Immunodiagnostics:

Cardiovascular system

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Immunodiagnostics

- These are used to assess patients with suspected immunological conditions.
- Clinical situations in which immune system components are assessed include;
  - Infection
  - Immunodeficiency: qualitative and quantitative measurements
  - Allergy: sensitization to allergens
  - Autoimmune disease: autoantibodies and T-cell autoreactivity
  - Transplantation: HLA matching

Immunodiagnostics

There are many immunodiagnostic tests that can be used to assist in the diagnosis of infectious, endocrine, and neoplastic diseases as well as to measure blood drug concentrations. These include;

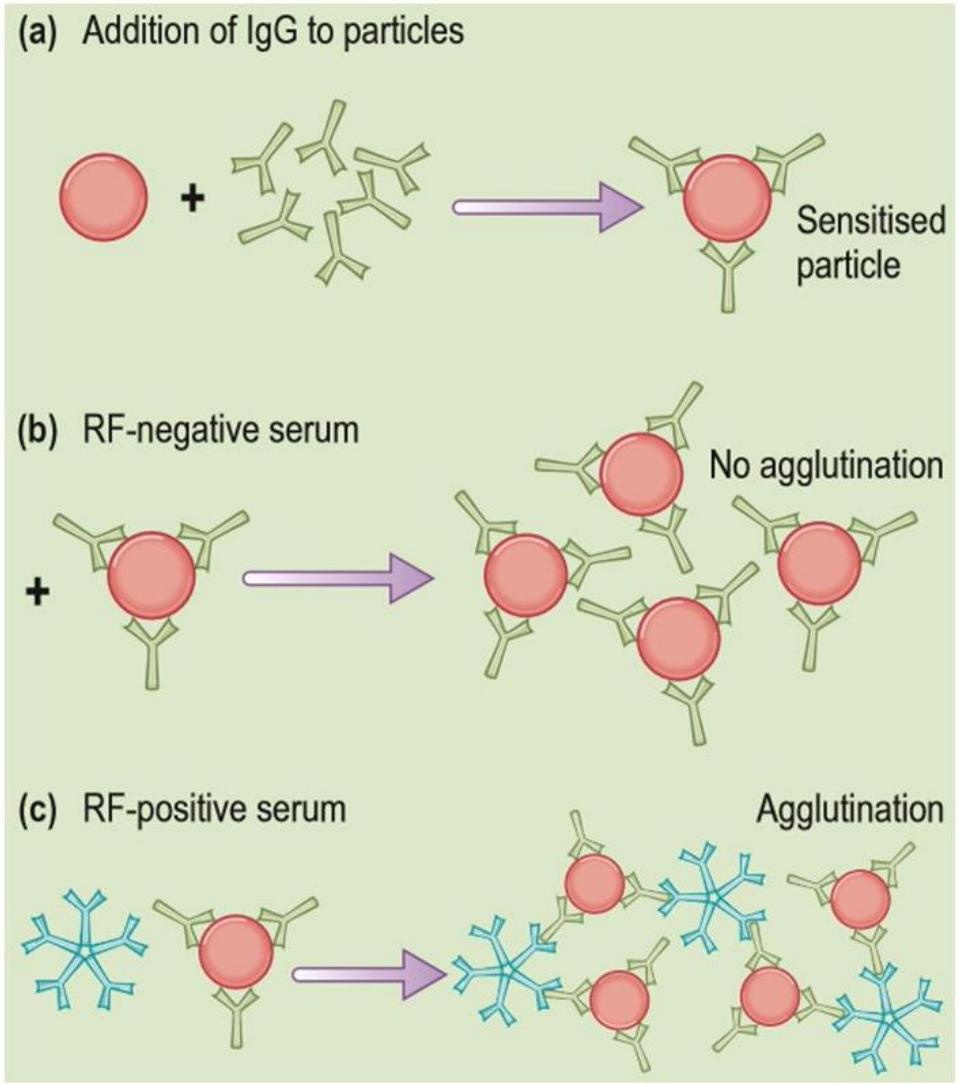
1. Agglutination and precipitation methods
    a) Agglutination of antigen-coated particles
    b) Nepholometry
    c) Immunoprecipitation
    d) Radial immunodiffusion
2. Western blotting
3. ELISA
4. ELISPOT
5. Immunofluorescence
6. Immunohistochemistry
7. Flow cytometry
8. Nitroblue Tetrazolium Test (NBT)
9. T-Cell Receptor Excision Circle

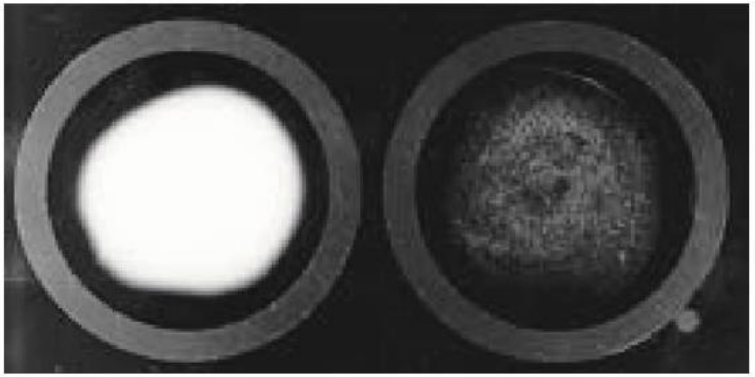
Agglutination and Precipitation

- These methods can be used for the detection of antibodies or antigens. These include;

Method	Use
Agglutination of antigen-coated particles	Detection of specific antibodies
Nephelometry	Quantification of proteins in serum, plasma or cerebrospinal fluid
Immunoprecipitation	Isolation of specific antigen from a mixture of antigens
Radial Immunodiffusion	Quantification of antigen

Agglutination



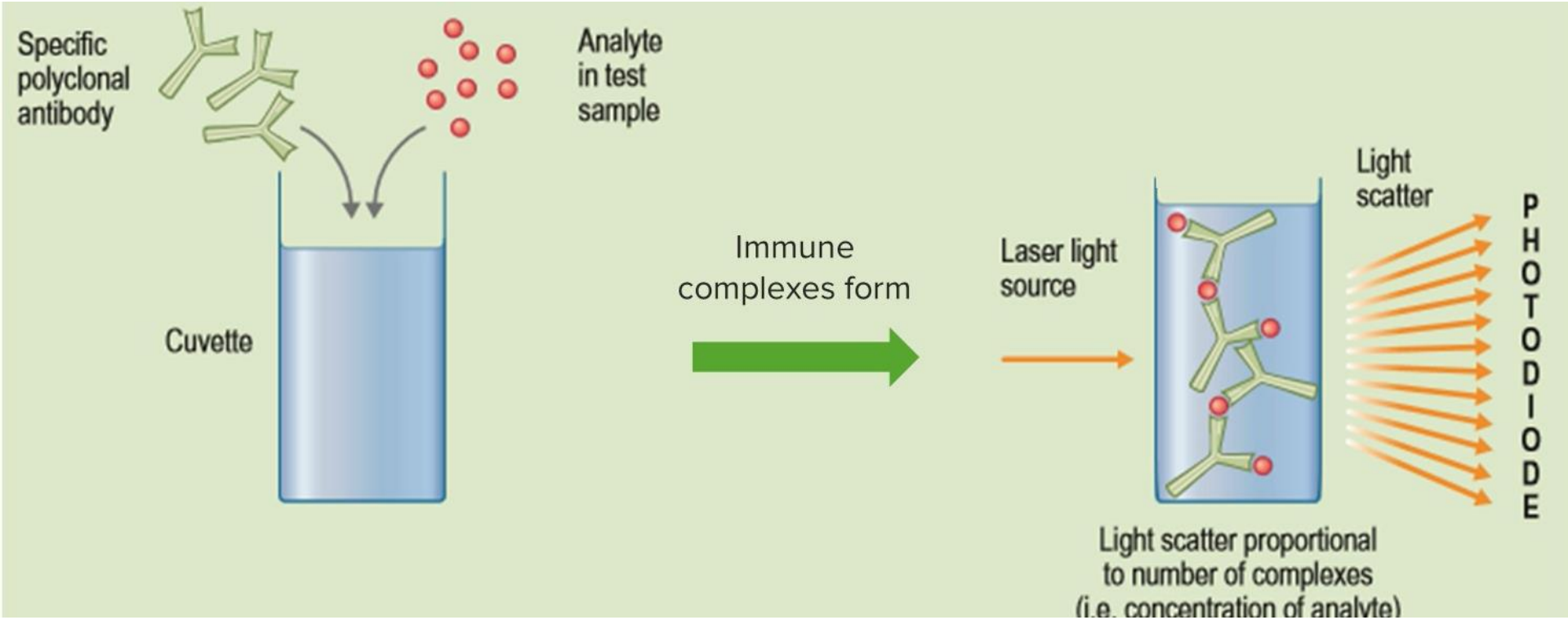


Negative Positive

Example: detection of rheumatoid factors (autoantibodies to Fcγ)

