Aseptic Technique

A GMP/GTP Training Module

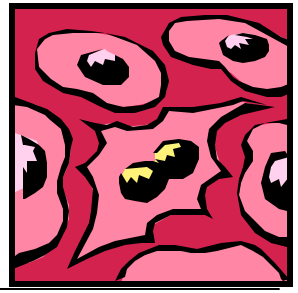
Center for CAGT Cell and Gene Therapy

BCM Methodist Texas Children's Hospital

Aseptic Technique

- The GMP Facility manufactures products for clinical use
- These products must meet a number of requirements, one of which is that they are sterile
- In order to produce sterile components it is necessary to follow aseptic technique

Aseptic Technique



- Use of aseptic technique protects the product and the operator from exposure to infectious agents and contaminants
- The technique is a way of working that provides this protection
- The following presentation provides basic information on aseptic technique

Aseptic Technique

- The idea behind aseptic technique is to PREVENT contamination
- It is not possible to “clean up” an already contaminated product by using aseptic technique
- In order to maintain sterility, it is necessary to start with a sterile product & handle it in an aseptic manner

Before Starting

- All reagents and materials to be used should be in-date, barcoded, and should have a Certificate of Analysis on file
- All equipment to be used should have been cleaned according to SOPs and that cleaning should be documented
- All equipment, including pipettors, should have been calibrated
- Waste traps should contain some bleach to act as a decontaminant

Before Starting

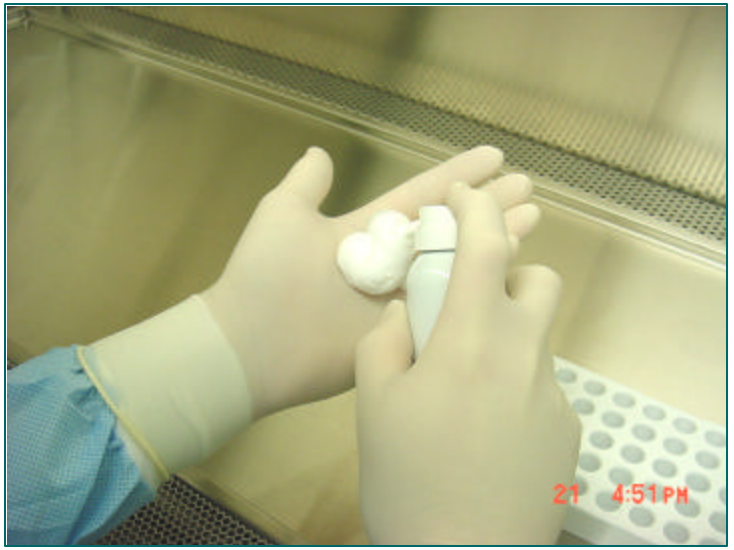
- **Collect all of the reagents and materials that you will need onto a workspace close to the biological safety cabinet**
- **Make sure that you have a biohazard trash container, sharps container and waste trap available – we recommend that waste traps be located on the floor OUTSIDE the BSC**
- **Make sure that you have disinfectant spray bottles containing 3M Quat and 70% ethanol available**

Before Starting

- **Make sure that you are wearing the required personal protective equipment**
  - **Blue labcoat**
  - **Gloves**
  - **Eye protection if necessary**
  - **Sleeve covers available (if you will work with more than one product)**

