Laboratory methods Immunology

- The goal is to identify abnormal function of immune system, which can explain symptom or disease
- Some errors in immune functions not necessary cause symptoms or disease of the patients
- Some symptoms/disease are not necessarily related to measureable errors of the immune system – the technology is always imperfect and maybe less sensitive
- Technologies are quickly developing and the clinical science has difficulties to handle with

Tests of immunity

– Number of immune cells – populations, sub-, precursors

– Function tests – change of phenotype, CD expression

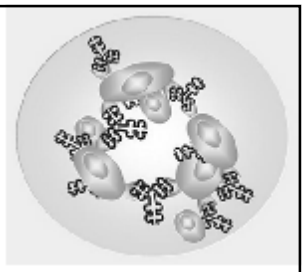
– Cell products - Igs, cytokines

– Nucleus changes

Immunologic Test Methods

- ❑ Agglutination
- ❑ Precipitation
- ❑ Electrophoresis, immunofixation
- ❑ Turbidimetry, nephelometry
- ❑ Enzyme- linked imunosorbent assay (ELISA)
- ❑ Immunoblotting

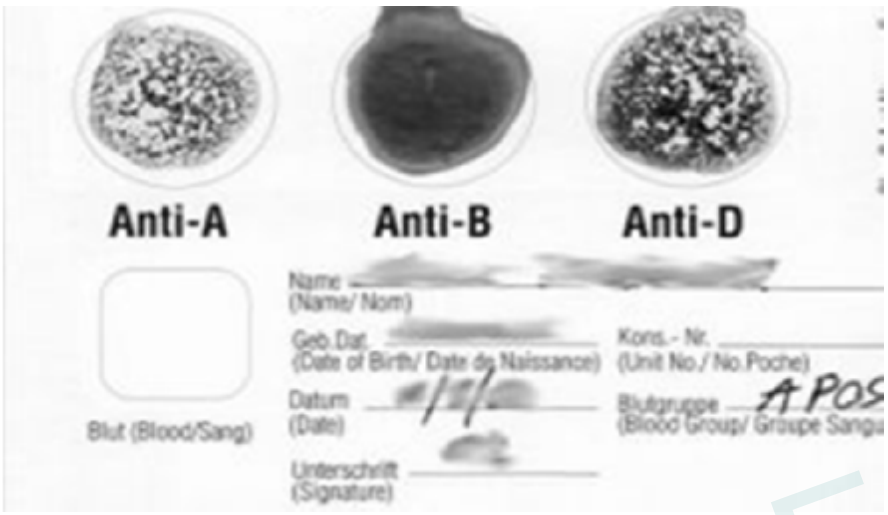
Aagglutination



- Clustering of particles bearing on its surface the antigen which reacts with antibody in the tested sample
- Can be seen as clot-agglutinate
- Evaluation – qualitative, Semi-quantitative

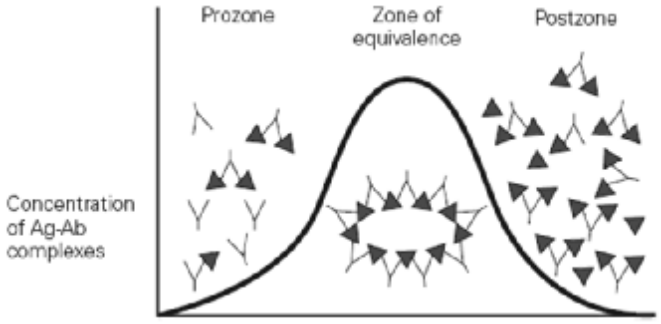
Serum from individuals of type	Red blood cells from individuals of type: O	A	B	AB
	Express the carbohydrate structures: R–GlcNAc–Gal (Fuc)	R–GlcNAc–Gal–GalNAc (Fuc)	R–GlcNAc–Gal–Gal (Fuc)	R–GlcNAc–Gal–GalNAc (Fuc) + R–GlcNAc–Gal–Gal (Fuc)
O — Anti-A and anti-B antibodies	no agglutination	agglutination	agglutination	agglutination
A — Anti-B antibodies	no agglutination	no agglutination	agglutination	agglutination
B — Anti-A antibodies	no agglutination	agglutination	no agglutination	agglutination
AB — No antibodies to A or B	no agglutination	no agglutination	no agglutination	no agglutination

