The Assessment of Immunocompetence

To determine the status of the immune system

***Immunocompetence**: is the ability of the body to produce an effective immune response against antigen*

The Assessment of *Immunocompetence*

for DIAGNOSIS, PROGNOSIS and THERAPEUTIC MONITORING of:

1. Congenital or acquired immunodeficiencies
2. Malignancies
3. Autoimmune disorders
4. Immunosuppression induced by drugs or radiation
5. In the reconstitution of the immune system after bone marrow or other lymphoid tissue transplantation
6. After vaccination
7. In clinical or basic research

The main clinical manifestations related to altered immunocompetence

-Increased susceptibility to infection

-Inability to overcome infectious events despite antibiotic therapy

-Dissemination of localized infections

-Occurrence of opportunistic infections

-Increased development of tumors

-Development of autoimmune diseases

The INTEGRITY of the IMMUNE SYSTEM relies on the presence of an adequate number of functionally competent cells and the appropriate concentration of factors

to assess it

- **Evaluate the number of cells**
- **measure the concentration of factors**

and…

- Evaluate the functionality of cells and factors

HOW DOES THE LABORATORY INVESTIGATE THE IMMUNOCOMPETENCE ?

Immunological competence can be evaluated through several tests

Data from these tests must be interpreted in the clinical context of the patient and are aimed at

Clinical Diagnosis

Prognosis

Therapeutic monitoring

Laboratory Tests have a biological and methodological

VARIABILITY

Biological : age, sex, race, nutritional status, daily changes, medications, infections

Methodological : type of equipment, reagents, operator

What kind of ***Biological Speciemen ?***

Blood (serum and plasma)

Biopsy from lymphoid tissues (bone marrow, lymph nodes, spleen)

Cerebrospinal fluid
Bronchoalveolar lavage
Ascitic fluid

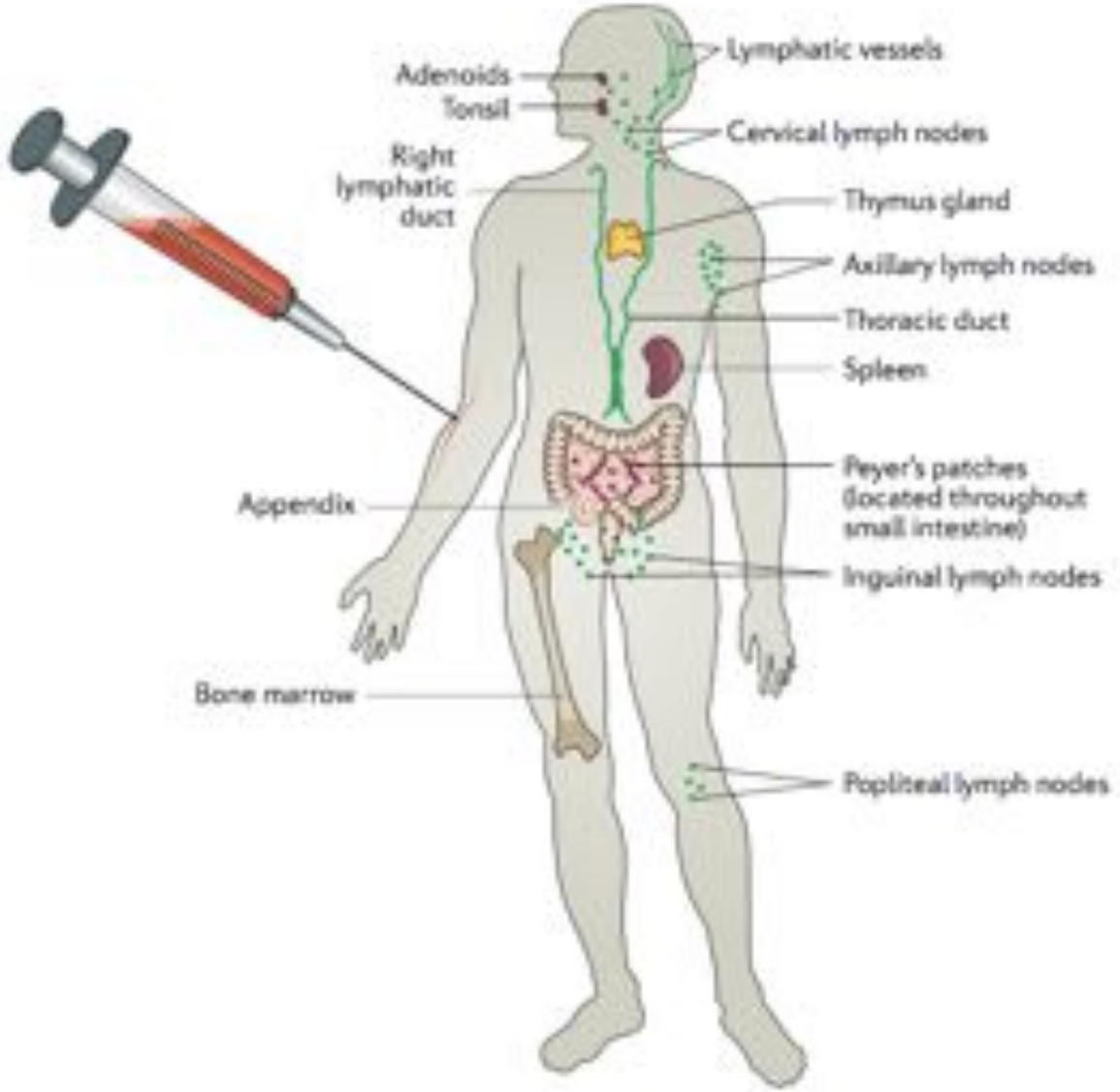
The tissue of origin of the cells and factors represents a "limit" of the laboratory tests:

eg.: Tissue protein levels reflect those in the blood, while cellular components do not reflect the distribution found in other lymphoid tissues such as spleen, lymph nodes, bone marrow.

Proportions of lymphocyte populations in different lymphoid tissues

Lymphocytes and subsets	Lymphocyte markers	Lymphocyte Percentage		
		Blood	Lymph node	Spleen
T lymphocyte	CD3+	70-80	70-80	30-40
Helper T lymphocyte	CD3+CD4+	40-57	50-60	50-60
Cytotoxic T lymphocyte	CD3+CD8+	14-31	15-20	10-15
B lymphocyte	CD19+	6-15	20-25	40-45
NK cells	CD16+CD56+	5-19	poche	10
NKT cells	CD3+CD16+	10	poche	10

The blood is a window for global immune system



Assays are performed mostly ***in vitro*** and in some cases ***in vivo***

For ***in vitro*** assays
blood samples obtained by venipuncture

with anticoagulant (EDTA, heparin)
to evaluate cells

no anticoagulant
for humoral components

Assessment of immunocompetence

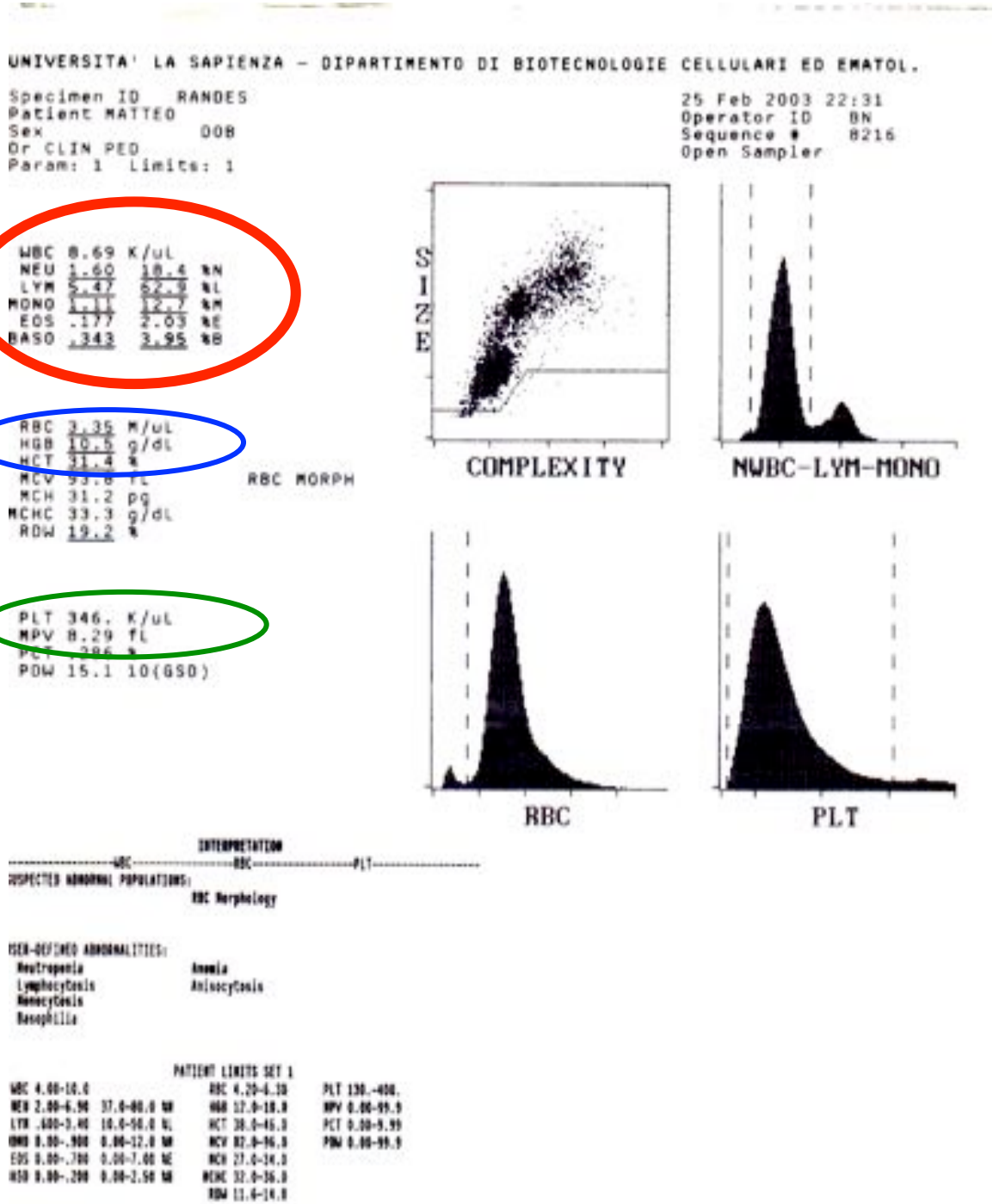
-***Complete blood count*** (CBC) with differential count of leukocytes

-Enumeration of lymphocyte populations and subsets

-Cell-mediated immunity *:* lymphocyte function
Intradermal reaction (DTH)
test for Phagocytosis

-Humoral immunity : Determination of immunoglobulin concentration (*antibody-mediated immunity*)
Complement protein concentration and CH50 Test

Complete blood count (CBC) report



UNIVERSITA' LA SAPIENZA - DIPARTIMENTO DI BIOTECNOLOGIE CELLULARI ED EMATOL.

Specimen ID RANDES
Patient MATTEO
Sex DOB
Dr CLIN PED
Param: 1 Limits: 1

25 Feb 2003 22:31
Operator ID BN
Sequence # 8216
Open Sampler

WBC	8.69	K/uL	
NEU	1.60	18.4	%N
LYM	5.47	62.9	%L
MONO	1.11	12.7	%M
EOS	.177	2.03	%E
BASO	.343	3.95	%B

RBC	3.35	M/uL
HGB	10.5	g/dL
HCT	31.4	%
MCV	93.8	fL
MCH	31.2	pg
MCHC	33.3	g/dL
RDW	19.2	%

RBC MORPH

PLT	346.	K/uL
MPV	8.29	fL
PCT	.286	%
PDW	15.1	10(GSD)

COMPLEXITY

NWBC-LYM-MONO

RBC

PLT

INTERPRETATION

WBC RBC PLT

SUSPECTED ABNORMAL POPULATIONS:
RBC Morphology

USER-DEFINED ABNORMALITIES:
Neutropenia
Lymphocytosis
Monocytosis
Basophilia
Anemia
Anisocytosis

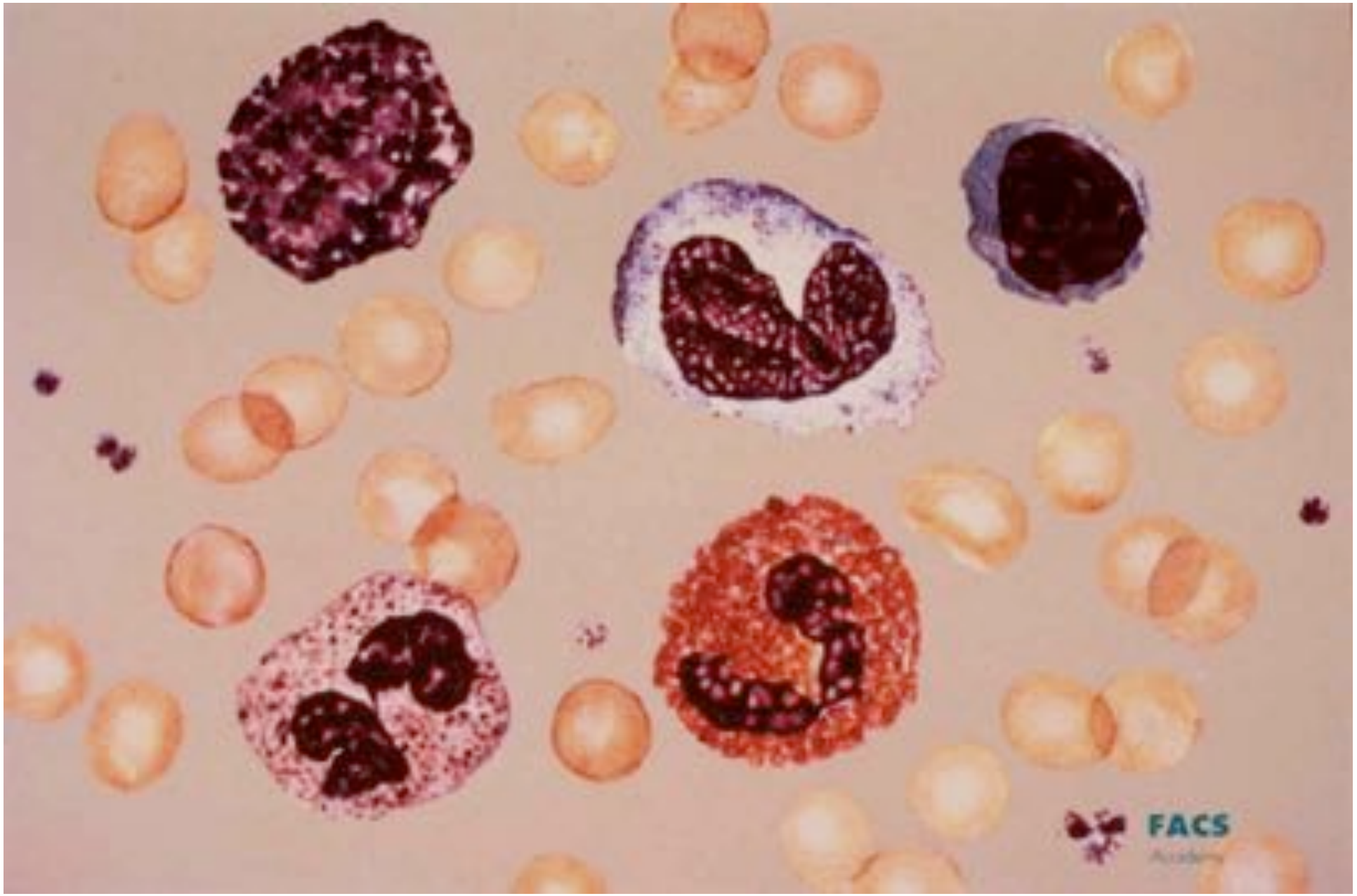
PATIENT LIMITS SET 1

WBC	4.00-10.0		RBC	4.20-6.30	PLT 130.-400.
NEU	2.00-6.90	37.0-80.0	%N	HGB 12.0-18.0	MPV 0.00-99.9
LYM	.600-3.40	16.0-50.0	%L	HCT 38.0-46.0	PCT 0.00-9.99
MONO	0.00-.900	0.00-12.0	%M	MCV 82.0-96.0	PDW 0.00-99.9
EOS	0.00-.700	0.00-7.00	%E	MCH 27.0-34.0	
BASO	0.00-.200	0.00-2.50	%B	MCHC 32.0-36.0	
				RDW 11.6-14.0	

The Complete Blood Count (CBC): Reference Range

quantitative (*numerical*) and morphological information about the three circulating cell types

CELL		ABSOLUTE COUNT	WBC differential count %
Red Blood Cells		**4.200.000-5.400.000/mm^3**	
White Blood cells		**4500 – 8500/mm^3**	
PMN neutrophil		**2700-6000/mm^3**	**60-70%**
PMN eosinophil		**45-260/mm^3**	**1-3%**
PMN basophil		**20-85/mm^3**	**0.5-1%**
Monocyte		**135-510/mm^3**	**3-6%**
Lymphocyte		**900-3000/mm^3**	**20-35%**
Platelet		**200.000 – 400.000/ mm^3**	



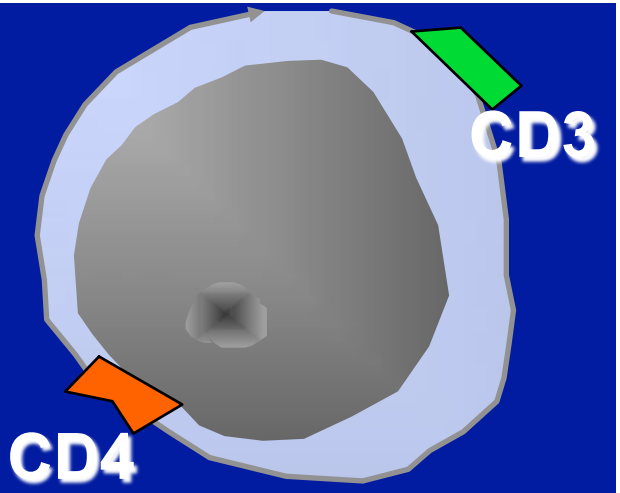
Peripheral blood smear

SPECIFIC TESTS

CELL	COUNT	FUNCTION
T Lymphocyte	MAb and Flow Cytometry	Proliferation response of mitogen stimulated cells
T Lymphocyte subsets	MAb and Flow Cytometry	Cytokine production Cytotoxicity Suppression
B Lymphocyte	MAb and Flow Cytometry	Serum protein electrophoresis Serum Ig levels
NK Cell	MAb and Flow Cytometry	Cytotoxicity Cytokine production
Neutrophil	CBC	Respiratory burst
Monocyte/Macrophage	CBC	Intracellular Killing of microbe

IMMUNOPHENOTYPING :

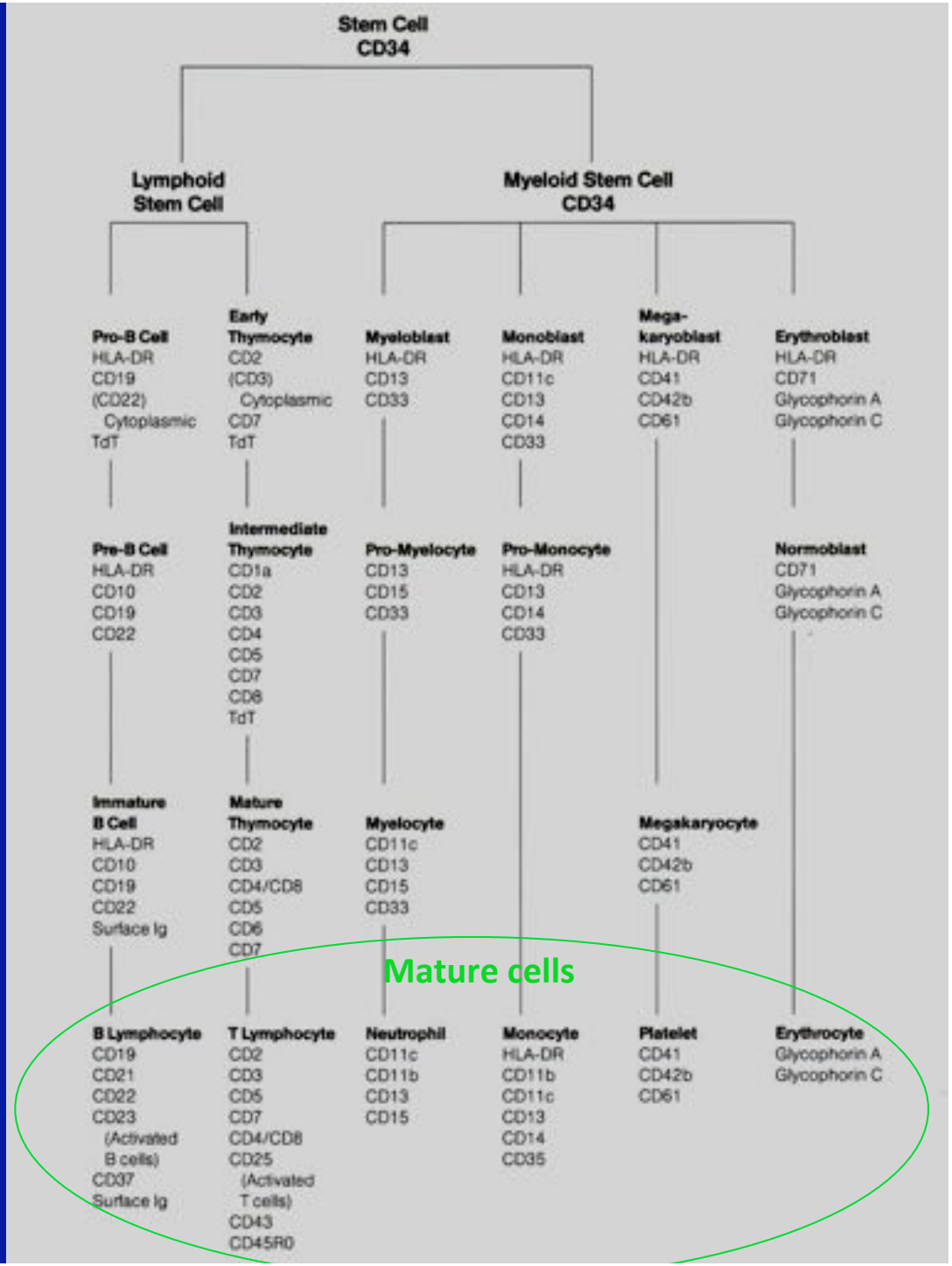
enumeration of different lymphocyte populations and subpopulations based on the expression of different antigen/ marker

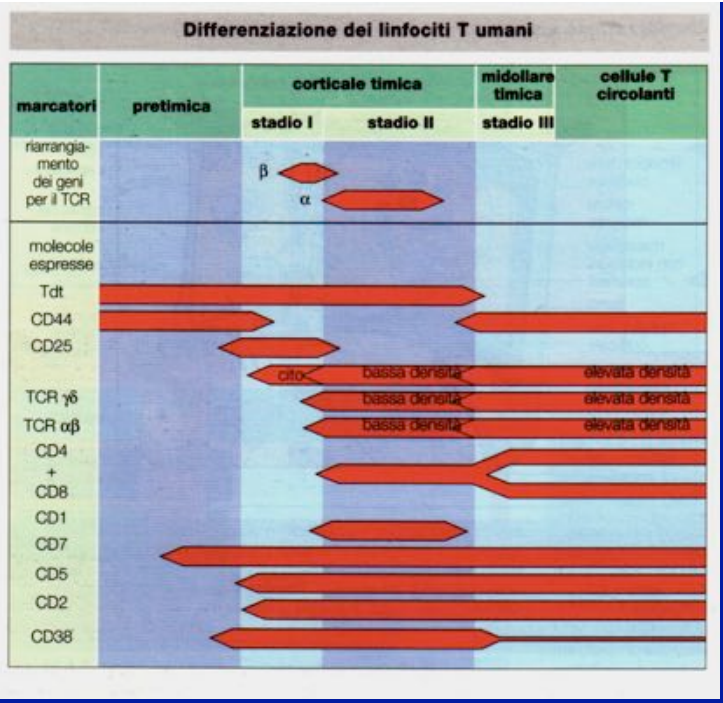


- Expression of one or more antigens on a cell type represents the method to determine if cell belongs to a lineage and / or to a defined stage of differentiation

