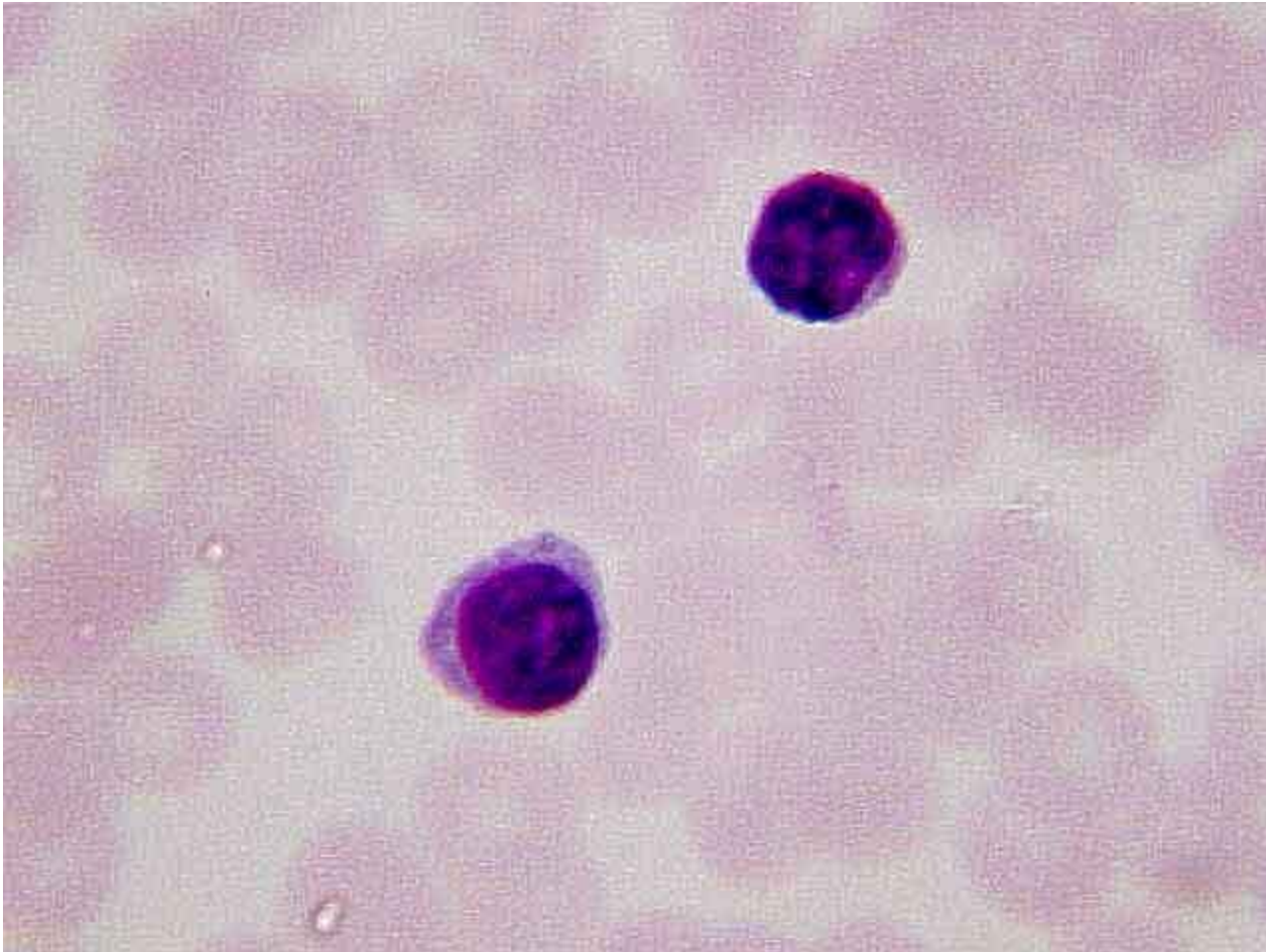


# Lymphocytes



## **How to Obtain Non-coagulating Blood**

- Heparin (5-10 IU/ml): activates antithrombin III, blocks the action of thrombin
- EDTA: binds  $\text{Ca}^{2+}$
- Citrate: binds  $\text{Ca}^{2+}$

# CD antigens

- Antigens expressed on leukocytes
- Abbreviation of Cluster of Differentiation
- Each antigen is designated: CD+ a number (CD1- CD340..)
- The antigens were designated after they were recognized and characterized – no system exists.