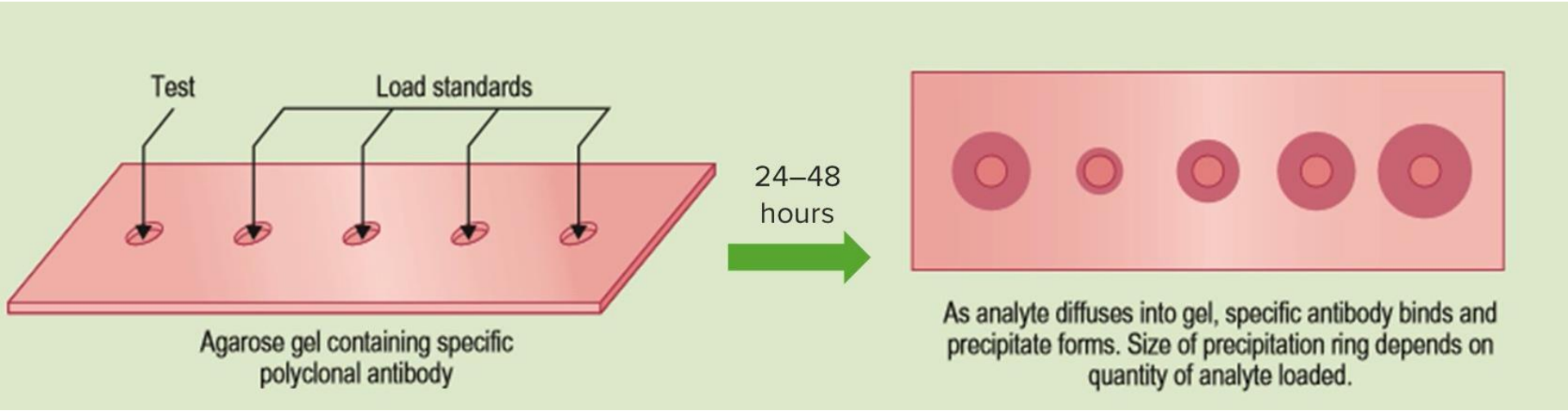
Nephelometry

- The presence of an analyte in a test sample can be detected

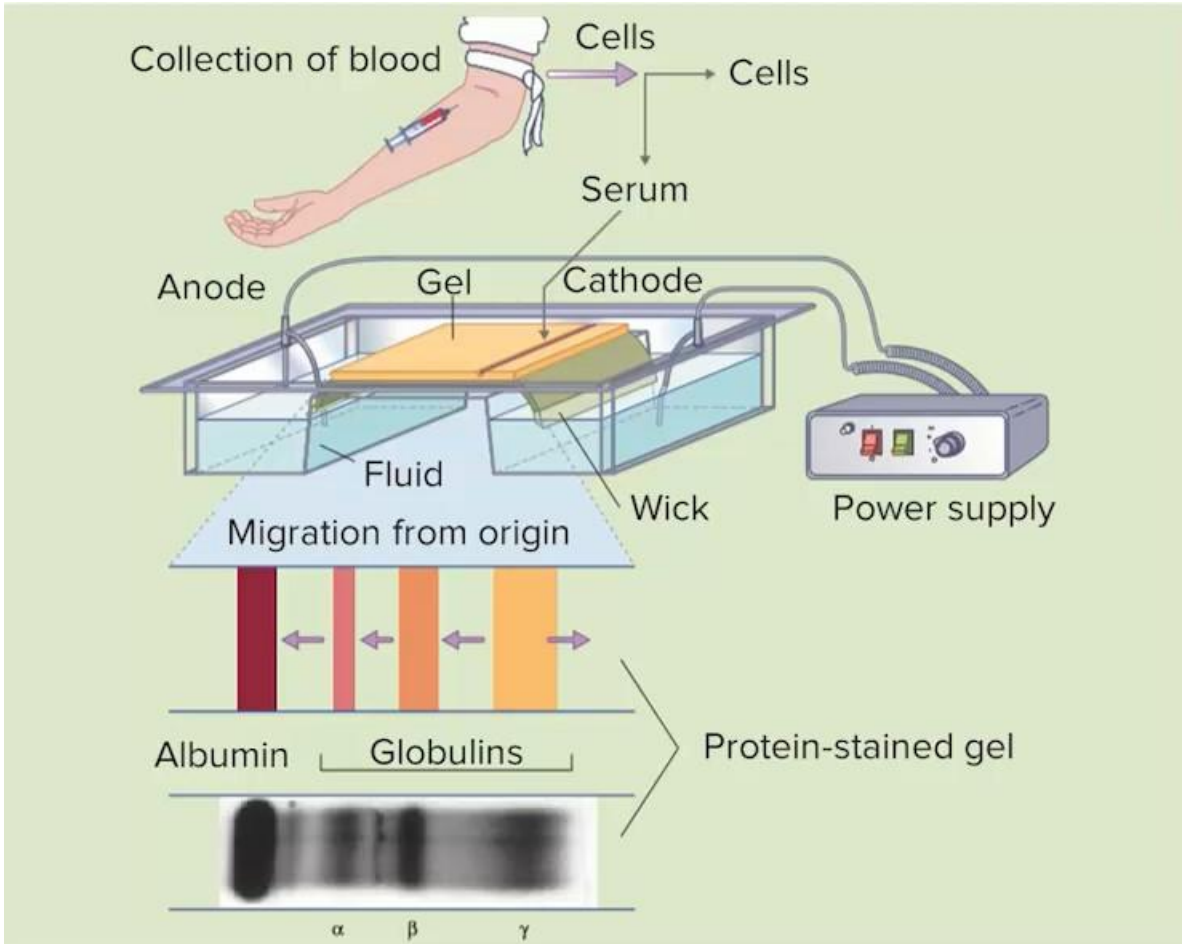


Immunoprecipitation

Radial immunodiffusion



Separation of serum protein by electrophoresis

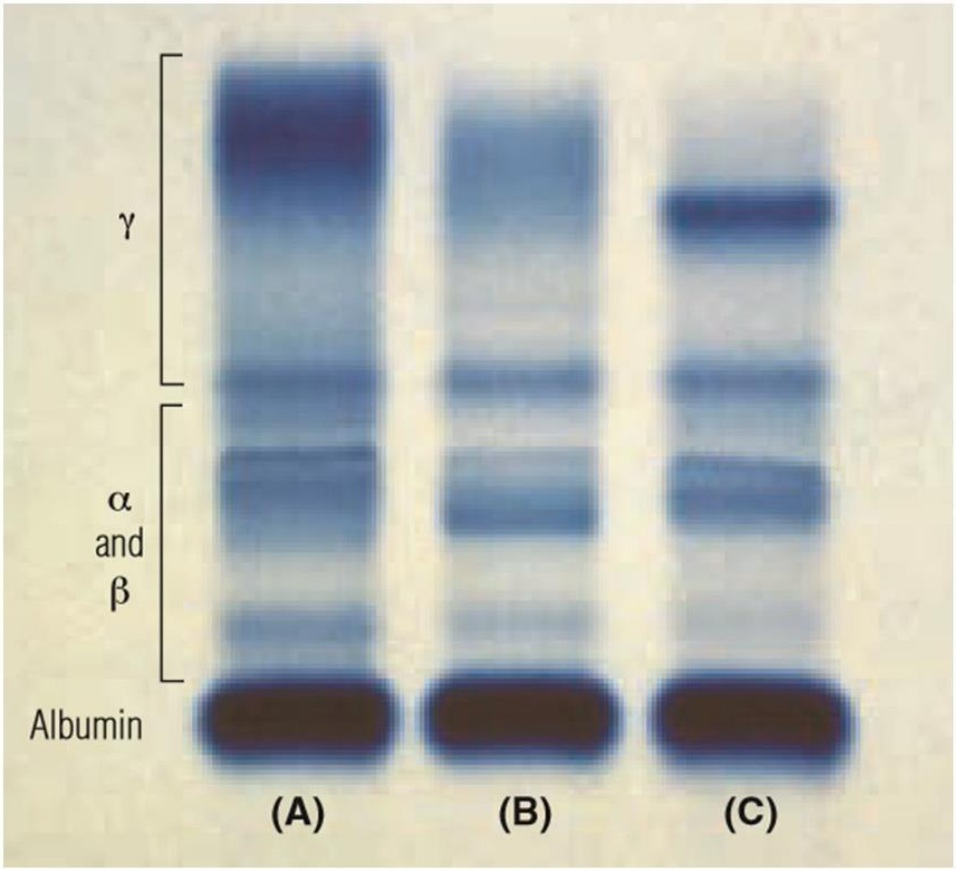


Example of electrophoretic analysis

(A) Patient with polyclonal expansion of B-cells resulting in increased γ-globulins

