**Cell Culture Media**

The culture medium provides the necessary nutrients, growth factors, and hormones for cell growth, as well as regulating the pH and the osmotic pressure of the culture. Initially, natural media was used to perform cell culture experiments that were obtained from tissue extracts and body fluids. Later on, the need for standardization, media quality, and increased demand led to the development of defined media. There are three basic classes of media i.e. basal media, reduced-serum media, and serum-free media, which differ in their requirement for supplementation with serum.

Serum is vitally important as a source of growth and adhesion factors, hormones, lipids and minerals for the culture of cells in basal media. In addition, serum also regulates cell membrane permeability and serves as a carrier for lipids, enzymes, micronutrients, and trace elements into the cell. However, using serum in media has a number of disadvantages including high cost, problems with standardization, specificity, variability, and unwanted effects such as stimulation or inhibition of growth and/or cellular function on certain cell cultures.

1. **Basal Media**: The majority of cell lines grow well in basal media, which contain amino acids, vitamins, inorganic salts, and a carbon source such as glucose, but these basal media formulations must be further supplemented with serum.
2. **Reduced-Serum Media**: Another strategy to reduce the undesired effects of serum in cell culture experiments is to use reduced-serum media. Reduced-serum media are basal media formulations enriched with nutrients and animal-derived factors, which reduce the amount of serum needed.
3. **Serum-Free Media**: Serum-free media (SFM) circumvents issues with using animal sera by replacing the serum with appropriate nutritional and hormonal formulations. Serum-free media formulations exist for many primary cultures and cell lines including recombinant protein producing lines, various hybridoma cell lines, few insect lines (Sf9 and Sf21) and for cell lines that act as hosts for viral production (VERO, MDCK, MDBK), and others. One of the major advantages of using serum-free media is the ability to make the medium selective for specific cell types by choosing the appropriate combination of growth factors.

**Following factors must be taken care of while preparing cell culture media**:

* **pH**: Most normal mammalian cell lines grow well at pH 7.4, and there is very little variability among different cell strains. However, some transformed cell lines have been shown to grow better at slightly more acidic environments (pH 7.0–7.4), and some normal fibroblast cell lines prefer slightly more basic environments (pH 7.4–7.7). Insect cell lines such as Sf9 and Sf21 grow optimally at pH 6.2.
* **CO2**: The growth medium controls the pH of the culture and buffers the cells in culture against changes in the pH. Usually, this buffering is achieved by including an organic (e.g., HEPES) or CO2-bicarbonate based buffer. Because the pH of the medium is dependent on the delicate balance of dissolved carbon dioxide (CO2) and bicarbonate (HCO3–), changes in the atmospheric CO2 can alter the pH of the medium. Therefore, it is necessary to use exogenous CO2 when using media buffered with a CO2-bicarbonate based buffer, especially if the cells are cultured in open dishes or transformed cell lines are cultured at high concentrations. While most researchers usually use 5–7% CO2 in air, 4–10% CO2 is common for most cell culture experiments. However, each medium has a recommended CO2 tension and bicarbonate concentration to achieve the correct pH and osmolality.
* **Temperature**: The optimal temperature for cell culture largely depends on the body temperature of the host from which the cells were isolated, and to a lesser degree on the anatomical variation in temperature (e.g., temperature of the skin may be lower than the temperature of skeletal muscle). Overheating is a more serious problem than underheating for cell cultures; therefore, often the temperature in the incubator is set slightly lower than the optimal temperature. Different cell cultures require varied range of temperature for carrying out the cell culture successfully and few examples are depicted as under:

1. Most **human and mammalian cell lines** are maintained at 36°C to 37°C for optimal growth.
2. **Insect cells** are cultured at 27°C for optimal growth; they grow more slowly at lower temperatures and at temperatures between 27°C and 30°C. Above 30°C, the viability of insect cells decreases, and the cells do not recover even after they are returned to 27°C.
3. **Avian cell lines** require 38.5°C for maximum growth. Although these cells can also be maintained at 37°C, they will grow more slowly.
4. Cell lines derived from **cold-blooded animals** (e.g., amphibians, cold-water fish) tolerate a wide temperature range between 15°C and 26°C.

Since the cell culture conditions vary for each cell type, it is very important to study the cell line of interest thoroughly and design the experiment accordingly.

Reference: A handbook on Cell Culture Basics