Tissue Culture

- History
- Animal Cell Culture
- Equipment
- Cell Culture Media
- Aseptic Technique
- General Methods of Analysis
- Measurement of Cell Characteristics
- Cell Separation
Aseptic Technique
In spite of introduction of antibiotics, contamination of cultures by microorganisms (e.g., bacteria, yeast, fungi, mycoplasma) remains a major problem in tissue culture.

- Cultures are checked on the microscope every time they are handled.
- Cultures are kept antibiotic-free for at least part of the time
- Reagents are checked sterility prior to use
- Bottles of media, etc., are not shared with other people or used for different cell lines
- Standards of sterile technique are maintained at all times.
# Aseptic Technique: Do & Don’t

## TABLE 5.1. Good Aseptic Technique

<table>
<thead>
<tr>
<th>Subject</th>
<th>Do</th>
<th>Don’t</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laminar-flow hoods</td>
<td>Swab down before and after use.</td>
<td>Clutter up the hood.</td>
</tr>
<tr>
<td></td>
<td>Keep minimum amount of apparatus and materials in hood.</td>
<td>Leave the hood in a mess.</td>
</tr>
<tr>
<td></td>
<td>Work in direct line of sight.</td>
<td></td>
</tr>
<tr>
<td>Contamination</td>
<td>Work without antibiotics.</td>
<td>Open contaminated flasks in tissue culture.</td>
</tr>
<tr>
<td></td>
<td>Check cultures regularly, by eye and microscope.</td>
<td>Carry infected cells.</td>
</tr>
<tr>
<td></td>
<td>Box open plates.</td>
<td>Leave contaminations unclaimed; dispose of them safely.</td>
</tr>
<tr>
<td>Mycoplasma</td>
<td>Test cells routinely.</td>
<td>Carry infected cells.</td>
</tr>
<tr>
<td>Importing cell lines</td>
<td>Get from reliable source.</td>
<td>Try to decontaminate items.</td>
</tr>
<tr>
<td></td>
<td>Check for mycoplasma.</td>
<td>Get from source far removed from originator.</td>
</tr>
<tr>
<td></td>
<td>Validate origin.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Keep records.</td>
<td></td>
</tr>
<tr>
<td>Exporting cell lines</td>
<td>Check for mycoplasma.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Validate origin.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Send data sheet.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Triple wrap.</td>
<td></td>
</tr>
<tr>
<td>Glassware</td>
<td>Keep stocks separate.</td>
<td>Use for chemicals,</td>
</tr>
<tr>
<td>Flasks</td>
<td>Vent briefly if stacked.</td>
<td>Stack too high.</td>
</tr>
<tr>
<td>Media and reagents</td>
<td>Swab bottles before placing them in hood.</td>
<td>Share among cell lines.</td>
</tr>
<tr>
<td></td>
<td>Open only in hood.</td>
<td>Share with others.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pour.</td>
</tr>
<tr>
<td>Pipettes</td>
<td>Use plugged pipettes.</td>
<td>Use the same pipette for different cell lines.</td>
</tr>
<tr>
<td></td>
<td>Change if contaminated.</td>
<td>Share with other people.</td>
</tr>
<tr>
<td></td>
<td>Use plastic for agar.</td>
<td>Overfill disposal cylinders.</td>
</tr>
</tbody>
</table>
Contamination

**Culture media** provide an excellent environment for the growth of microbial contaminants. **Common cell culture procedures** provide ample opportunities for contamination.

Characteristics of microbial contamination:
- Sudden change in pH
  - usually a decrease with most bacterial infection
  - very little change with yeast until contamination is heavy
  - Sometimes an increase in pH with fungal contamination
- Cloudiness in medium
- Specific phenomena under microscope
- **Mycoplasma** (tiny prokaryotic cells with no cell wall, 0.3-0.5 μm) infections cannot be detected by the naked eye other than by signs of deterioration in the culture.
  - Traditionally detected using Hoechst 33258
Note that Microbial infection may be confused with precipitates of media constituents or with cell debris, but can be distinguished by their regular morphology.
General Methods in Cell Culture

- Cell Quantification
  - Hemacytometer
  - Electronic Particle Counting
- Determination of Cell Viability
  - Dye Exclusion Test
  - Dye Uptake
  - MTT Assay
- Subculture of Cells
- Storage of Cells
Cell Quantification

- **Hemacytometer**
  - An optically flat chamber used to determine the concentration of a cell suspension with the aid of a microscope
  - Hemacytometer counting is cheap and gives you the opportunity to see what you are counting.
  - Slow
  - Prone to error both in the method of sampling and sampling size.