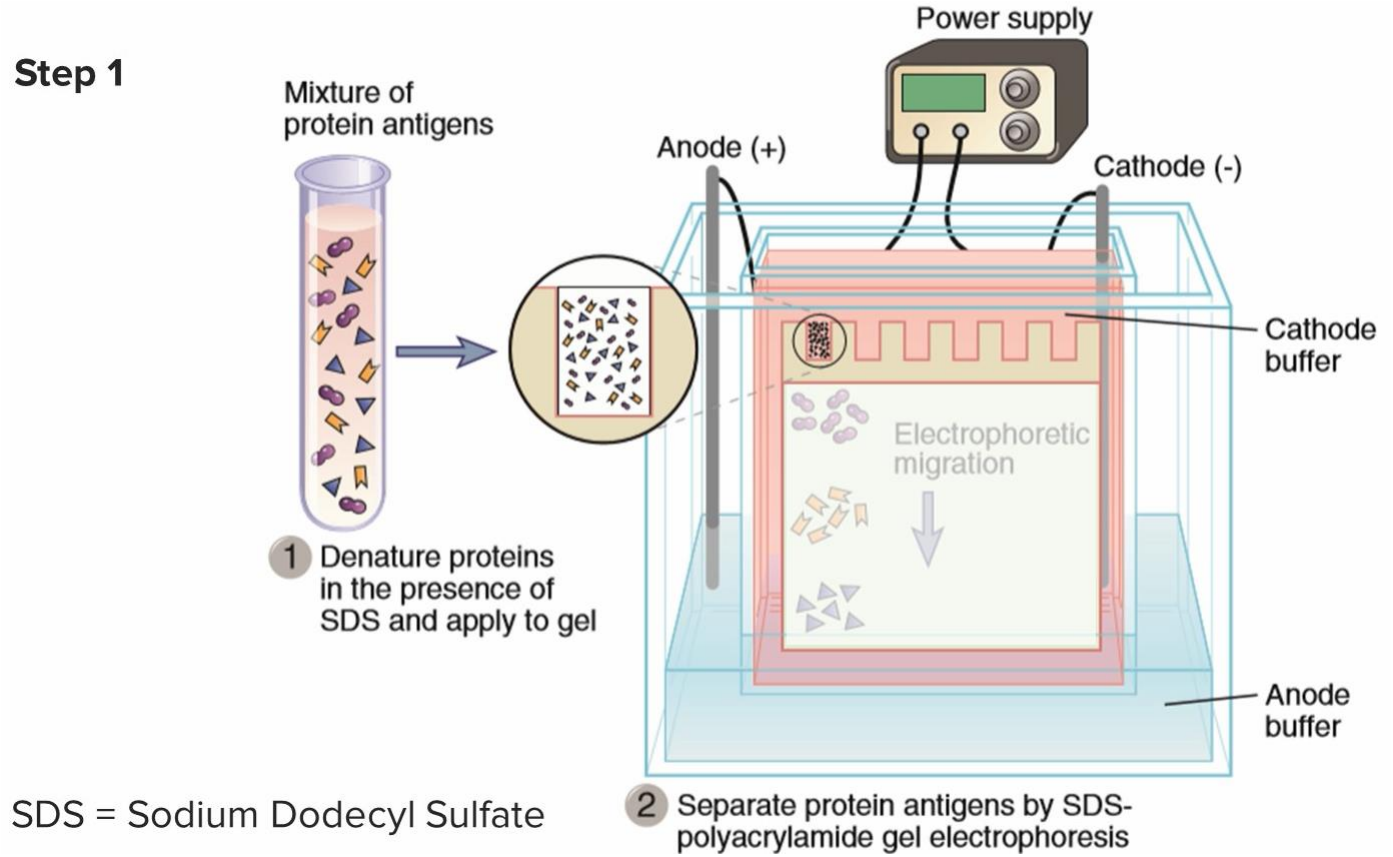
(B) Normal control serum

(C) Patient with a B-cell malignancy



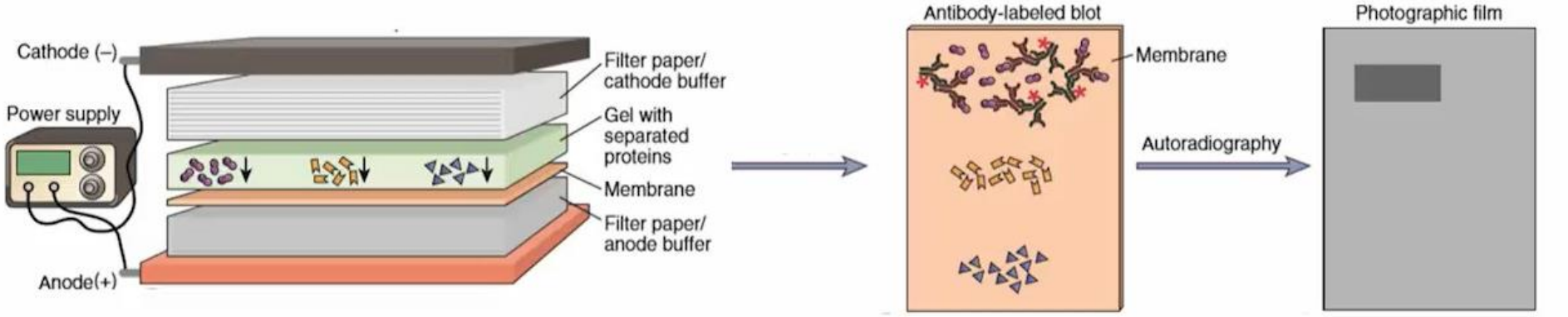
Western blotting

- Technique used for the analysis of proteins



Western blotting

Step 2

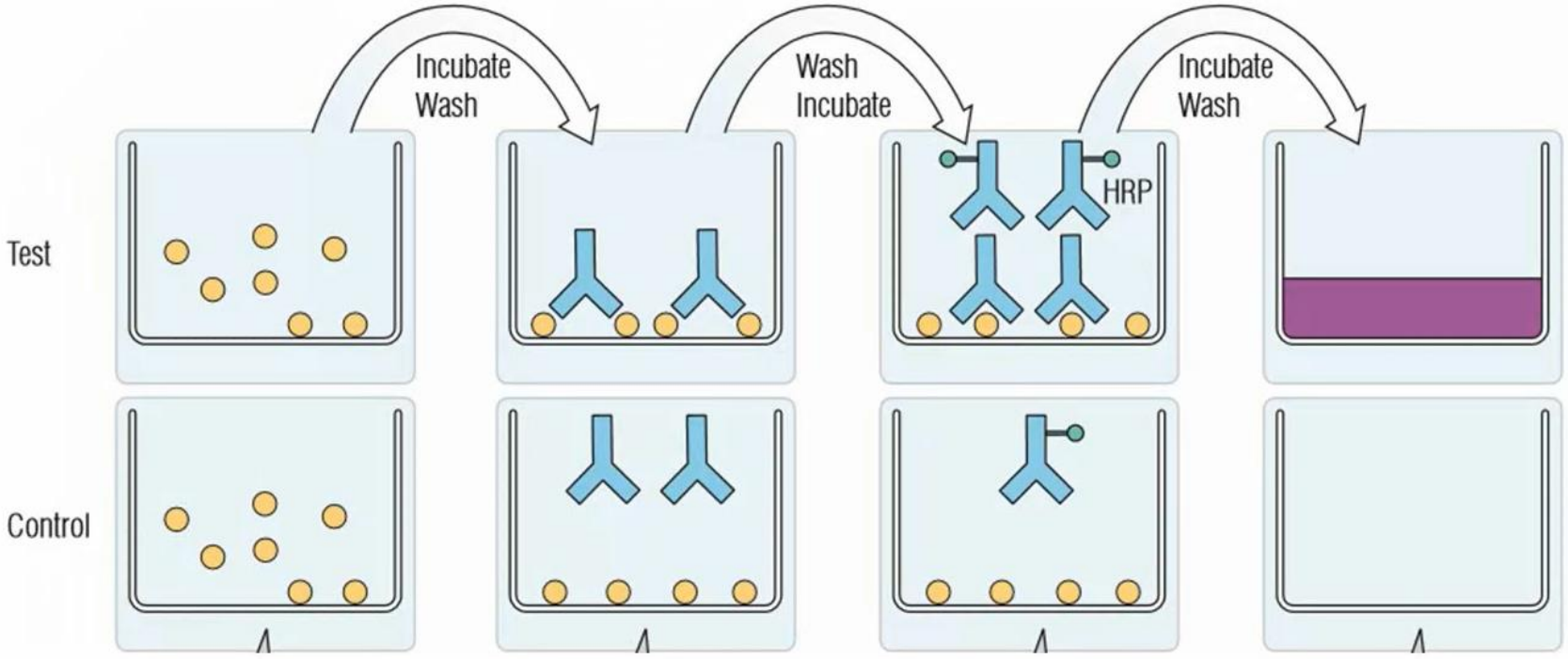


Electrophoretic transfer proteins to membrane

Label proteins in membrane using a primary antibody specific for the antigen of interest, and a secondary one specific for the primary antibody and tagged with an enzyme.
A substrate is added which emits light when cleaved by the enzyme.

Use antibody-labeled membrane with light-emitting substrate added to expose film.

ELISA



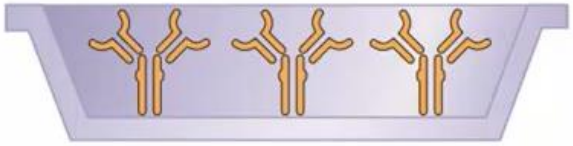
Antigen binds to plastic plate. Excess is washed off.

Test antibody is added and plate washed.

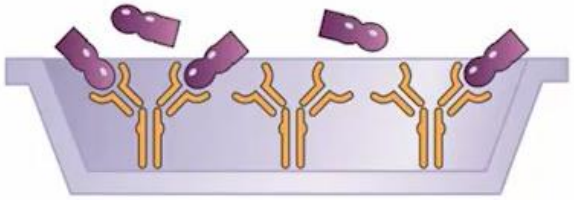
Ligand to the test antibody is added. In this case the ligand is another antibody coupled to the enzyme horseradish peroxidase (HRP).

Substrate (chromagen) is added and a colored product is produced by enzyme reaction. The amount of color is proportional to the amount of antibody bound to antigen.

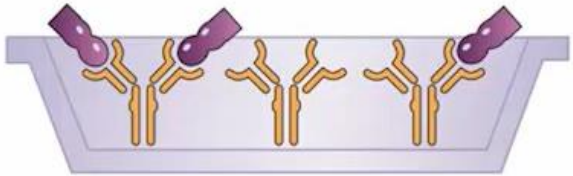
Sandwich immunoassay



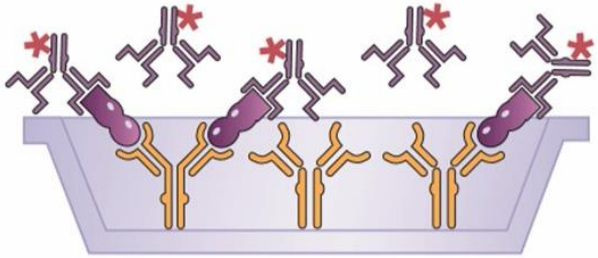
Bind first antibody to well of microtiter plate



Add varying amount of antigen ( )

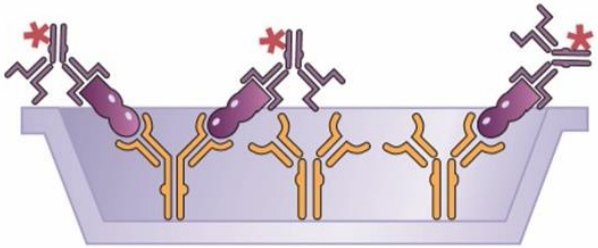


Remove unbound antigen by washing

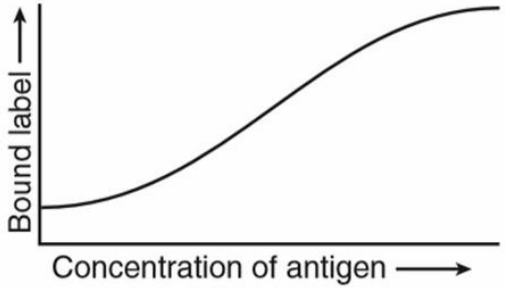


Add labeled second antibody specific for non-overlapping epitopes of antigen

(Labels include radioactive isotope, or an enzyme that causes a substrate to change color or emit light (chemiluminescence))

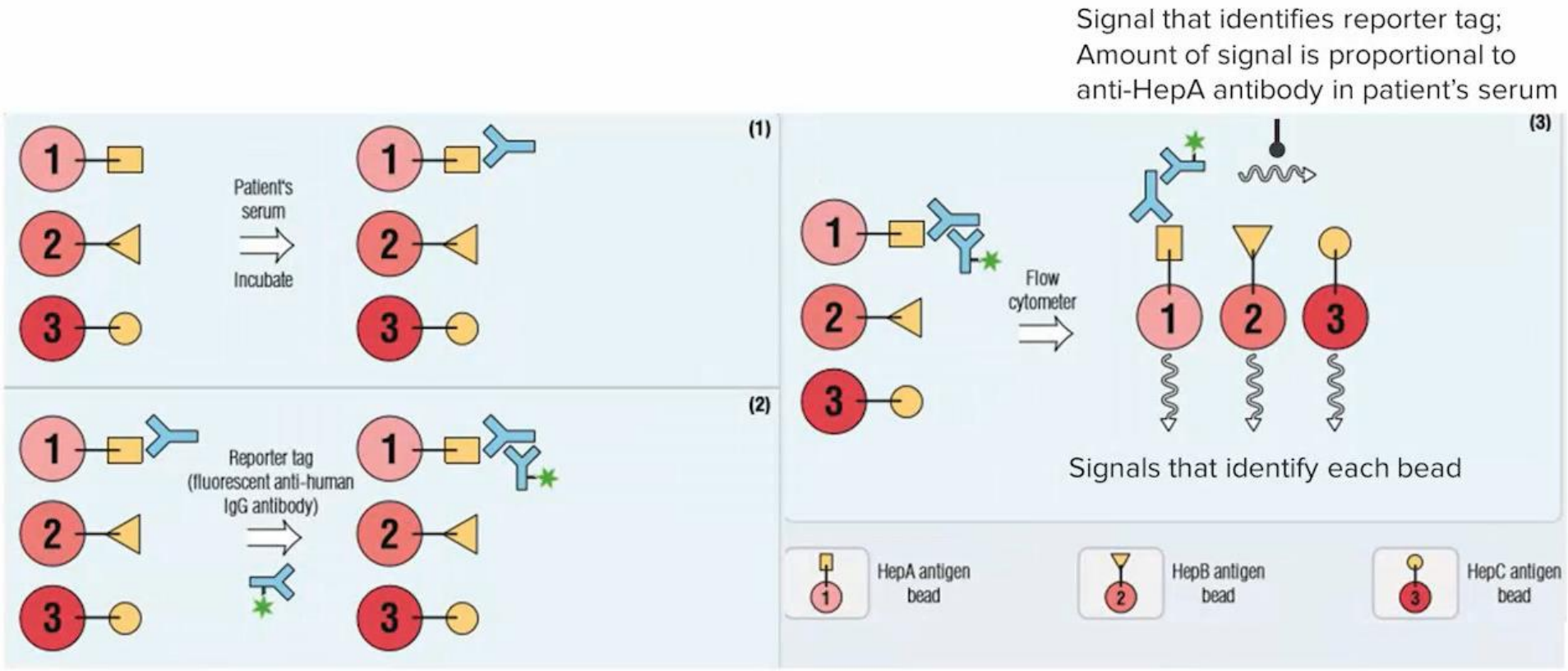


Remove unbound labeled second antibody by washing measure amount of second antibody bound

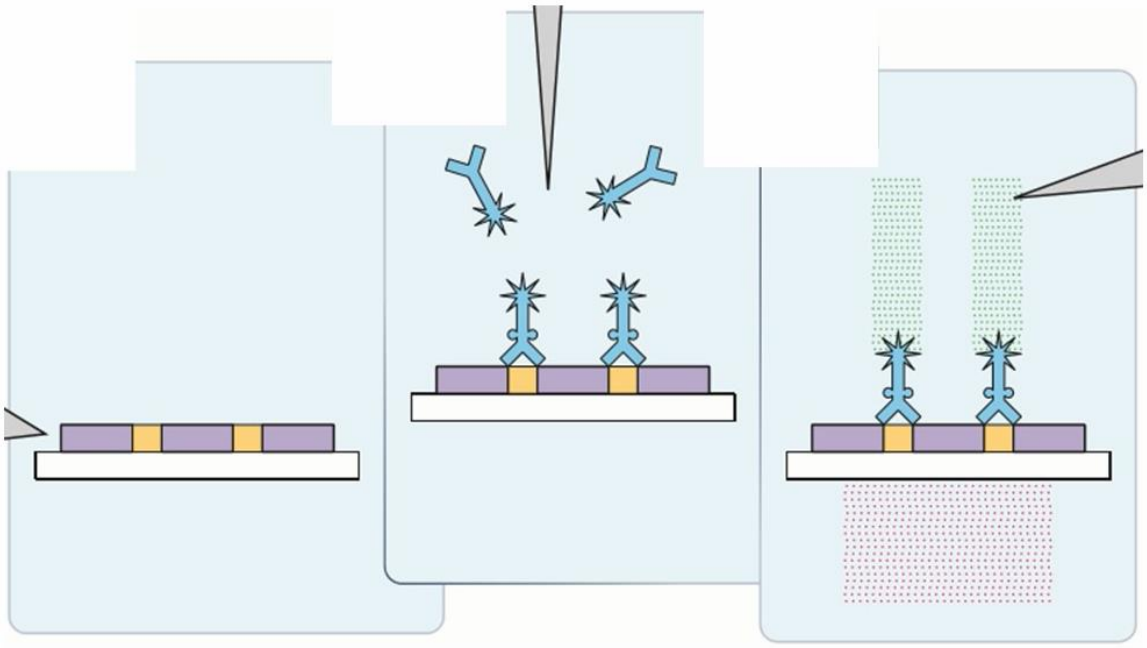


Determine amount of bound second antibody as a function of the concentration of antigen added (construction of a standard curve)