# The most important CD antigens of lymphocytes

Marker:	Cell Subpopulation
CD3 , CD4, CD8	T-lymfocytes
CD19, CD20, CD21	B-lymfocytes
CD16/CD56	NK cells
CD14/DR	monocytes
HLA-DR; CD25; CD69	activation markers

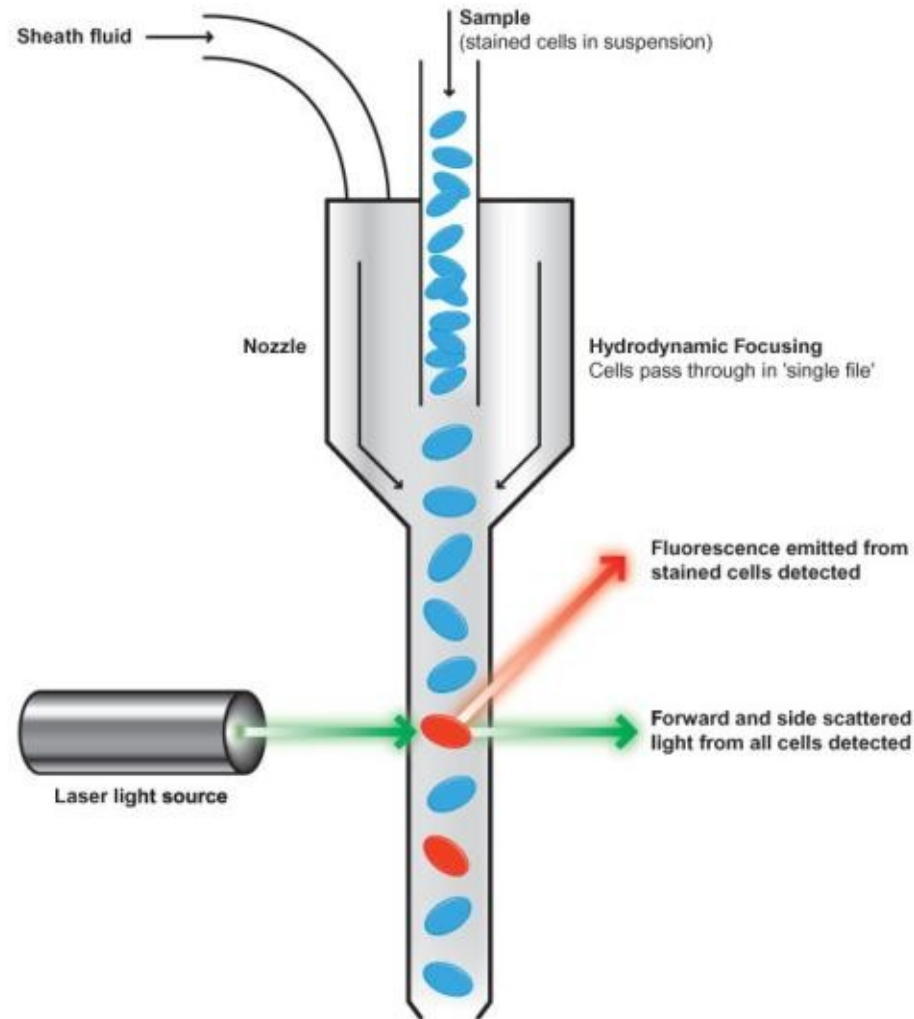
# Flow cytometer –FACS

Fluorescence-Activated Cell Scanner (Sorter)

