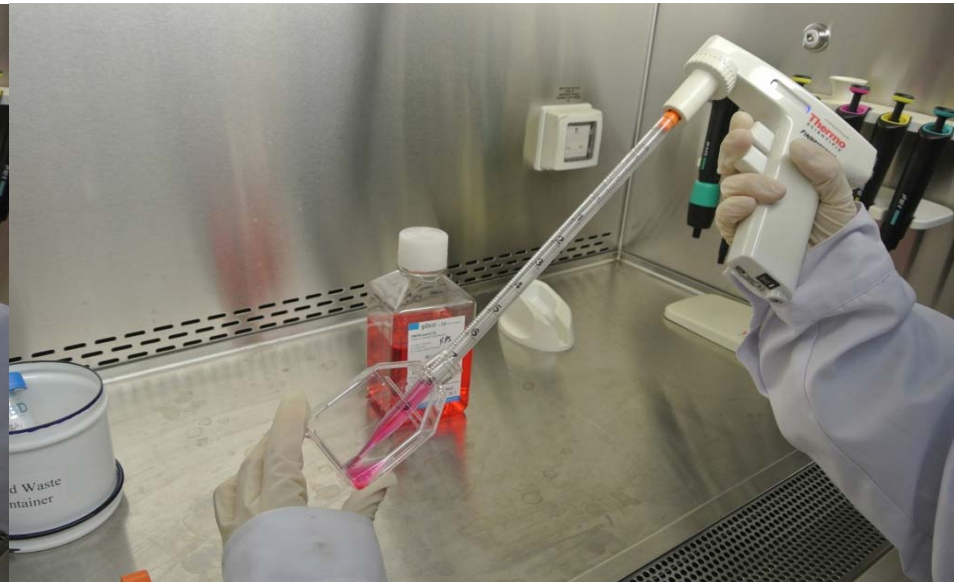
- Sterile Handling
  - Always wipe your hands and your work area with 70% alcohol.
  - Wipe the outside of the containers, flasks, plates, and dishes with 70% alcohol before placing them in the cell culture hood.



Aseptic Technique

Sterile Handling

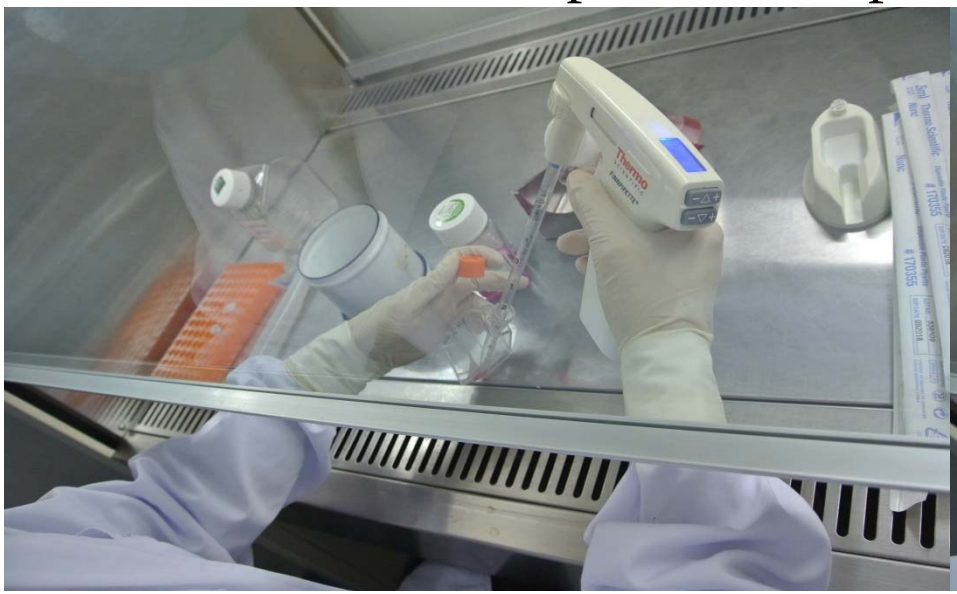
- Use sterile glass or disposable plastic pipettes and a pipettor to work with liquids.
- Always cap the bottles and flasks after use and seal multi-well plates with tape or place them in resealable bags to prevent microorganisms and airborn contaminants from gaining entry.