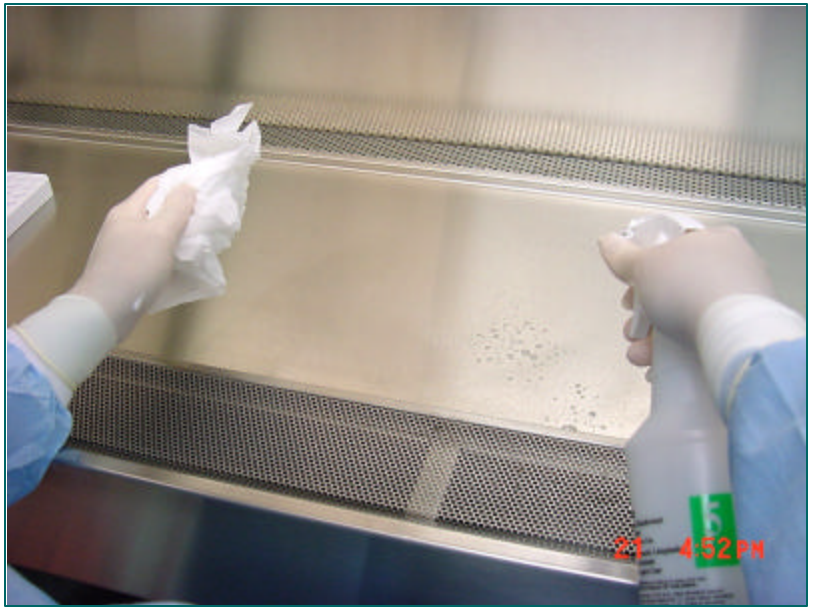
Before Starting

- **Page QA/QC if you require environmental monitoring during production**
- **Use alcohol foam to clean gloves before using the BSC**



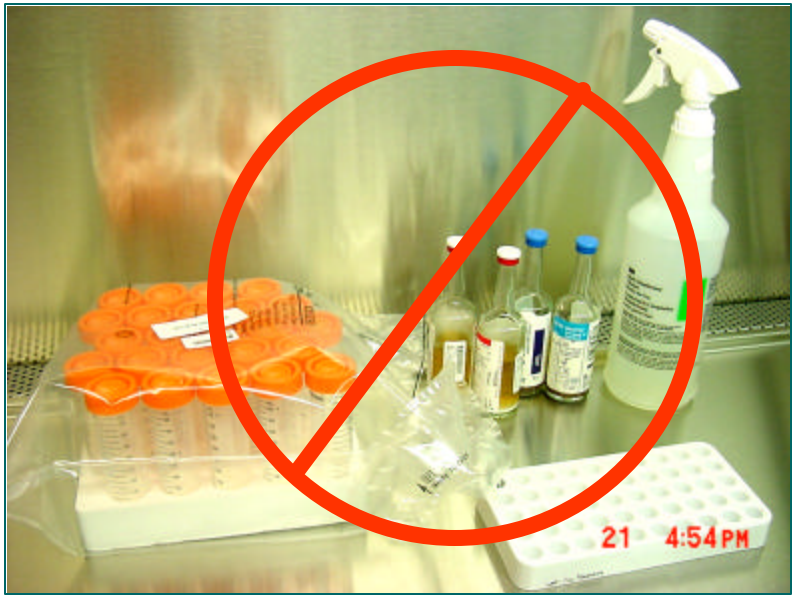
Before Starting

- **Wipe down the working surface in the BSC with 3M Quat or 70% ethanol and leave to dry**
- **Disinfect any equipment e.g. racks, before placing them into the BSC**



Starting Work

- **Do not clutter up the working area in the BSC – bring in only what is necessary**
- **Be careful not to block the air vents at the back and front of the BSC**



Starting Work

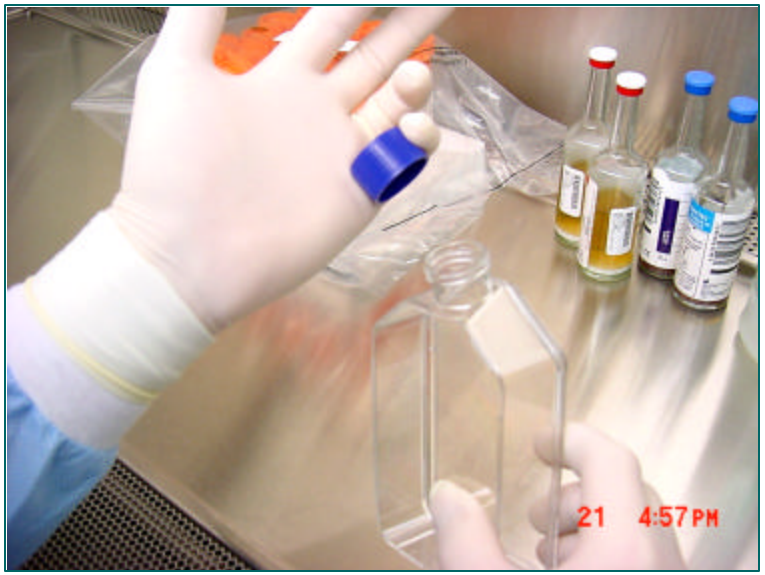
- **When you start working, be sure that you work well inside the BSC and not directly above the front air vents**
- **Keep your nose and mouth away from the window opening**