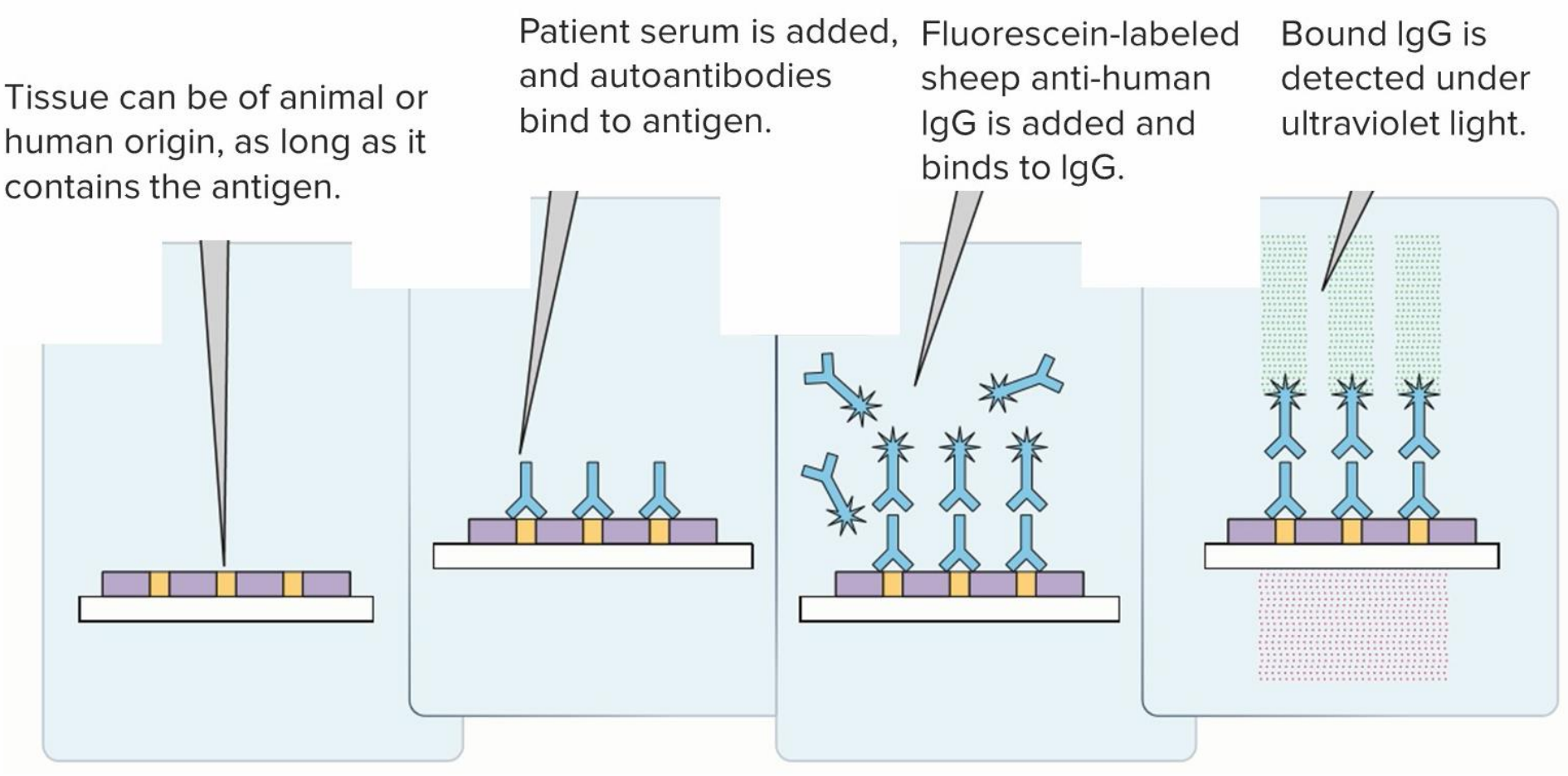
Fluorescent microsphere assay



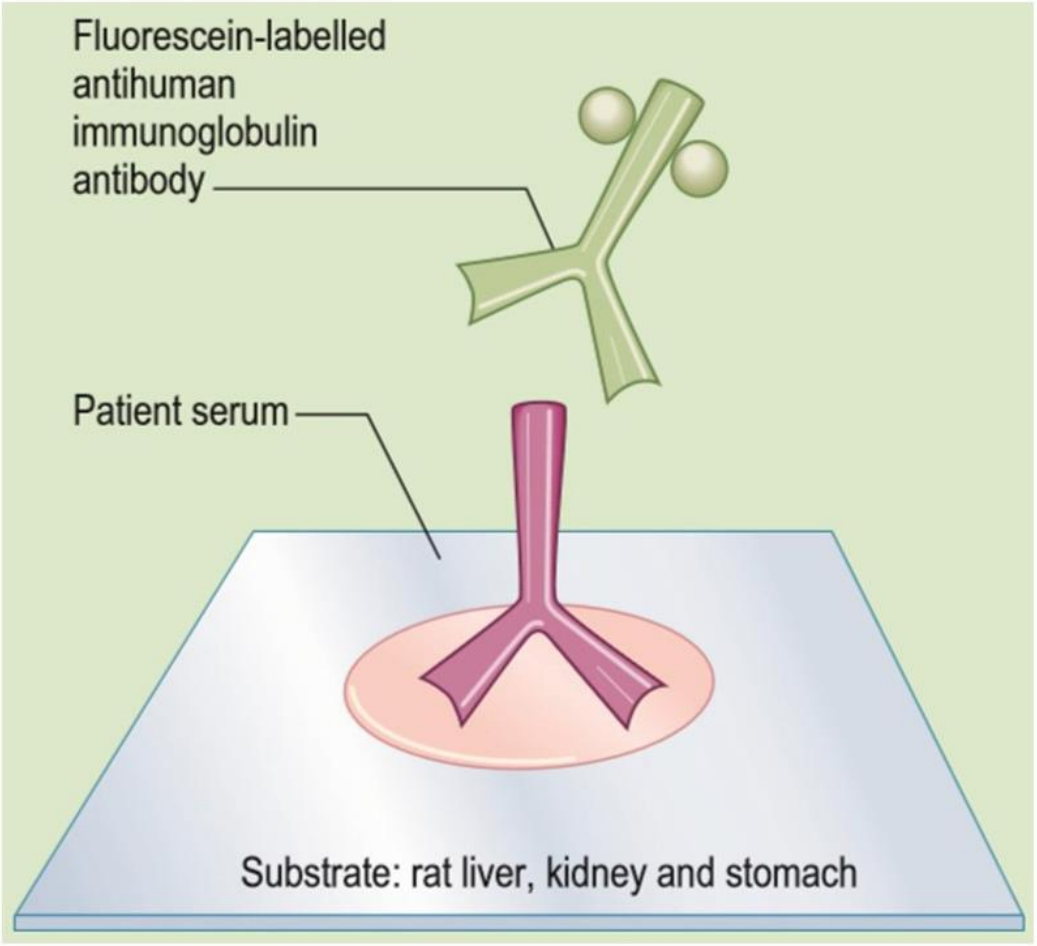
Direct Immunofluorescence

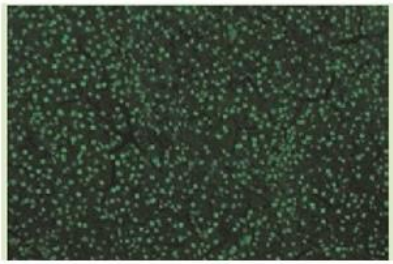


Indirect Immunofluorescence

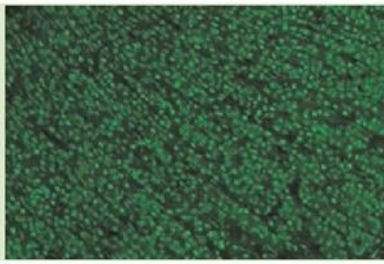


Example: measurement of anti-nuclear antibodies in patients with SLE

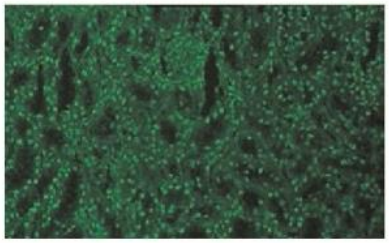




Liver

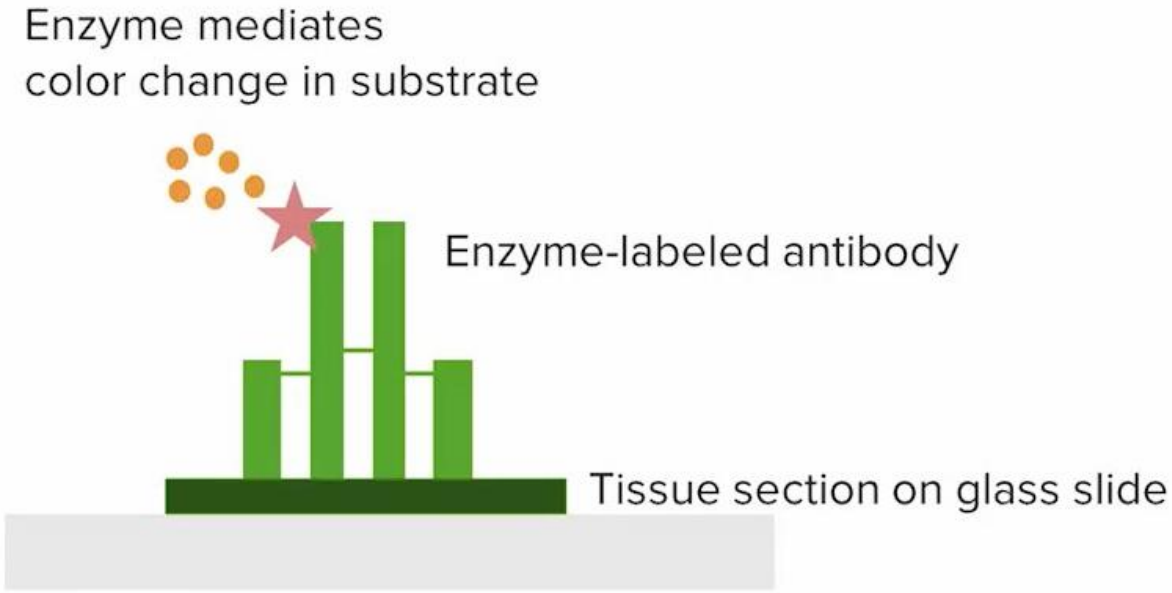


Kidney