Figure A-8 Immunobiology, 6/e, © Garland Science 2005]



Precipitation Methods

- demonstration of reaction antigen **(soluble)** – antibody by precipitation, in zone of equivalence generates precipitation line



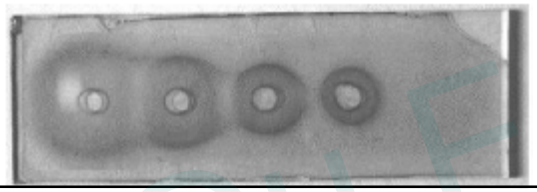
Precipitation Methods

Gel: hydrated polysacharides *(agarose)*

- **Ag *(Ab) or both diffuse through gel***
- **precipitation lineages or rings are formed in zone of equivalence**

- simple and unexpensive technique

-substantial delay in obtaining of results *(days)*



Measurement of **precipitation by light scattering**

the turbidity

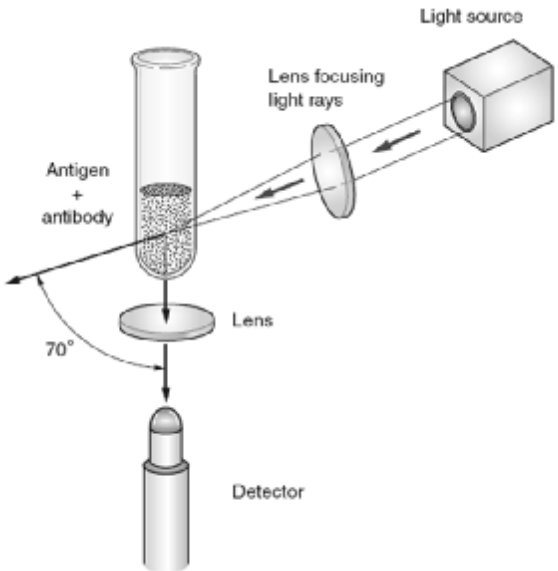
- measure of the turbidity (cloudiness) of a solution
- A detection device is placed in direct line with the incident light
- collecting light after it has passed through the solution
- It measures the reduction in light intensity due to reflection, absorption, or scatter

Measurement of precipitation by light scattering

Nephelometry

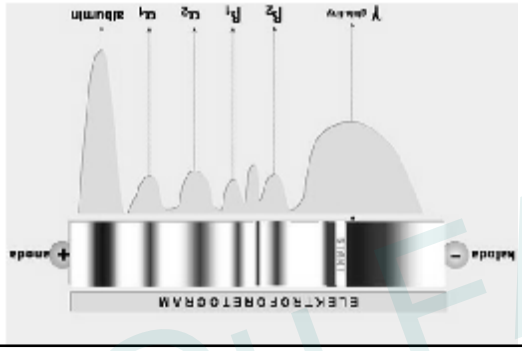
- measures the light scattered at a particular angle from the incident beam as it passes through a suspension
- The amount of light scattered is an index of the solution's concentration
- Beginning with a constant amount of Ab, increasing amounts of Ag result in an increase in Ag-Ab complexes