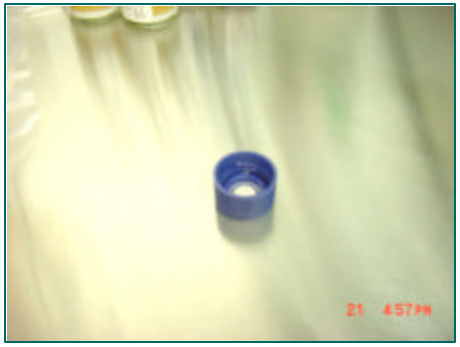
Working Tips

- Tighten the caps on culture flasks before moving them to the BSC
- Wipe down culture flasks with spray disinfectant before bringing them into the BSC
- Decontaminate your gloves using alcohol foam frequently during the culture procedure

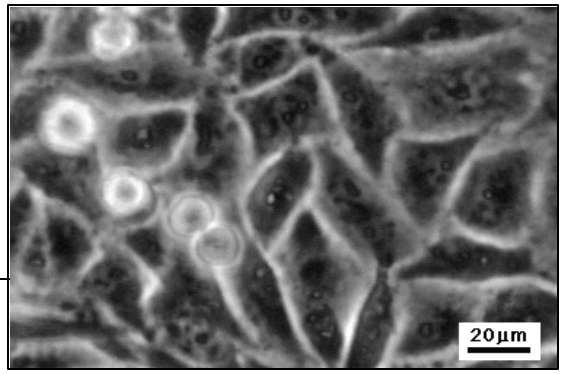
Working Tips

- Be careful not to touch the necks of culture flasks when removing the caps
- Be careful not to touch the inside of the cap
- Place the cap either face up, or face down on an alcohol wipe





Working Tips



- Handle cells gently when pipetting and resuspending
- Do not over-expose cells to enzymes when dispersing a monolayer into a suspension
- Avoid extreme temperature changes

Working Tips

- Resuspend in medium that can maintain the correct pH of the culture
- Bicarbonate-buffered medium requires a 5% CO_2 atmospheres to maintain its pH
- Most media contain pH indicators and are a reddish orange at the correct pH

Working Tips

- A culture that becomes cloudy rapidly after incubating is probably contaminated
- This can be checked by sterility cultures and microscopic examination
- QA/QC recommends that all contaminated cultures should be sterility tested to identify the contaminating organism
- Cultures that contain many cells usually turn yellow, but the supernatant medium remains clear rather than cloudy, as with contaminated cultures

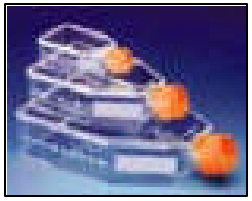
Working Tips

- Cultures that contain white or gray threads, or cotton wool-like floating particles usually are contaminated with fungus or mold
- This can be checked by sterility cultures and microscopic examination
- QA/QC recommends that all contaminated cultures should be sterility tested to identify the contaminating organism
- Fungus and mold is difficult to cure effectively

Working Tips

- **Contaminated cultures should normally be discarded after a sterility culture has been submitted**
- **If it is essential to maintain a contaminated culture, it should be moved to a separate quarantine incubator – see QA/QC**
- **The incubator in which it was grown should also be cleaned, including wiping down the exterior of any flasks that were sharing the incubator**

Working Tips



- When returning a flask from the BSC to the incubator, tighten the cap first
- When the flask is in the incubator, the cap may then be loosened to allow for gas exchange
- Avoid medium entering the neck of the flask during transport
- Any medium that spills in the incubator should be sprayed with disinfectant and immediately wiped up