- **Electronic Particle Counting**
  - Machine-counting
  - More reproducible than hemacytometer
Determination of Cell Viability

- **Dye Exclusion Test** – viable cells do not take up certain dyes, whereas dead cells are permeable to these dyes
  - *Trypan blue*; cell suspension is mixed with dye (> 3 min and < 10 min)
  - Concerns with membrane integrity

- **Dye Uptake**
  - *diacetyl fluorescein* -> fluorescein (live cells fluoresce green)
  - *Propidium iodide* (PI) (dead cells fluoresce red)
  - *Ethidium bromide* (dead cells fluoresce red)
Determination of Cell Viability

- MTT Assay – based on the concept that (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium (MTT), a yellow water-soluble tetrazolium dye, is reduced by live, but not dead, cells to a water-insoluble, purple formazan product.
Subculture of Cells

Fig. 12.2. Growth Curve and Maintenance. Semilog plot of cell concentration versus time from subculture, showing the lag phase, the exponential phase, and a plateau, and indicating times at which subculture and feeding should be performed.
Subculture of Cells

- Adhesive Cells
  - removal of medium
  - trypsinization
  - removal of trypsin
  - re-suspend in medium
  - cell counting
  - seeding in new dish/flask
  - cells adhesion and spreading

- Suspension Cells
Storage of Cells

Long-term storage of cells at low $T$ is now routine.

- **Ice crystals** can form within cells during freezing
- **Cryoprotective agents**
  - 5-10% Dimethylsulfoxide (DMSO)
  - 10-15% Glycerol
- In general, **slow freezing** and **rapid thawing** is recommended for maximum survival.
  - Rate of cooling (generally 1 °C/min)
    - *cell dehydration* versus *intracellular freezing*
  - Rate of thawing
- **SLOWLY** add medium to the thawed cell suspension to avoid osmotic damage
Measurement of Cell Characteristics

- Cell Morphology
- Cell Number and Viability
- Measuring Cell Motility
- Cell Function
- Mechanical Properties of Individual Cells
Cell Morphology

- The form and structure of cells is collectively described as morphology
- Fibroblastic-like (elongated and branched)
- Epithelial-like (rounded or cobblestone-like)
- Quantitative cell morphology

\[
\text{shape index} = \frac{4\pi A}{P^2}
\]

where \( A \) is the area of the cell and \( P \) the perimeter

- Cell orientation; assigning a major axis
Cell Migration

- Boyden filter transmigration assay
  - Pore size of the membrane (~3-8 μm)
- Zigmond chamber
  - Able to see migrating cells; improved stability of chemokine gradient
- Dunn chemotaxis chamber
  - Avoid the edge effects associated with the linear bridge of Zigmond chamber
- Under agarose assay
Cell Function

- Cell proliferation – BrdU assay, etc.
- Cell differentiation – DNA microassay, etc.
- Apoptosis – TUNEL, etc.
- Quantification mRNA expression - RT-PCR, etc.
- Quantification protein expression – protein (western) blotting, etc.
- Quantification of enzyme activity – zymography, etc.
- Localization of protein expression – Immunofluorescence, etc.
Gene Expression at the mRNA level

Fig. 3.39. Representation of the detection of active viruses using RT–PCR.
Gene Expression at the protein level
Mechanical Properties of Individual Cells

- Indentation with a pipette or atomic force microscope tip
- Aspiration by negative pressure at the end of a micropipette
- Compression with optical tweezers
- Detachment by shear stress

- Contractile forces generated by cells can be illustrated by “traction force microscopy”
### TABLE 9.4
A summary of devices and methods used to study the effects of physical forces on animal cells in culture. From [139].