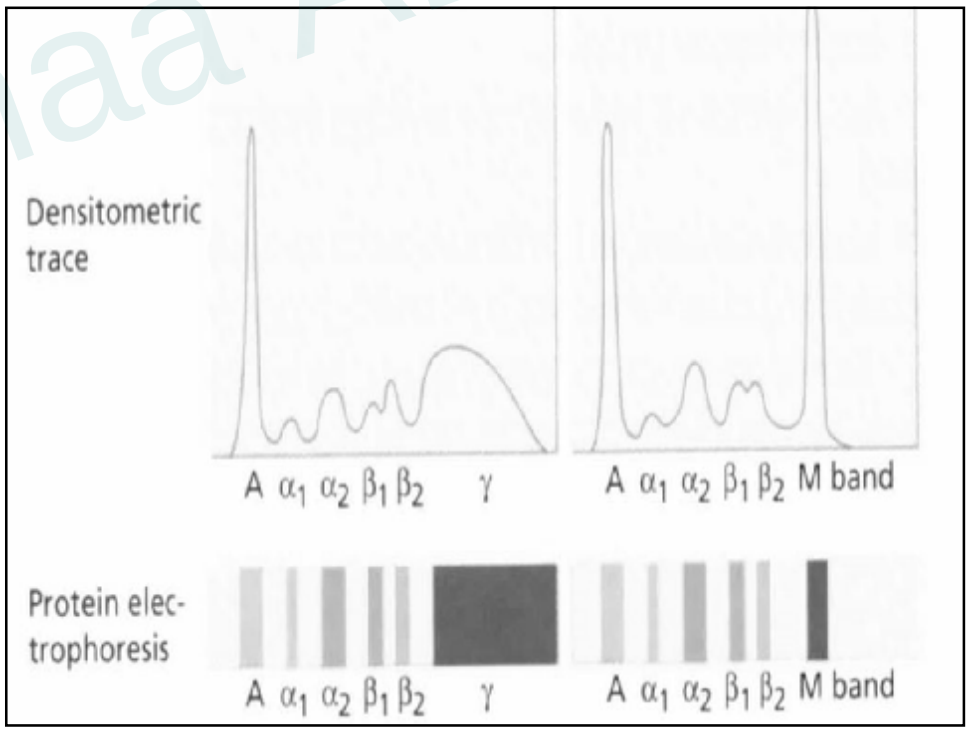
Nephelometry



Electrophoresis

- **Protein separation in electric field - size and charge**
- **agarose, polyacrylamide**

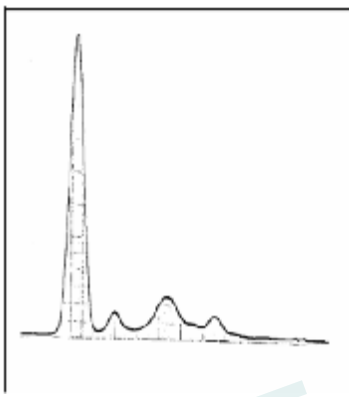




Agammaglobulinemia

	%	g/dl
Albumin	66.0	3.96
Alpha-1	6.0	0.36
Alpha-2	18.0	1.08
Beta	7.9	0.47
Gamma	2.0	0.12
Total Protein		6.0

IgG = 75 mg/dl
IgA = 0 mg/dl
IgM = 0 mg/dl



ELISA •

Flow cytometry

- **Cyto ~ cell**, **Metry ~ measure**
- FACS (fluorescent analysed cell sorting)
- measuring various properties of cells while in a fluid stream
- (biological, chemical, physical)
- (pH, size, granularity, viability etc.)
- **Flow ~** cells in motion,

Flow cytometry

- Measurement of several parameters at the same time:
- number of cells
- size (FSC)
- granularity (SSC) of cells
- fluorescent signal (FL) (2 or multiple depending on number of lasers)

staining of cells with mononuclear antibodies against:

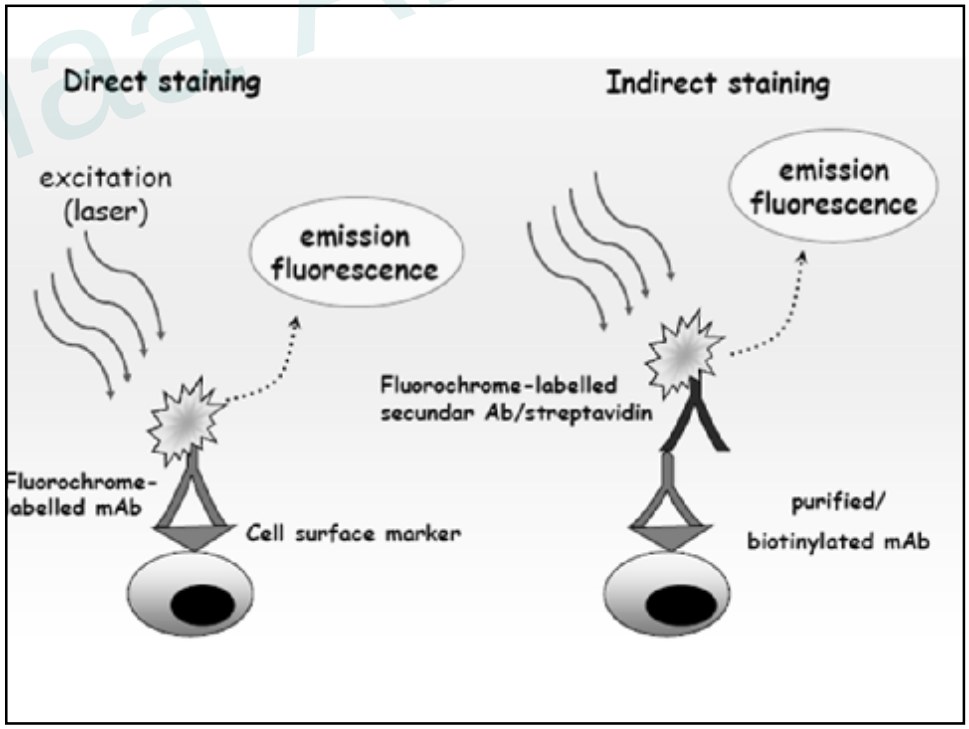
- cell surface molecules
- cytoplasmatic molecules
- nuclear molecules

Flow cytometry

- **Material:**

whole blood, bioptic samples of bone marrow, separated cell subpopulations, or other cell suspensions obtained by tissue desintegration

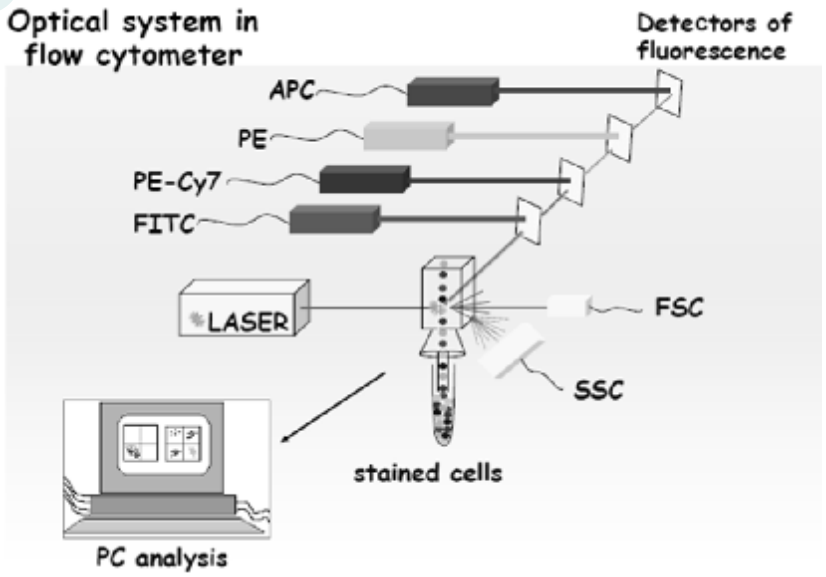
- **Immunofluorescent staining of cells:**
- Direct or indirect