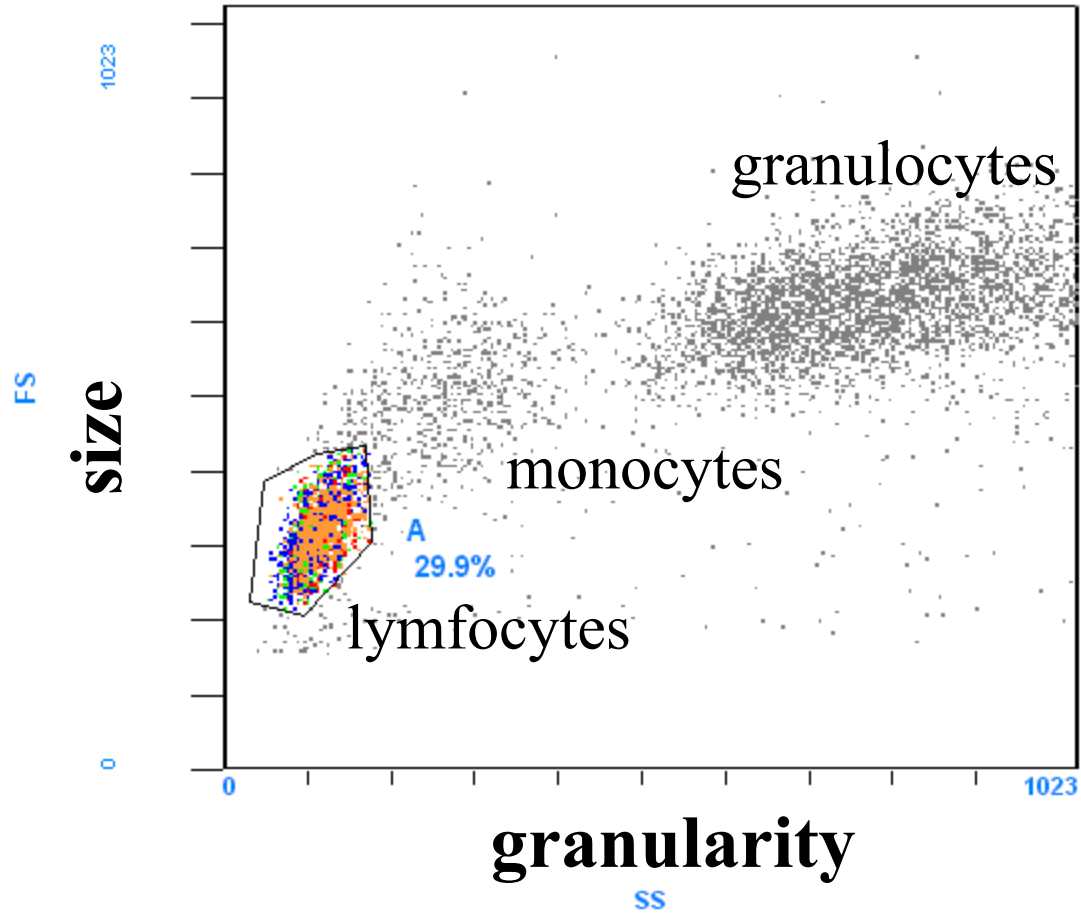
# Flow cytometer



# Flow cytometry

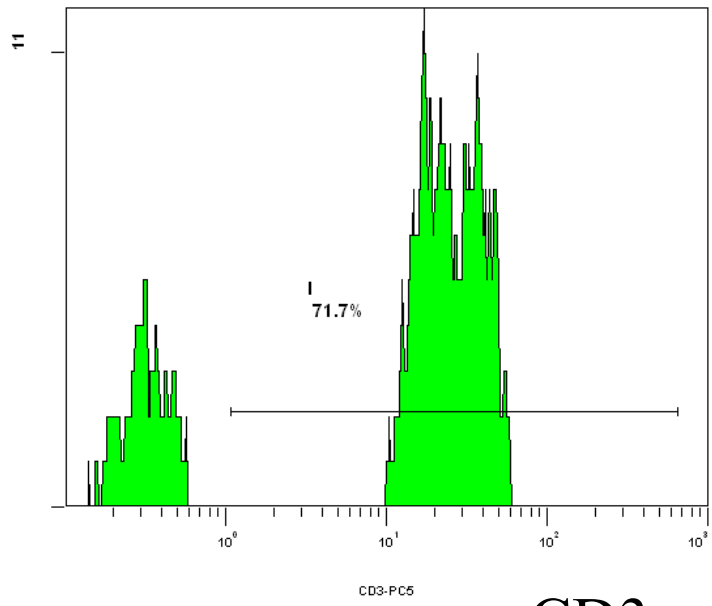


(F1)[Ungated] Z0051674.LMD : SS/FS



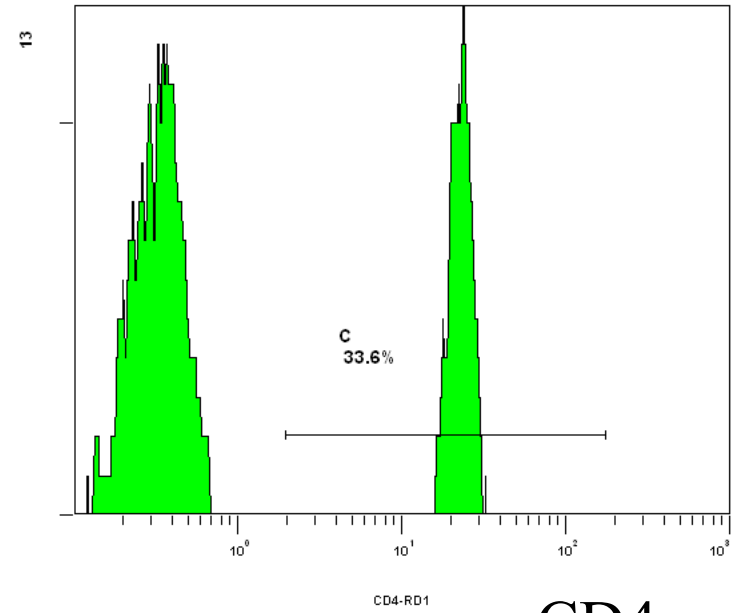


[F1][A] 20051674.LMD : FL4 LOG