- *By immunofluorescence and flow cytometry using monoclonal antibodies (Mab) conjugated to fluorescent probes and directed to surface, cytoplasmic or nuclear antigen it is possible to identify and enumerate populations*

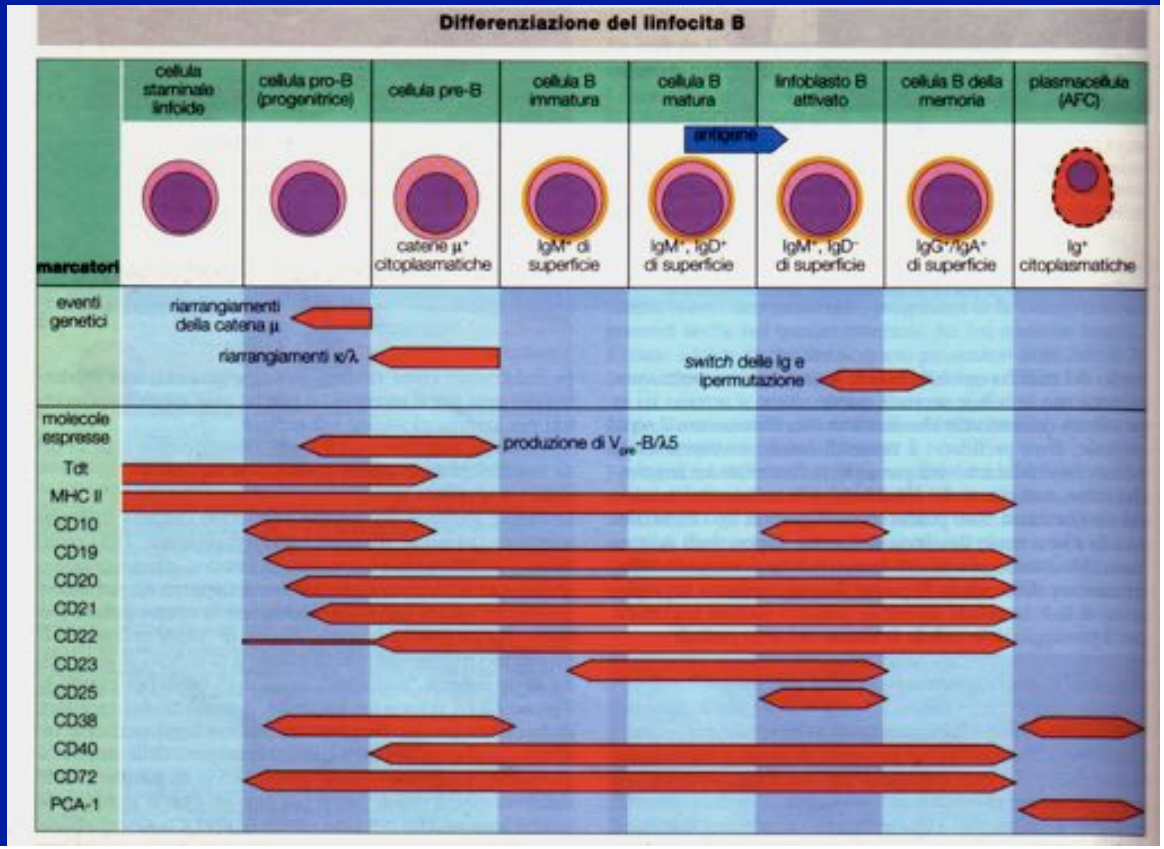
The hematopoietic cell types can be identified by monoclonal antibodies directed against antigens exclusively, or in specific combination, expressed on a given cell type



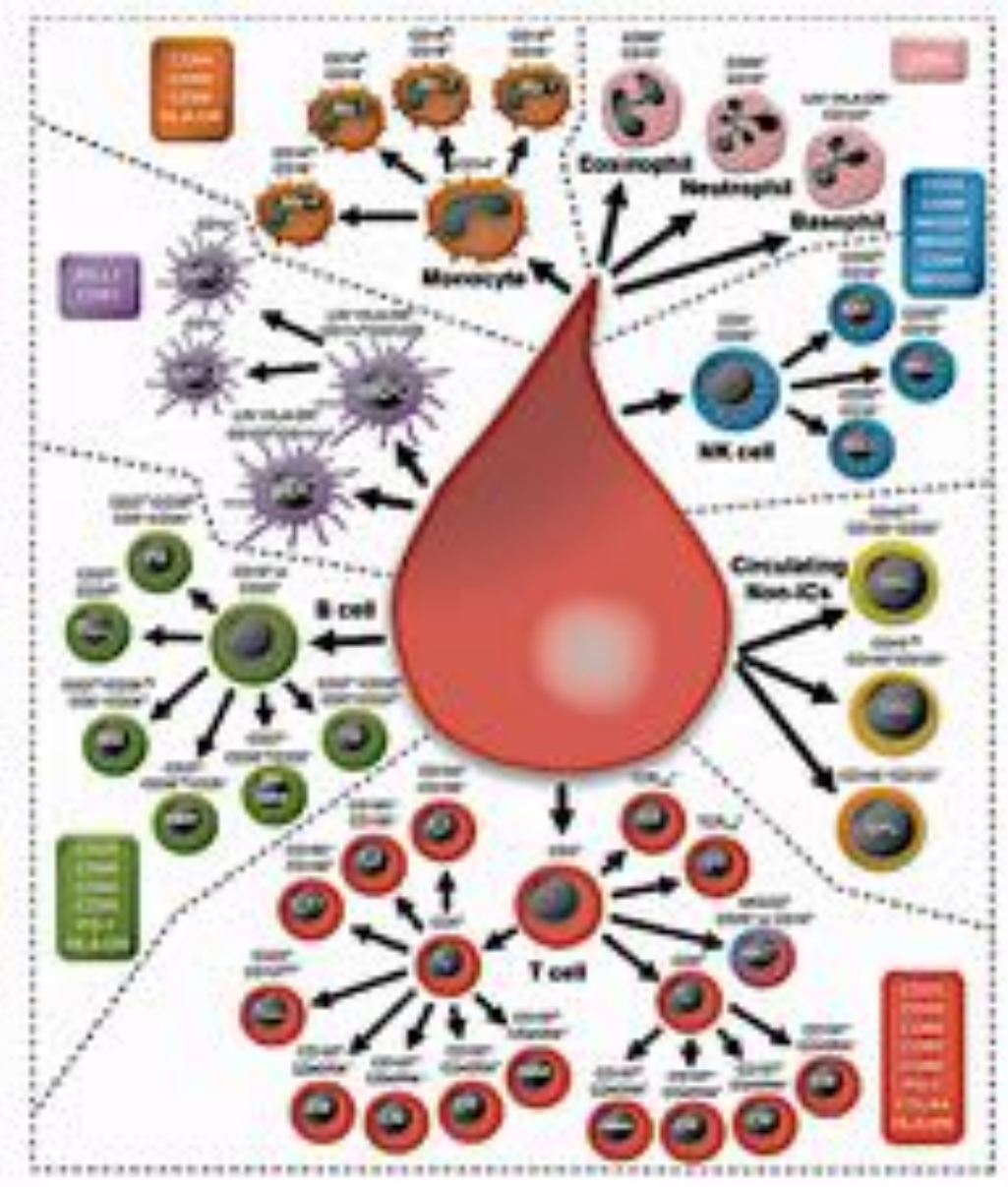


Maturational stages of T and B lymphocytes

Expression of antigens associated with different stages of development



IMMUNE CELLS



LYMPHOID LINEAGE :

lymphocytes

T	55-84 %
B	5-17 %
NK	5-15 %

CD4+/CD8+ ratio = 0.6-2.8

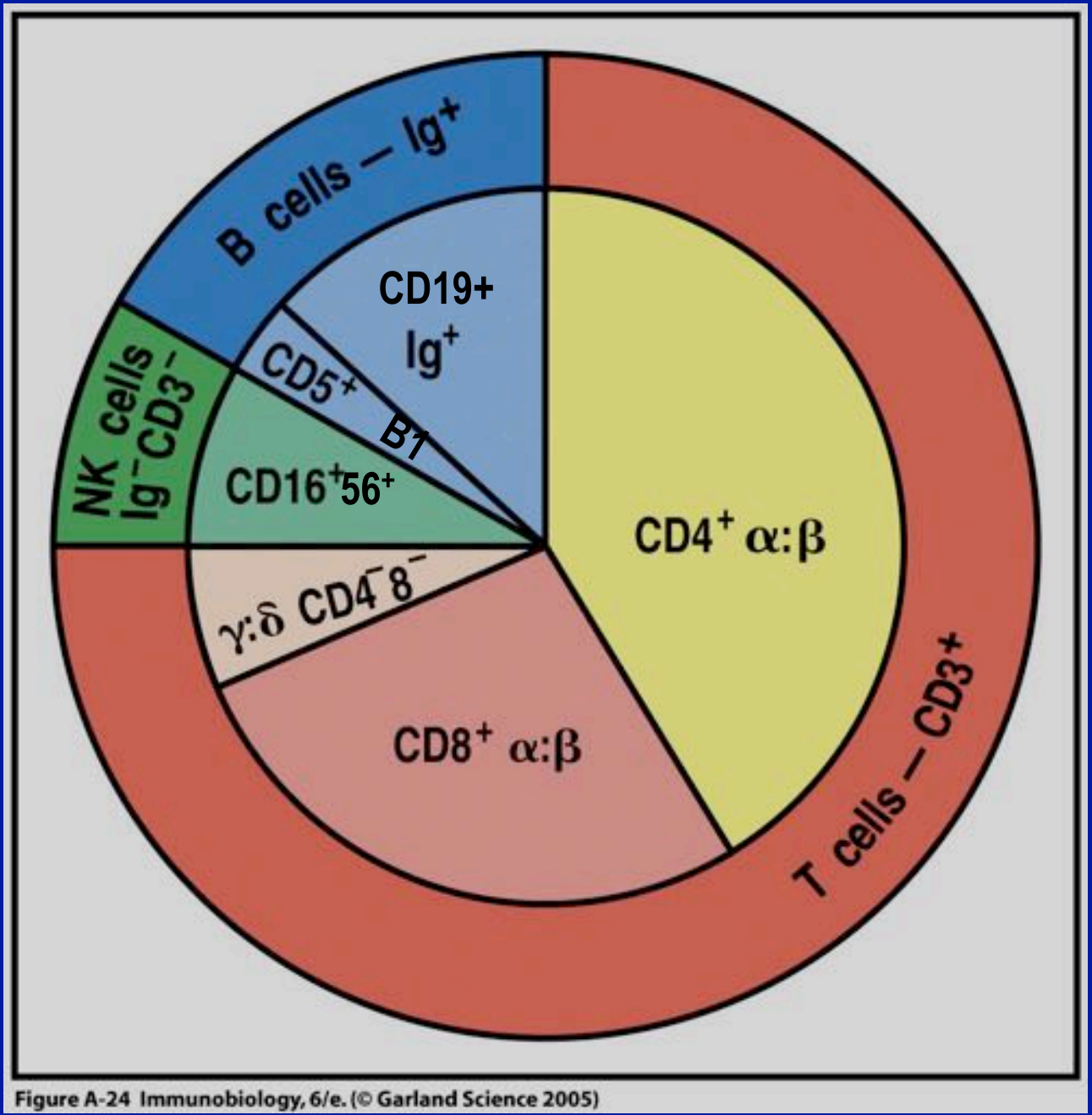
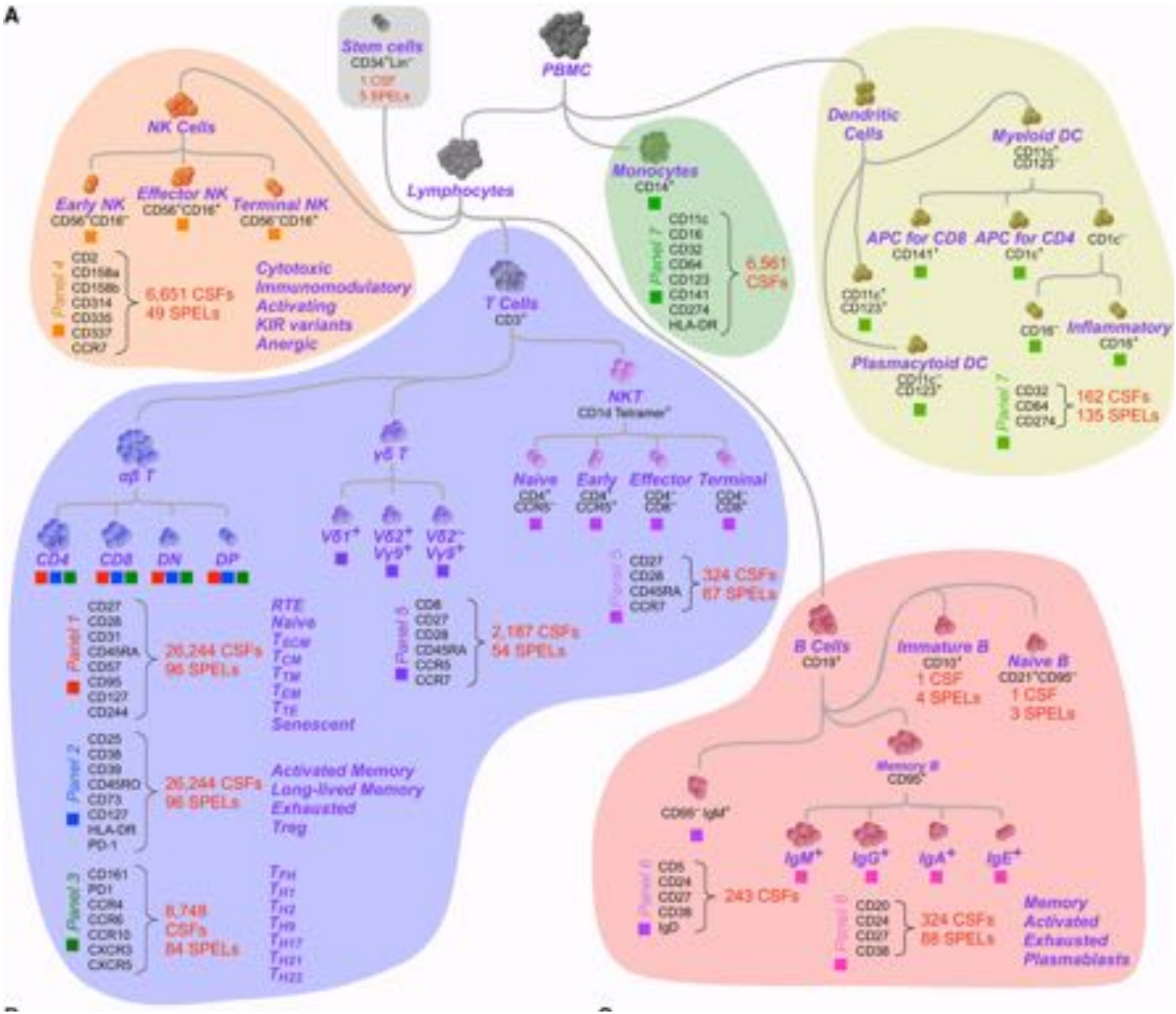


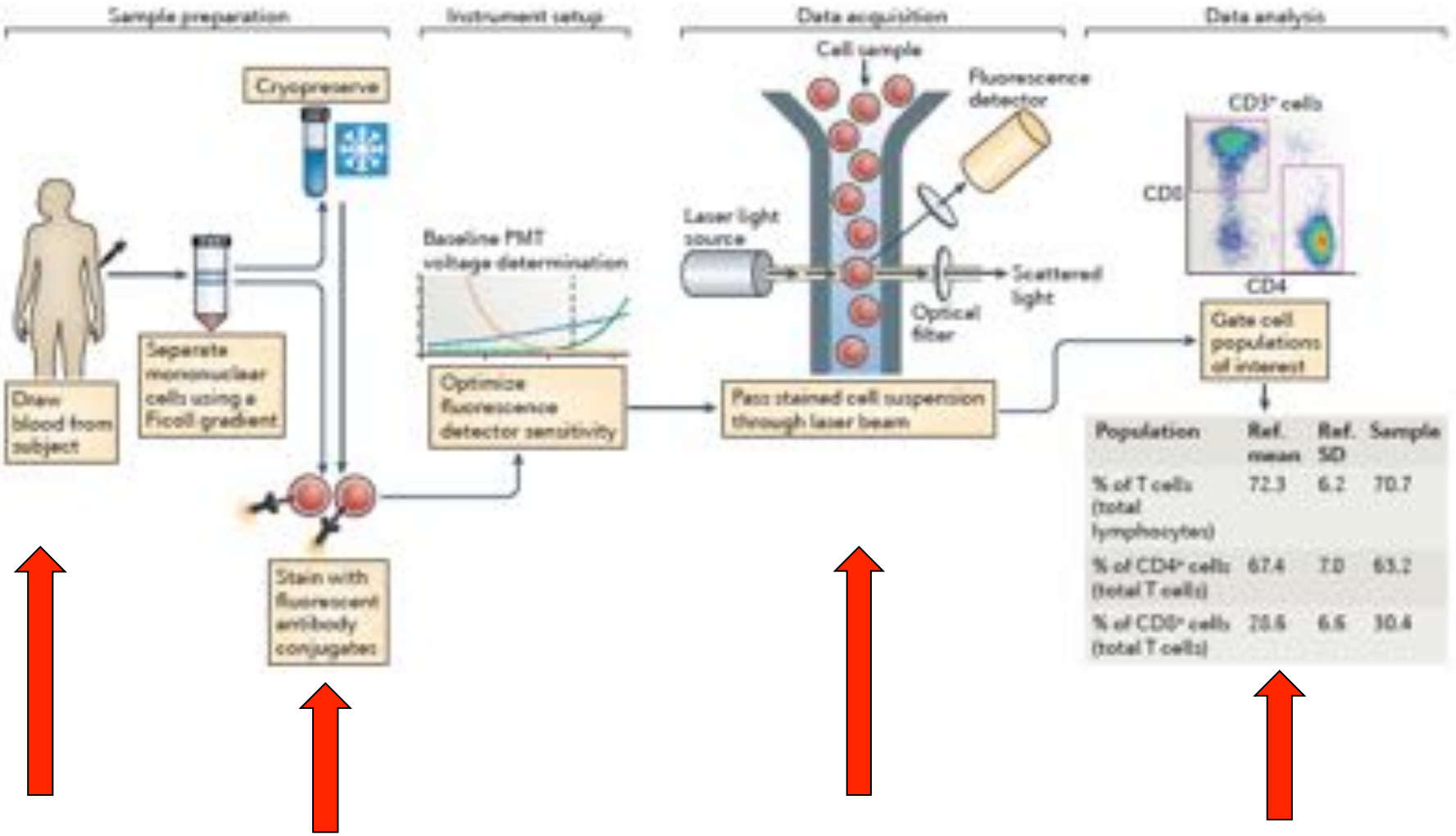
Figure A-24 Immunobiology, 6/e. (© Garland Science 2005)



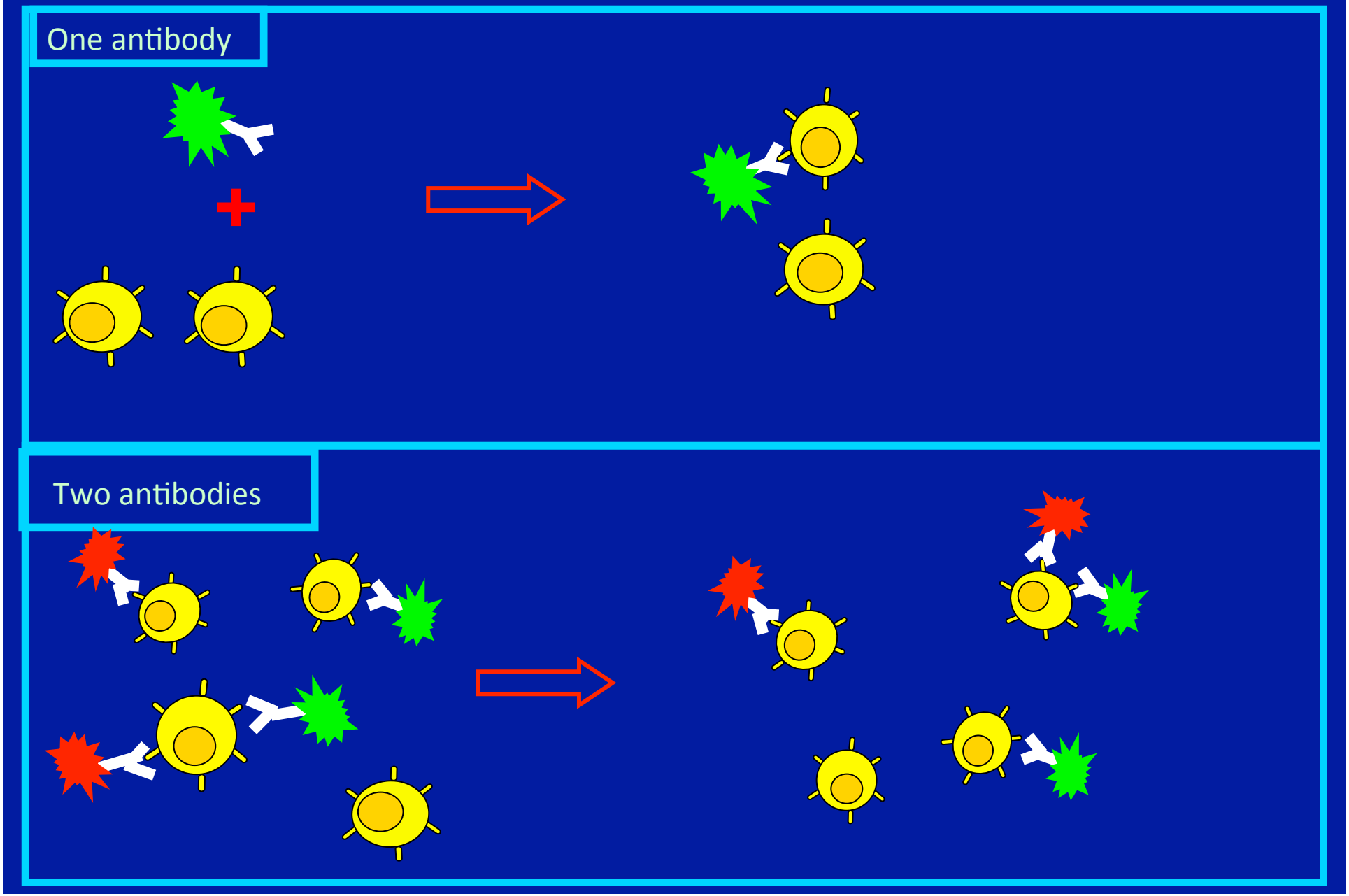
IMMUNOPHENOTYPING
of
PERIPHERAL BLOOD LYMPHOCYTE

by immunofluorescence with antibodies directed against lymphocyte antigens and Flow Cytometry (FCM)

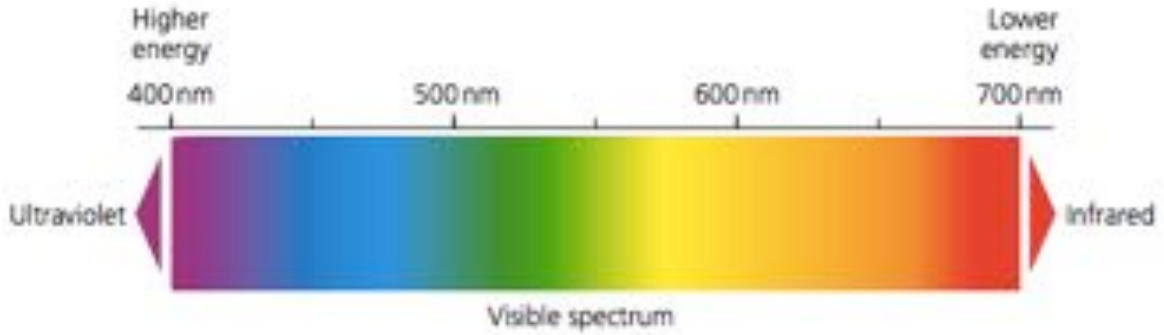
Anti-CD45	**lymphocyte**
Anti-CD3	
Anti-CD4	**T lymphocyte**
Anti-CD8	
Anti-CD19	**B lymphocyte**
Anti-CD56/CD16	**NK cell**