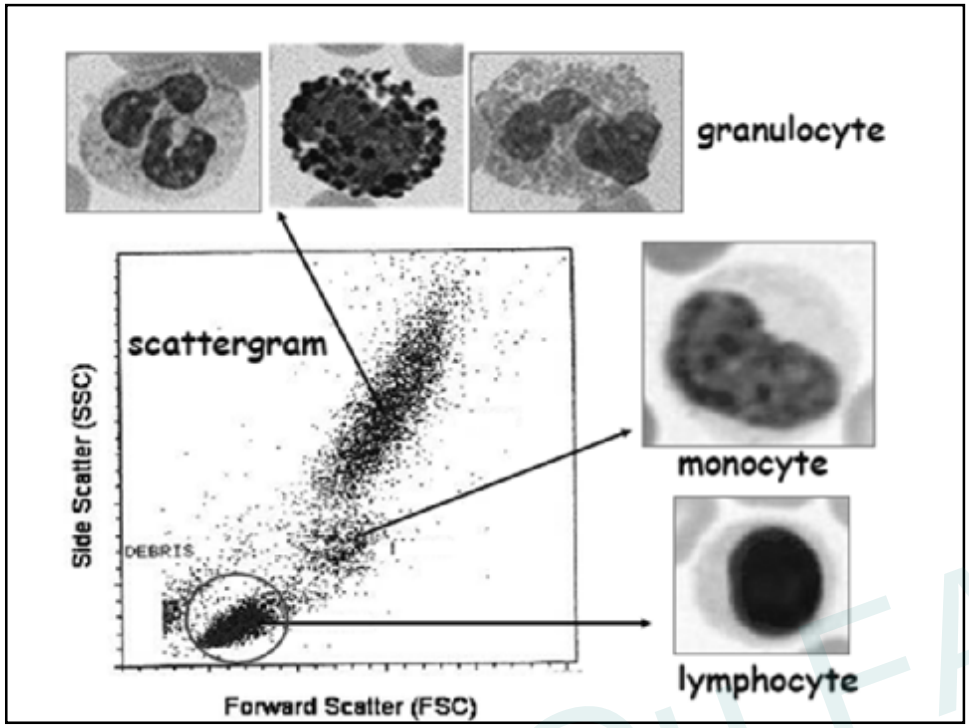


Flow cytometry

Principle of flow cytometry

- One-by-one stream of cells moves rapidly through the flow cytometer
- Cells pass through a focused beam of light from a laser
- Photons scattered in all directions
- Photo detectors capture scattered light and generate digital signals to define cellular characteristics
  –Size, internal complexity, antigen makeup
- Information stored and analyzed by computer

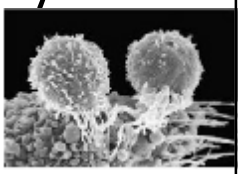




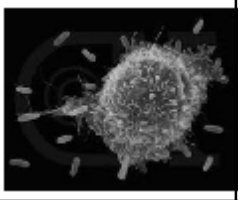
Application of cytometric analysis

- phenotypisation of cells (diagnostic of primar immunodeficiency, autoimmune diseases, leukemie and lymphoms, etc.)
- functional tests of leukocytes and thrombocytes (proliferating activity – measurement of DNA content)
- Detection of viruses, bacteries and parasites, analysis of chromosomes, assessment of enzymatic activity, measurement of intracellular calcium

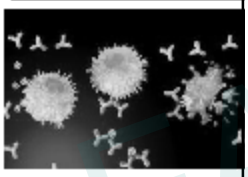
Functional tests of lymphocytes



- Proliferation
- Expression of activated markers
- Cytotoxicity



- Cytokine secretion
- Production of antibodies



Functional tests of phagocyting cells

- Phagocytosis
- Tests of oxidative burst
- Determination of adhesive molecules expression
- Testing of chemotaxis
- Bactericidal test

Test of oxidative burst

- Examination of phagocyte`s ability to build 02 radicals (activation of NADPH oxidase)

Measurement by flow cytometry

- DHR-123 test: Full blood + phorbol esters + dihydrorhodamin rhodamin (effect of 02 radicales measurement of fluorescenct intensity