<table>
<thead>
<tr>
<th>Name of device/method of developing force</th>
<th>Primary force</th>
<th>Spatial distribution of forces</th>
<th>Fluid flow in and out of device?</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parallel-disk viscometer</td>
<td>Shear stress</td>
<td>Shear stress directly proportional to radial position</td>
<td>No</td>
<td>Possible presence of secondary flows</td>
</tr>
<tr>
<td>Cone-and-plate viscometer</td>
<td>Shear stress</td>
<td>Ideally, shear stress is not a function of position</td>
<td>No</td>
<td>Possible presence of secondary flows</td>
</tr>
<tr>
<td>Parallel-plate flow chamber</td>
<td>Shear stress</td>
<td>Shear stress is not a function of position; pressure varies linearly as a function of position, though this variation is often ignored as it is very small</td>
<td>Yes</td>
<td>Possible presence of not fully developed laminar flow where fluid enters device</td>
</tr>
<tr>
<td>Radial flow chamber</td>
<td>Shear stress</td>
<td>Shear stress varies as a function of position, being greatest near the center of the device</td>
<td>Yes</td>
<td>Presence of not fully developed laminar flow where fluid enters device</td>
</tr>
<tr>
<td>Uniaxial stretch</td>
<td>Tensitional stress</td>
<td>Greatest at the center of the device</td>
<td>No</td>
<td>May also generate shear stress due to motion of cells relative to fluid</td>
</tr>
<tr>
<td>Biaxial stretch</td>
<td>Tensitional stress</td>
<td>Greatest at the edge of the device</td>
<td>No</td>
<td>May also generate shear stress due to motion of cells relative to fluid</td>
</tr>
<tr>
<td>Compression of gas phase by addition of an inert gas (such as helium)</td>
<td>Pressure</td>
<td>Uniform</td>
<td>No</td>
<td>Change in concentration of dissolved gas due to nonideal behavior</td>
</tr>
<tr>
<td>Direct compression of liquid phase</td>
<td>Pressure</td>
<td>Uniform</td>
<td>No</td>
<td>No gas phase present</td>
</tr>
</tbody>
</table>
Cell Separation

It is often desired to purify cells of interest.

**Depletion** (or **ablation**): removal of unwanted cell types
**Enrichment** (or **purification**): positive selection for a desire cell type

Examples:
- Removal of contaminating tumor cells in an autologous bone-marrow transplant
- Removal of contaminating fibroblasts in a cartilage biopsy
- Selection of epithelial hepatic cells, CD34+ bone-marrow cells.
Basis for Cell Separation

There are many cellular properties that can be used as the basis for separation.

- Physical Properties
  - Size – size exclusion
  - Density - centrifugation
  - Light-scatter – flow cytometry

- Biochemical Properties
  - Cytoplasmic marker
  - Surface marker
Characterizing Cell Separation

There are two primary measures used to characterize cell-separation processes.

- **Yield**: the number of desired cells following separation divided by the number of the same cells in the starting material.
- **Purity**: the fraction of the desired cells in the end product.

\[ N_{in} = D_{in} + C_{in} \quad N_{out} = D_{out} + C_{out} \]

\[ \text{purity} = \frac{D_{out}}{N_{out}} \quad \text{yield} = \frac{D_{out}}{D_{in}} \]
Practice of Cell Separation

There are several cell-separation methods that can be broadly grouped into two categories.

- Treating Population of Cells
  - Centrifugal elutriation
  - Discontinuous centrifugation
  - Immunoaffinity-based cell separation

- Treating Cells Individually
  - Flow cytometry
  - Scanning cytometry
Centrifugal Elutriation
This method is based on cell size and density
Discontinuous Centrifugation

Cells can be placed in suspension ($\rho_1$) above a denser fluid ($\rho_2$). If a centrifugal force is then applied, cells that are denser than $\rho_2$ will collect at the bottom, while cells that have densities in between $\rho_1$ and $\rho_2$ will collect at the interface.
Immunoaffinity-Based Cell Separation

These methods rely on specific binding of surface proteins on the desirable cell type to its ligand (e.g., antibody or lectins). The ligand is then bound to a solid support (e.g., magnetic beads) for separation.

- Magnetic beads
- Solid surface-panning
- Column chromatography
Flow Cytometry

used to analyze and separate cells based on their light-scattering or fluorescent characteristics

Mechanical vibration breaks the jet of fluid into tiny droplets containing a single cell

Every droplet that fulfills sort criteria is charged.

When passing between parallel charged plates, charged droplets are swing out of their trajectories and collected.

Cells can be sorted at fairly high speed (> $10^4$/s)