Sample preparation
Instrument setup
Data acquisition
Data analysis
Cryopreserve
Draw blood from subject
Separate mononuclear cells using a Ficoll gradient
Stain with fluorescent antibody conjugates
Baseline PMT voltage determination
Optimize fluorescence detector sensitivity
Cell sample
Fluorescence detector
Laser light source
Scattered light
Optical fiber
Pass stained cell suspension through laser beam
CD3+ cells
CD8
CD4
Gate cell populations of interest
Population | Ref. mean | Ref. SD | Sample
% of T cells (total lymphocytes) | 72.3 | 6.2 | 70.7
% of CD4+ cells (total T cells) | 67.4 | 7.0 | 63.2
% of CD8+ cells (total T cells) | 28.6 | 6.6 | 30.4

IMMUNOFLUORESCENCE: *staining with fluorophore-conjugated Mab*



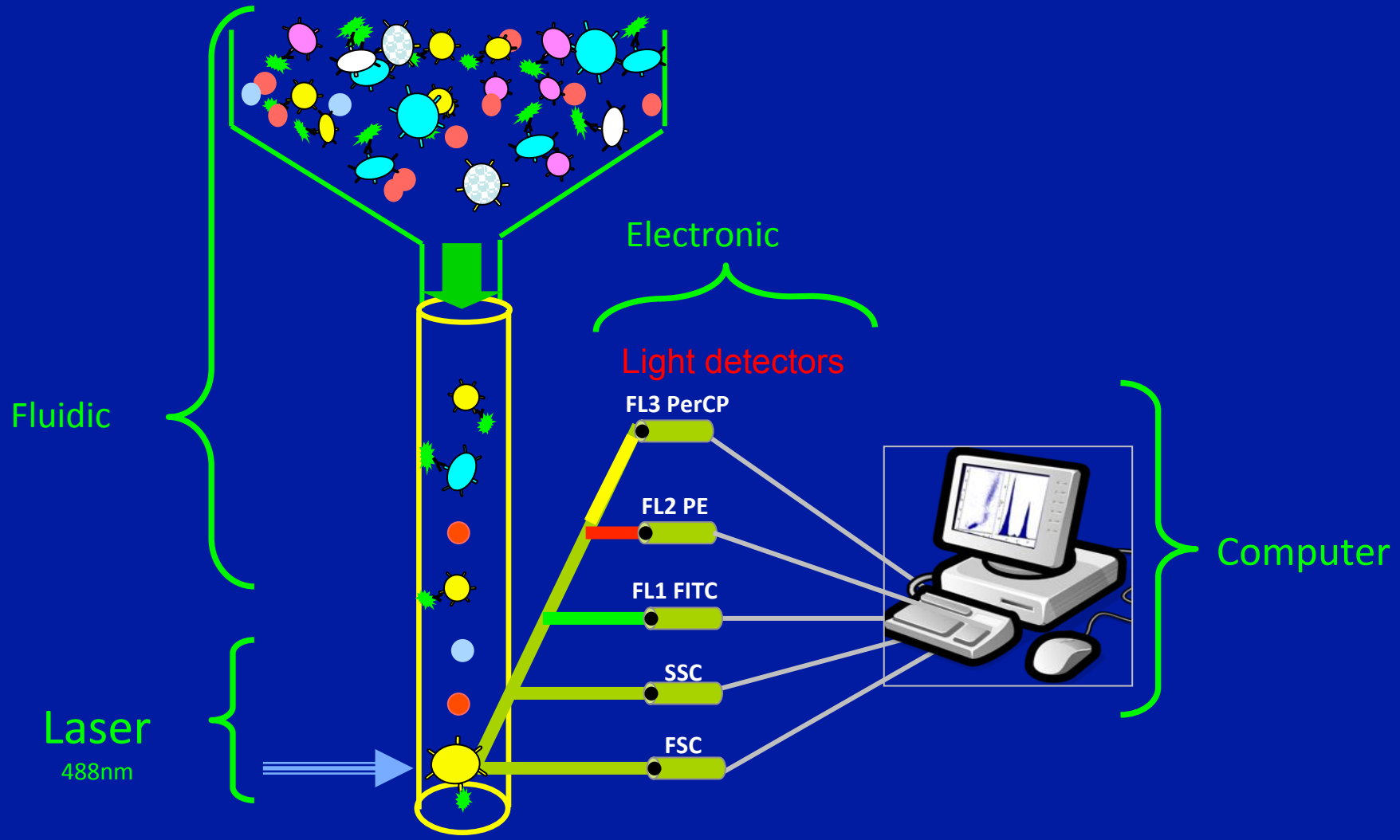
Fluorescent probe / Fluorophore



Single Dyes for Flow Cytometry/Microscopy

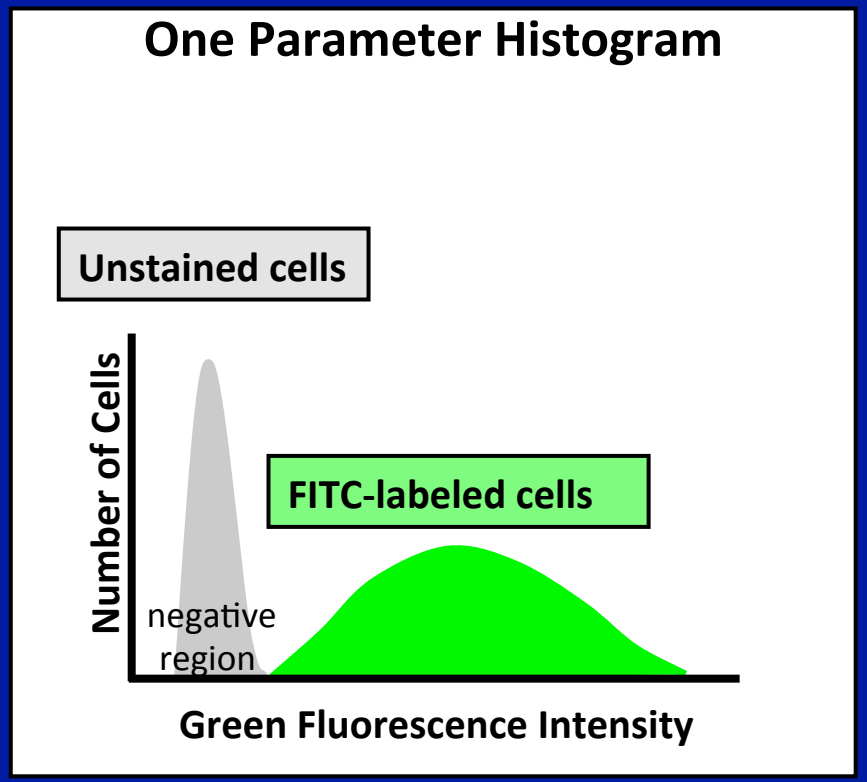
Fluorochrome	Fluorescence color	Maximal absorbance (nm)	Maximal emission (nm)	Spectrally similar dyes
Alexa Fluor® 405		401	421	Cascade Blue , DyLight® 405
Alexa Fluor® 488		495	519	Cy2, DyLight® 488, FITC
Alexa Fluor® 647		650	665	APC, Cy5, DyLight® 649
Alexa Fluor® 700	Infrared	702	723	Alexa Fluor® 647
APC		650	661	Alexa Fluor® 647, Cy5
Cy5		649	670	
DyLight® 405		400	420	Alexa Fluor® 405, Cascade Blue
DyLight® 488		493	518	Alexa Fluor® 488, FITC
DyLight® 549		562	576	Alexa Fluor® 546, Alexa Fluor® 555, Cy3, TRITC
DyLight® 649		654	673	Alexa Fluor® 647, Cy5
DyLight® 680		692	712	Alexa Fluor® 650, Cy5.5
DyLight® 750	Near Infrared	752	778	Alexa Fluor® 750
DyLight® 800	Infrared	777	794	IR Dye® 800
FITC		490	525	Alexa Fluor® 488, Cy2, DyLight® 488
Pacific Blue™		410	455	
PerCP		490	675	
PE		490; 565	578	
Texas Red®		596	615	
TRITC		596	570	Alexa Fluor® 488, Cy3

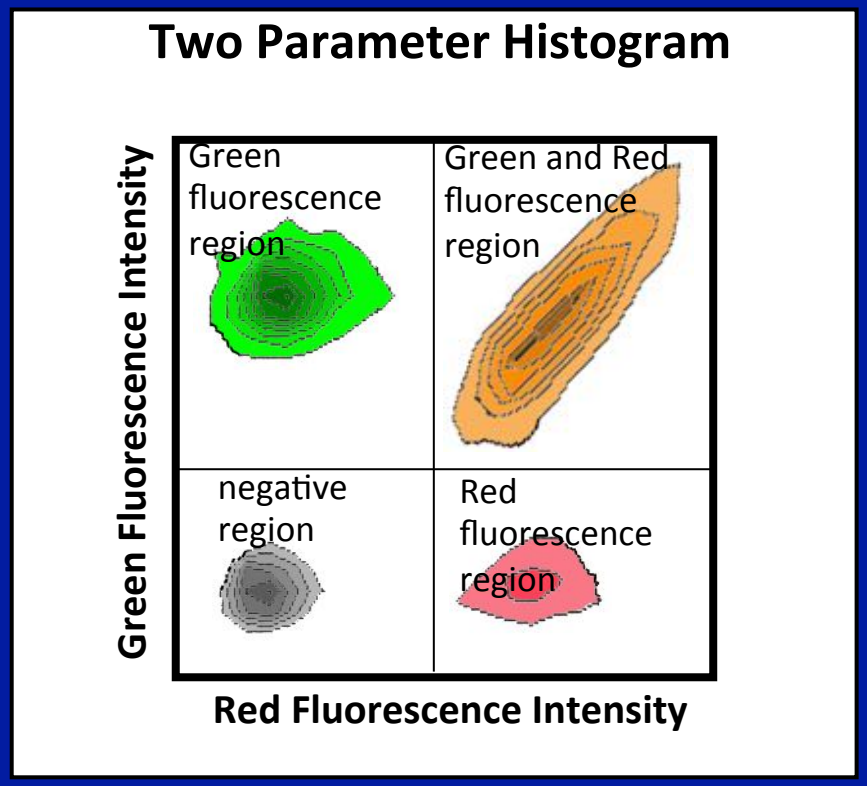
Flow Cytometry



Types of FCM Cytogram

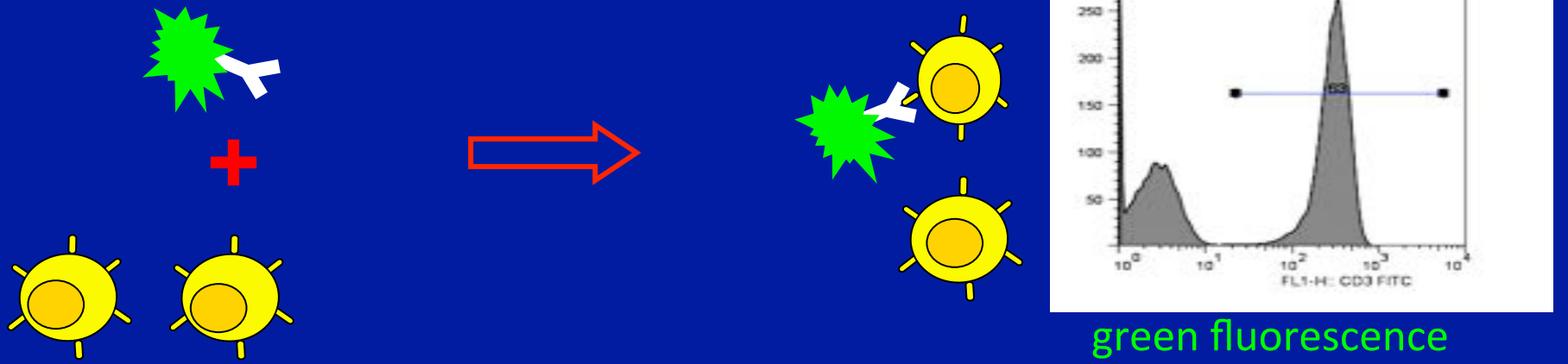
representation of fluorescence distribution





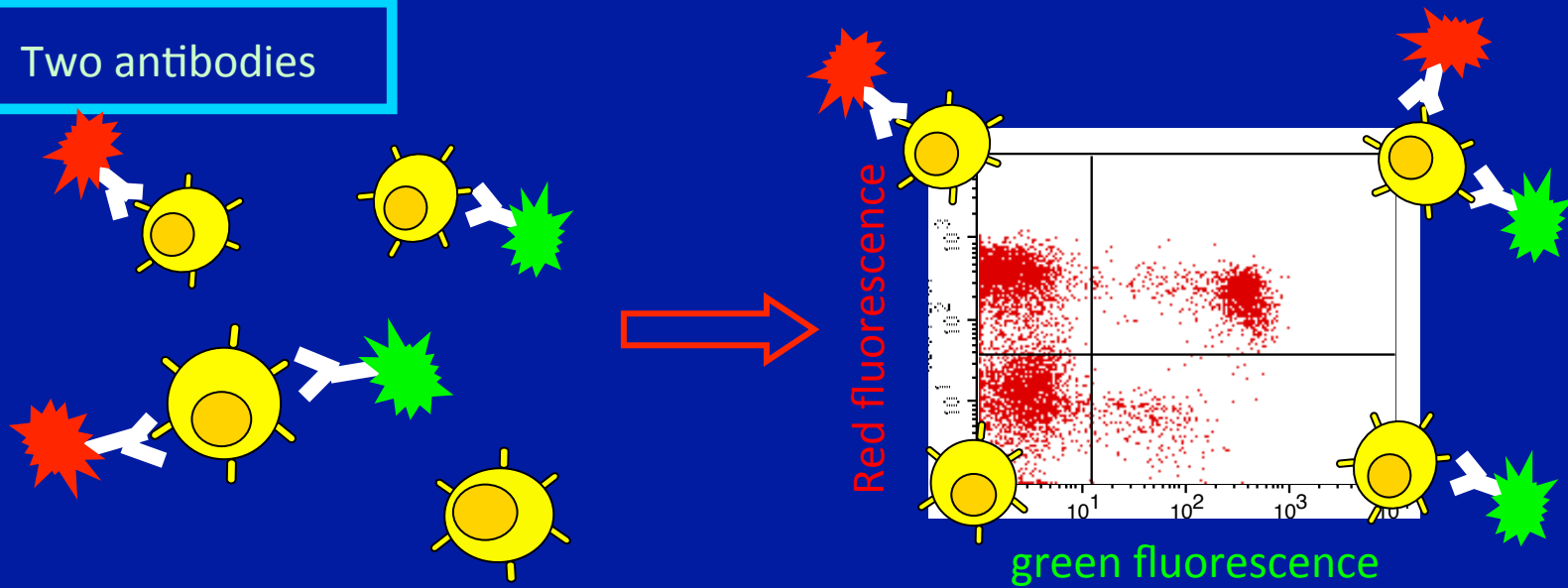
IMMUNOFLUORESCENCE and distribution of fluorescence

One antibody



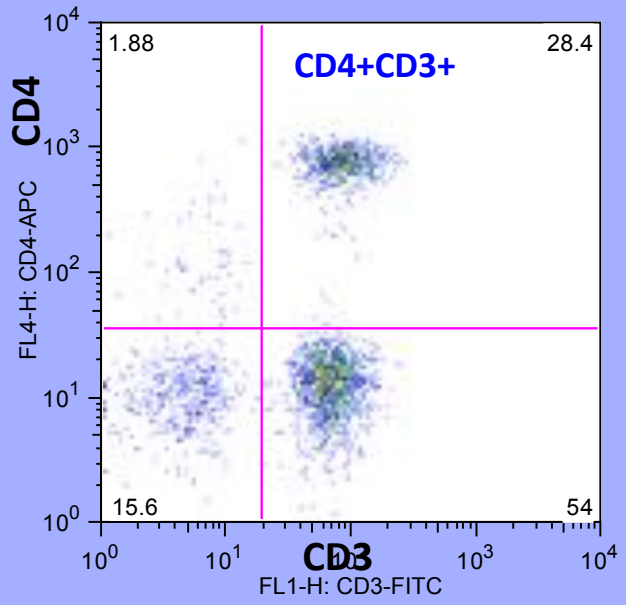
green fluorescence

Two antibodies

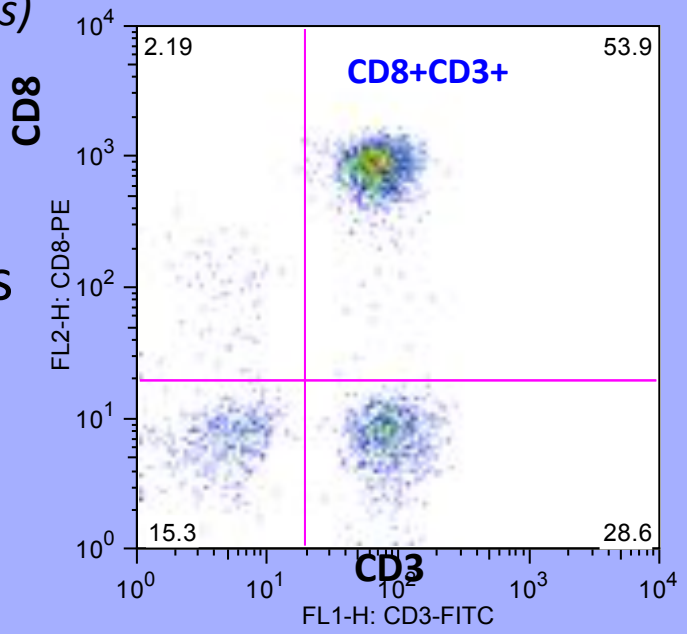


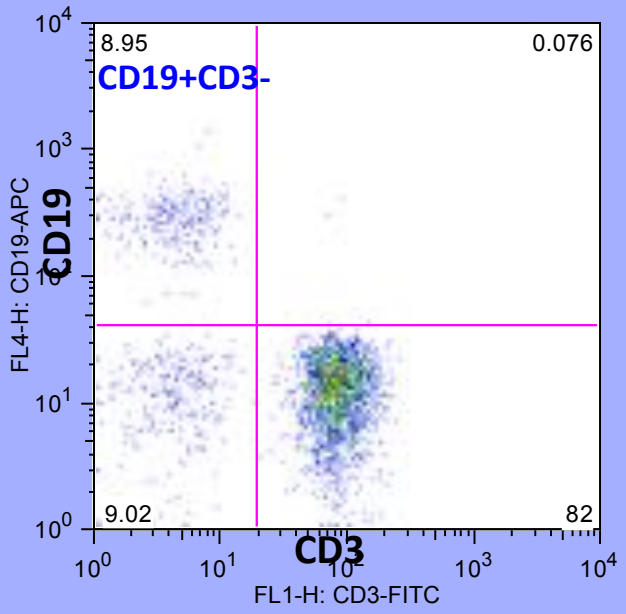
IMMUNOPHENOTYPING: example of **lymphocyte cytograms**

(fluorescence distributions)

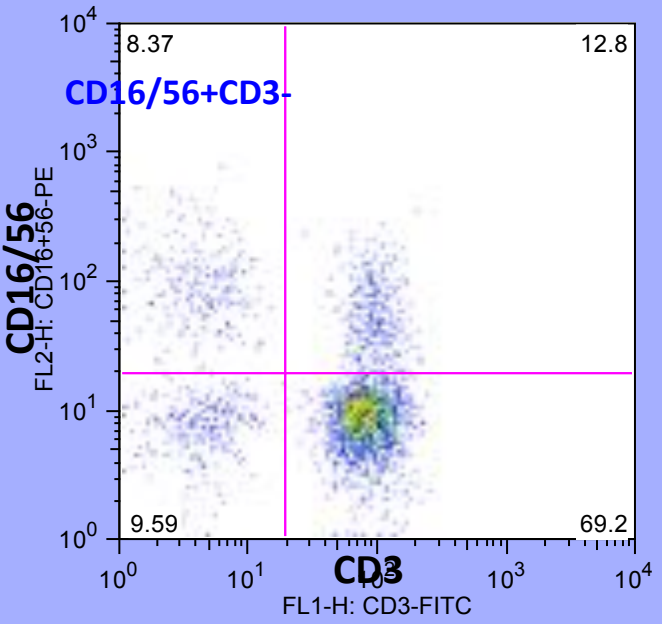


CD4 / CD8 subsets

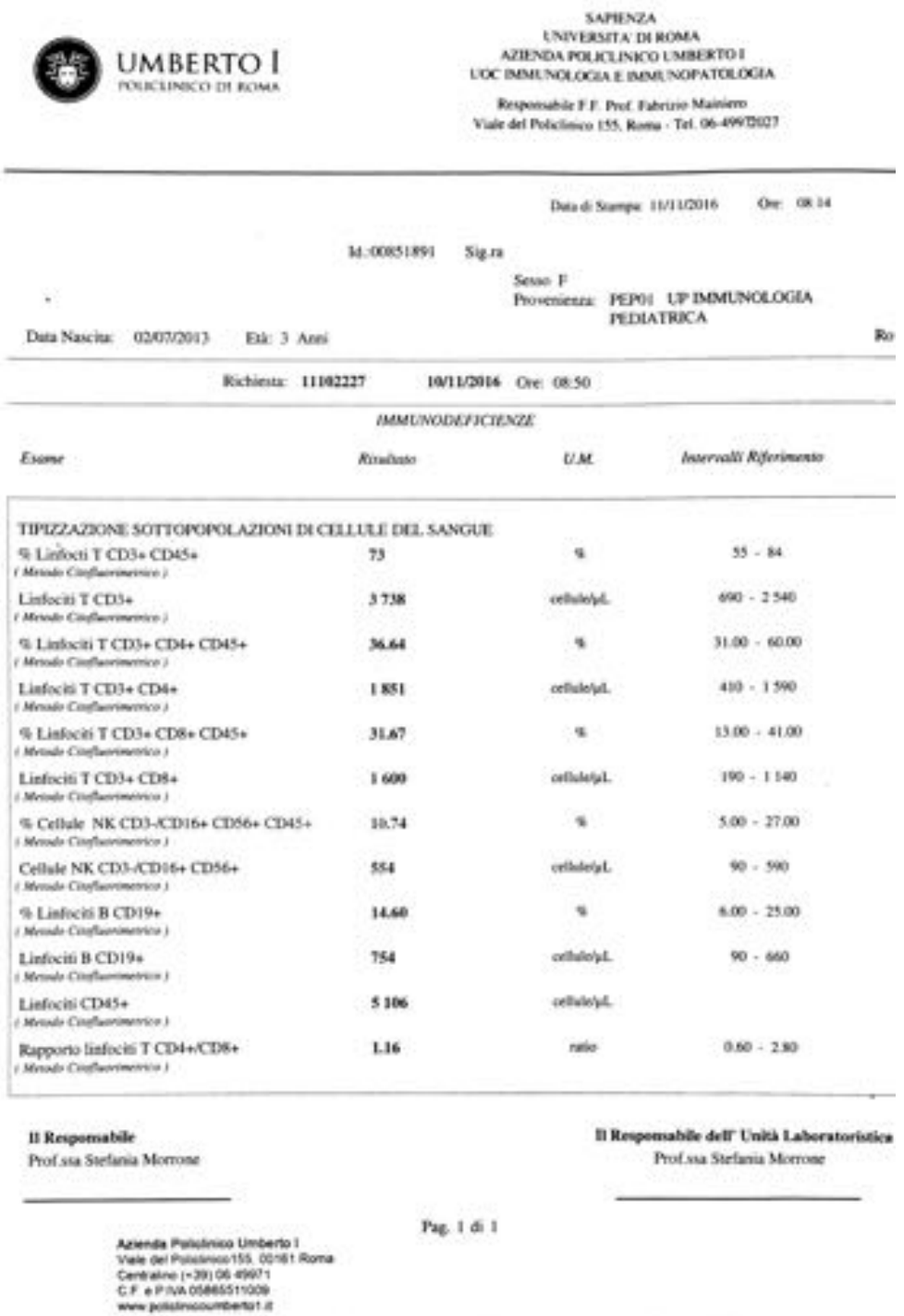




T/ B / NK lymphocytes



Immunophenotyping Report



UMBERTO I
POLICLINICO DI ROMA

SAPIENZA
UNIVERSITA' DI ROMA
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UOC IMMUNOLOGIA E IMMUNOPATOLOGIA
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Data di Stampa: 11/11/2016 Ore: 08:14