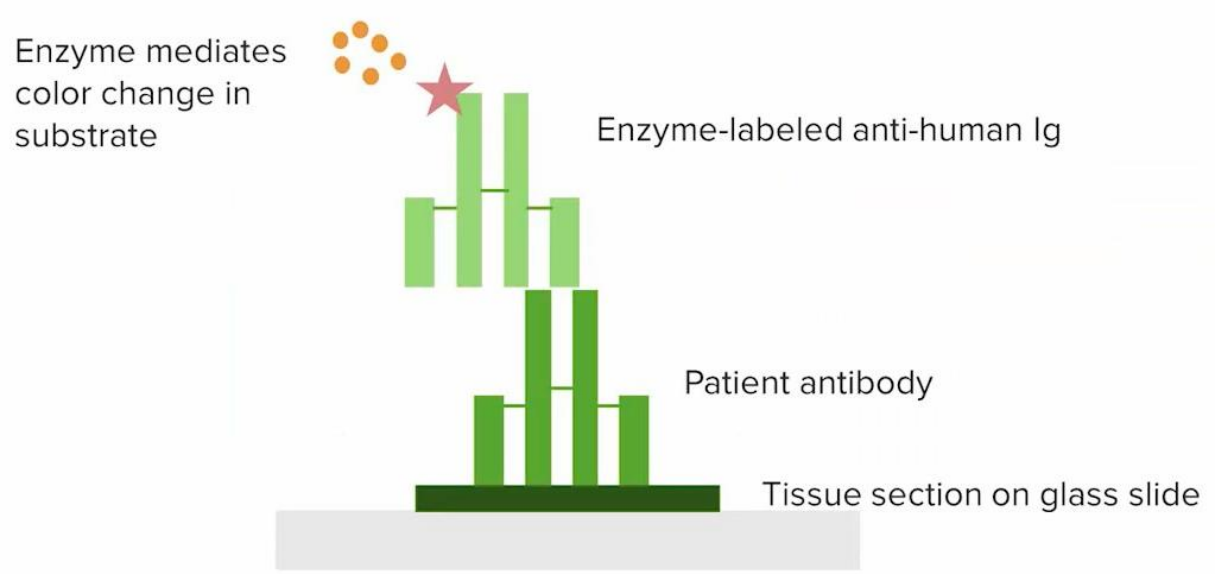
Stomach

Direct Immunohistochemistry

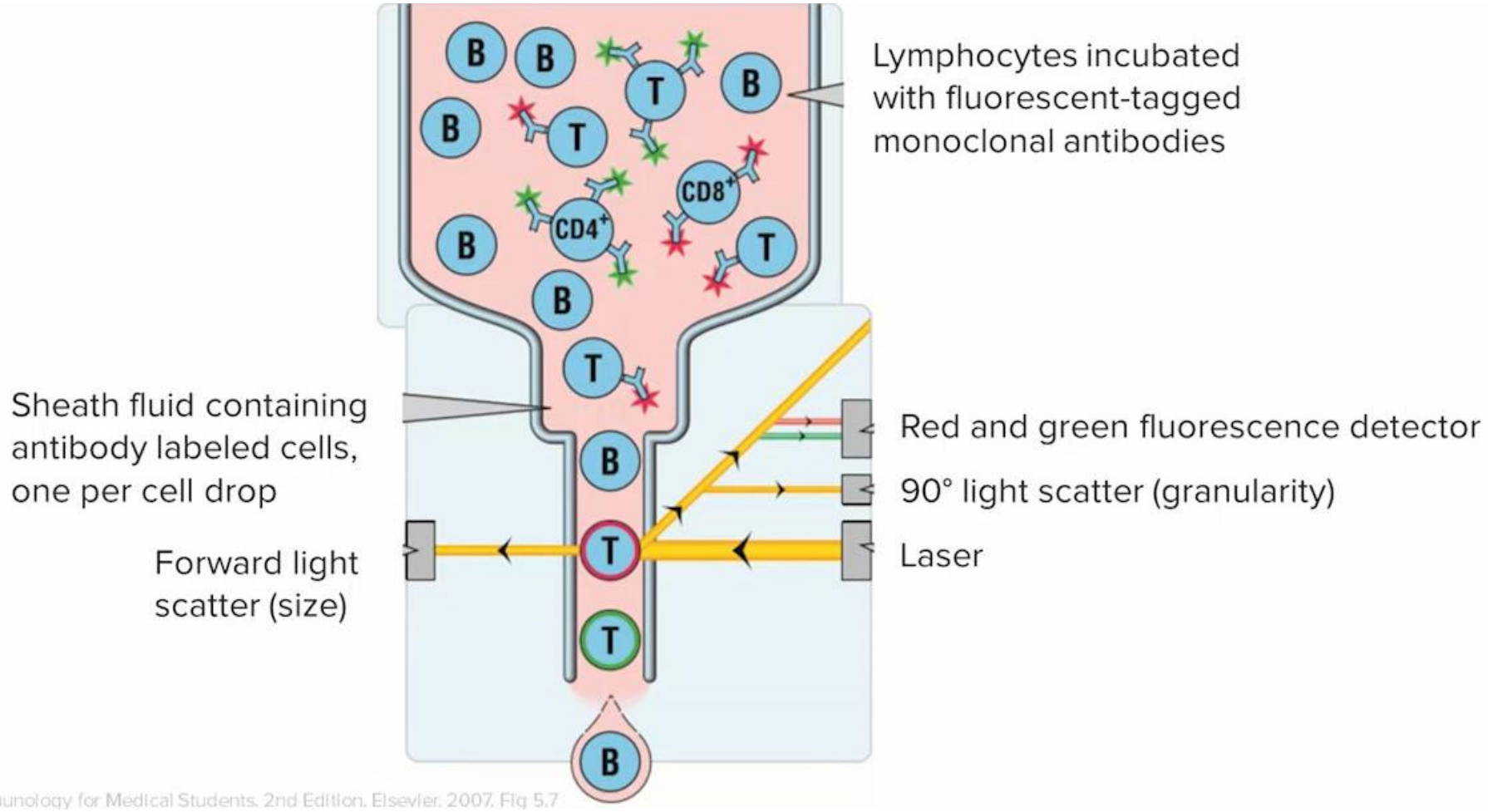


Insoluble substrate deposited

Indirect Immunohistochemistry



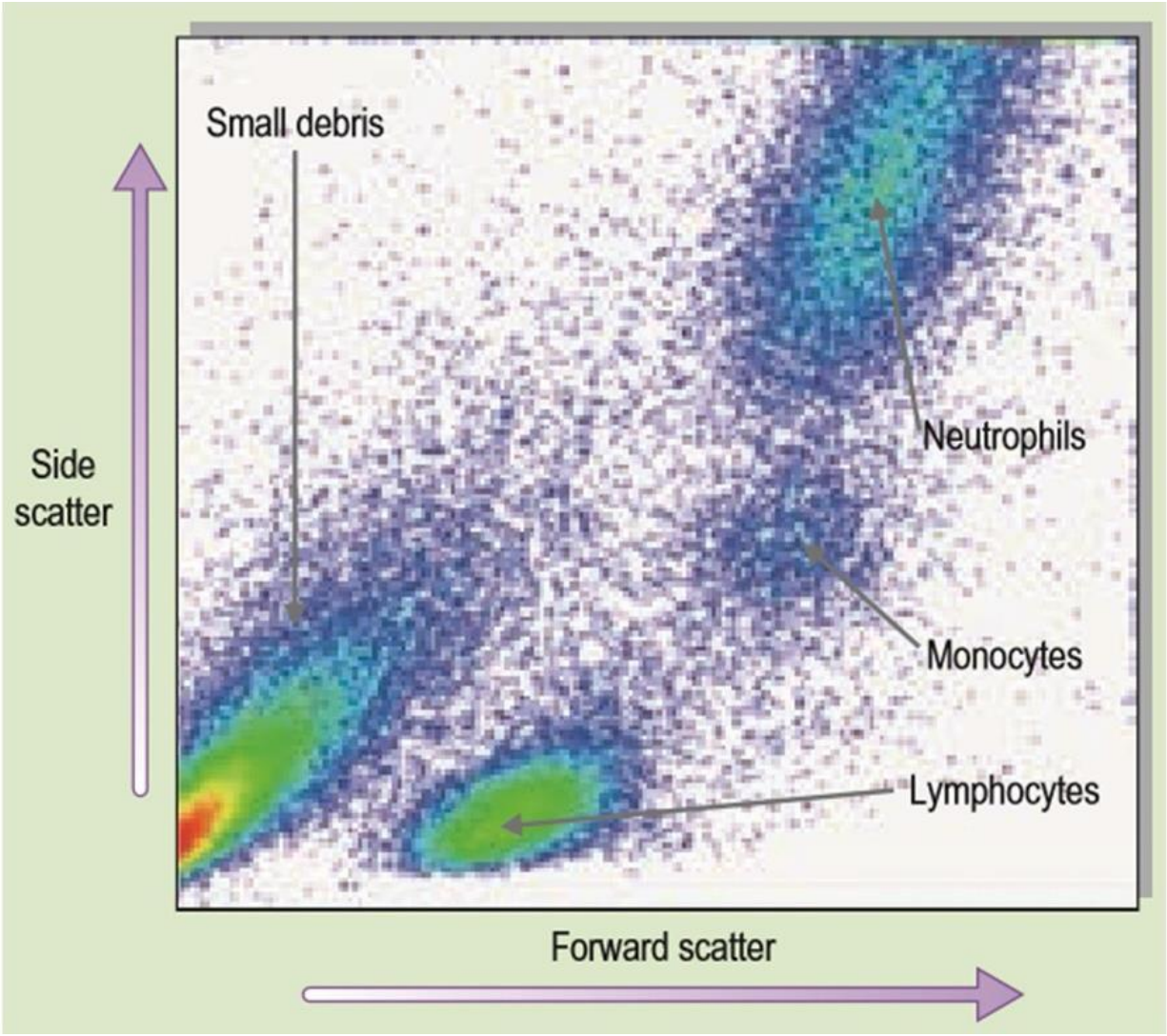
Flow cytometry



unology for Medical Students. 2nd Edition. Elsevier. 2007. Fig 5.7

Flow cytometry

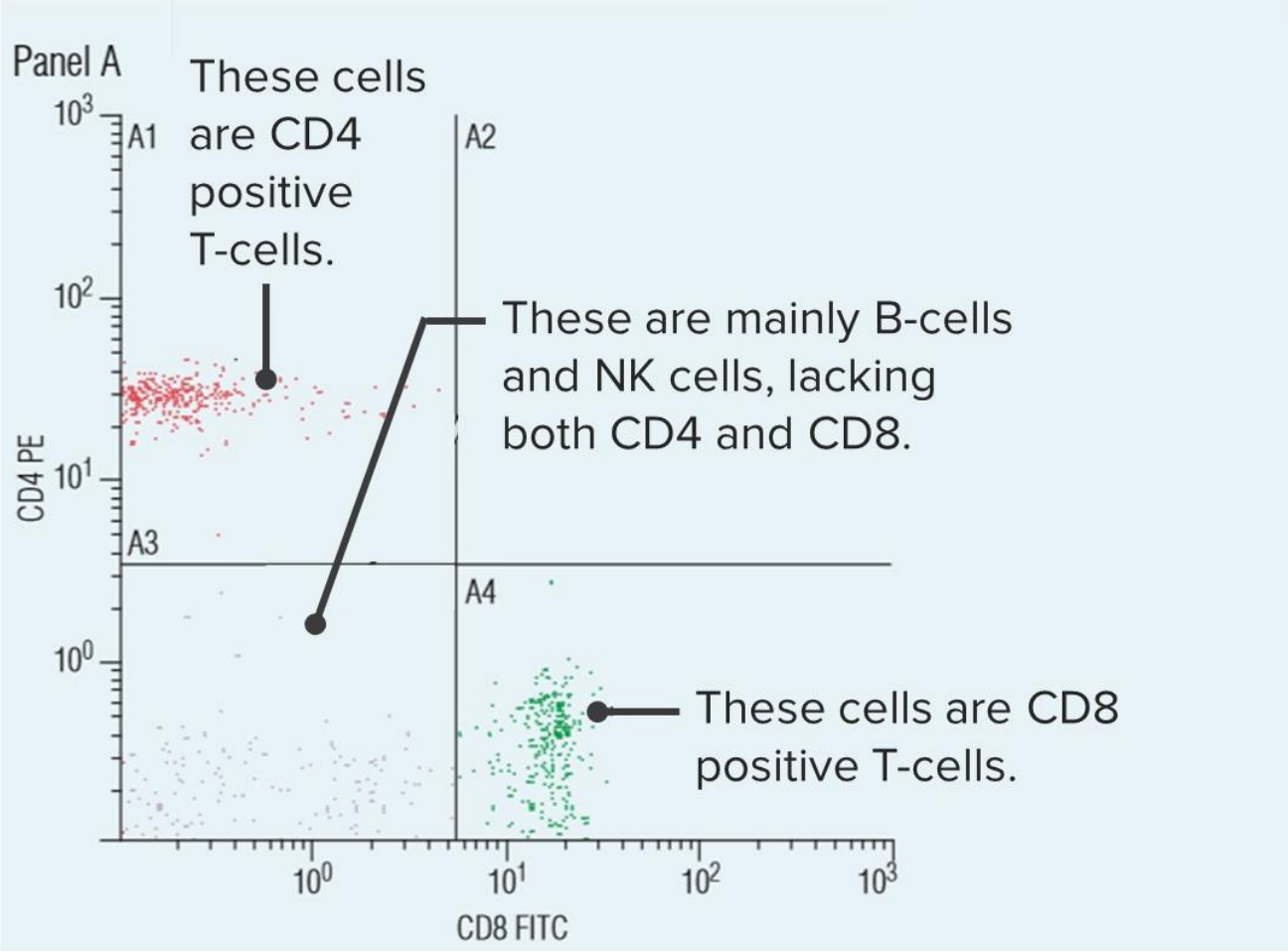
Forward scatter and side (90°) scatter allows detection of different cell types.



