BIOASSAY

By: Dr. Mayur Savaliya,
Lecturer, Clinical Research,
IISHLS, Indus University.

BIOLOGICAL STANDARDIZATION OF VACCINES

- **Biological standardisation** may be defined as the quantitative estimation of active substances by means of their biological actions in cases where chemical or physical methods are inadequate or unavailable.

CELL CULTURE TECHNOLOGY

- Cell culture technology refers to the use of specific systems and emergent trends for the in vitro culture of cells under controlled environmental conditions.
- It is a multidisciplinary area, involving basic concepts of cellular and molecular biology, as well as applied engineering concepts, which has gained prominence in modern biotechnology.
- Since its establishment in the early 19th century, considerable progress has taken place, making this technology a major industrial platform for the production of therapeutic products such as recombinant proteins, monoclonal antibodies and the cell-derived ones.

ANIMAL CELL CULTURES

- Cell culture refers to the process by which cells are grown in a controlled artificial environment.
- In animal cell culture technique, cells are removed from an animal and grown subsequently in a favourable environment.
- For animal cell culture the cells are taken from the organ of an experimental animal. The cells may be removed directly or by mechanical or enzymatic action.
- The cells can also be obtained by previously made cell line or cell strain.
- Examples of cells used to culture are fibroblast, lymphocytes, cells from cardiac and skeletal tissues, cells from liver, breast, skin, and kidney and different types of tumor cells.

TYPES OF ANIMAL CELL CULTURE

1) PRIMARY CELL CULTURE

a) Adherent cells
b) Suspension cells

2) CELL LINES

a) Finite cell lines
b) Continuous cell lines

GROWTH REQUIREMENTS

- The culture media used for animal cell cultures are generally quite complex, and culture condition widely varies for each cell type.
- However, media generally include amino acids, vitamins, salts (maintain osmotic pressure), glucose, a bicarbonate buffer system (maintains a pH between 7.2 and 7.4), growth factors, hormones, O_2 and CO_2.
- To obtain best growth, addition of a small amount of blood serum is usually necessary, and several antibiotics, like penicillin and streptomycin are added to prevent bacterial contamination.
- Temperature varies on the type of host cell. Most mammalian cells are maintained at 37^{o}C for optimal growth, while cells derived from cold- blooded animals tolerate a wider temperature range (i.e. 15^{o}C to 26^{o}C). Actively growing cells of log phage should be used which divide rapidly during culture.

- One of the most important uses of cell culture is in research and production of vaccines.
- The ability to grow large amounts of virus in cell culture eventually led to the creation of the polio vaccine, and cells are still used today on a large scale to produce vaccines for many other diseases, like rabies, chicken pox, hepatitis B, and measles.
- In early times, researchers had to use live animals to grow poliovirus, but due to the development of cell culture technique they were able to achieve much greater control over virus production and on a much larger scale which eventually develop vaccines and various treatments.
- However, continuous cell lines are not used in virus production for human vaccines as these are derived from malignant tissue or possess malignant characteristics.

ALTERNATIVES TO ANIMAL SCREENING

- Alternatives to animal screening are the development and implementation of test methods that avoid the use of live animals.
- There is widespread agreement that a reduction in the number of animals used and the refinement of testing to reduce suffering should be important goals for the industries involved.
- Two major alternatives to in vivo animal testing are in vitro cell-culture techniques and in silico computer simulation. However, some claim they are not true alternatives because simulations use data from prior animal experiments and cell cultures often require animal derived products, such as serum or cells.
- Another alternative is so-called micro-dosing, in which the basic behaviour of drugs is assessed using human volunteers receiving doses well below those expected to produce whole-body effects.
- While micro-dosing produces important information about pharmacokinetics and pharmacodynamics, it does not reveal information about toxicity or toxicology.

- Guiding principles for more ethical use of animals in testing are the Three Rs(3 Rs) first described by Russell and Burch in 1959. These principles are now followed in many testing establishments worldwide.
- **Replacement** refers to the preferred use of non-animal methods over animal methods whenever it is possible to achieve the same scientific aim.
- **Reduction** refers to methods that enable researchers to obtain comparable levels of information from fewer animals, or to obtain more information from the same number of animals.
- **Refinement** refers to methods that alleviate or minimize potential pain, suffering, or distress, and enhance animal welfare for the animals used.

PATCH-CLAMP TECHNIQUE

- The **patch clamp technique** is a laboratory technique in electrophysiology used to study ionic currents in individual isolated living cells, tissue sections, or patches of cell membrane.
- The technique is especially useful in the study of excitable cells such as neurons, cardiomyocytes, muscle fibres, and pancreatic beta cells.
- Patch clamping can be performed using the voltage clamp technique.
- In this case, the voltage across the cell membrane is controlled by the experimenter and the resulting currents are recorded.

MOLECULAR BIOLOGY

- **Molecular biology** is the branch of biology that concerns the molecular basis of biological activity in and between cells, including synthesis, modification, mechanisms and interactions.

TECHNIQUES

- MOLECULAR CLONING
- POLYMERASE CHAIN REACTION
- GEL ELECTROPHORESIS
- MICROARRAYS
- ALLELE-SPECIFIC OLIGONUCLEOTIDES

TRANSGENIC ANIMALS

- A transgenic animal is one whose genome has been altered by the transfer of a gene or genes from another species or breed.
- Transgenic animals are routinely used in the laboratory as models in biomedical research. Over 95 per cent of those used are genetically modified rodents, predominantly mice. They are important tools for researching human disease, being used to understand gene function in the context of disease susceptibility, progression and to determine responses to a therapeutic intervention.
- Mice have also been genetically modified to naturally produce human antibodies for use as therapeutics.

- Transgenic farm animals are also being explored as a means to produce large quantities of complex human proteins for the treatment of human disease. Such therapeutic proteins are currently produced in mammalian cell-based reactors, but this production process is expensive.
- Only two biomedical products have so far received regulatory approval. The first is human antithrombin III, a therapeutic protein produced in the milk of transgenic goats, which is used to prevent clots in patients with hereditary antithrombin deficiency receiving surgery or undergoing childbirth.
- The second product is a recombinant human C12 esterase inhibitor produced in the milk of transgenic rabbits. This is used to treat hereditary angioedema, a rare genetic disorder which causes blood vessels in the blood to expand and cause skin swellings.

IMMUNOASSAY

- An **immunoassay** is a biochemical test that measures the presence or concentration of a macromolecule or a small molecule in a solution through the use of an antibody or an antigen.
- The molecule detected by the immunoassay is often referred to as an "analyte" and is in many cases a protein, although it may be other kinds of molecules, of different size and types, as long as the proper antibodies that have the adequate properties for the assay are developed.
- Analytes in biological liquids such as serum or urine are frequently measured using immunoassays for medical and research purposes.
- Immunoassays come in many different formats and variations. Immunoassays may be run in multiple steps with reagents being added and washed away or separated at different points in the assay.

- Multi-step assays are often called separation immunoassays or heterogeneous immunoassays.
- Some immunoassays can be carried out simply by mixing the reagents and sample and making a physical measurement. Such assays are called homogeneous immunoassays, or less frequently non-separation immunoassays.
- The use of a calibrator is often employed in immunoassays. Calibrators are solutions that are known to contain the analyte in question, and the concentration of that analyte is generally known.
- Comparison of an assay's response to a real sample against the assay's response produced by the calibrators makes it possible to interpret the signal strength in terms of the presence or concentration of analyte in the sample.