Id.:00851891 Sig.ra

Sesso: F
Provenienza: PEP01 UP IMMUNOLOGIA PEDIATRICA

Data Nascita: 02/07/2013 Età: 3 Anni Ro

Richiesta: **11102227** **10/11/2016** Ore: 08:50

IMMUNODEFICIENZE

Esame	*Risultato*	*U.M.*	*Intervalli Riferimento*
TIPIZZAZIONE SOTTOPOPOLAZIONI DI CELLULE DEL SANGUE			
% Linfoci T CD3+ CD45+ *(Metodo Citofluorimetrico)*	73	%	55 - 84
Linfociti T CD3+ *(Metodo Citofluorimetrico)*	3 738	cellule/µL	690 - 2 540
% Linfociti T CD3+ CD4+ CD45+ *(Metodo Citofluorimetrico)*	36.64	%	31.00 - 60.00
Linfociti T CD3+ CD4+ *(Metodo Citofluorimetrico)*	1 851	cellule/µL	410 - 1 590
% Linfociti T CD3+ CD8+ CD45+ *(Metodo Citofluorimetrico)*	31.67	%	13.00 - 41.00
Linfociti T CD3+ CD8+ *(Metodo Citofluorimetrico)*	1 600	cellule/µL	190 - 1 140
% Cellule NK CD3-/CD16+ CD56+ CD45+ *(Metodo Citofluorimetrico)*	10.74	%	5.00 - 27.00
Cellule NK CD3-/CD16+ CD56+ *(Metodo Citofluorimetrico)*	554	cellule/µL	90 - 590
% Linfociti B CD19+ *(Metodo Citofluorimetrico)*	14.60	%	6.00 - 25.00
Linfociti B CD19+ *(Metodo Citofluorimetrico)*	754	cellule/µL	90 - 660
Linfociti CD45+ *(Metodo Citofluorimetrico)*	5 106	cellule/µL	
Rapporto linfociti T CD4+/CD8+ *(Metodo Citofluorimetrico)*	1.16	ratio	0.60 - 2.80

Il Responsabile
Prof.ssa Stefania Morrone

Il Responsabile dell' Unità Laboratoristica
Prof.ssa Stefania Morrone

Pag. 1 di 1

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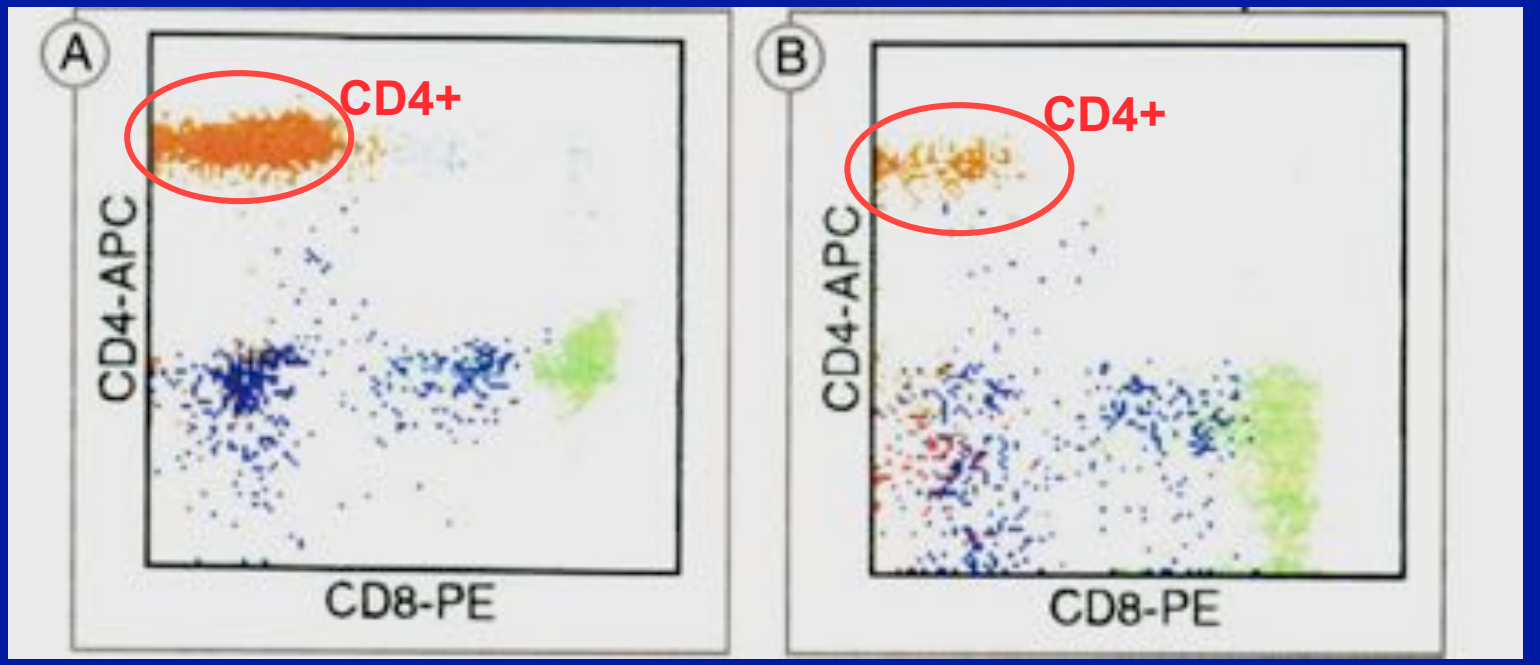
Flow cytometric analysis of T lymphocyte CD4+ and CD8+ in patient with HIV infection

Normal subject

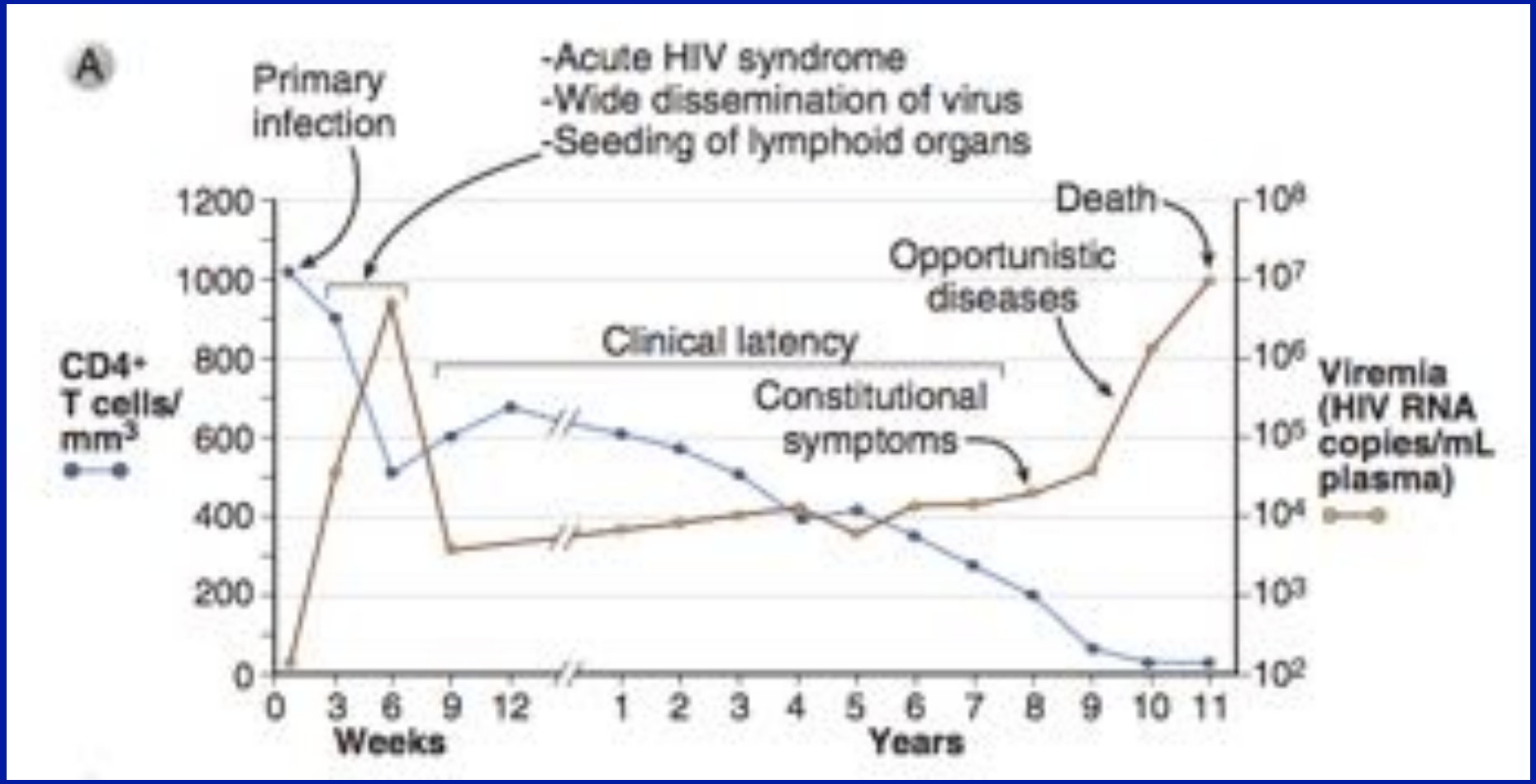
HIV+ patient



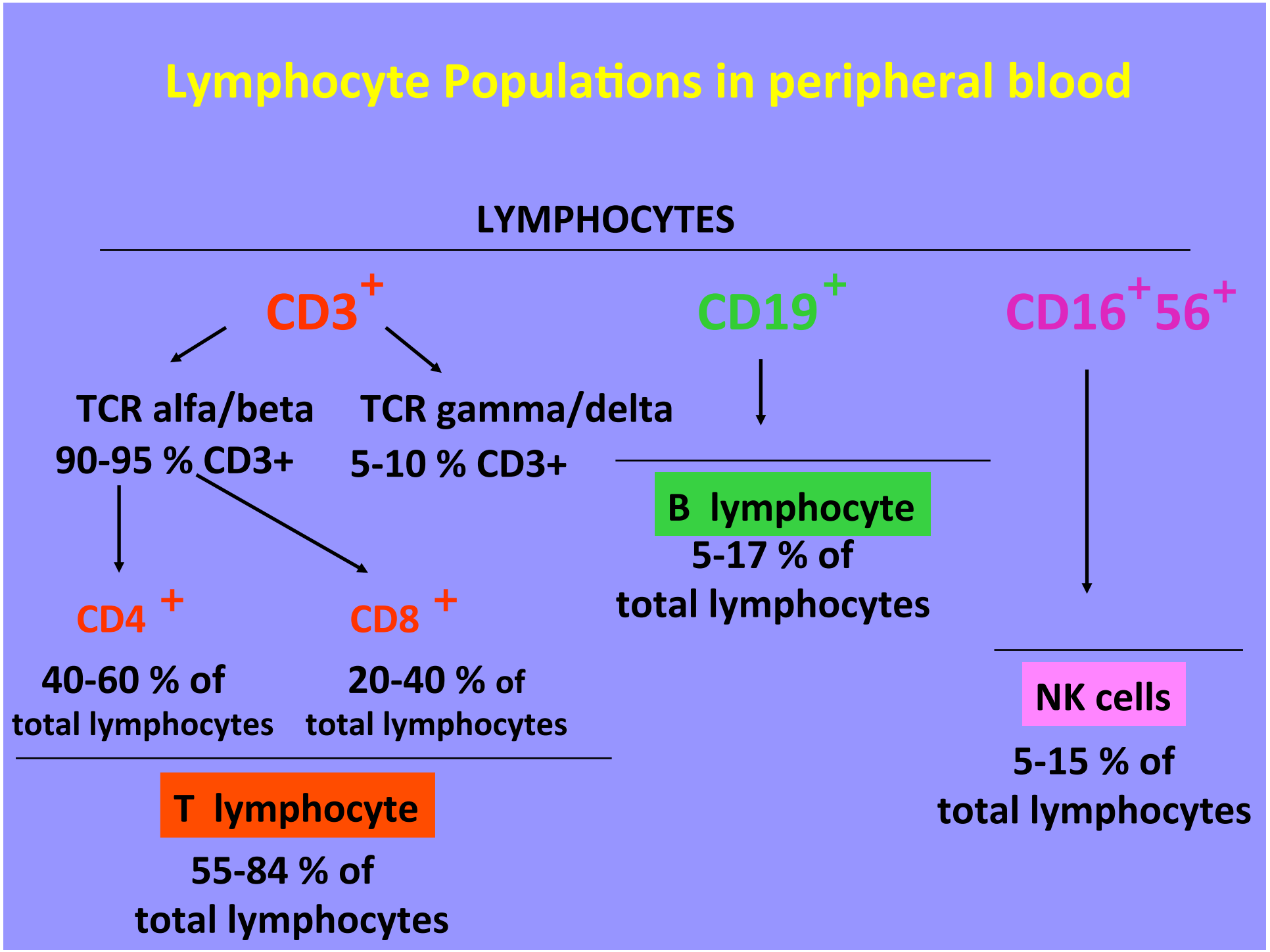
1395 CD4+ T lymphocyte/mm ^{3}

66 CD4+ T lymphocyte/mm ^{3}

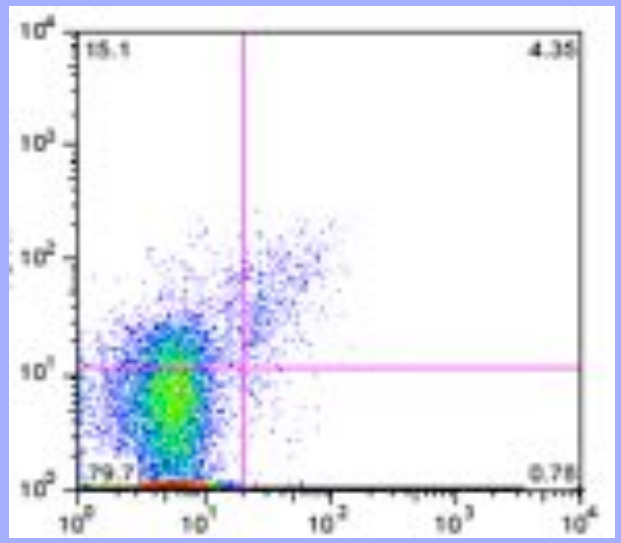
CLINICAL COURSE OF HIV INFECTION



Lymphocyte Populations in peripheral blood



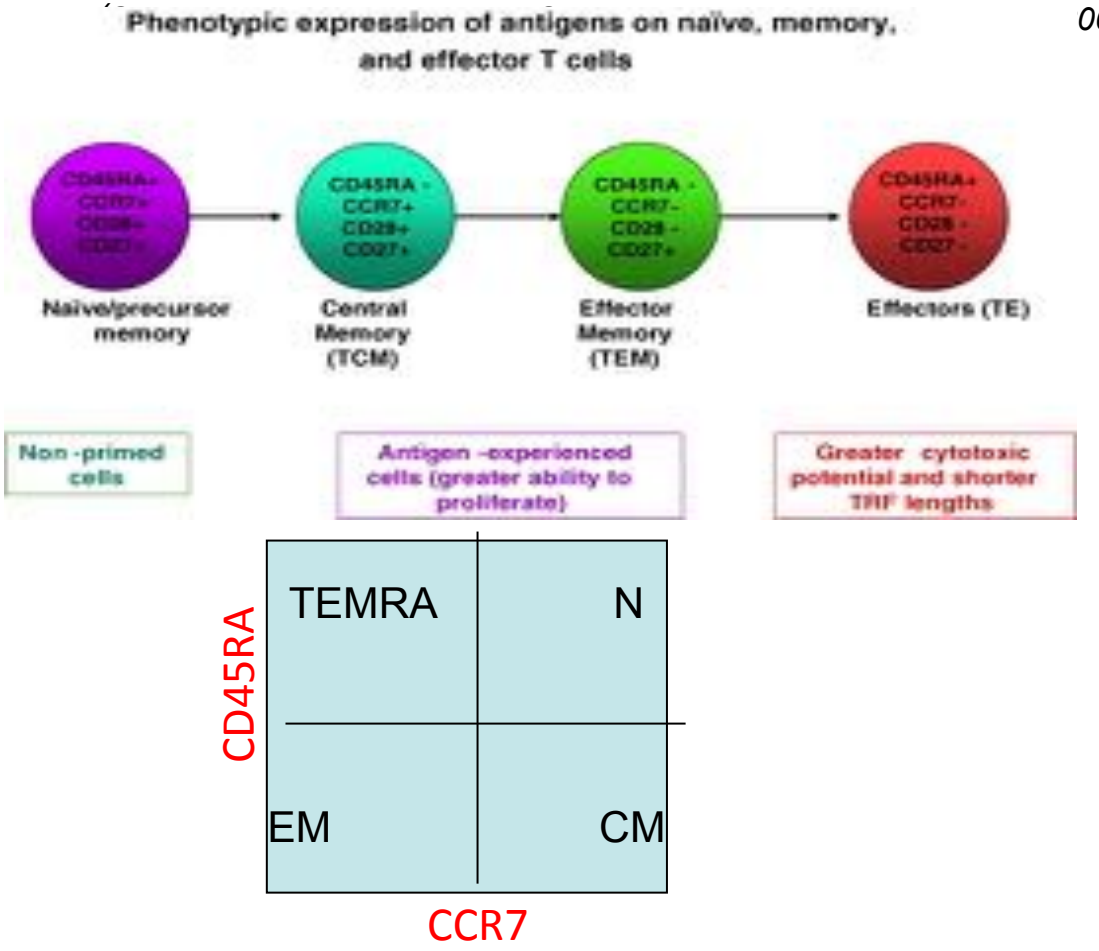
T reg lymphocyte CD4+CD25+FoxP3+



Naive/memory phenotype

Four functional T-cell compartments are defined in humans by the expression of CD45RA and CCR7:

- **Naïve** precursor (CD45RA+ CCR7+),
- **CM** central memory (CD45RA-CCR7+),
- **EM** effector memory (CD45RA- CCR7-)
- **TEMRA** Terminally *differentiated effector* memory (CD45RA+ CCR7−)



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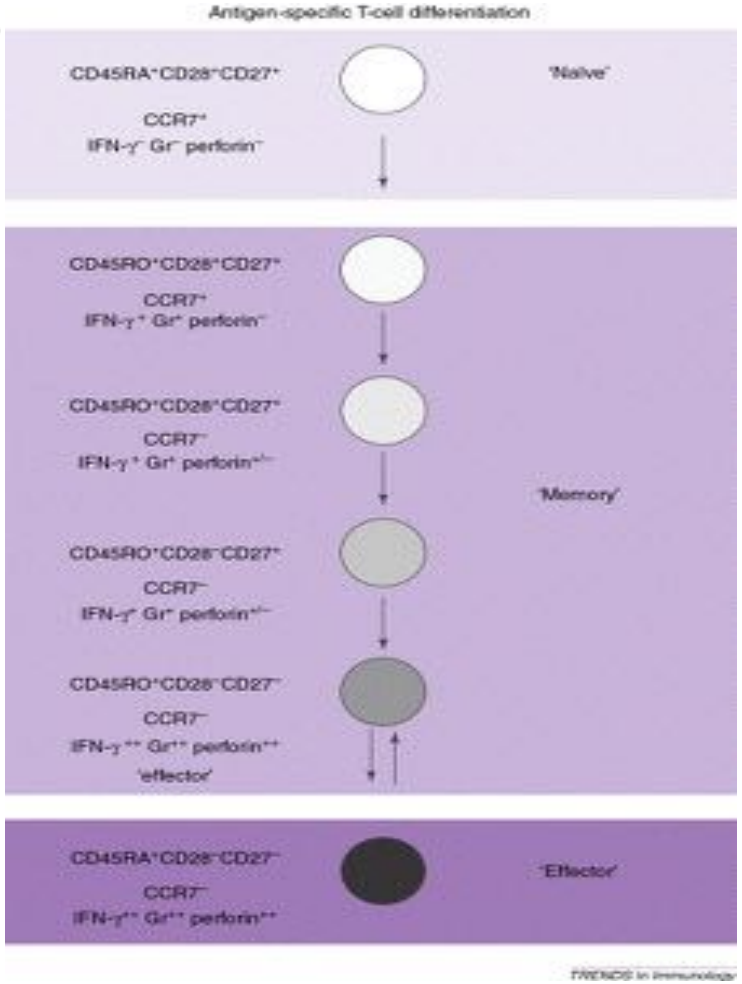
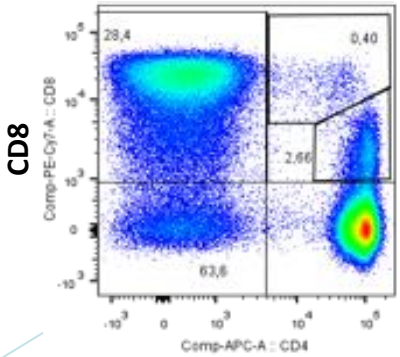


Naïve - memory

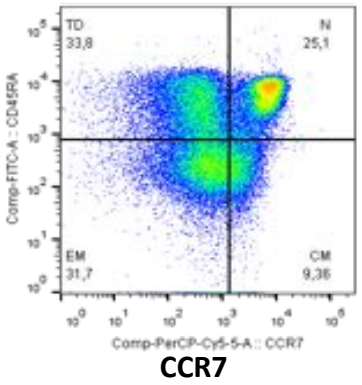
T Cells



CD8

CD4

CD8+

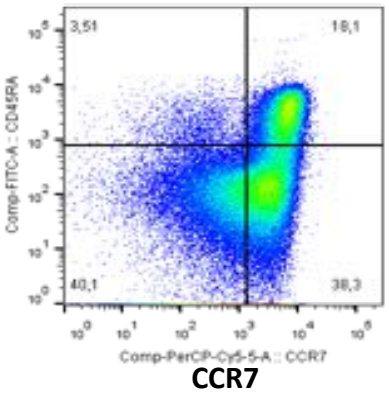


CD45RA

CCR7

TEMRA	N
EM	CM

CD4+

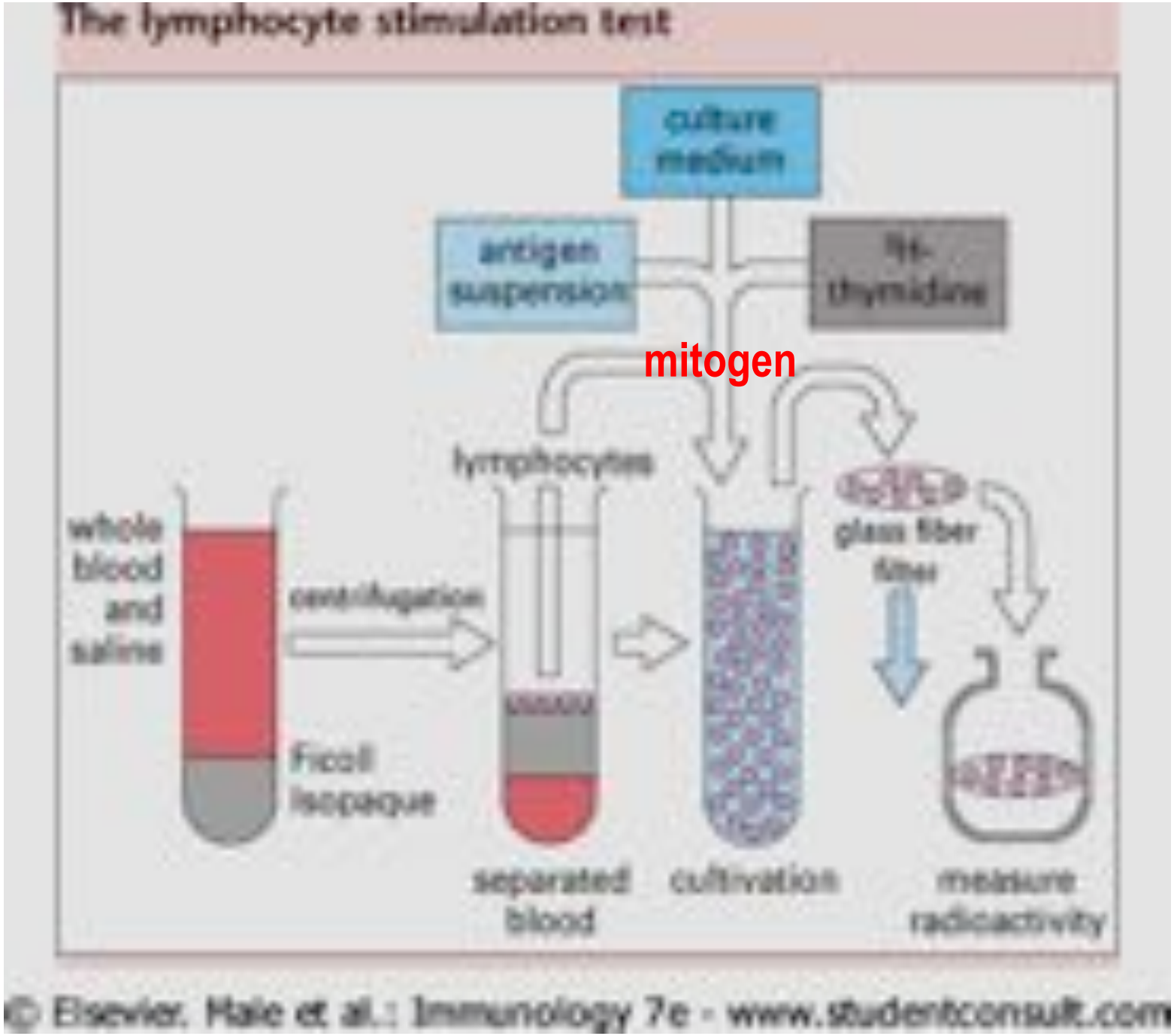


CCR7

MITOGEN and lymphocyte proliferation

Mitogen	Responding cells
Phytohemagglutinin (PHA) (red kidney bean)	T cells
Concanavalin (ConA) (Jack bean)	T cells
Pokeweed mitogen (PWM) (Pokeweed)	T and B cells
Lipopolysaccharide (LPS) (*Escherichia coli*)	B cells (mouse)