Flow cytometry

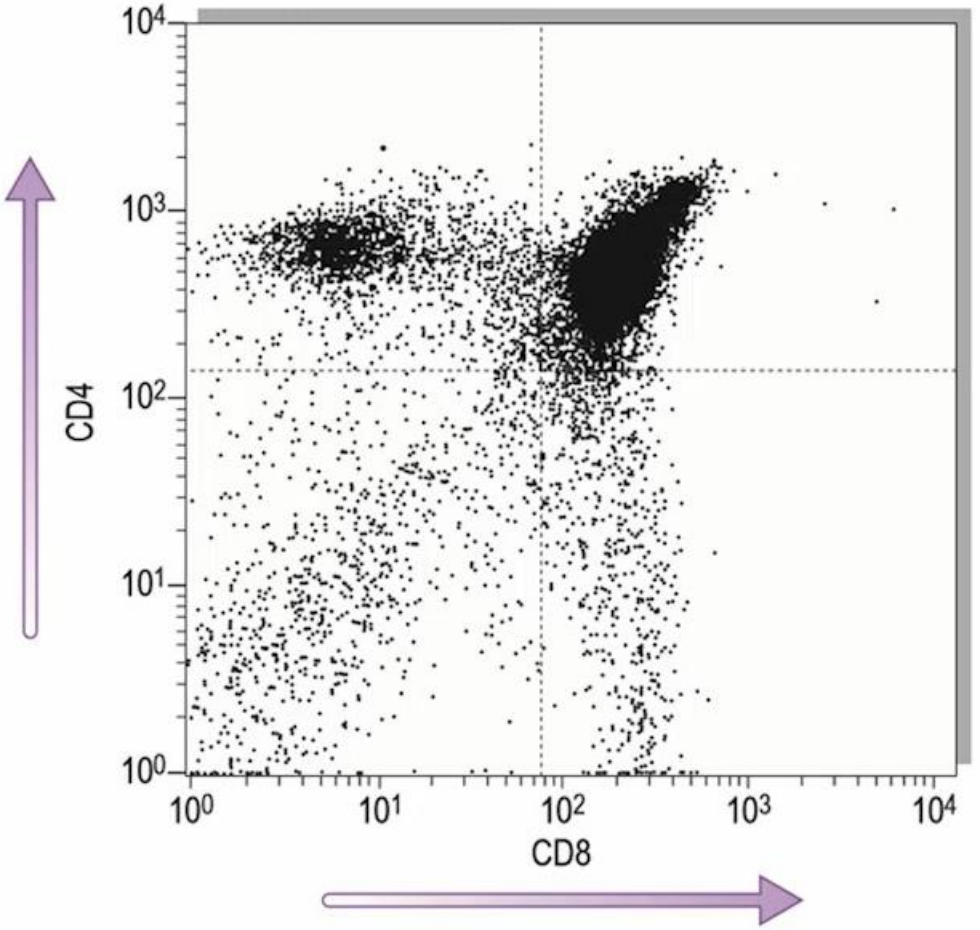
Detection of cell surface markers (e.g. CD4 and CD8 T-cells)

Anti-CD8-fluorescein isothiocyanate



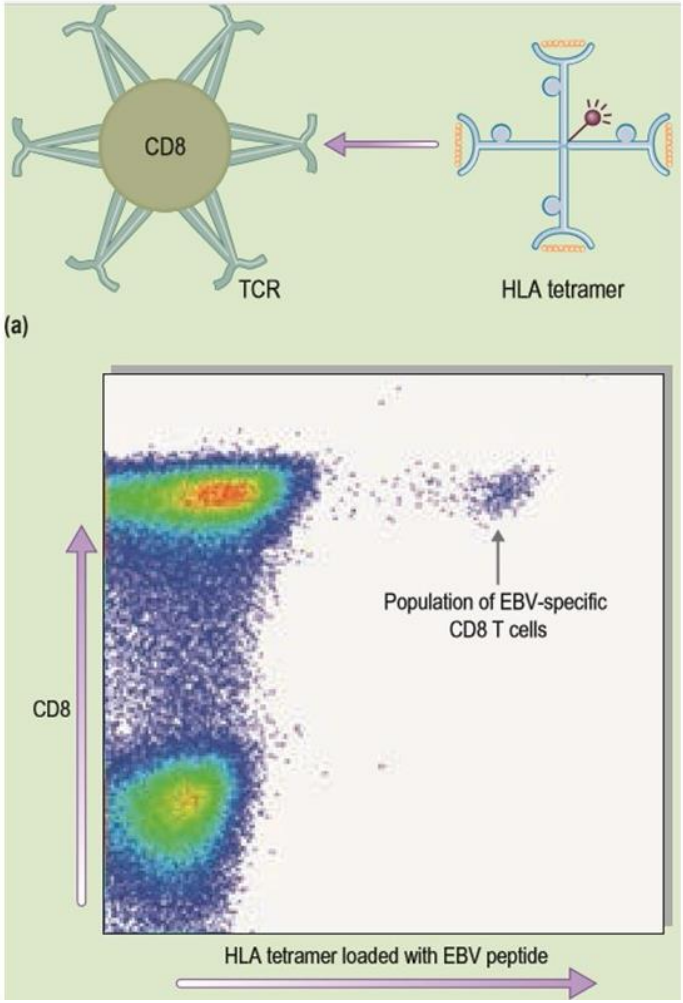
Flow cytometry

Detection of
'double positive'
thymocytes



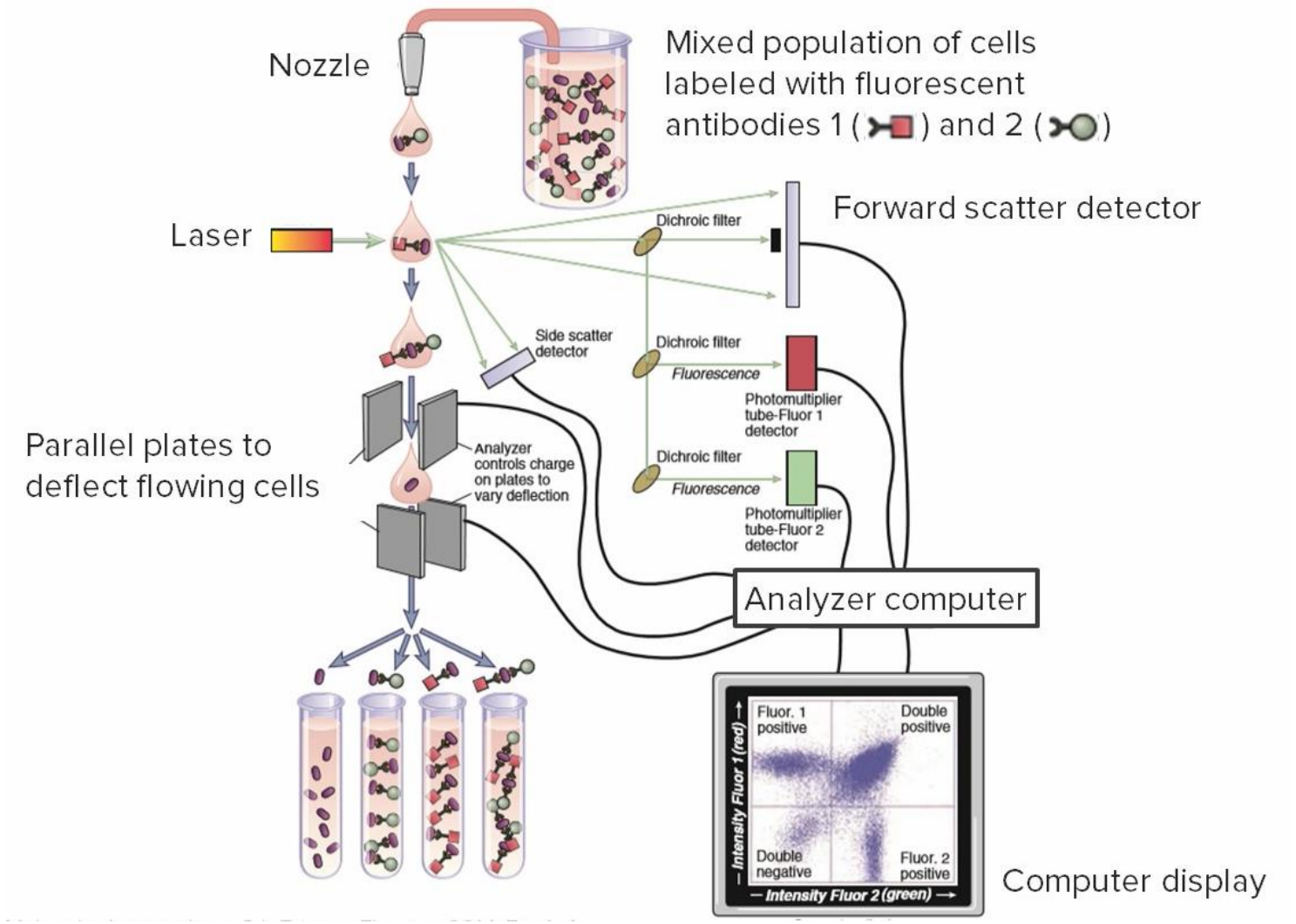
Flow cytometry

Detection of specific T-cells using pMHC tetramers



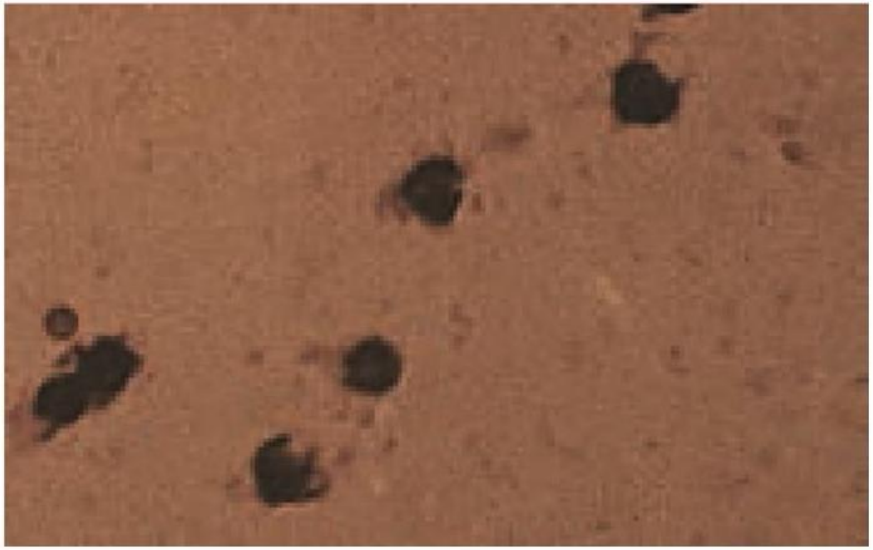
Fluorescently-labeled tetramer of 4 identical HLA molecules loaded with a peptide from Epstein-Barr virus

Fluorescence-activated cell sorter

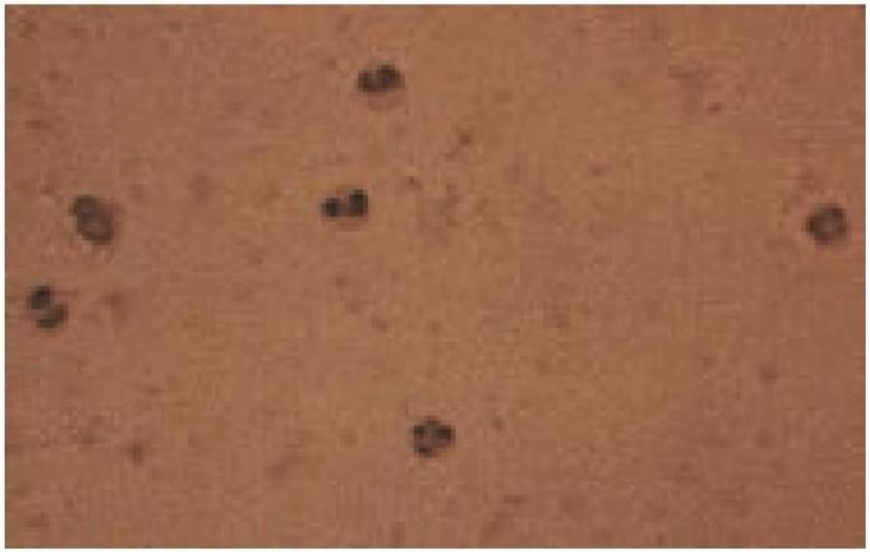


Nitroblue Tetrazolium Test (NBT)

Pale-yellow color of NBT changes to dark blue in neutrophils stimulated with phorbol myristate acetate (PMA) to induce reactive oxygen species.