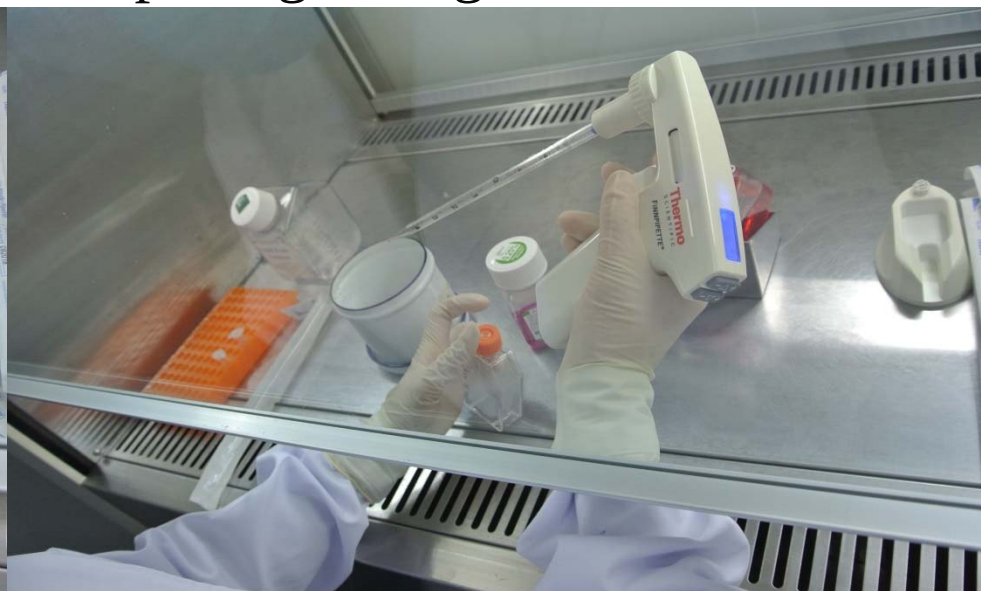


Aseptic Technique

Sterile Handling

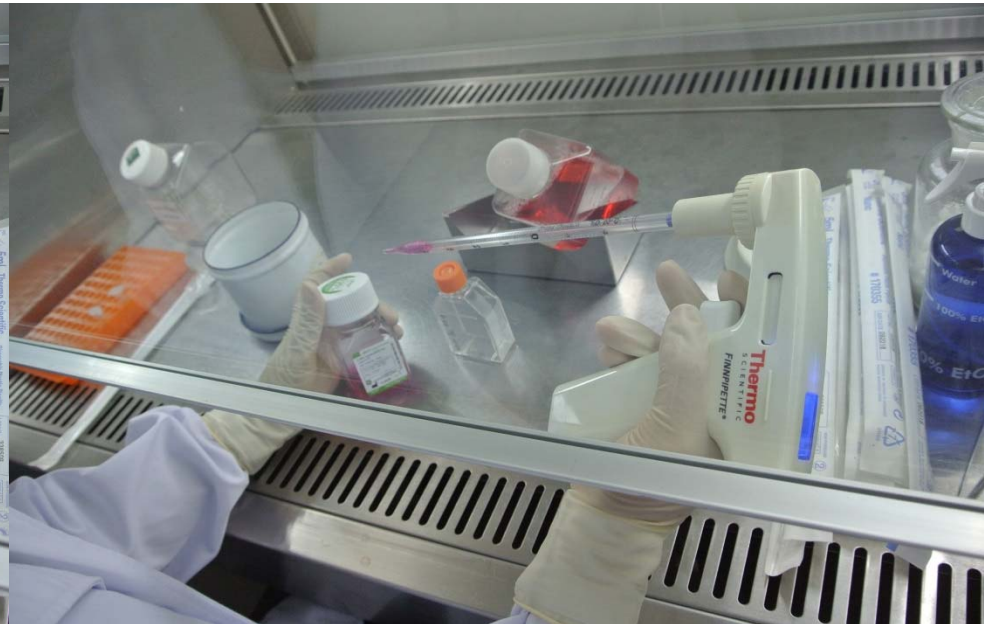
- Never uncover a sterile flask, bottle, petri dish, etc. until the instant you are ready to use it and never leave it open to the environment. Return the cover as soon as you are finished.
- If you remove a cap or cover, and have to put it down on the work surface, place the cap with opening facing down.





Aseptic Technique

- Sterile Handling
  - Not to talk, sing, or whistle when you are performing sterile procedures.
  - Perform your experiments as rapidly as possible to minimize contamination




Thank you !