Lab#4 Introduction to Cell culture and media preparation

Cell Culture is the art of growing cells *in vitro*. It is most common method for viral cultivation and growth of viruses.

Types of cultures and cell lines

The more differentiated the cell line, the slower it will grow.

Cell cultures are described in two ways:

- **Classification by origin of the cells**

Primary Cells

Primary cells are freshly isolated cells that are directly derived from the tissue of origin. The tissue source of the majority of the primary cell cultures is either laboratory animals or human pathology specimens. Healthy young or embryonic animals are preferable to adults.

Continuous cell lines

Cell lines are continuously growing subcultures of the primary cells. Most cell lines can be propagated for a limited number of cell generations (*finite cell line*), beyond which they either die out or give rise to continuous cell lines.

Continuous cell lines are often manipulated to become transformed cell lines expressing particular phenotype with many propertied of cancer cells. Some of these cell lines have actually been derived from tumors or transformed spontaneously in culture by mutation. Cells can be purposefully transformed by a chemical or by a tumor-inducing virus, which carries a gene that induces overproduction of an aberrant protein needed for growth. As a result, the transformed cells have their function, morphology, and growth characteristics altered. Below are some characteristics of transformed cells:

1. Growth to high cell density
2. Lower requirement for growth factors and serum
3. More anchorage independence (do not adhere tightly to tissue culture dishes).
4. Ability to proliferate indefinitely.

- **Classification by manner of growth**

Suspension cells grow suspended in the growth medium. They are able to survive and proliferate without attachment to the culture vessel. Cells cultured from blood, spleen or bone marrow, especially immature cells, tend to grow in suspension. The advantages of suspension growth are the large numbers of cells that can be achieved, and the ease of harvesting.

Adherent cells grow in monolayer, attached to the surfaces of the culture vessel. Cells that derive from ectodermal or endodermal embryonic cell layers tend to grow adherently. The advantage of adherent growth is the ability of the cells to adhere and spread on surfaces such as coverslips, making microscopy, hybridizations, and functional assays.

Cell Medium

Most labs either buy the medium *already prepared and bottled,* or as a *powder that must be rehydrated and filter sterilized.* Of course, the latter choice is less expensive, and especially makes sense if the medium is being used in bulk.

Commercially prepared medium has an expiration date, which should be nonrigidly adhered to. Use expired medium for cell washes.

Cell media look alike, since most contain phenol red or another dye as a pH indicator. But they are not alike, so don't just use what medium is available. Check the *formulation* you need, and order it from a company that guarantees its media to be mycoplasma-free.

Medium with a phenol red pH indicator will look

Lemon yellow below	pH 6.5
Yellow at	pH 6.5
Orange at	pH 7.0
Red at	pH 7.4
Pink at	pH 7.6
Purple at	pH 7.8

Most cells grow best when medium is around pH 7.4.

Yellowish medium is *acidic* and can indicate
- An overgrown culture
- Bacterial contamination
- Too much CO_2 in the incubator

Purplish medium is *alkaline* and can indicate
- A sparse and non-growing culture
- Mold contamination
- Too little CO_2 in the incubator

Some cells require the addition of other components to the prepared medium. L-Glutamine is a common addition, as some cells will quickly exhaust the glutamine in the medium.
Cell culture medium contains many heat labile components so you should store the medium in the cold. However, medium should be warmed to 37°C before being added to cells. Never shock the cells by the addition of cold medium.

Antibiotics

Antibiotics are used standardly in many labs but should not be needed if aseptic technique is being properly done. The half-life of many antibiotics is quite short at 37 °C. With valuable cells or cells prone to contamination because of a lot of manipulation, it is sometimes too worrying not to include antibiotics in the medium.

Antifungal agents amphotericin B (Fungizone) and Mycostatin (Nystatin) are not recommended for routine use, because they can affect the membrane permeability of all eukaryotic cells.

Antibiotics for cell culture are usually obtained in a sterile vial. Rehydrate with sterile water or solvent.

Two standard antibiotics for cell culture

Gentamicin 5–10 mg/ml stock, final 50 µg/ml
Penicillin (10,000 units)/**Streptomycin** (10 mg/ml) stock, final 100 units/ml and 100 µg/ml

Aliquot in 1-ml tubes and store at –20°C. Add 1 ml of either solution to 99 ml of medium. If you use large quantities of media, make up 5-ml aliquots to add to 500-ml bottles of medium.

Serum

Serum supplies needed growth factors and nutrients. Some cells, particularly transformed cells, have a very low serum requirement of around 0.5%. Some cell lines have been "trained" to survive in medium with low serum. As the needed components for serum are defined, more and more cells can be cultured with supplements and individually added components. This is a fortunate situation for you if your cells can be cultured without serum.

