ELISPOT (Enzyme Linked Immunospot technique)

This technique is used for the detection of secreted proteins, such as cytokines and growth factors. It is therefore used primarily in immunology research in the following areas (**Applications of the test**):

- Transplantation
- Vaccine development (IFNγ)
- Th1/Th2, T-cell regulation analysis
- Monocyte and Dendritic cell analysis
- Autoimmune disease
- Cancer – tumor antigens
- Allergy
- Viral infection monitoring and treatment

Principles of the test:

ELISpot assays employ the sandwich enzyme-linked immunosorbent assay (ELISA) technique.

procedure

1. Monoclonal or polyclonal antibody specific for the chosen analyte is pre-coated onto a PVDF (polyvinylidene difluoride)-backed microplate. (e.g Anti- IL-2 antibody)
2. Target cells (e.g T cells) are pipetted into the wells and the microplate is placed into a humidified 37 °C CO_2 incubator for a specified period of time.
3. During this incubation period, the immobilized antibody (anti-IL2) binds secreted analyte (IL-2) from the target cells (TH2 cells).

4. Washing away any cells (other T cells) and unbound substances (e.g. IL-4).
5. Biotinylated polyclonal antibody specific for the chosen analyte is added to the wells.
6. Washing step to remove any unbound biotinylated antibody.
7. Alkaline-phosphatase conjugated to streptavidin is added.
8. Unbound enzyme is subsequently removed by washing
9. Substrate solution (BCIP/NBT) is added.
10. A blue-black colored precipitate forms and appears as spots at the sites of cytokine localization, with each individual spot representing an individual analyte-secreting cell.
11. The spots can be counted with an automated ELISpot reader system or manually, using a stereomicroscope.