Figure A-35 Immunobiology, 6/e. (© Garland Science 2005)


The lymphocyte stimulation test
culture medium
antigen suspension
³H-thymidine
mitogen
lymphocytes
whole blood and saline
centrifugation
Ficoll isopaque
glass fiber filter
separated blood
cultivation
measure radioactivity
© Elsevier. Male et al.: Immunology 7e - www.studentconsult.com

Lymphocyte PROLIFERATION TEST :

mitogen stimulated lymphocytes incorporate thymidine

Dose/Response curve

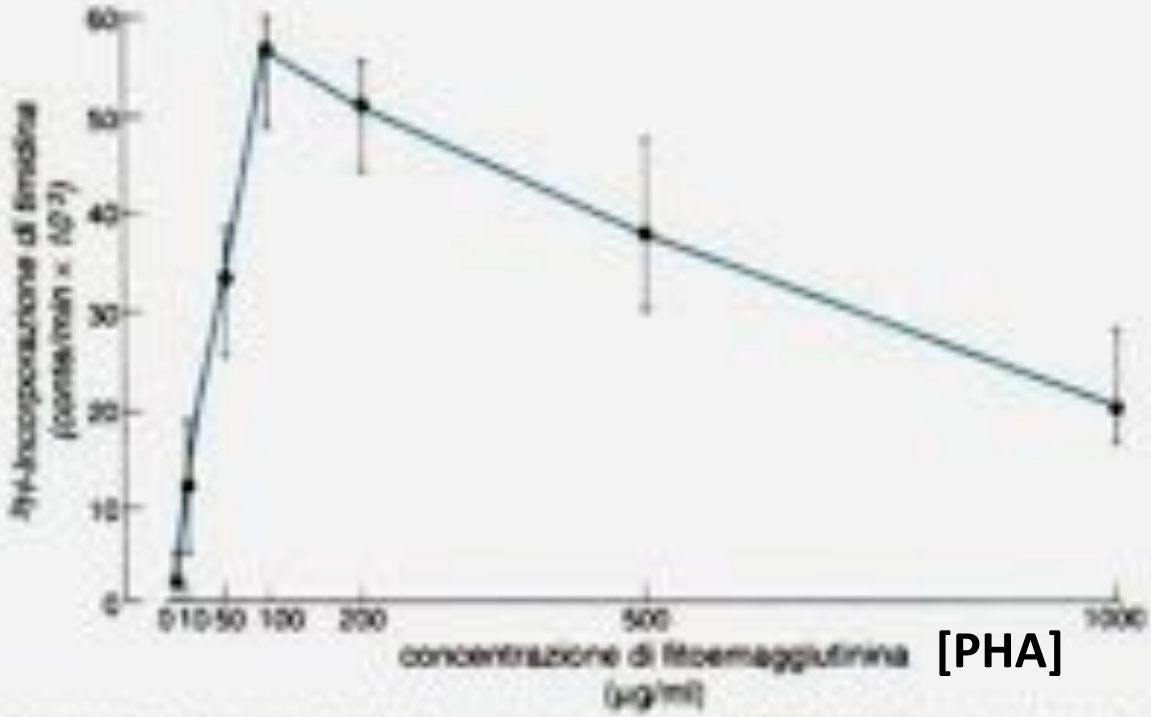


Figura 15-6. Curva di risposta in funzione della dose di stimolazione di 10^5 linfociti con mitogeni. Curva dose-risposta di un gruppo di dieci adulti normali i cui linfociti del sangue periferico sono stati stimolati con varie concentrazioni di fitoemagglutinina per 72 ore. I linfociti sono stati marcati con 2 μCi di timidina triziata per 6 ore prima della raccolta. Le conte per minuto di timidina triziata incorporata sono state determinate in uno spettrometro di scintillazione e riportate come media delle determinazioni di 10 individui ±1DS. La risposta massima si ottiene con 100-200 μg/ml di fitoemagglutinina.

PROLIFERATION Curve

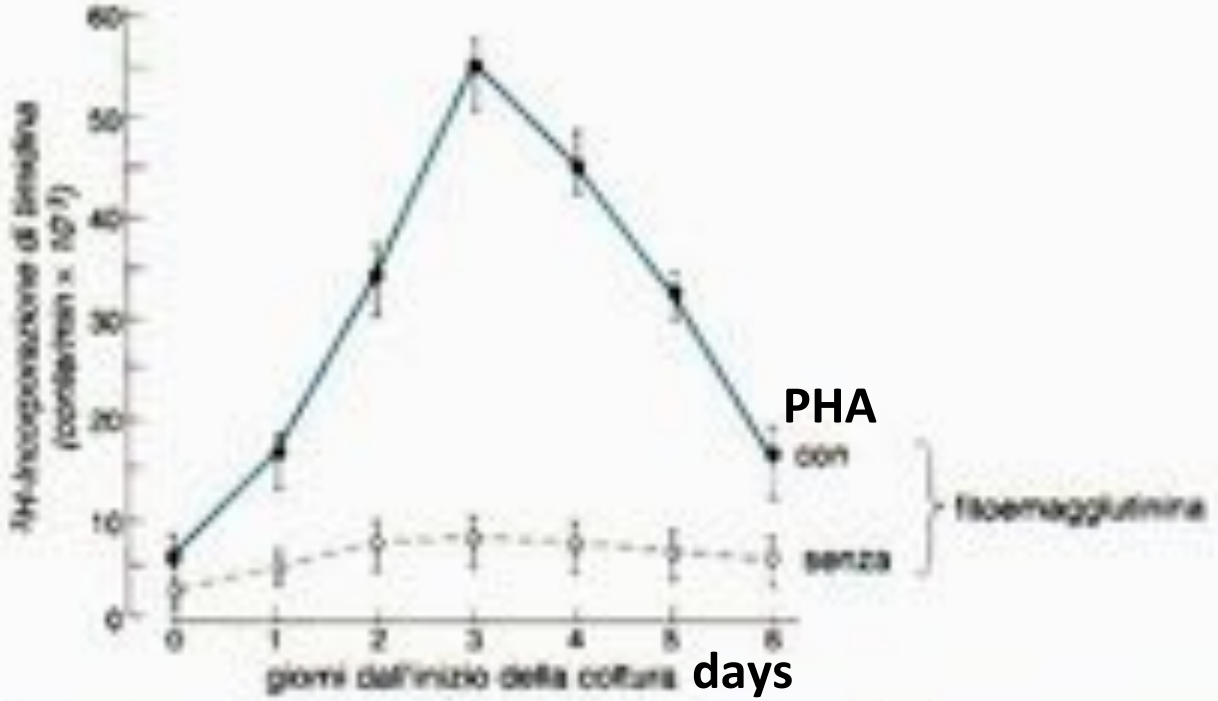


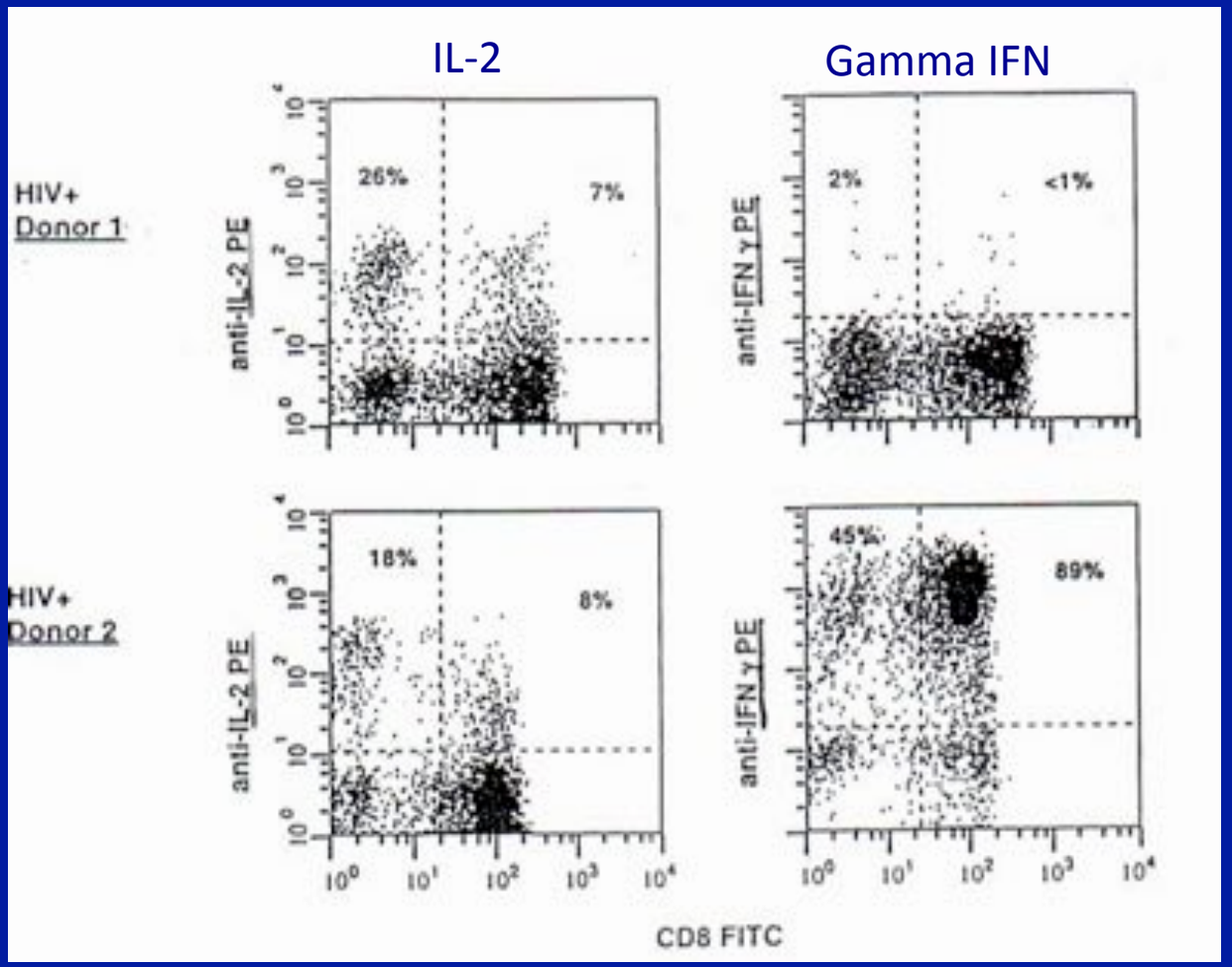
Figura 15-7. Curva di risposta in funzione del tempo di stimolazione di 10^5 linfociti con mitogeni. Curva in funzione del tempo dei linfociti del sangue periferico di dieci adulti normali, stimolati in tessuti di coltura per vari periodi di tempo a una concentrazione ottimale di fitoemagglutinina (100 µg/ml). Le colture sono state marcate con timidina triziata per 6 ore, il giorno della raccolta. La massima risposta si ottiene dopo 3 giorni dall'inizio della coltura. I risultati sono riportati come media ± 1 DS della conta per minuto.

ADDITIONAL TESTS

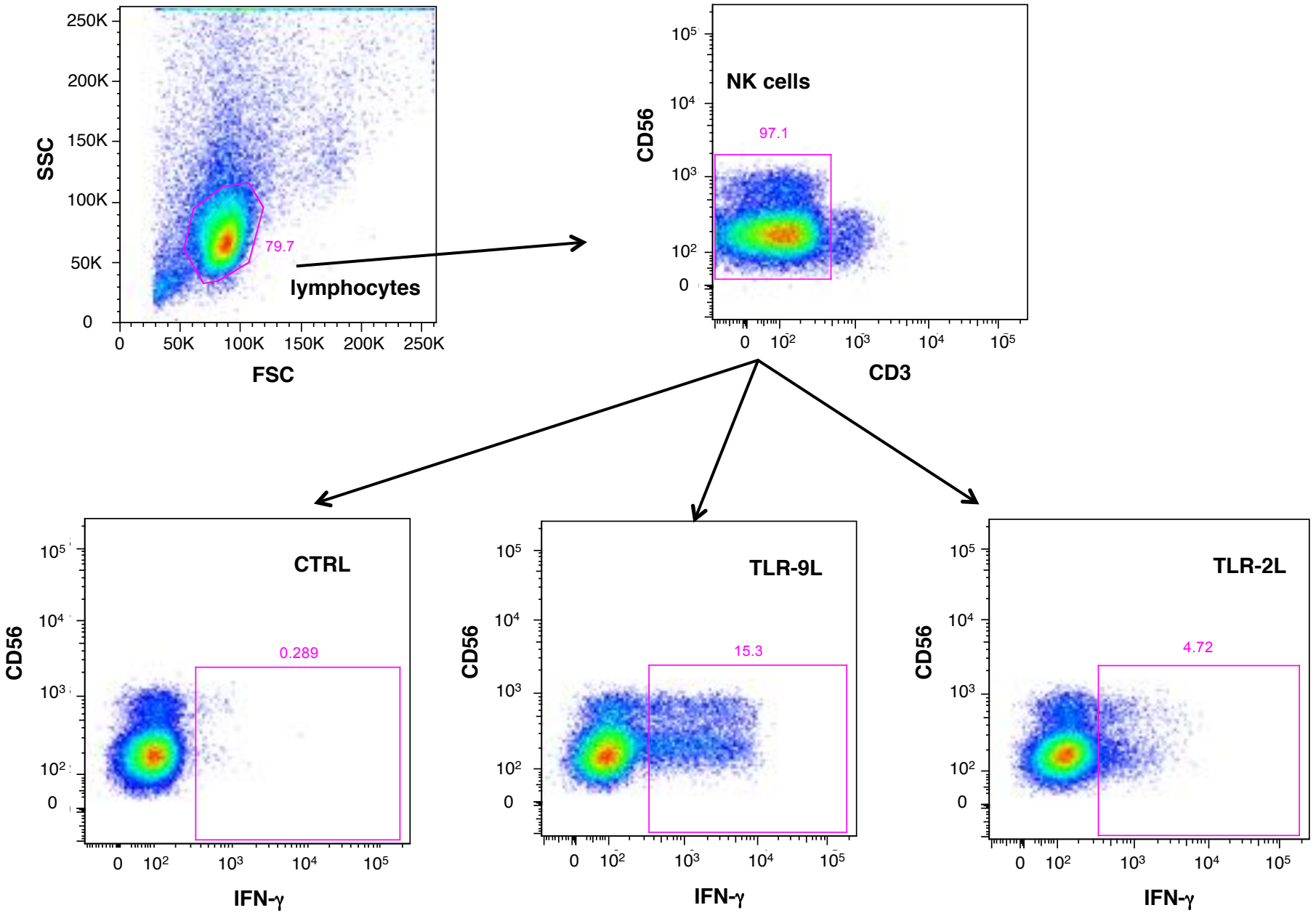
Tabella 19-4. Test aggiuntivi di competenza T-cellulare non disponibili correntemente per una valutazione clinica.

Produzione di linfochine
IL-2, IL-3, IL-5
Interferon gamma
IL-4
TNF
Recettori per linfochine
IL-1
IL-2
IL-4
Interferon gamma
Citotossicità linfocitaria
MHC-ristretta
MHC-non ristretta
Antigene-specifica
Lectina (PHA) dipendente
Citotossicità cellulo-mediata anticorpo-dipendente (ADCC)

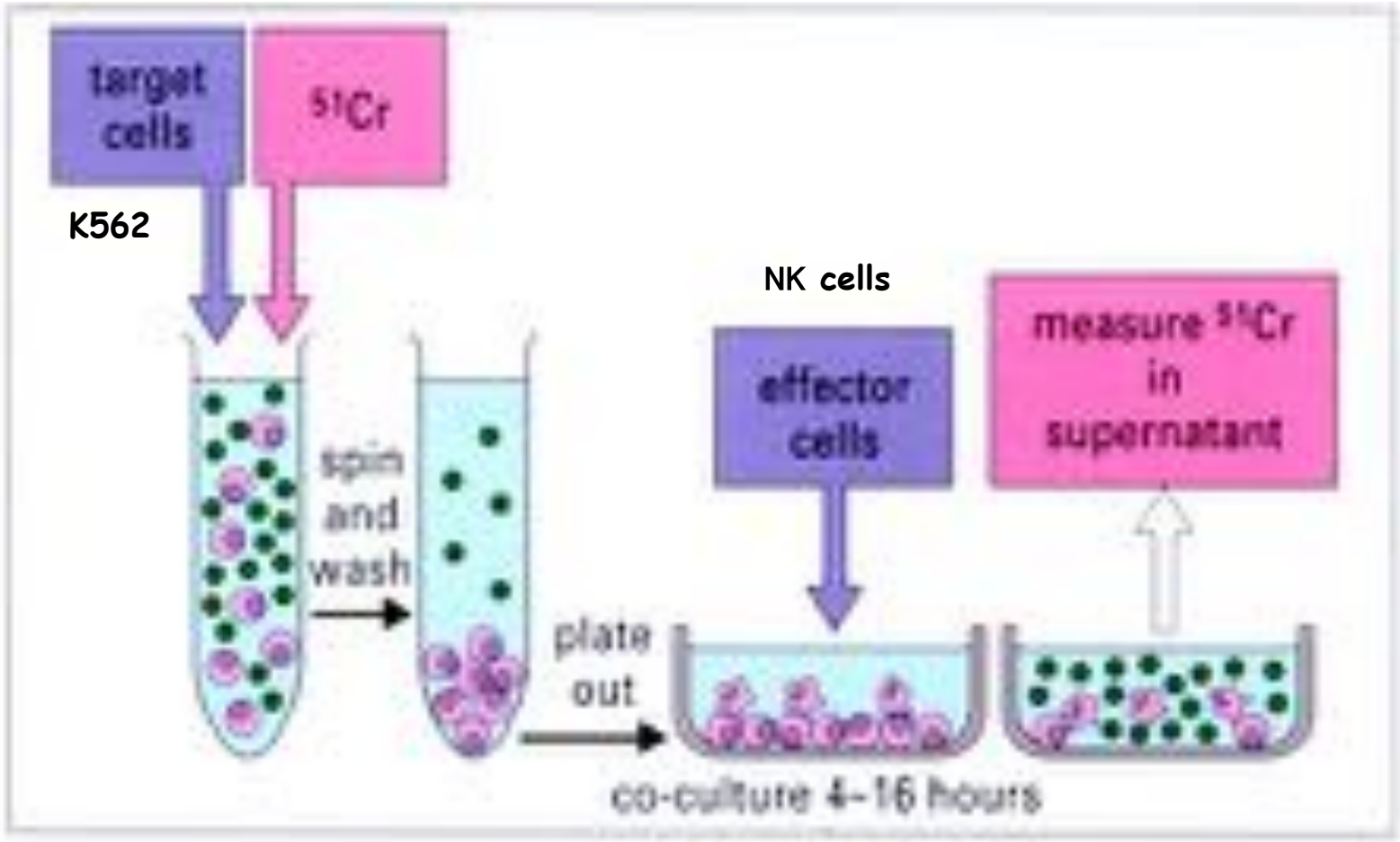
CD8+ T LYMPHOCYTES from HIV PATIENTS ARE ABLE TO PRODUCE CYTOKINES *in vitro* ?



IFN-γ production by purified NK cells upon TLRs stimulation



Cytotoxicity by ^{51}Cr release assay



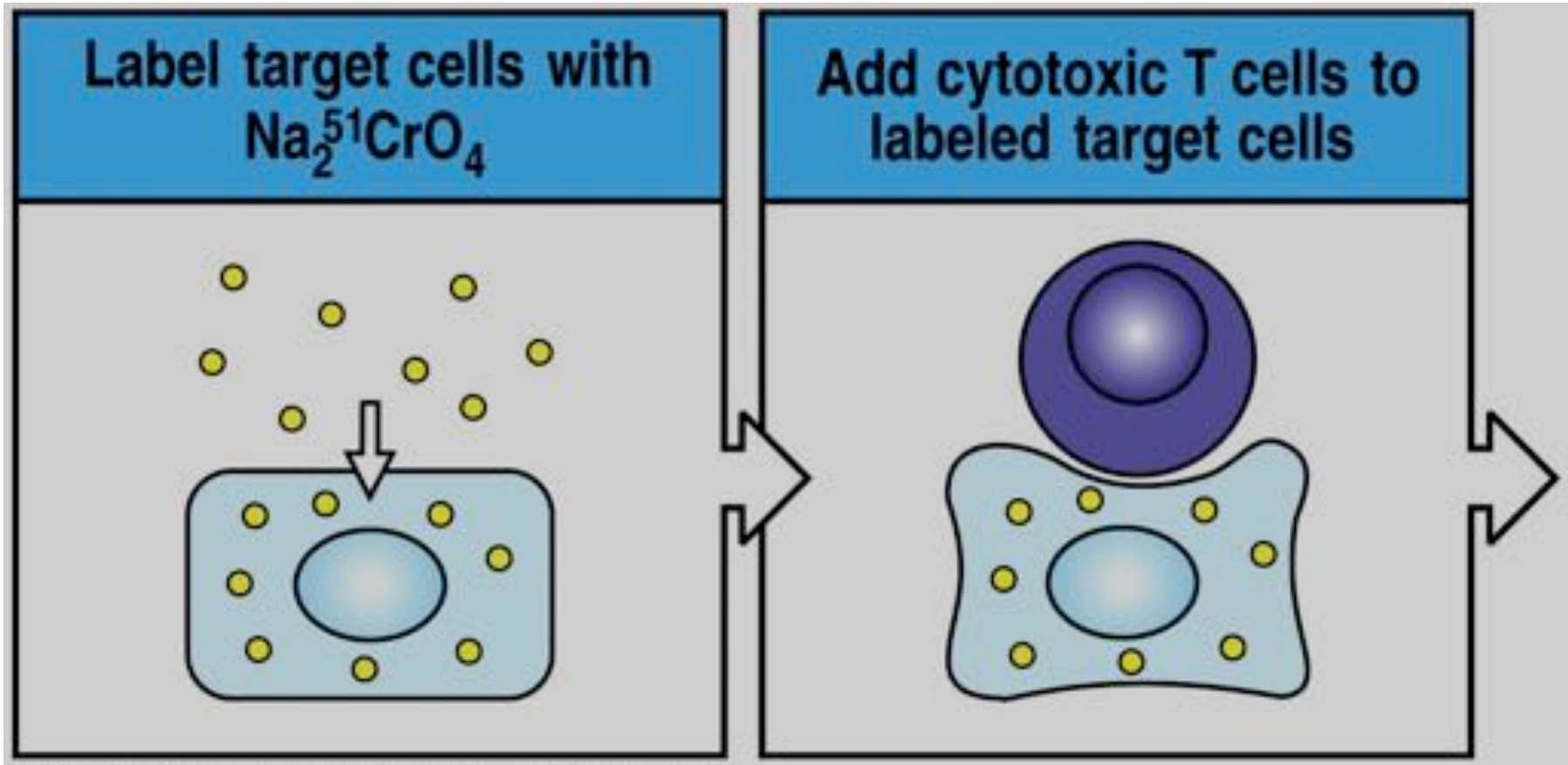


Figure A-38 part 1 of 2 Immunobiology, 6/e. (© Garland Science 2005)