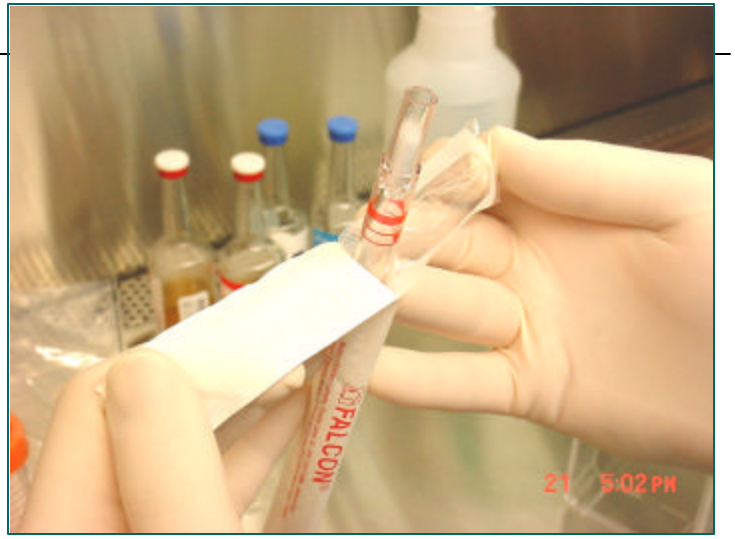
Working Tips

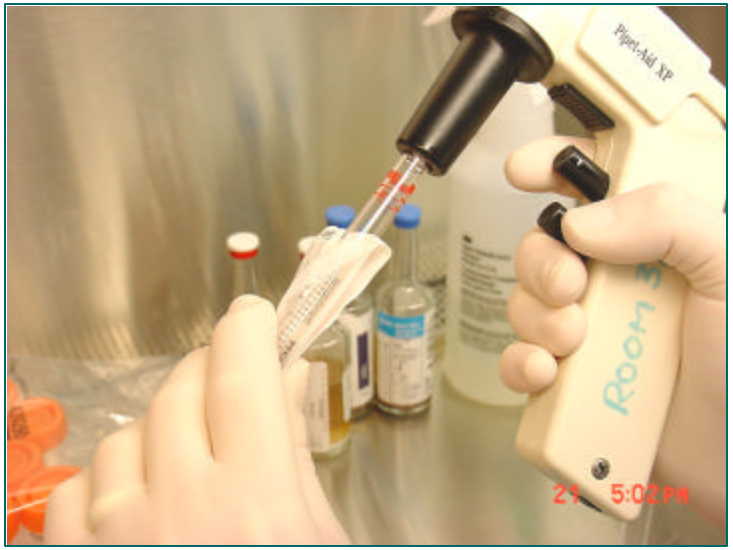
- QA/QC recommends that medium that is prepared/supplemented for cell culture should be sterility tested before use whenever possible
- Random bottles from new lots should be submitted to QA/QC for testing as a check on the manufacturer
- Remember to assign an expiration date to newly supplemented medium, and to indicate the nature and amount of the supplement(s)
- Initial and date the changes to the label.

Serological Pipets

When working with serological pipets

- **Transfer pipet to the BSC before opening**
- **Open package by grasping each side of the closure and peeling back the wrapper from the wide end**
- **Do not touch the unwrapped area of the pipet**
- **Plug the unwrapped end firmly into the Pipet Aid device**





Serological Pipets

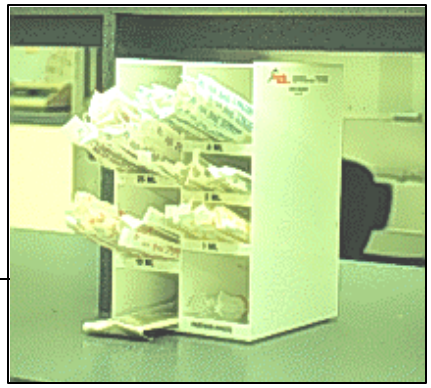
- **When working with serological pipets**
  - **Remove the wrapper by pulling it from the tip end of the pipet, taking care not to touch the pipet**

Serological Pipets

- **Be careful not to let the pipet touch items inside the BSC**

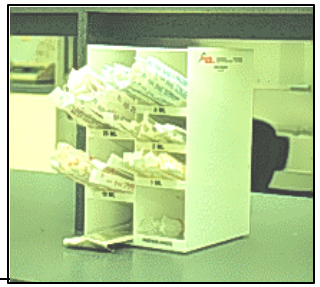
- **When pipetting liquids, insert the pipet tip beneath the surface of the liquid, taking care not to let the pipet barrel touch the neck of the container**