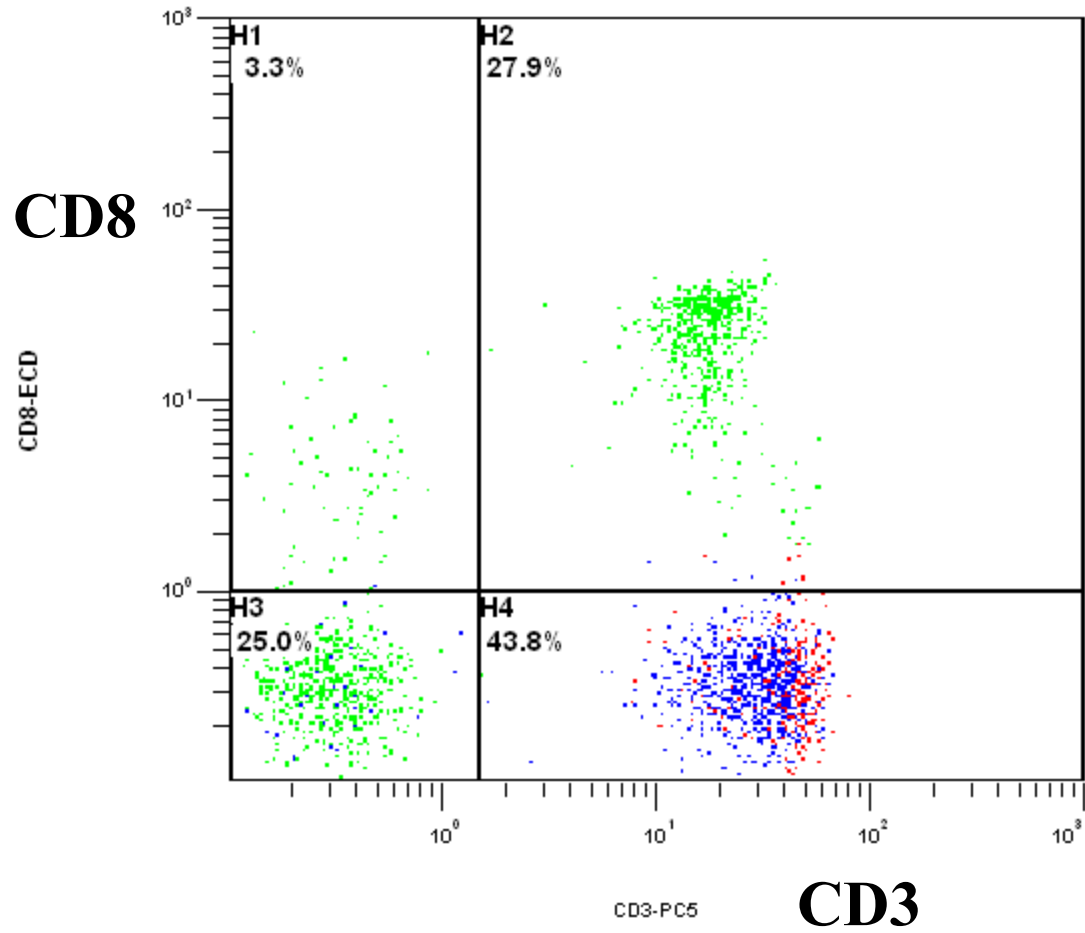
CD3

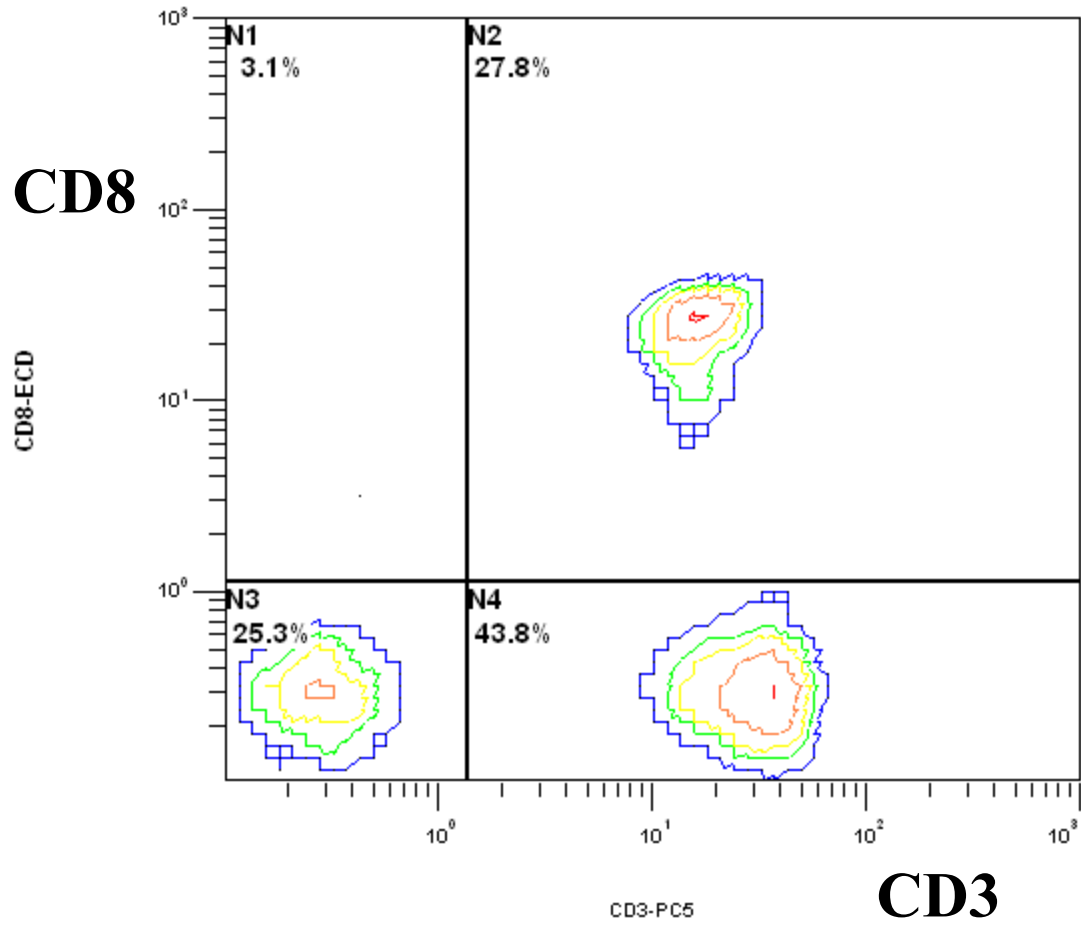
[F1][A] 20051674.LMD : FL2 LOG



CD4

(F1)[A] 20051674.LMD : FL4 LOG/FL3 LOG

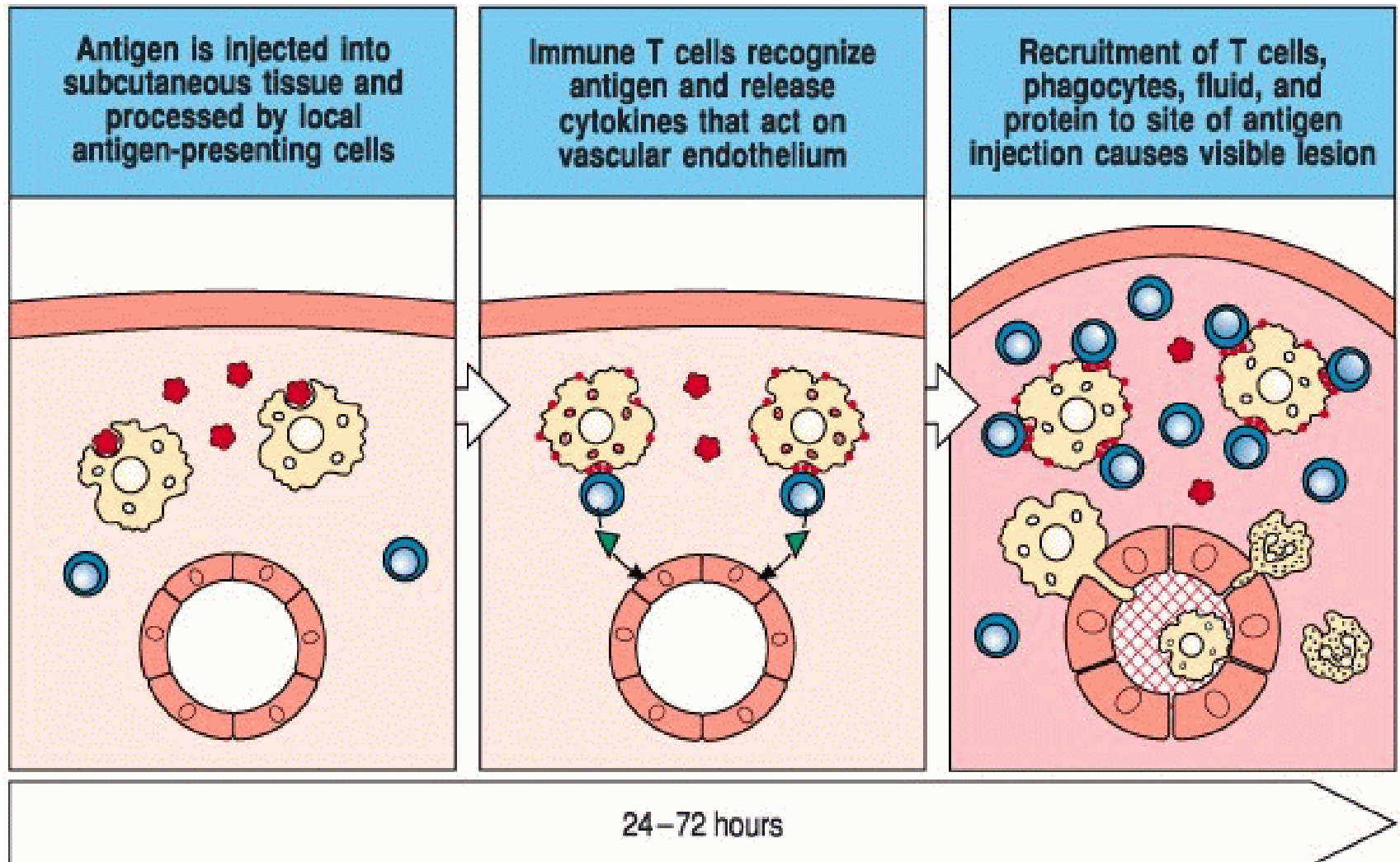




# Laboratory investigation of lymphocytes

- Enumeration of lymphocyte subsets by monoclonal antibodies against typical cell-surface markers -  
CD3 (T-lymphocytes), CD4 (helper T-lymphocytes),  
CD8 (cytotoxic T-lymphocytes),  
CD19 or CD20 (B-lymphocytes), CD16 (NK cells).
- Evaluation of function - lymphocyte proliferation tests - determines response to various stimuli:
  - specific (antigen, anti CD-3)
  - non-specific - polyclonal mitogens: PHA (Phytohaemagglutinin), ConA (Concanavaline A), PWM (Pokeweed mitogen)