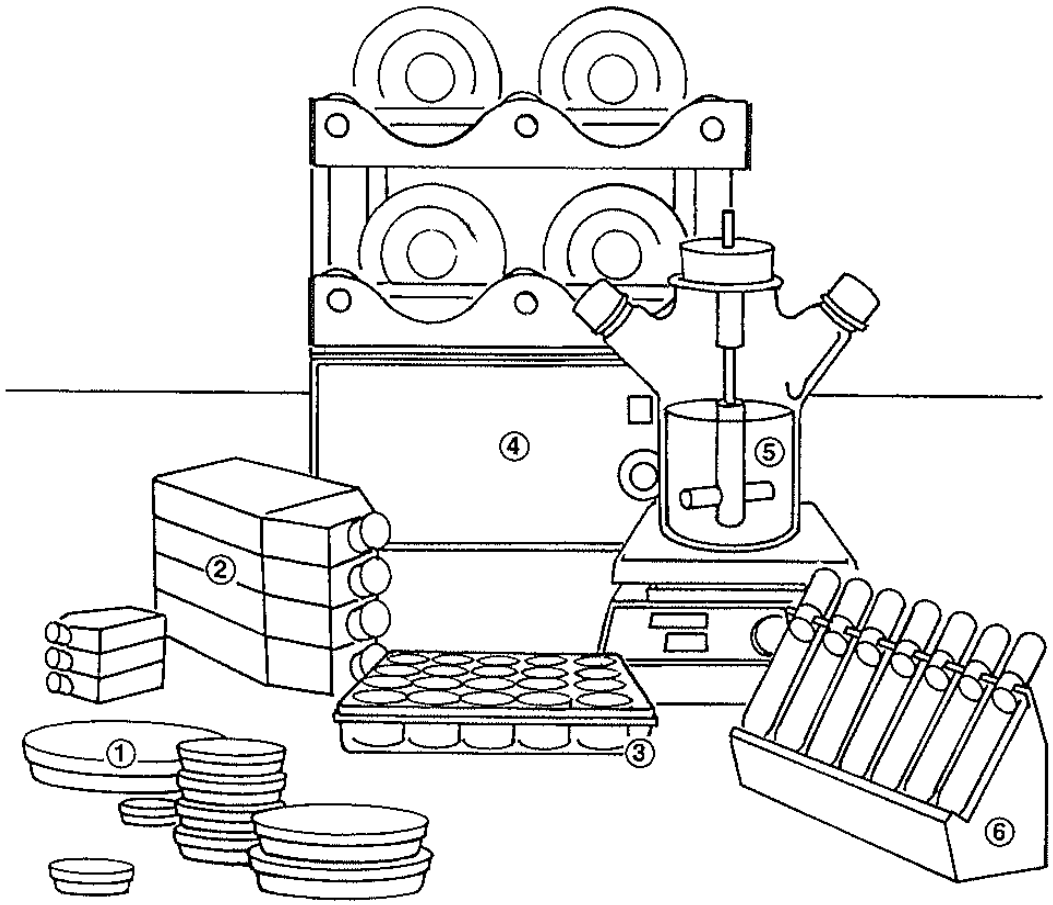
Serum is very expensive. Always aliquot and freeze serum, and add it to medium just before use. Store unused portions of thawed aliquots in the refrigerator, where it will be fine for several weeks.

These are the serum variables you must consider:

- **The percentage of serum.** Most cells require 5–20% in the medium for good growth.
- **The type of serum.** Some cells like horse serum. The standard for tissue culture cells is calf serum. Some cells require the more expensive fetal calf (also known as fetal bovine) serum, and some cells (usually human) require serum of their own species, the most expensive serum proposition of all.
- **Whether or not the serum is heat-inactivated.** Serum is subjected to heat to inactivate components such as complement.

To heat-inactivate serum

Thaw the frozen serum at room temperature. This may take 5 or 6 hours for a 500-ml bottle of serum. Incubate the thawed serum at 65°C for 30 minutes. Aliquot the serum. Aliquot for the percentage of serum and the amount of medium you usually use. Freeze and store. Thaw aliquots in a 37°C water bath as needed.



Cell culture containers. Key: (1) *Dishes.* Generally used for adherent cells, most are treated to maximize attachment of cells. The 100-mm size, which holds 10 ml, should not be confused with bacterial dishes of the same size, as bacterial dishes are not treated for cell attachment. The 60-mm size holds 4–5 ml, and the 35-mm, 1–2 ml. (2) *Flasks.* Available with straight or canted necks: Straight necks minimize sloshing, which is good for suspension cultures, and canted necks permit easier access to the culture surface, which is useful when manipulating adherent cells. However, either neck type can be used for either adherent or suspension cultures. 25 cm^2 (50 ml), 75 cm^2 (250 ml), 175 cm^2 (750 ml) are common sizes. (3) *Multiple-well tissue culture plates.* A standard size of 86 X 128 mm is divided into 6-, 12-, 24-, 48-, or 96-well sizes, and is compatible with automatic diluters and plate readers. These are used for hybridoma and monoclonal antibody work, titrations, toxicity testing, and any experiment that requires a comparison of different cell treatments. They can be used for adherent and nonadherent cells. Plates are usually purchased treated, and some sizes are available with flat or round-bottomed wells: Most instruments require flat-bottomed wells. (4) *Roller bottles.* Usually used for maximum yield. Adherent or suspension cells can be grown in roller bottles. Can be used in an open or closed system. (5) *Spinner bottles.* Designed to spin gently, without subjecting cells to harsh mixing. Used for suspension and microcarrier cell cultures, as well as for insect cultures. Different gas mixtures can be added, and the bottles can be used in an open or closed system. (6) *Tubes.* For adherent cells. Round bottoms allow adherence on all parts of the tube. Leighton tubes have one side flattened, permitting microscopic observation.