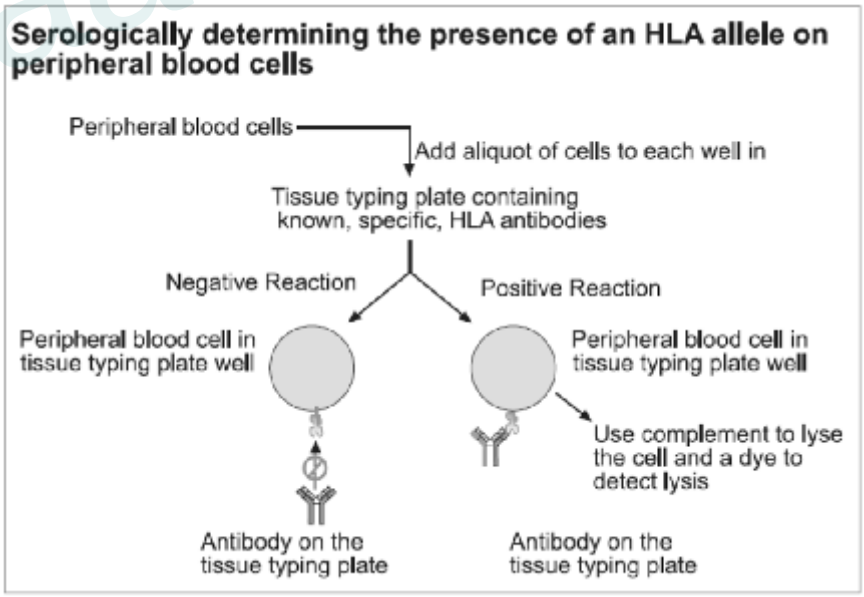
HLA Typing

- Transplantation
- Determining HLA haplotypes for the gathering of forensic evidence
- Studies of anthropology
- Disease incidence
- Drug reactions
- Research into mechanisms underlying cancer and autoimmune diseases

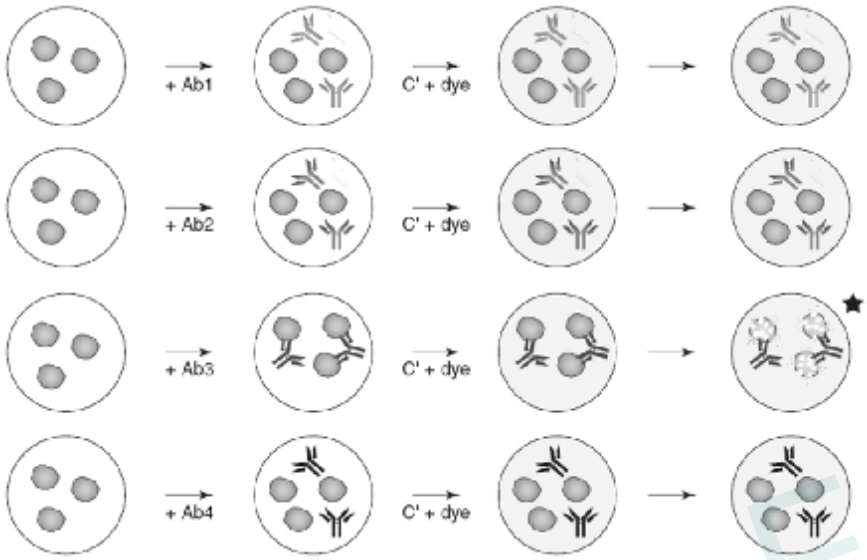
Serological techniques

Complement-Dependent Cytotoxicity (CDC)

- The peripheral blood leukocytes (PBLs) are mixed with separate antibody samples in a parallel series of microwells.
- complement (Rabbit serum + visible dye (such as trypan blue) that is excluded from viable cells.
- If the cells express HLA recognized by a given anti-HLA antibody, they are lysed by the complement and take up the dye.



Complement-Dependent Cytotoxicity (CDC)



PCR-SSP
(Sequence Specific Primers)

allele-specific primers are used to amplify patient DNA

amplification products are characterized by gel electrophoresis and ethidium bromide staining.

- PCR-SSP is highly sensitive such that tests for multiple alleles can be conducted in the same sample.