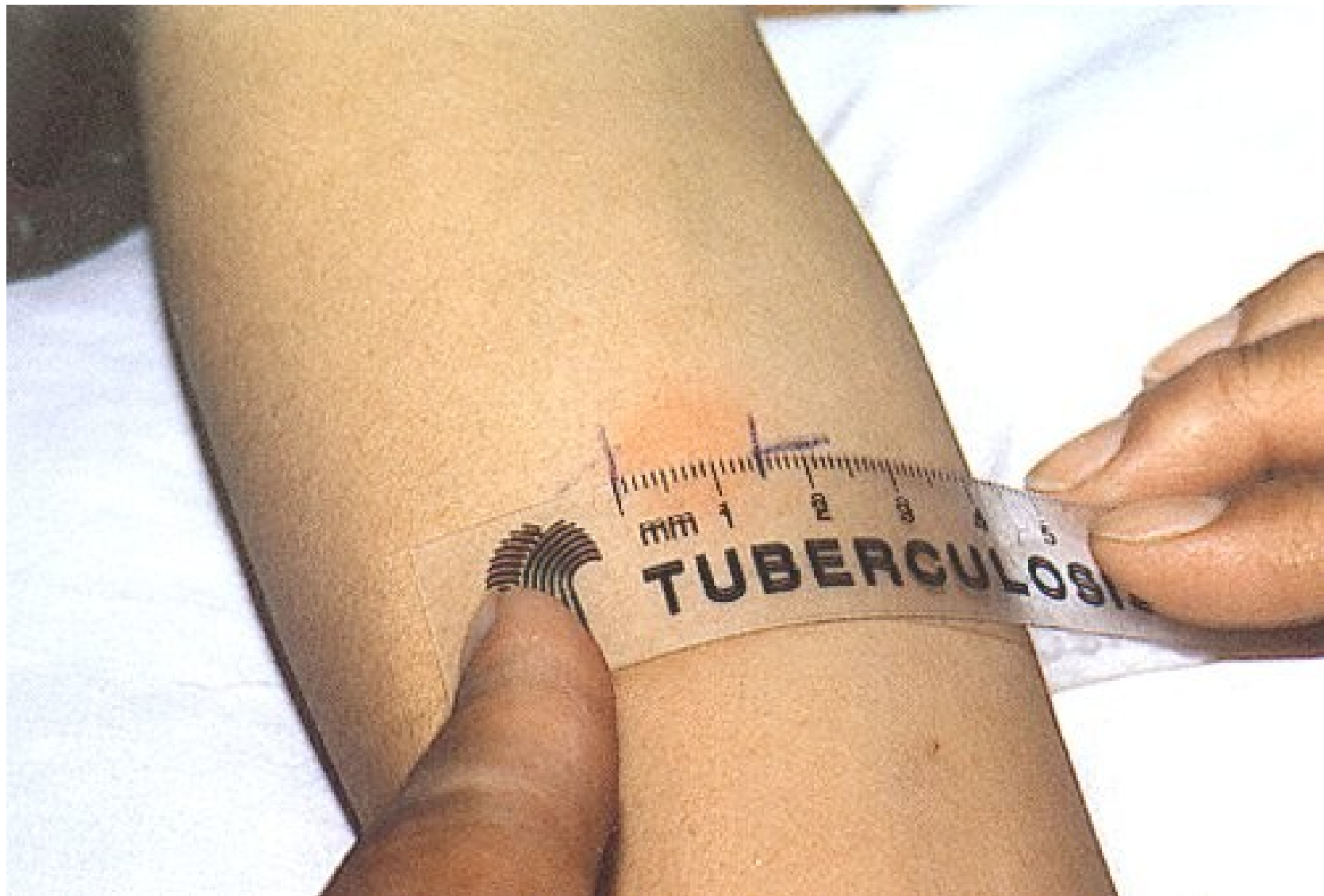
# Delayed-type of hypersensitivity test



# Tuberculin test







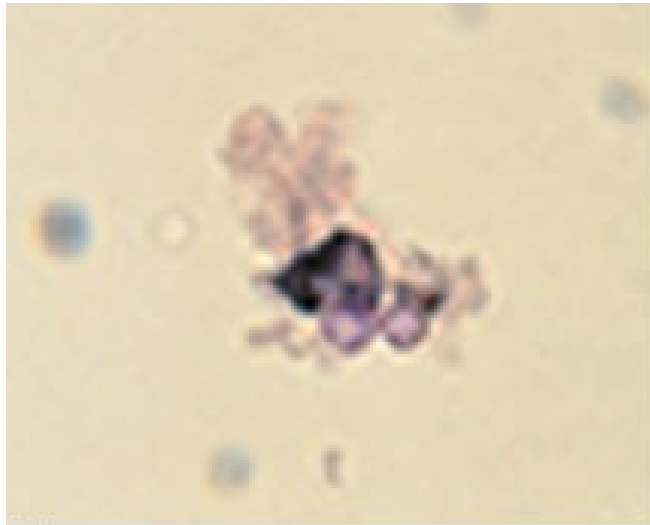


# Cell-Mediated Immunity (CMI) tests

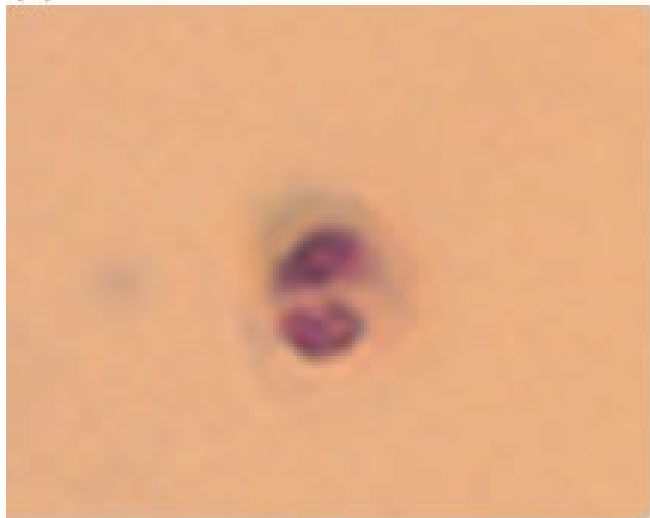
- Uses the principle of delayed type of hypersensitivity
- Common anamnestic antigens are used (tuberculin, candidin, trichofitin, staphylococcal antigens...)
- In normal situation induration should be formed by 48 hours.
- If the patient does not react to majority of antigens a deficiency of T- cell mediated immunity is suspected.

# Investigation of function of neutrophils

- Baktericidal killing of various bacteria of fungi
- Chemotaxis (e.g. under agarose)
- Ingestion of inert particles
- Metabolic activity - production of reactive metabolites of oxygen
  - NBT test (reduction of colourless nitro-blue-tetrazolium into blue formazan)
  - “Burst test“ – tetrarrhodamide reduction – measured by flow cytometer.



(a)



(b)

NBT