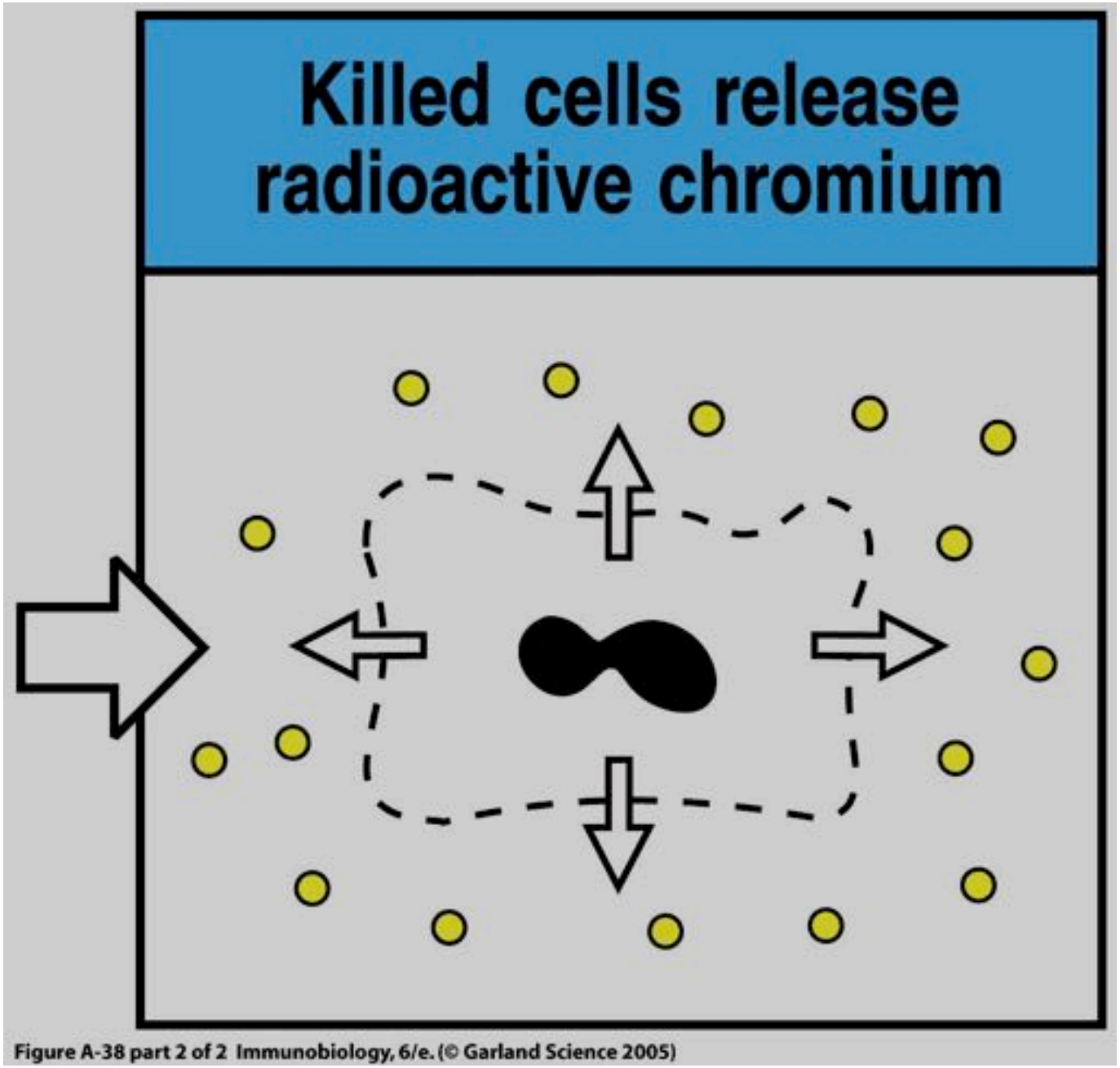


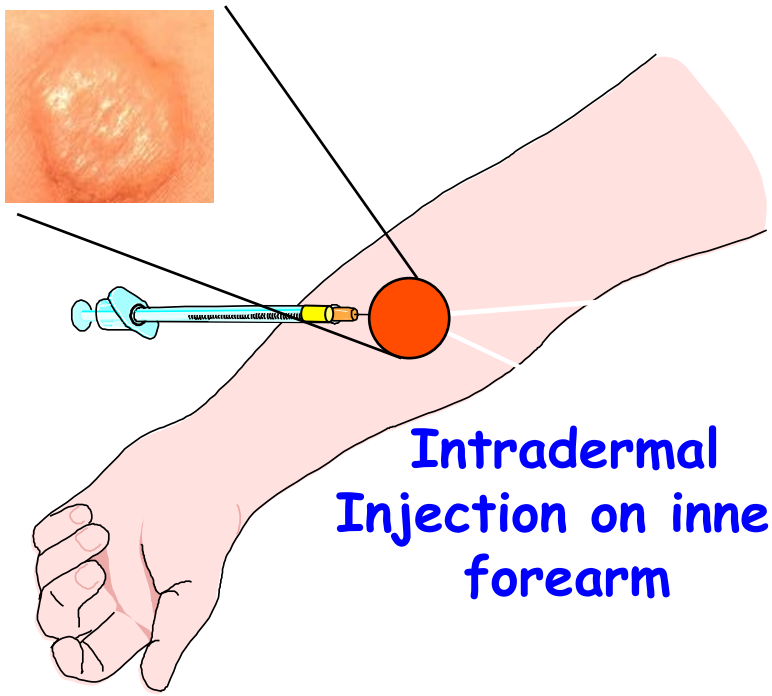
Figure A-38 part 2 of 2 Immunobiology, 6/e. (© Garland Science 2005)

Skin Test

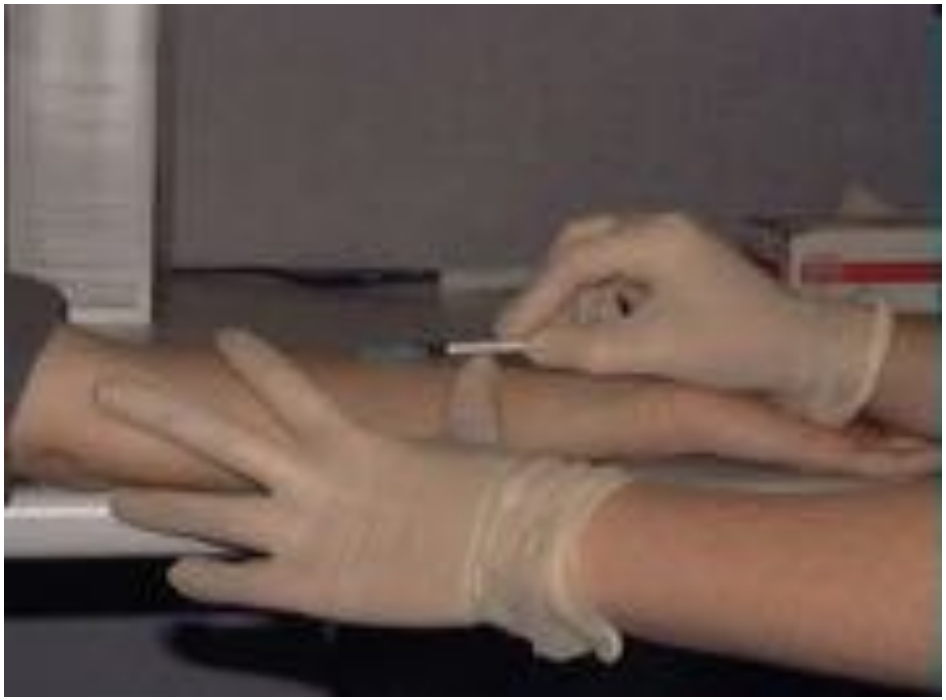
(extracts of various infectious microorganisms)

(Mantoux tubercolin test)

48-72 hours

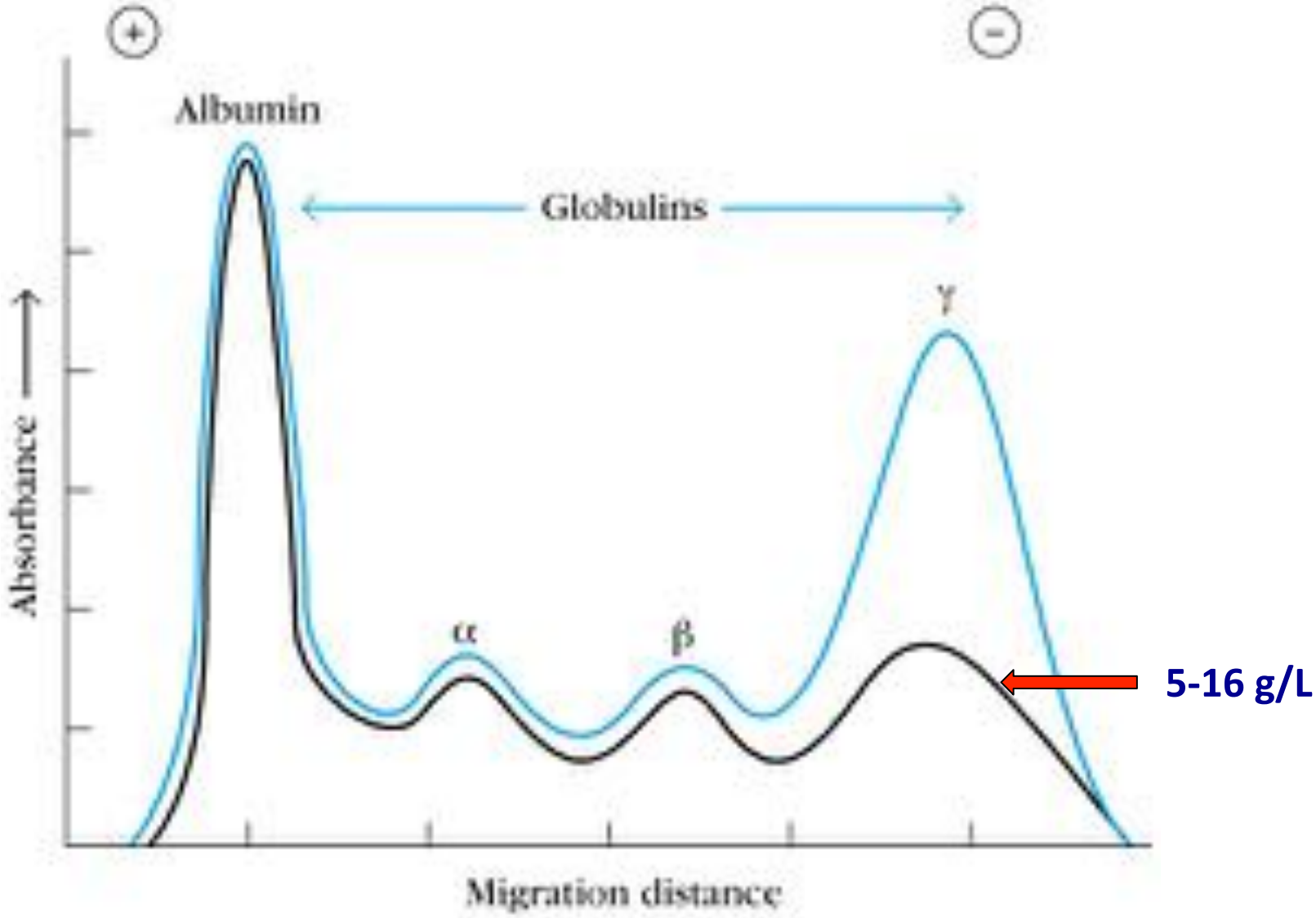


Intradermal Injection on inner forearm



Cells recruitment and activation → **Inflammation**

Antibody production by B cells



SERUM PROTEIN ELECTROPHORESIS

Immunoelectrophoresis

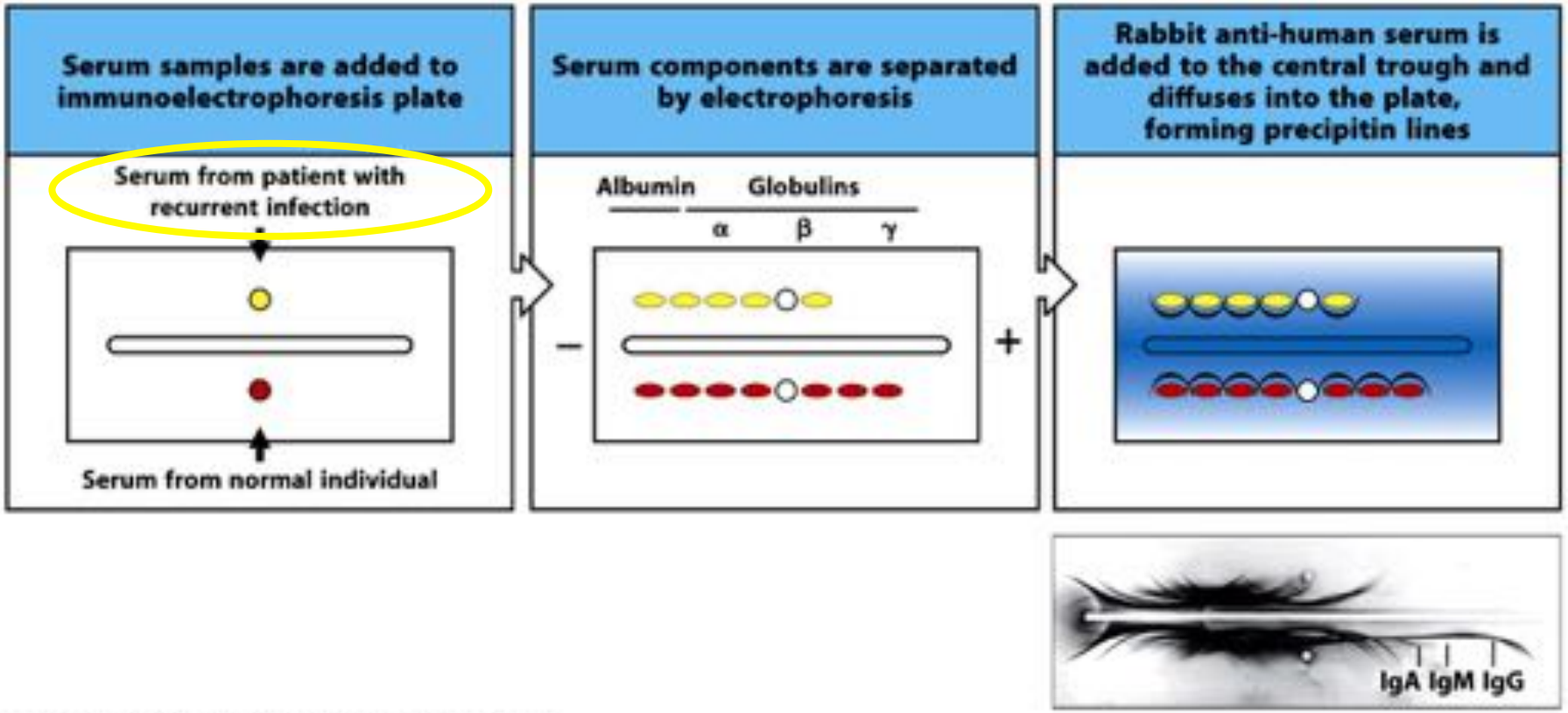
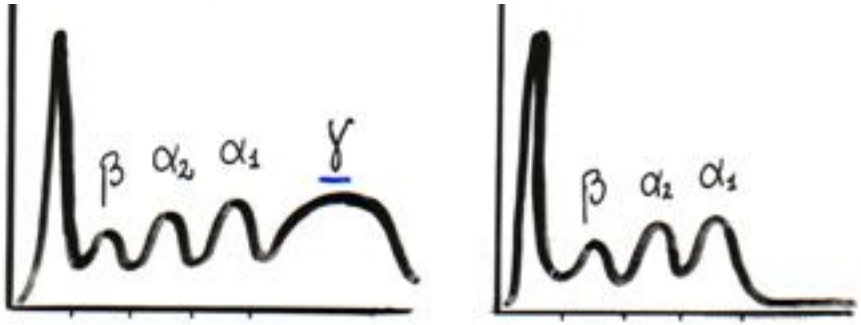


Figure 12-8 Immunobiology, 7ed. (© Garland Science 2008)

Common Variable Immunodeficiency



Normal **Immunodeficit**

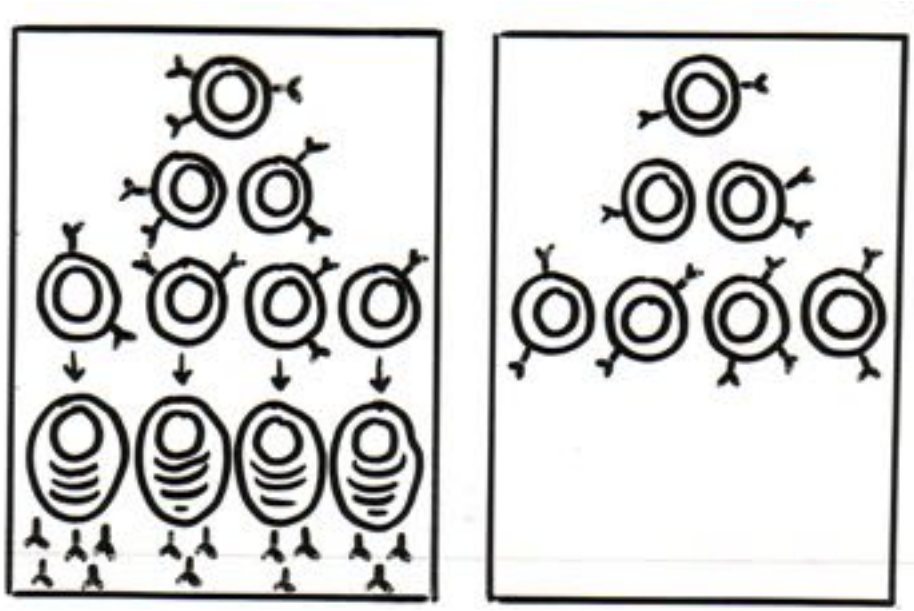


TABLE 3–13 Laboratory Evaluation for Common Variable Immunodeficiency (CVID)

Laboratory Test	Result/Comments
Serum protein electrophoresis	Marked decrease in the gamma globulin fraction; rarely it may be normal in the dysfunctional variant
Serum IgM, IgG, and IgA levels	Usually low, but may be normal in dysfunctional variant
CD19 and CD20 cells	Usually normal, may be increased; rarely, low normal but never absent
Response to polysaccharide and protein antigens	There is a failure to respond to these antigens; the expected 4-fold rise in titer following vaccination is not observed; defines the functional defect

IgA and IgG specific antibodies are biomarkers for Celiac Disease

(*an immune-mediated disorder*)

TABLE 15–2 Commonly Used Diagnostic Tests for Celiac Disease

Test	Advantages	Disadvantages
Tissue transglutaminase (TTG) IgA antibodies	Most reliable non-invasive test Inexpensive Widely available Easy sample collection High sensitivity and specificity	Falsely negative with IgA deficiency (3% of patients with celiac disease) May be negative if on low gluten diet
Gliadin antibodies (IgG and IgA)	Inexpensive Widely available Easy sample collection Positive in IgA deficiency May be more sensitive in children	Not as sensitive or specific as TTG IgA antibodies May be negative if on low gluten diet
Deamidated gliadin antibodies (IgG and IgA)	Widely available Easy sample collection Positive in IgA deficiency High sensitivity and specificity	Not as widely available as first 2 tests above More expensive than anti-gliadin antibody test
Small bowel biopsy	Reliable test, considered gold standard Reflects response to treatment	Requires endoscopy and biopsy Very expensive

Ig SERUM CONCENTRATION mg/ml

IgG	IgM	IgA	IgD	IgE
13.5	1.5	3.5	0.03	0.0005

IgG1	IgG2	IgG3	IgG4
9	3	1	0.5

IgA1	IgA2
3	0.5

In atopic subject: **RIST =** serum total IgE

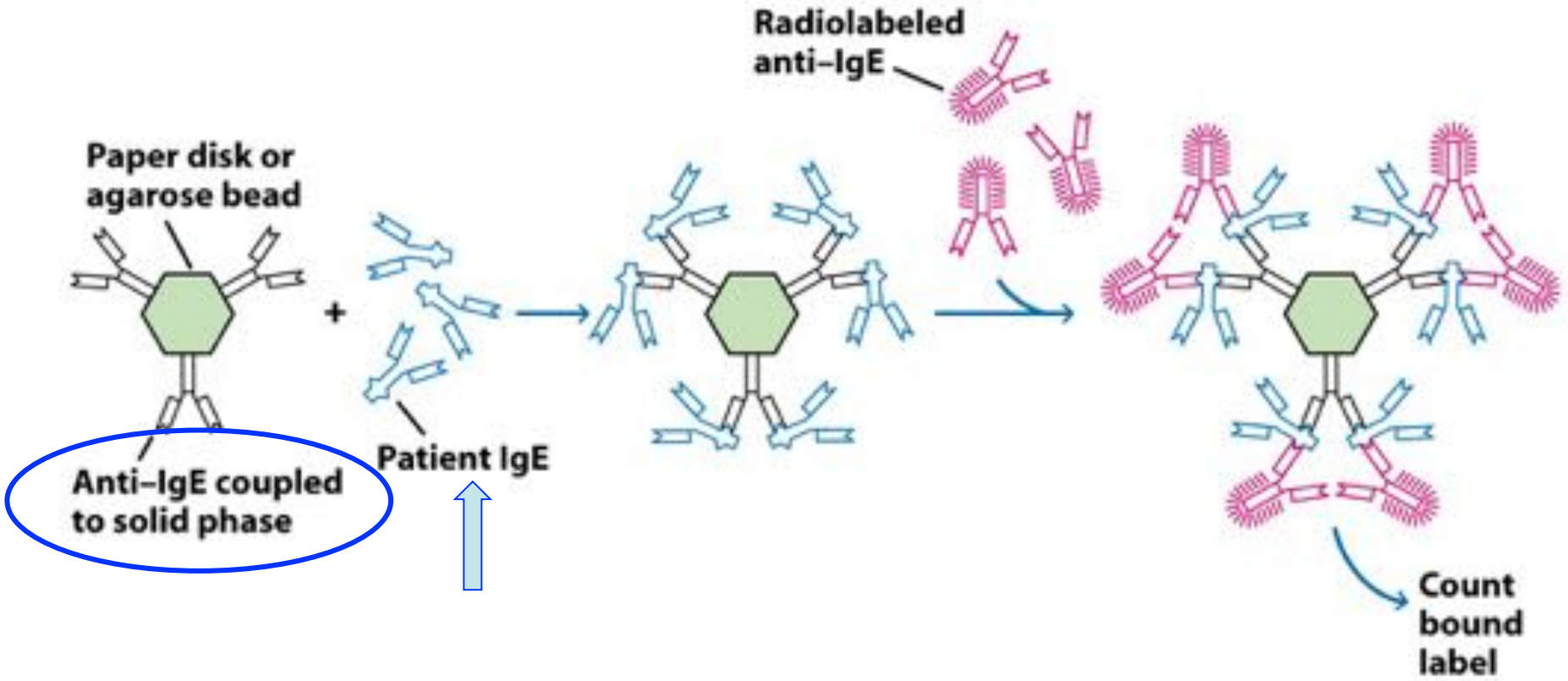


Figure 15-11a
Kuby IMMUNOLOGY, Sixth Edition

RAST = serum specific IgE

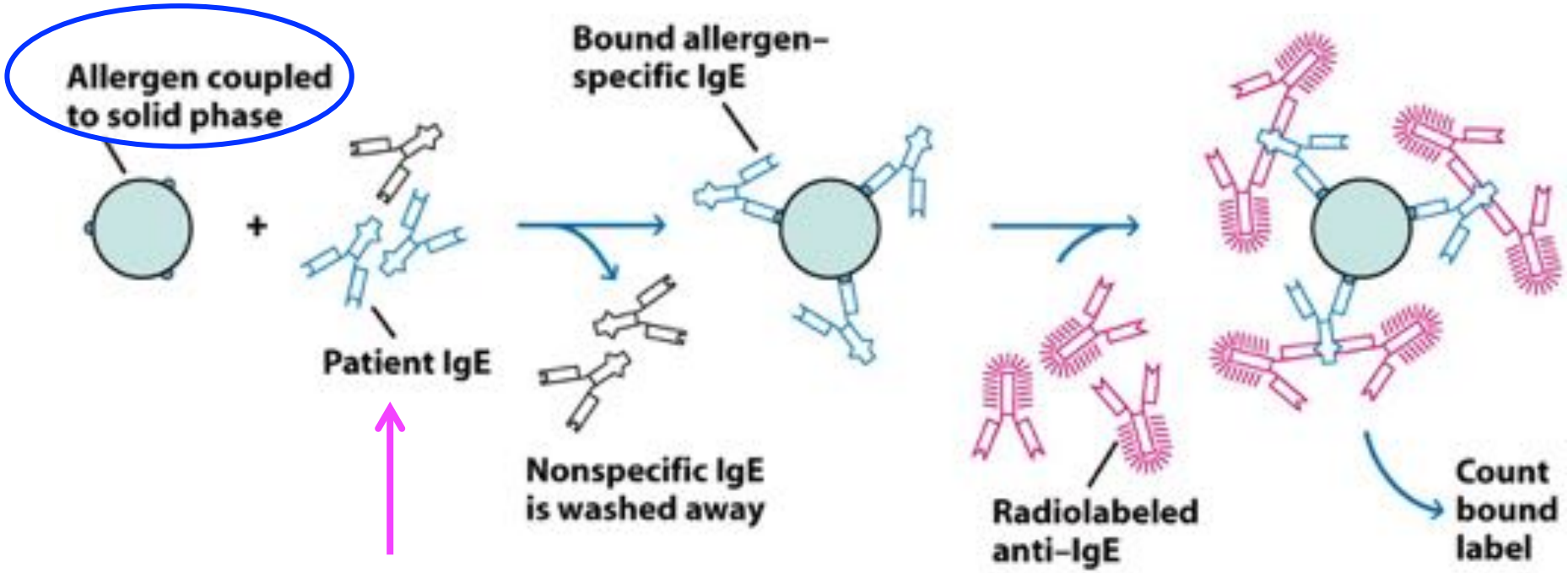


Figure 15-11b
Kuby IMMUNOLOGY, Sixth Edition

Methods for assaying the neutrophil functionality

Methods of assessing neutrophil polymorph function	
function	**test**
Mobilization from marrow stores	↑ blood WBC with adrenalin or steroids
Possession of LFA-1/CR3/gp150/90 antigens	monoclonal antibody markers
Adherence	adherence to glass wool columns
Directional migration	chemotaxis through filters
Ingestion of organisms	phagocytic index
Respiratory burst	NBT reduction
Intracellular killing	microbicidal tests

ROS production

Tabella 24-1 Valutazione della fagocitosi.

