Biology | Immunology

ELISPOT assay (Dot ELISA)

General Aim
The quantification of the number of cytokine secreting cells.

Method
EliSPOT

Learning Objectives (ILOs)
- To understand the concept of ELISPOT assay.
- To practice the steps of ELISPOT assay.

Theoretical Background/Context
- ELISPOT, or enzyme linked immunospot, is a technique that was developed for the detection and quantification of cells that secrete proteins, such as cytokines and growth factors.
- It is most commonly used in immunology research. For example: Transplantation, vaccine development (IFNγ), viral infection monitoring and treatment, Th1/Th2, T cell regulation and monocyte and dendritic cell analyses. It can also be used to study autoimmune diseases, allergies and cancer.

Principle of Work
- ELISPOT is performed using a PVDF (or nitrocellulose) membrane 96-well plate, which is pre-coated with an antibody specific to the secreted protein. Samples (including cells) are added to the plate. Cells are then stimulated to secrete its proteins. The secreted protein binds to the coating antibody. A detection antibody is then added. It is specific to the bound protein. Detection of the antibody complex can be done either enzymatically by the production of a colored substrate, or, fluorescently. Fluorescent tags can identify more than one secreted protein at a time.
- Each secreting cell appears as a spot of color or fluorescence, this allows quantitative evaluation of cells secreting proteins. The membrane can be analyzed either by manually counting the spots, or by an automated reader.