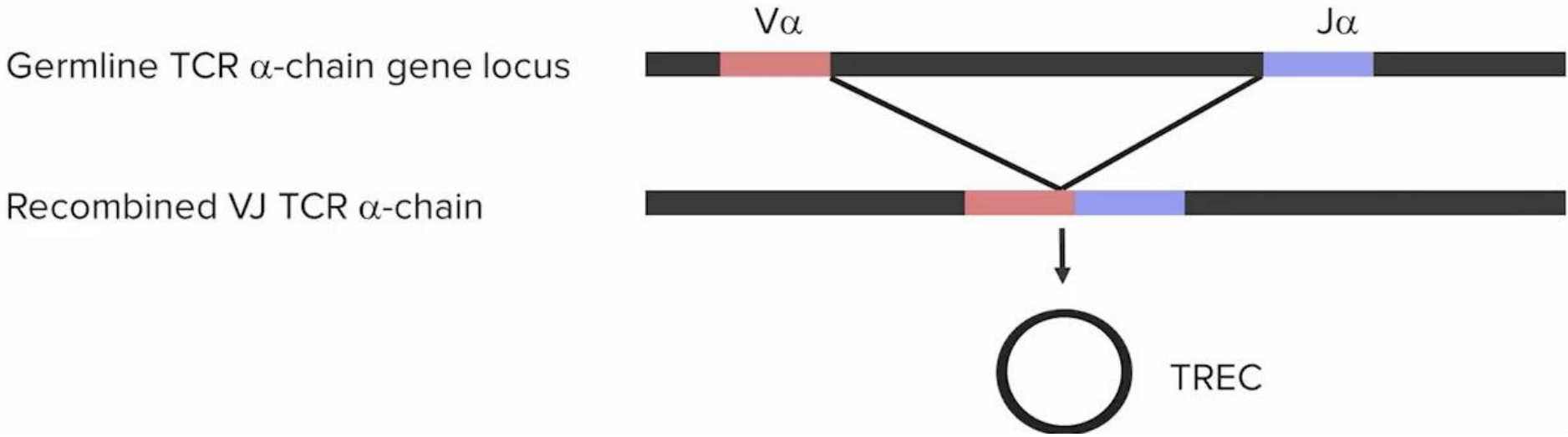
Normal donor



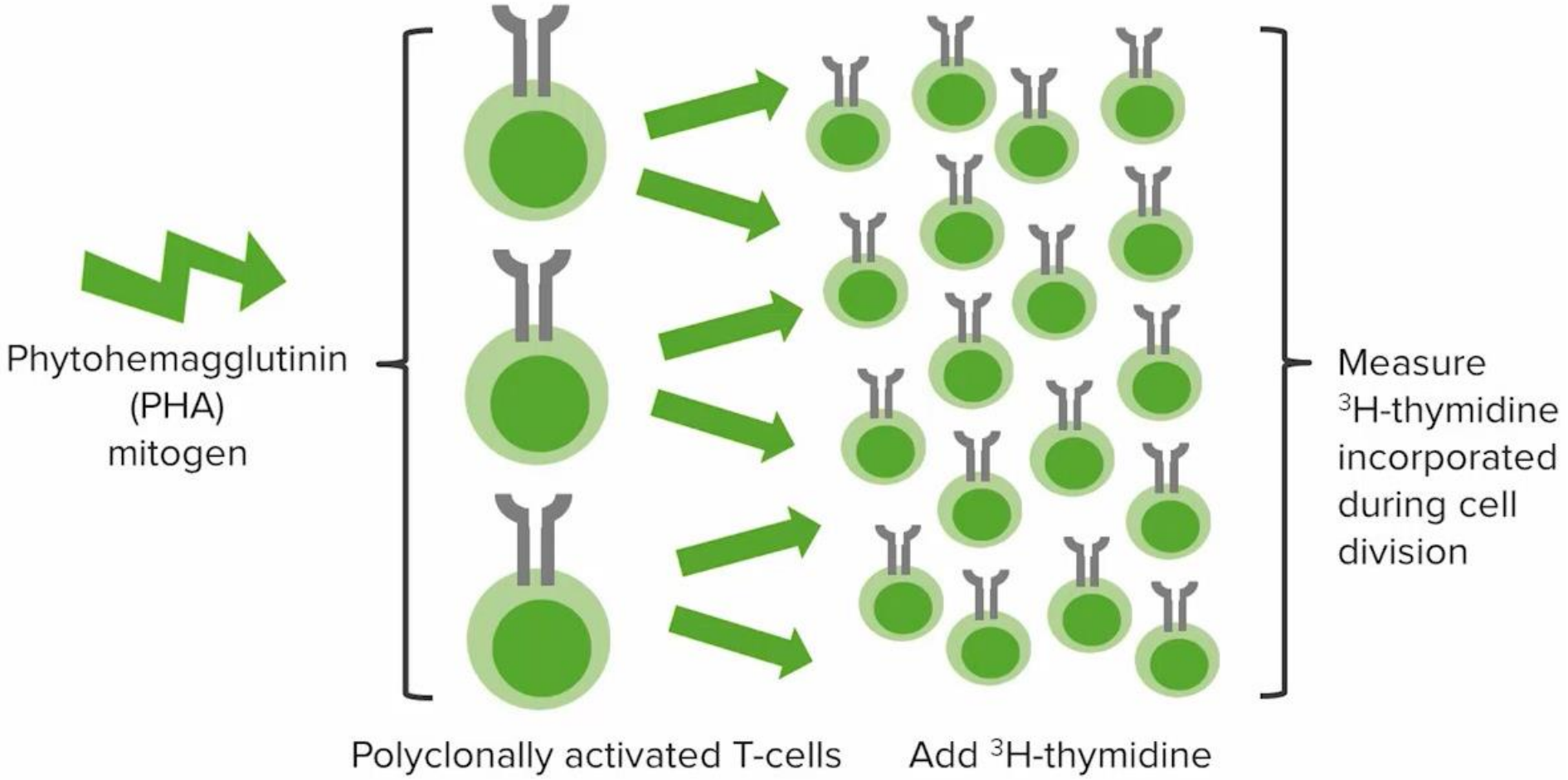
Patient with chronic granulomatous disease

T-cell receptor Excision Circle (TREC) assay

- TRECs are circular DNA molecules produced during TCR gene recombination
- Measured by PCR
- Used to quantify recent thymic emigrants as a measure of T-cell output from the thymus