Indagine	Commenti
Test al nitroblu tetrazolio (NBT)	Impiegato per la diagnosi e lo screening della malattia cronica granulomatosa e per l'identificazione dello stato di portatore.
Curva quantitava di killing intracellulare	Impiegato per la diagnosi della malattia cronica granulomatosa. Può essere eseguito con microrganismi isolati dallo stesso paziente.
Chemiotassi	Alterata in diverse condizioni associate a infezioni batteriche ricorrenti. Non permette una diagnosi specifica. Indagata con la metodica della camera di Boyden, con tecnica microscopica o radioattiva per valutare la migrazione cellulare. Il test della finestra di Rebuck consente di ottenere un risultato qualitativo *in vivo*.
Chemiluminescenza	Alterata nella malattia cronica granulomatosa e nel deficit di mieloperossidasi.
Test enzimatici	Deficit di enzimi specifici: glucosio-6-fosfato deidrogenasi e mieloperossidasi.
Glicoproteine di membrana	Deficitarie nei disordini dell'adesione leucocitaria (integrine) associati ad alterazione dell'adesione e dei movimenti leucocitari.

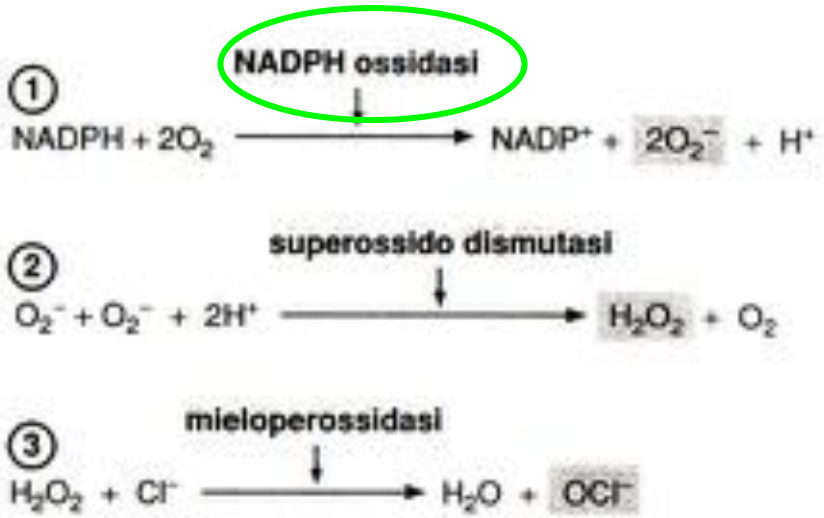


Figura 24-1. Schema della catena respiratoria (*burst*) con generazione di superossido (O_2^-), perossido di idrogeno (H_2O_2) e ione ipoclorito (OCl^-).

Nitroblue of tetrazolium TEST (NBT)

(Testing the intracellular killing)

Nitroblue of tetrazolium is a yellow compound

↓

after reduction (respiratory burst)

↓

dark blue FORMAZAN is formed

(determined by spectrophotometric reading)

DEGRANULATION ASSAY or FRUSTRATED PHAGOCYTOSIS

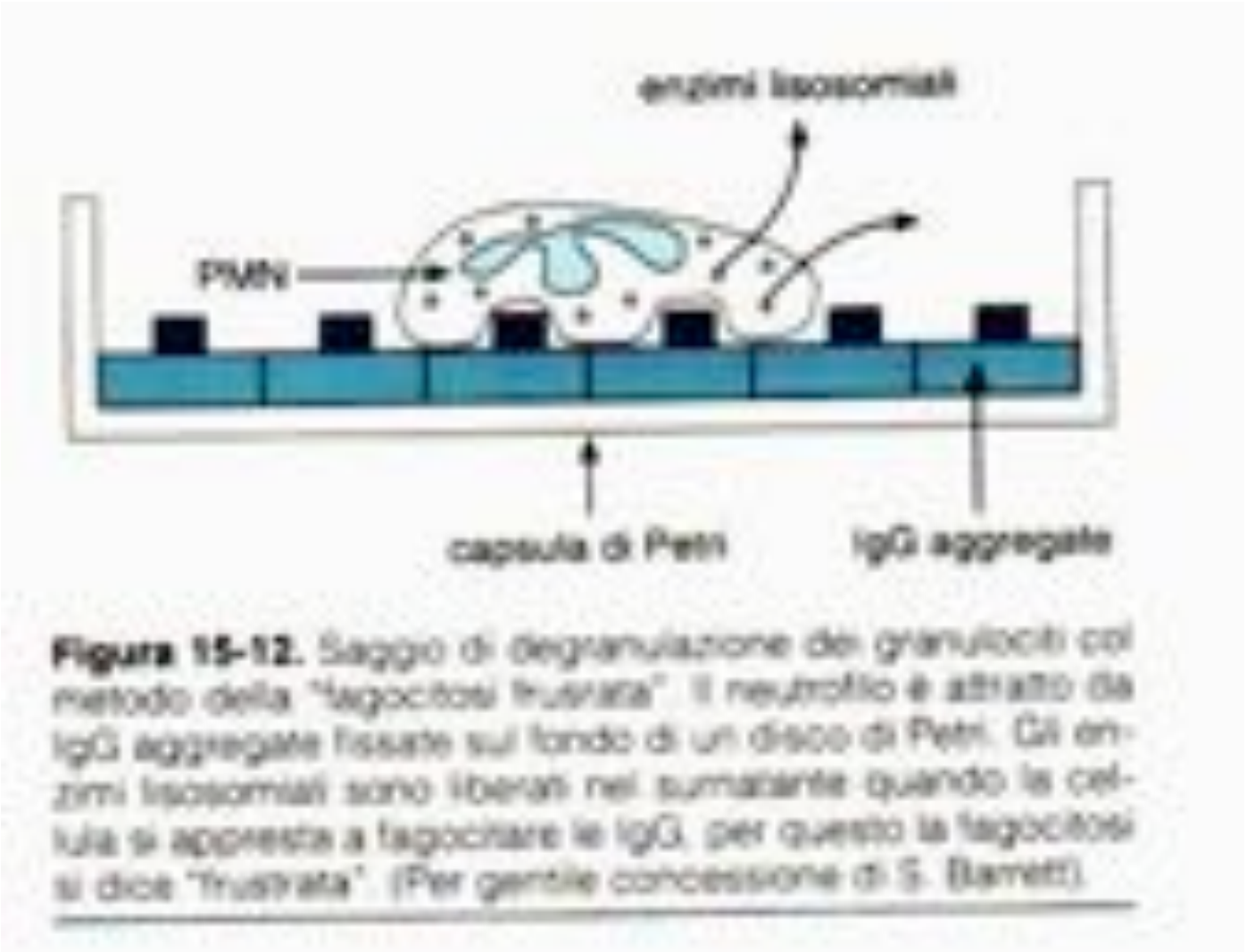


Figura 15-12. Saggio di degranulazione dei granulociti col metodo della "fagocitosi frustrata". Il neutrofilo è attratto da IgG aggregate fissate sul fondo di un disco di Petri. Gli enzimi lisosomiali sono liberati nel sumatante quando la cellula si appresta a fagocitare le IgG, per questo la fagocitosi si dice "frustrata". (Per gentile concessione di S. Barrett).

SPECIFIC TESTS

CELL	COUNT	FUNCTION
T Lymphocyte	MAb and Flow Cytometry	Proliferation response of mitogen stimulated cells
T Lymphocyte subsets	MAb and Flow Cytometry	Cytokine production Cytotoxicity Suppression
B Lymphocyte	MAb and Flow Cytometry	Serum protein electrophoresis Serum Ig levels
NK Cell	MAb and Flow Cytometry	Cytotoxicity Cytokine production
Complement	Serum level of components by ELISA and nephelometry	CH50 hemolytic assay
Neutrophil	CBC	Respiratory burst
Monocyte/Macrophage	CBC	Intracellular Killing of microbe

SERUM CONCENTRATION OF COMPLEMENT SYSTEM COMPONENTS

C3	**1000 - 1200**	**ug / ml**
C4	**300 – 600**	**“**
C1q	**70**	**“**
C1r	**50**	**“**
C1s	**50**	**“**
C2	**20**	**“**
C5	**80**	**“**
C6	**45**	**“**
C7	**90**	**“**
C8	**60**	**“**
C9	**60**	**“**
Factor B	**200**	**“**
Factor D	**1-2**	**“**
Properdin	**25**	**“**
Factor I	**35**	**“**
Factor H	**560**	**“**

Hemolytic assay or CH50

CH50 : defines the amount of Complement required to induce 50% lysis of sensitized erythrocytes.

It is expressed as the inverse of the serum dilution that gives 50% lysis

.

diluted **Serum Sample**

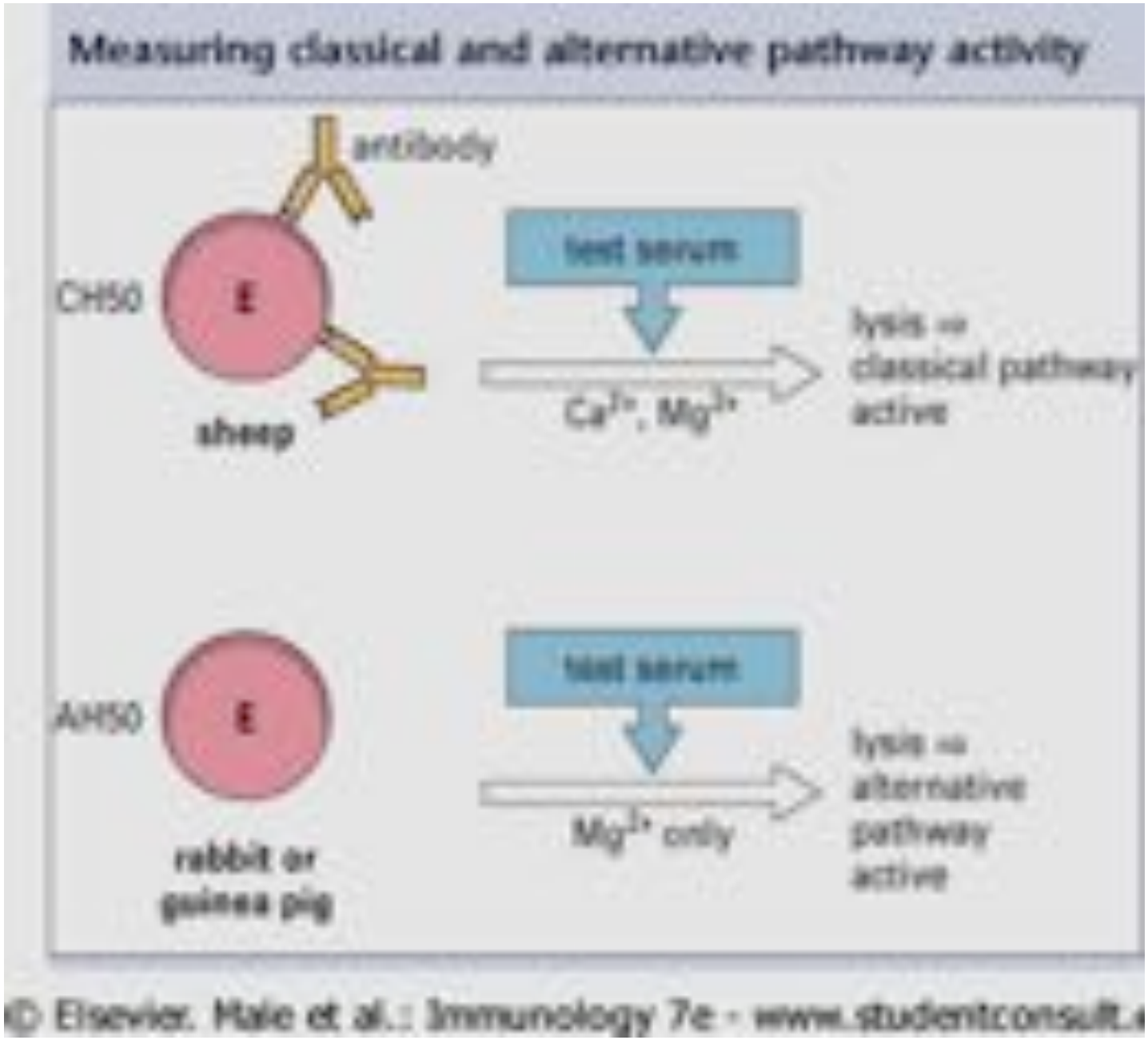
+

sheep erythrocytes sensitized with specific antibody

↓

Spectrophotometric measurement of hemoglobin release

Correlation between hemoglobin release and percentage of hemolysis



Relationship between the percentage of hemolysis and serum dilution

CH50 Normal values
between 50-200 units

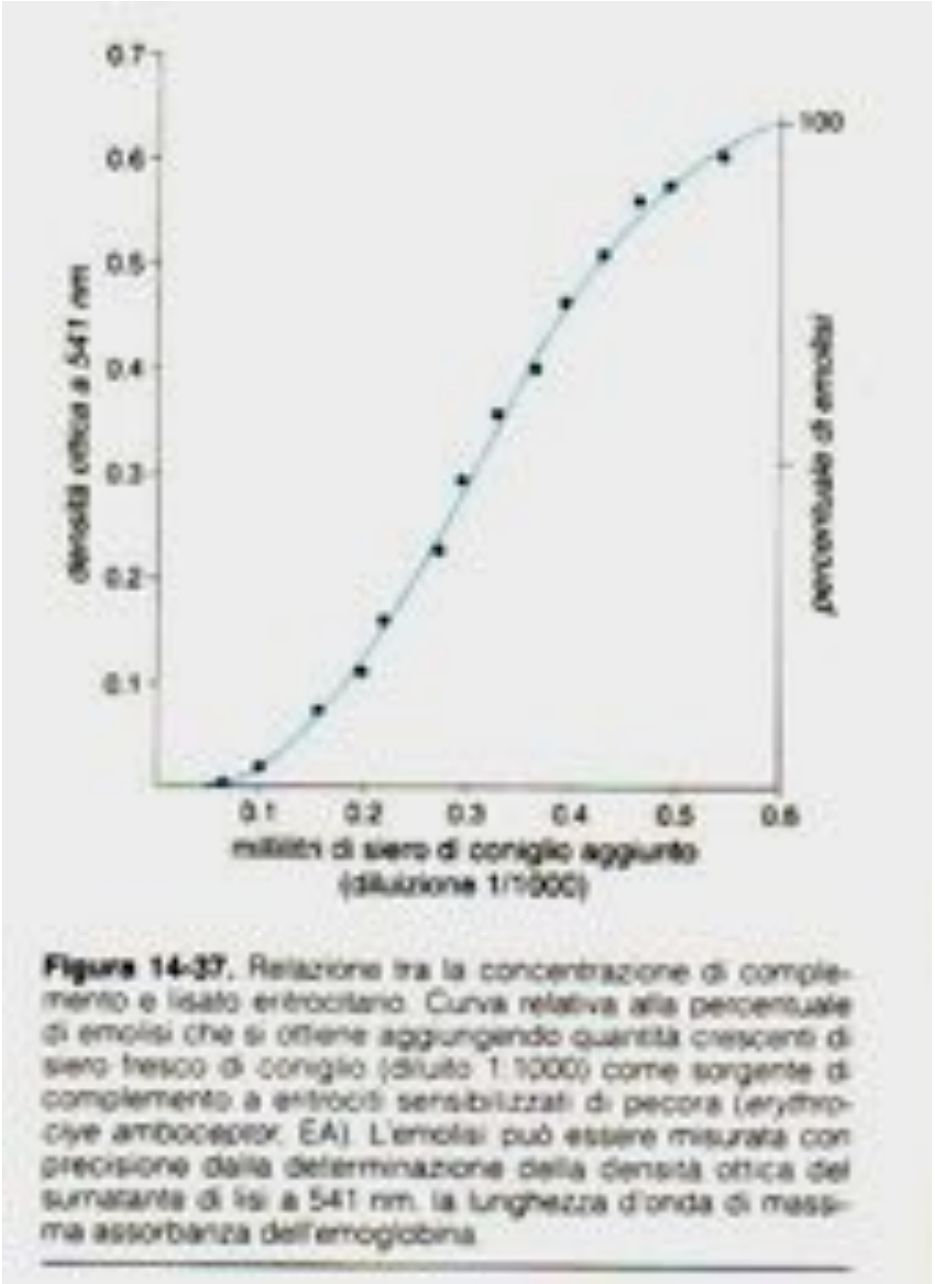


Figura 14-37. Relazione tra la concentrazione di complemento e lisato eritrocitario. Curva relativa alla percentuale di emolisi che si ottiene aggiungendo quantità crescenti di siero fresco di coniglio (diluito 1:1000) come sorgente di complemento a eritrociti sensibilizzati di pecora (*erythrocyte amboceptor*, EA). L'emolisi può essere misurata con precisione dalla determinazione della densità ottica del surnatante di lisi a 541 nm, la lunghezza d'onda di massima assorbanza dell'emoglobina.

The reduction of CH50 correlates with the reduction of C3 activity

Reduction of C activity is observed for:

1. **Consumption of C for the formation of immunocomplex**

2. **Decreased synthesis of C**

3. **Increased catabolism of C**

Diagnosis of systemic autoimmune diseases

Systemic autoimmune disorders are immuno-mediated diseases with predominant involvement of connective tissue with clinical manifestations such as inflammation of joints, skin, muscles. The connective tissue diseases (CTD) are characterized by the presence of particular types of

autoantibodies

SLE	Systemic Lupus Erythematosus
SSc	Systemic Sclerosis
MCTD	Mixed Connective Tissue Disease
PM-DM	Polymyositis-Dermatomyositis
SS	Sjogren Syndrome
RA	Rheumathoid Arthritis
UCTD	Undetermined Connective Tissue Disease

Autoantibodies are **markers**
in autoimmune diseases useful for

diagnosis

classification

prognosis

monitoring

The term **ANA** indicates

a group of antibodies (IgG or IgM)

directed against different nuclear antigens

Antinuclear antibodies are directed against :

n-DNA o ds-DNA

ssDNA

histone

extractable nuclear antigens (ENA):

RNP/Sm

Scl 70

SS-A / Ro

SS-B / La

PM-1

Jo-1

Ku

RANA

PCNA

When a SYSTEMIC AUTOIMMUNE DISEASE is suspected

The diagnostic approach will be

↓

Test ANA (antinuclear antibodies)

↓

Test anti-ENA (extractable nuclear antigens)
(to confirm the positivity for ANA)

ANA

represent a group of antibodies present in the serum of patients

the ANA are specific for self antigens contained in nucleus

The ANA are found in many systemic autoimmune diseases, but also in 5-10% of cases of organ-specific autoimmune diseases (thyroiditis, chronic atrophic gastritis, secondary amenorrhea, type I DM ...).

Low titer of ANA may be detected in normal population : prevalence has increased in females than in males and in the elderly than in the young.

Methods for the measurement of ANA and anti-ENA:

- **ANA**

assessment of the serum titer and the morphological pattern by

IFI = INDIRECT IMMUNOFLUORESCENCE

*using rat liver and kidney sections or the **Hep-2** human laryngeal carcinoma cell line that express human antigens present in all cell cycle phases.*

*For antibodies anti-DNAn/ds, using **Crithidia luciliae**, a protozoan, which contains a kinetoplast with DNA histone free (useful for the diagnosis of Lupus induced by drugs characterized by antibodies anti-histone)*

- **Anti-ENA**

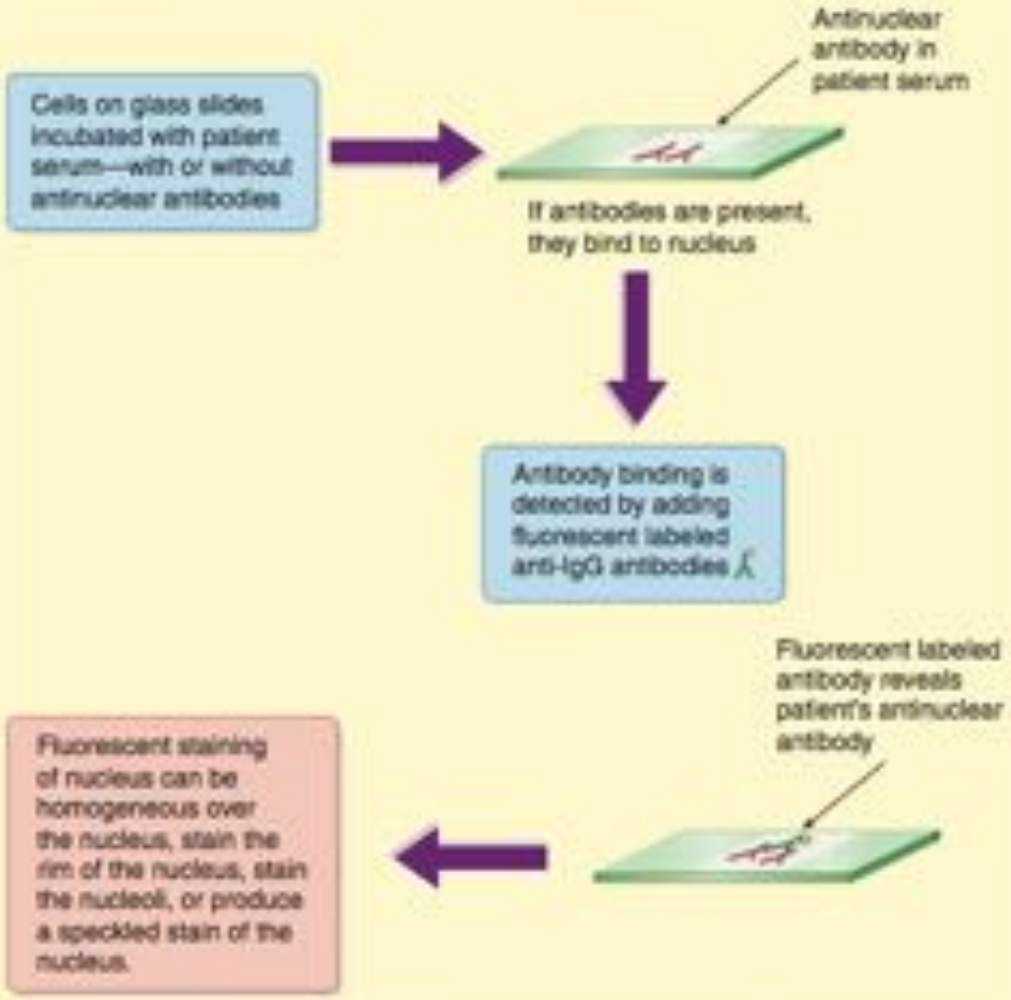
detected in serum by

ELISA or DOUBLE DIFFUSION in agarose gel

Antinuclear antibody (ANA) testing

Expense: Low

Manual with microscopic evaluation



If an antibody is detected, the patient's serum is progressively diluted until the staining is no longer detected. The final result includes the highest serum dilution producing a detectable response and the pattern of nuclear staining.