Serological Pipets



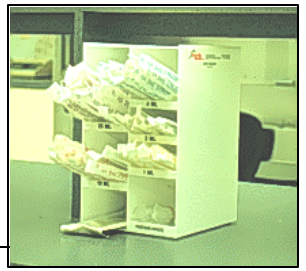
- **Draw up the liquid into the barrel,do not blow air thru the liquid**
- **Remove the pipet from the container, again avoiding touching the barrel against the container neck**

Serological Pipets



- Take care not to let liquid drip from the pipet when transferring
- Insert the pipet into the receiving vessel without touching the barrel to the neck
- Gently expel the liquid into the vessel. Do not blow bubbles. Expel the liquid against the vessel wall if necessary
- The same pipet may be used to resuspend cells in the liquid, but must then be discarded

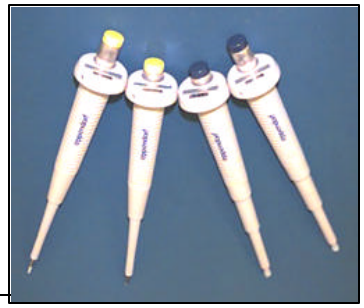
Serological Pipets



- **Remove the pipet from the Pipet Aid by grasping the barrel firmly and pulling**
- **Discard the pipet into the SHARPS container**

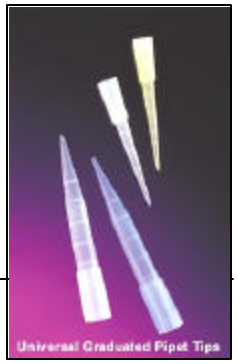
- **Disinfect gloves with alcohol foam**
- **If any liquid has dripped onto surfaces, clean it up with an alcohol swab, or disinfectant spray and gauze.**

Micro-Pipetting



- When using Eppendorf or similar pipets, individually wrapped tips should be handled like serological pipets
- Racked pipet tips can be used, but care must be taken to keep the tip box covered when tips are not being removed
- If you are in doubt about the sterility of a rack of pipet tips, discard them, and use a new rack
- Wipe down pipet barrels after use using an alcohol wipe

Micro-Pipetting

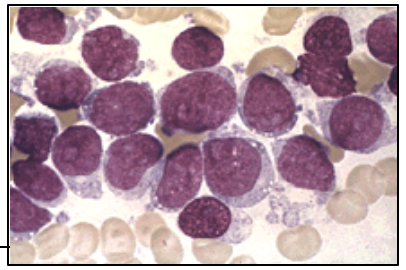


- The use of barrier tips is recommended for most applications
- If liquid enters the pipet barrel, stop using the pipetter until it can be properly cleaned and disinfected
- When using micro-pipetters for cell sampling, be sure to mix the cell suspension thoroughly

Universal Precautions

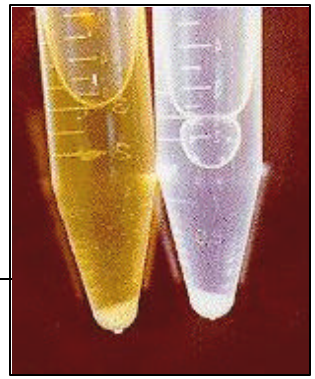
- All cell products should be regarded as potentially infectious
- Follow Universal Precautions when working with these products
- You will receive annual training on Universal Precautions

General Precautions



- Avoid creating aerosols when pipetting or transferring liquids
- Handle cells gently – do not shake or pipet suspensions vigorously
- If you feel that you may have touched a sterile item – discard it and use a new one
- Clean up any spills immediately after decontaminating with disinfectant

General Precautions



- Make sure caps are on tight before centrifuging cell suspensions
- If possible use aerosol containment or wrap parafilm aound the caps
- Avoid spinning cells too hard or for too long
- Pellet resuspension is best done gently, using small volumes of medium, followed by dilution of the suspension

Waste Reagents

- Vacuum aspiration is the easiest way to remove supernatants [SN]
- The SN should be aspirated into a waste container containing undiluted bleach
- If the container is not full at the end of the day, be sure to add more bleach – the liquid in an active waste container should normally be a clear yellow

Waste

- DO NOT empty full liquid waste containers
- The container should be disconnected from the vacuum apparatus and discarded into the biohazardous trash
- Discard old culture flasks into the biohazardous trash after tightening the caps
- All pipets should be discarded into the SHARPS containers
- DO NOT overfill waste containers