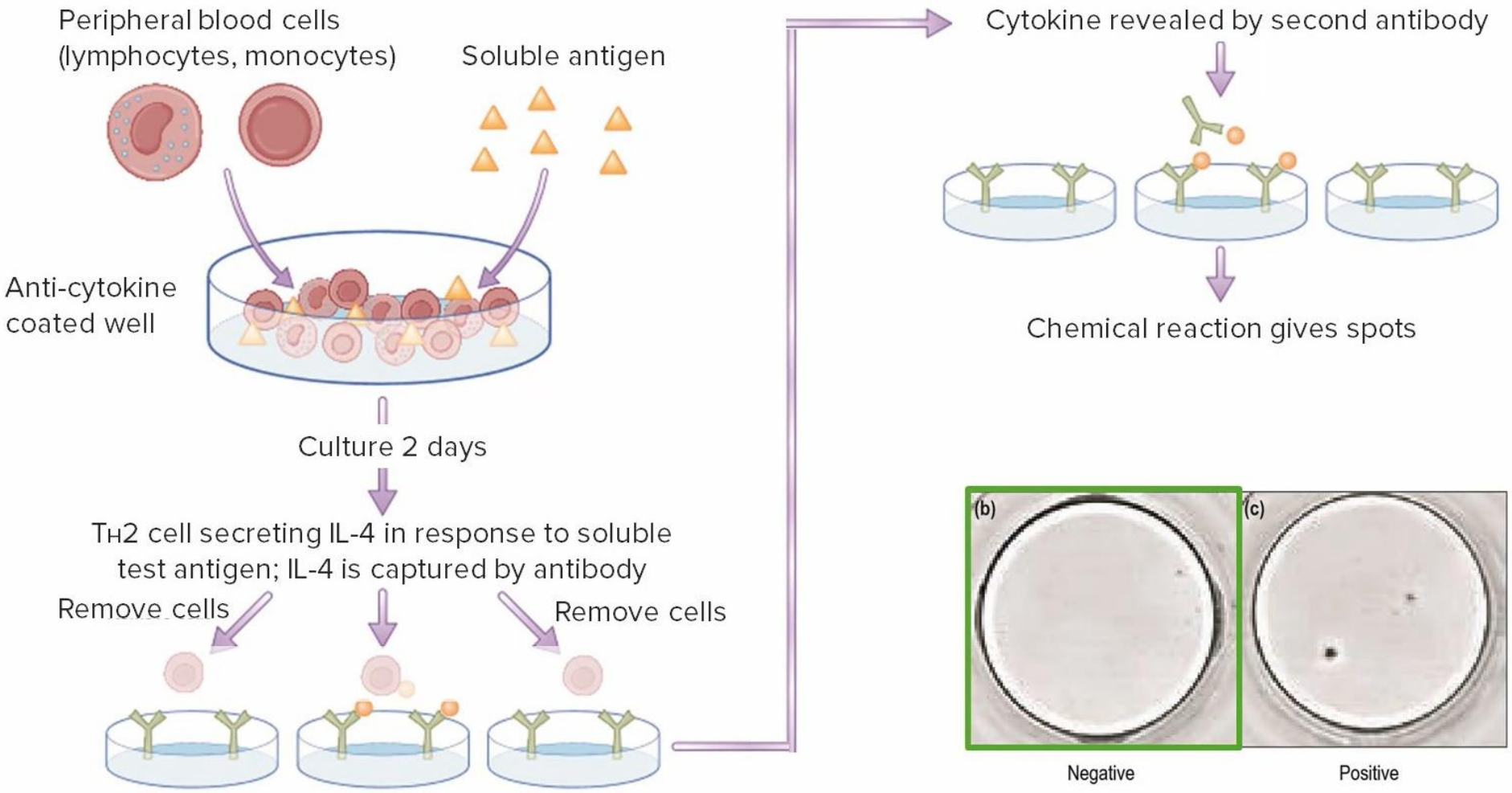


Lymphocyte Proliferation Assay



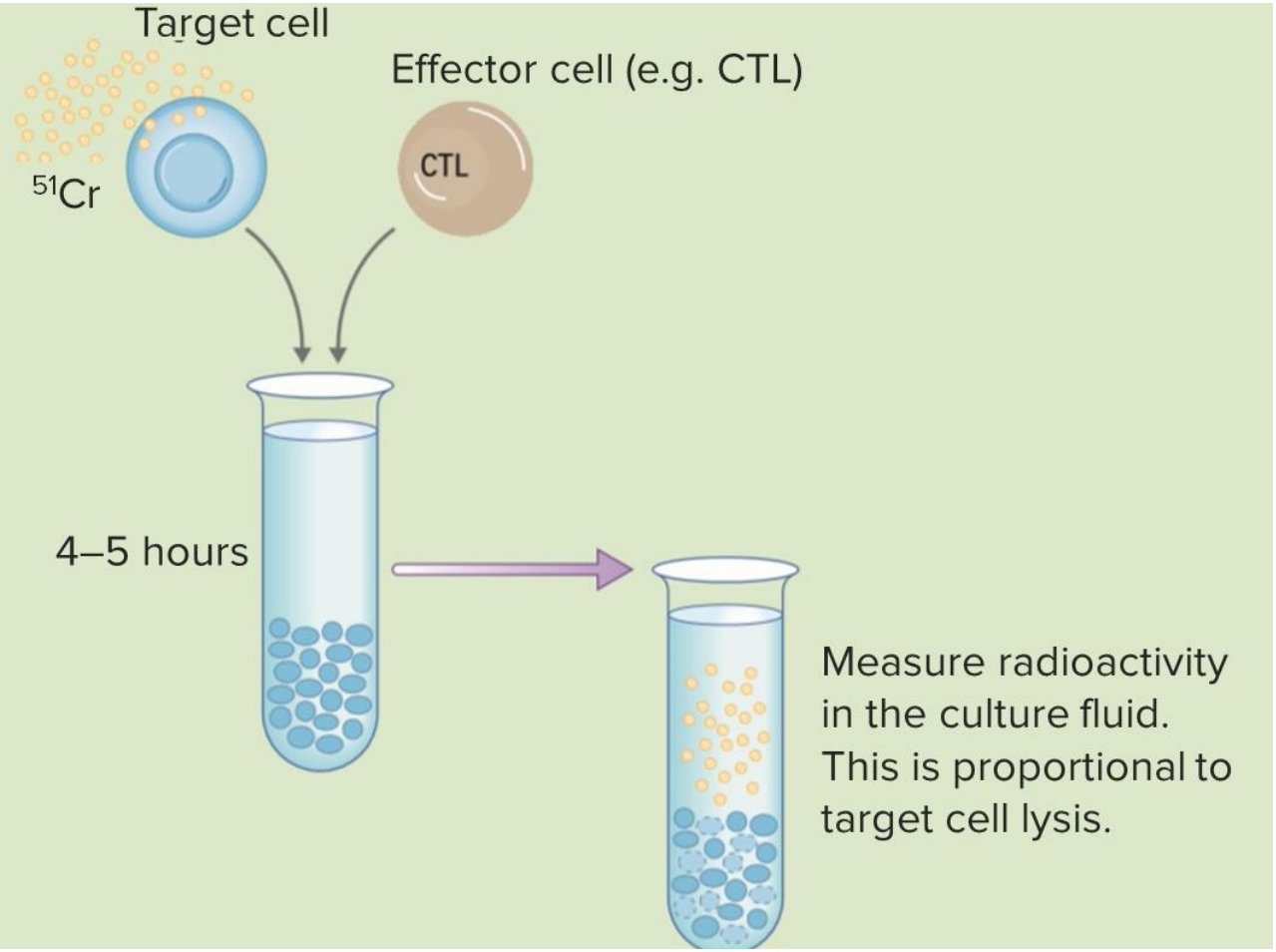
ELISPOT assay

To measure cytokine release



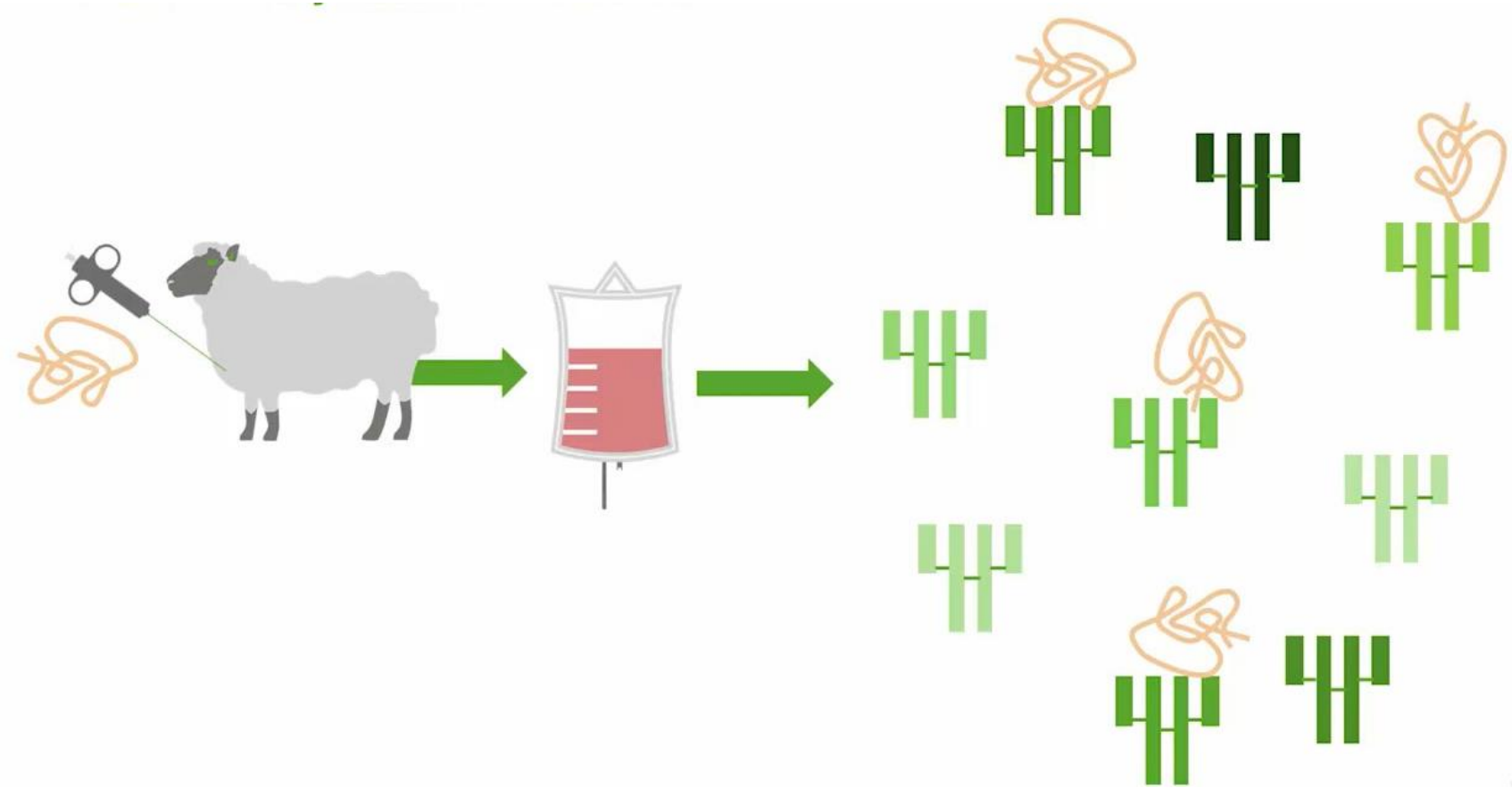
ELISPOT assay

To measure cytotoxic T-Cell activity



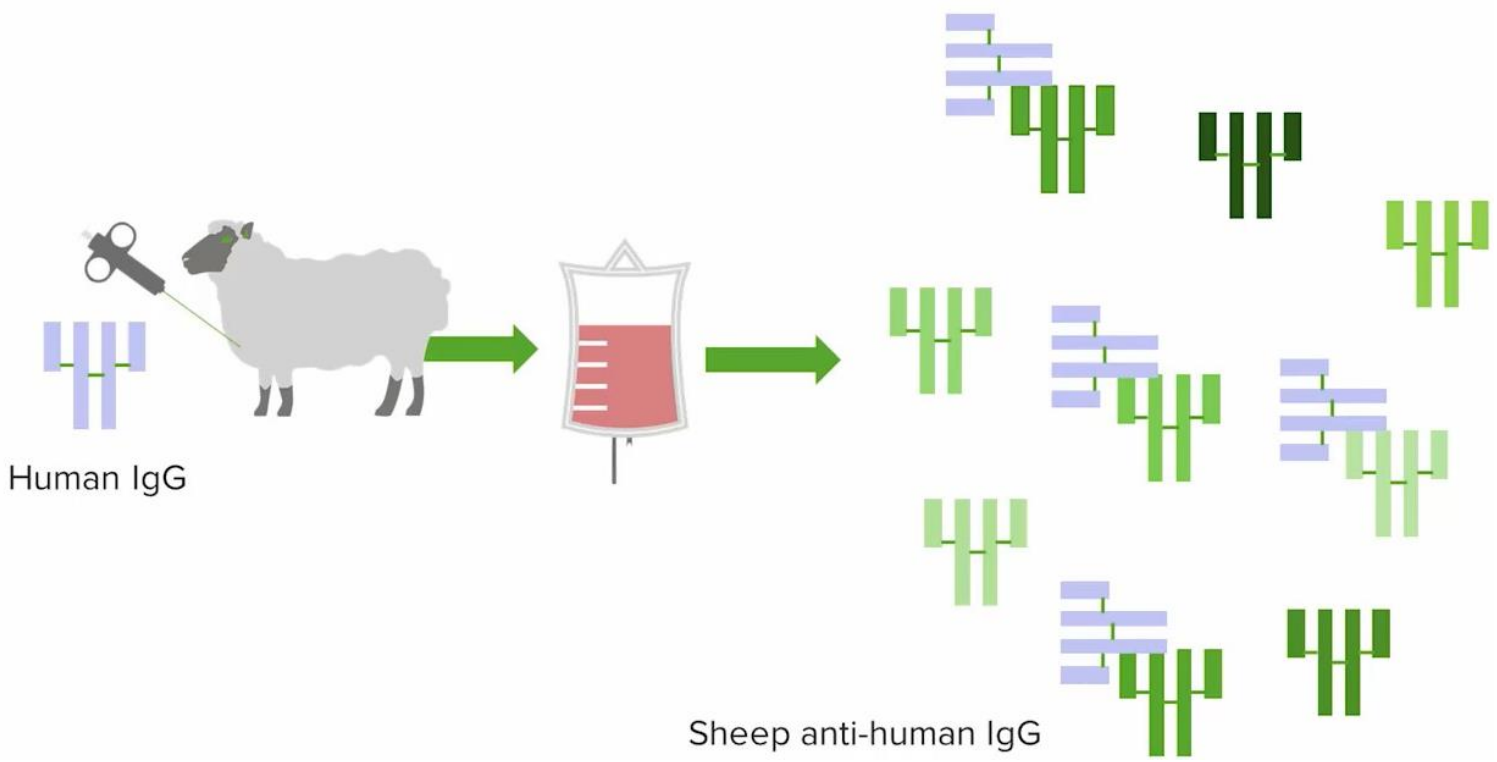
Production of antibodies

- Polyclonal antibodies



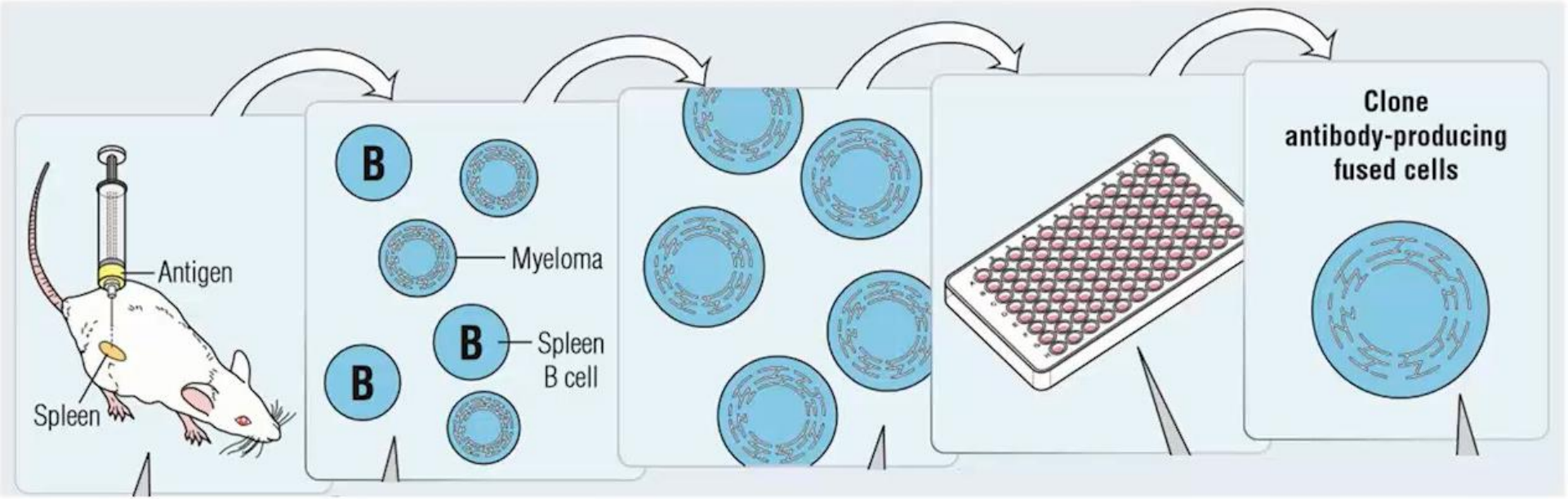
Production of antibodies

- Polyclonal antibodies



Production of antibodies

- Monoclonal antibodies – Hybridoma method



Immunize with antigen of choice. Remove spleen when the mouse is making an antibody response.

Fuse the immune spleen cells with a myeloma tumor cell.

The cells are cultured in a selective medium. Only fused cells survive after several days. Cells are diluted so that one cell is plated per well.

Cells are grown in individual culture plate wells, and culture supernatants from wells containing growing hybrid cells are screened for presence of desired antibody by ELISA.

This clone (hybridoma) is an immortal producer of the desired monoclonal antibody.

Production of antibodies

- Monoclonal antibodies – Phage Display