IFI and ANA Titer

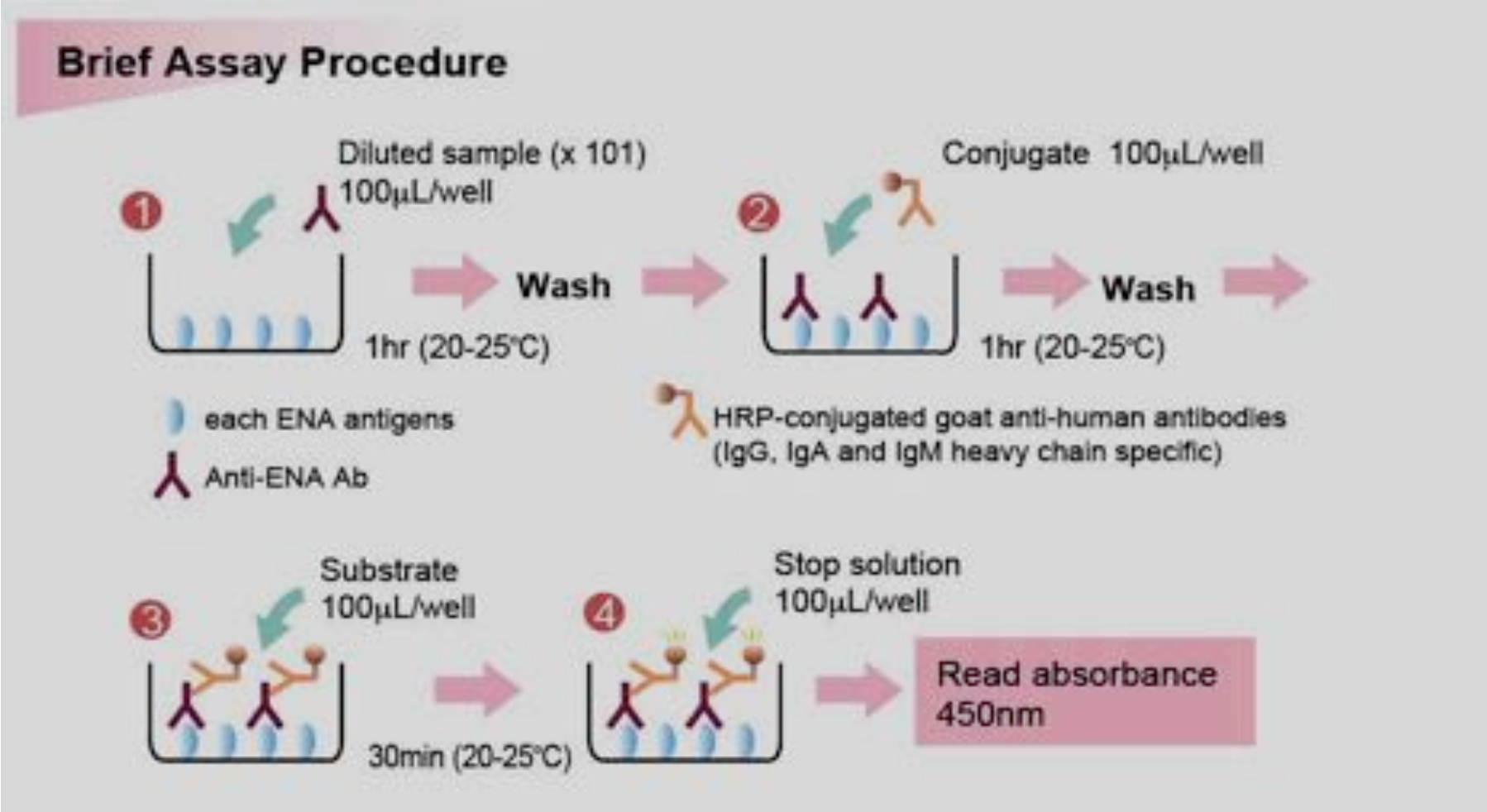
For diagnostic purposes the titer of **1:40** and **1:160** are considered as decision-making levels:

<1:40 negative (antinuclear antibodies in low titer 1:40 - 1:80 can be present in healthy subjects, in pregnant women, in women over 40 years, in the elderly)

> 1:40 and <1:160 low positive (in the absence of specific symptoms, diagnostic protocol must suggest monitoring in later times)

> / = 1:160 are considered **suggestive** of autoimmune disease

ELISA (Enzyme-Linked Immunosorbent Assay)for Anti-ENA Ab



Fluorescence Pattern

Fluorescence type	Antigen	Disease
Periphery	ds-DNA	LES
Homogeneous	Histone-DNA complex	LES and connective tissue disease
Speckled	Nuclear antigen not DNA type	LES ,MCTD, LES, SS,Sjogren
Nucleolar	Nucleolar RNA	Sclerodermia

homogeneous

peripheral or rim

speckled

nucleolar

Figura 33-1. Quadri all'immunofluorescenza degli anticorpi antinucleo.

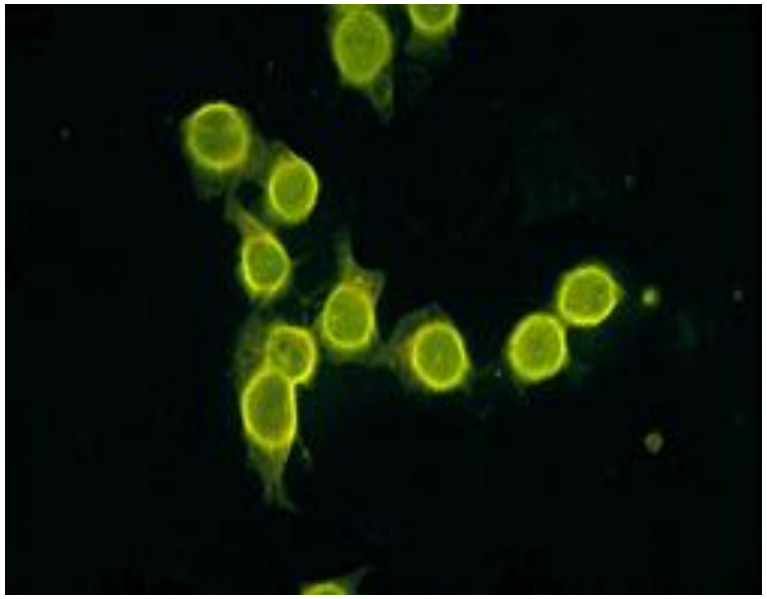
Tabella 33-1. Anticorpi antinucleo.

Quadro all'immunofluorescenza	Antigene	Malattia associata
Periferico	DNA a doppia elica	LES
Omogeneo	Complesso DNA-istone	LES, talvolta altre connettiviti
Punteggiato	Sm (antigene Smith)	LES
	RNP (ribonucleoproteina)	MCTD, LES sindrome di Sjögren, sclerodermia, polimiosite
	SS-A (Ro)	Sindrome di Sjögren, LES
	SS-B (La)	Sindrome di Sjögren, LES
	Jo-1	Polidermatomiosite
	Mi-2	Dermatomiosite
	Scl-70	Sclerodermia
	Centromero	Sclerodermia limitata
	RANA (antigene nucleare associato all'artrite reumatoide) (antigene nucleare indotto da EBV)	Artrite reumatoide
Nucleolare	RNA nucleolo-specifico	Sclerodermia
	PM-Scl	Polimiosite

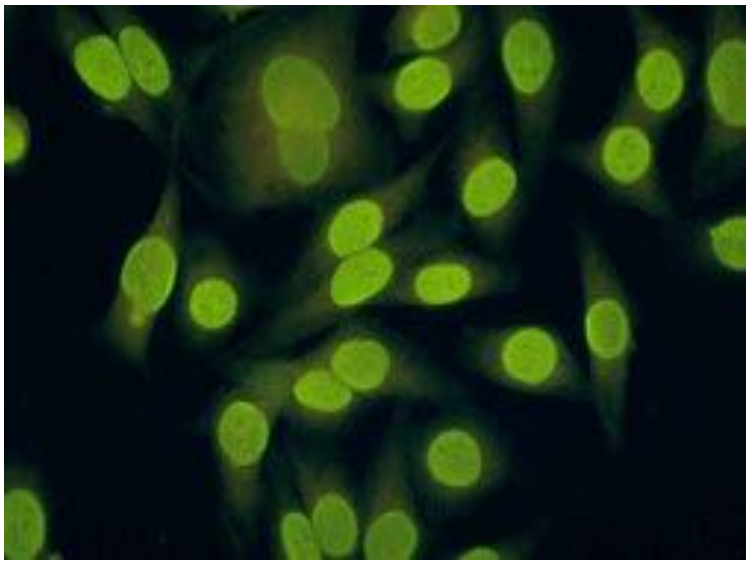
EBV = virus di Epstein-Barr, MCTD = malattia mista del tessuto connettivo, LES = lupus eritematoso sistemico.

ANTINUCLEAR ANTIBODY (ANA)

IFI on HEp-2



Peripheral Pattern: anti-dsDNA



Homogeneous Pattern : anti-DNA-Histone

Speckled Pattern: anti-ENA antibodies

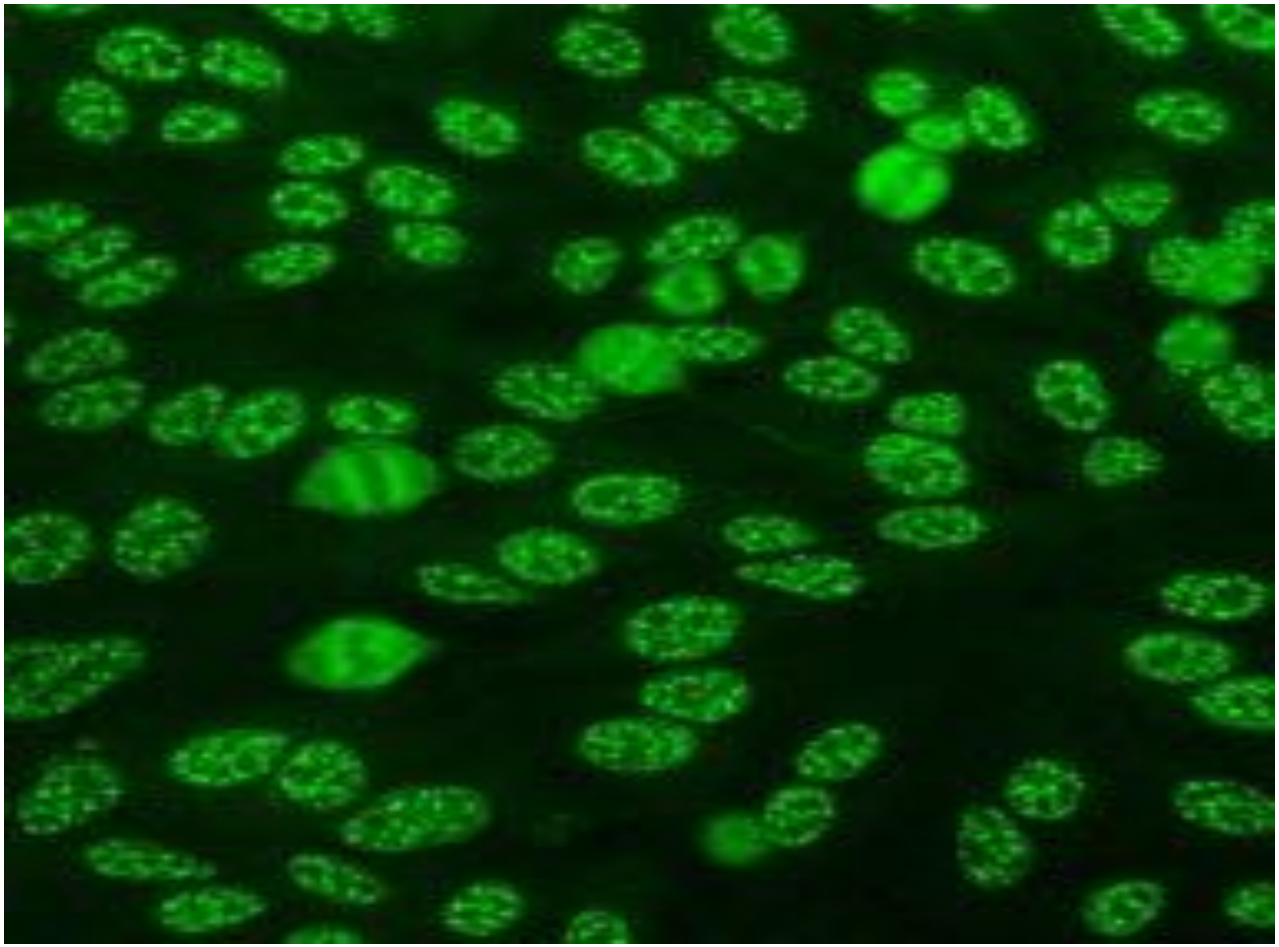


TABLE 3-1 Systemic Autoimmune Diseases: Diseases Associated with Positive Test Results for Antinuclear Antibodies (ANA)

Disease	% ANA Positive	Titer	Common Patterns
Systemic lupus erythematosus—active	95–98	High	H > S > R
Systemic lupus erythematosus—remission	80	Moderate-high	H > S
Mixed connective tissue disease	95	High	S > N
Scleroderma/CREST	80	High	S > C > N
Sjögren syndrome	60	Moderate-high	S > H
Polymyositis/dermatomyositis	50	Low-moderate	S > N
Rheumatoid arthritis	40	Low-moderate	S
Drug-induced lupus	100	Low-moderate	S
Pauciarticular juvenile chronic arthritis	70	Low-moderate	S

Note: ANA patterns on HEp-2 cells by indirect immunofluorescent technique (IIF). Patterns: H, homogeneous; S, speckled; R, rim; C, centromere; N, nucleolar. Titers: high = 1:1280 to 1:5120; moderate = 1:160 to 1:640; low = 1:40 to 1:80.

TABLE 3-2 Specific Organ Autoimmune Diseases: Diseases Associated with Positive Test Results for Antinuclear Antibodies (ANA)

Disease	% ANA Positive	Titer	Common Patterns
Graves disease	30	Low-moderate	S
Hashimoto thyroiditis	40	Low-moderate	S
Autoimmune hepatitis	60–90	Low-moderate	S
Primary biliary cirrhosis	70–90	Low-moderate	S

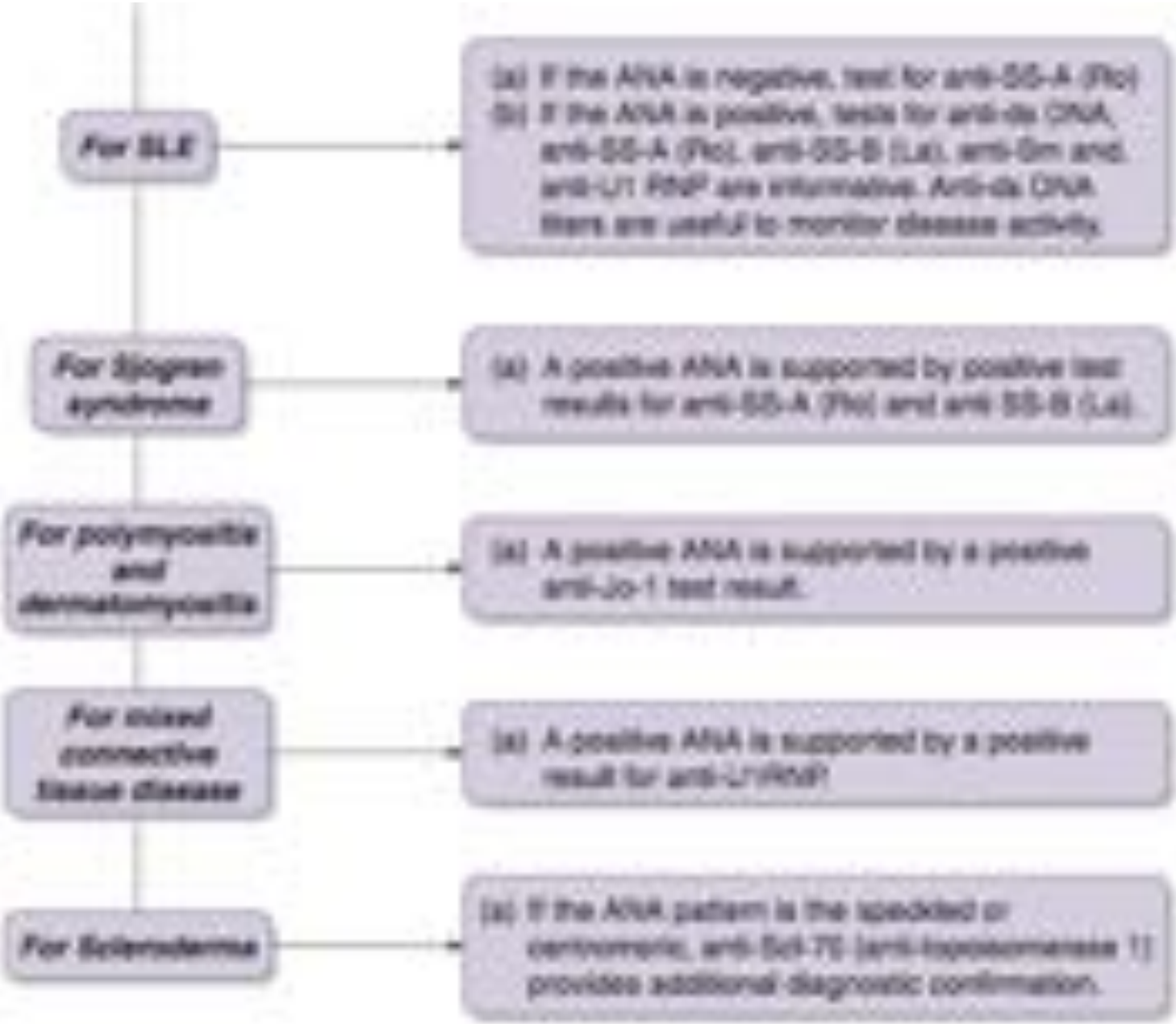
Patterns: H, homogeneous; S, speckled; R, rim; C, centromere; N, nucleolar. Miscellaneous causes: low-titer positive ANA patterns (mostly speckled) have been described in chronic infectious diseases such as infectious mononucleosis, hepatitis C infection, HIV, subacute bacterial endocarditis, and certain lymphoproliferative diseases.

The clinical features of the disease, the morphologic pattern of the ANA test, and the serum titer of the positive ANA test are established.

If the ANA is positive, the pattern of staining suggests the differential diagnosis. The results of specific antinuclear antibody tests often establish the diagnosis.
A negative ANA test can occur in rheumatoid arthritis, inflammatory muscle diseases, and when there are connective tissue manifestations in patients with selected chronic infectious diseases.

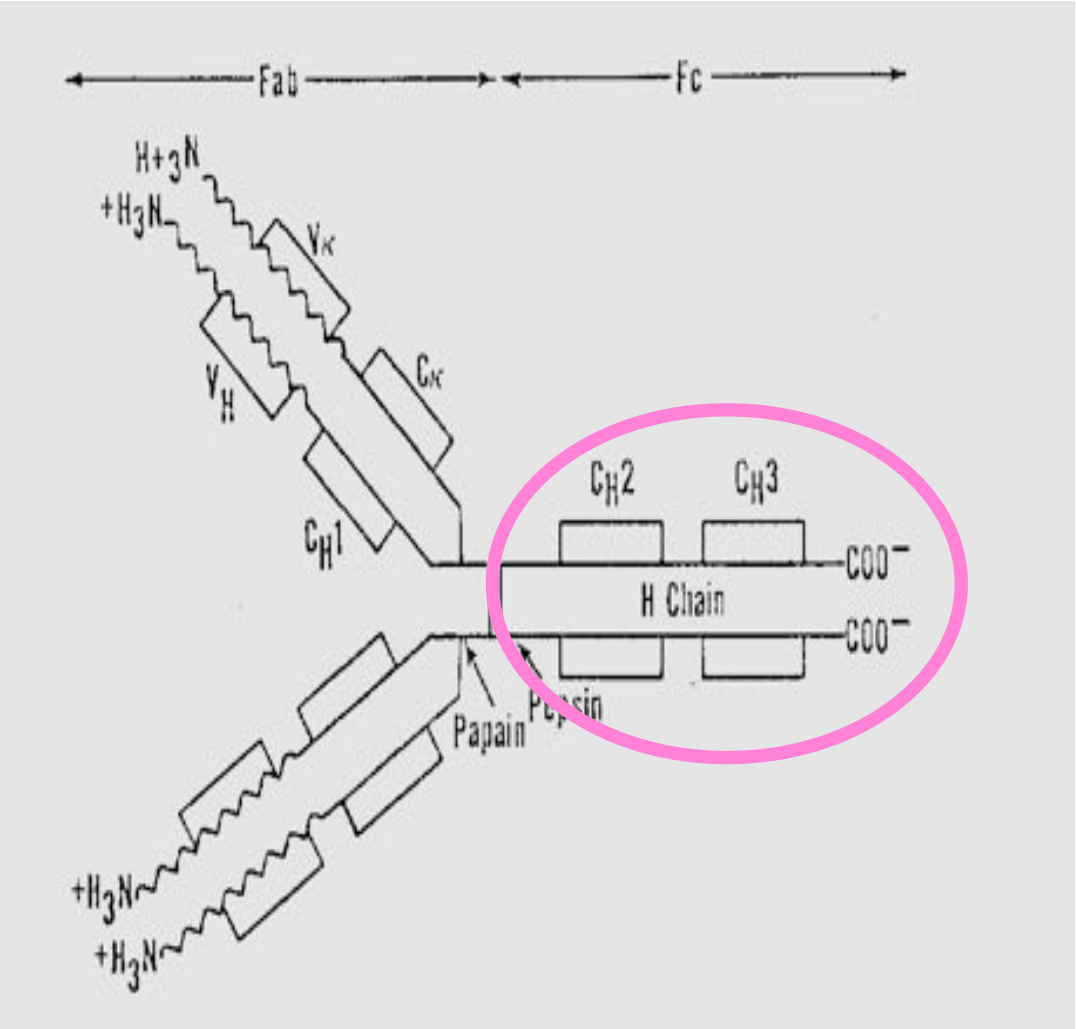
The following is an algorithm for the serologic evaluation of autoimmune connective tissue diseases.

If diagnosis is unknown and the ANA is positive, the following test panel is useful:
(a) anti-ds DNA
(b) anti-SS-A (Ro)
(c) anti-SS-B (La)
(d) anti-Sm
(e) anti-U1 RNP
(f) anti-Jo-1
(g) anti-Scl-70


For SLE
(a) If the ANA is negative, test for anti-SS-A (Ro)
(b) If the ANA is positive, tests for anti-ds DNA, anti-SS-A (Ro), anti-SS-B (La), anti-Sm and anti-U1 RNP are informative. Anti-ds DNA titers are useful to monitor disease activity.
For Sjogren syndrome
(a) A positive ANA is supported by positive test results for anti-SS-A (Ro) and anti SS-B (La).
For polymyositis and dermatomyositis
(a) A positive ANA is supported by a positive anti-Jo-1 test result.
For mixed connective tissue disease
(a) A positive ANA is supported by a positive result for anti-U1RNP.
For Scleroderma
(a) If the ANA pattern is the speckled or centromeric, anti-Scl-70 (anti-topoisomerase 1) provides additional diagnostic confirmation.

RHEUMATOID FACTOR (RF)

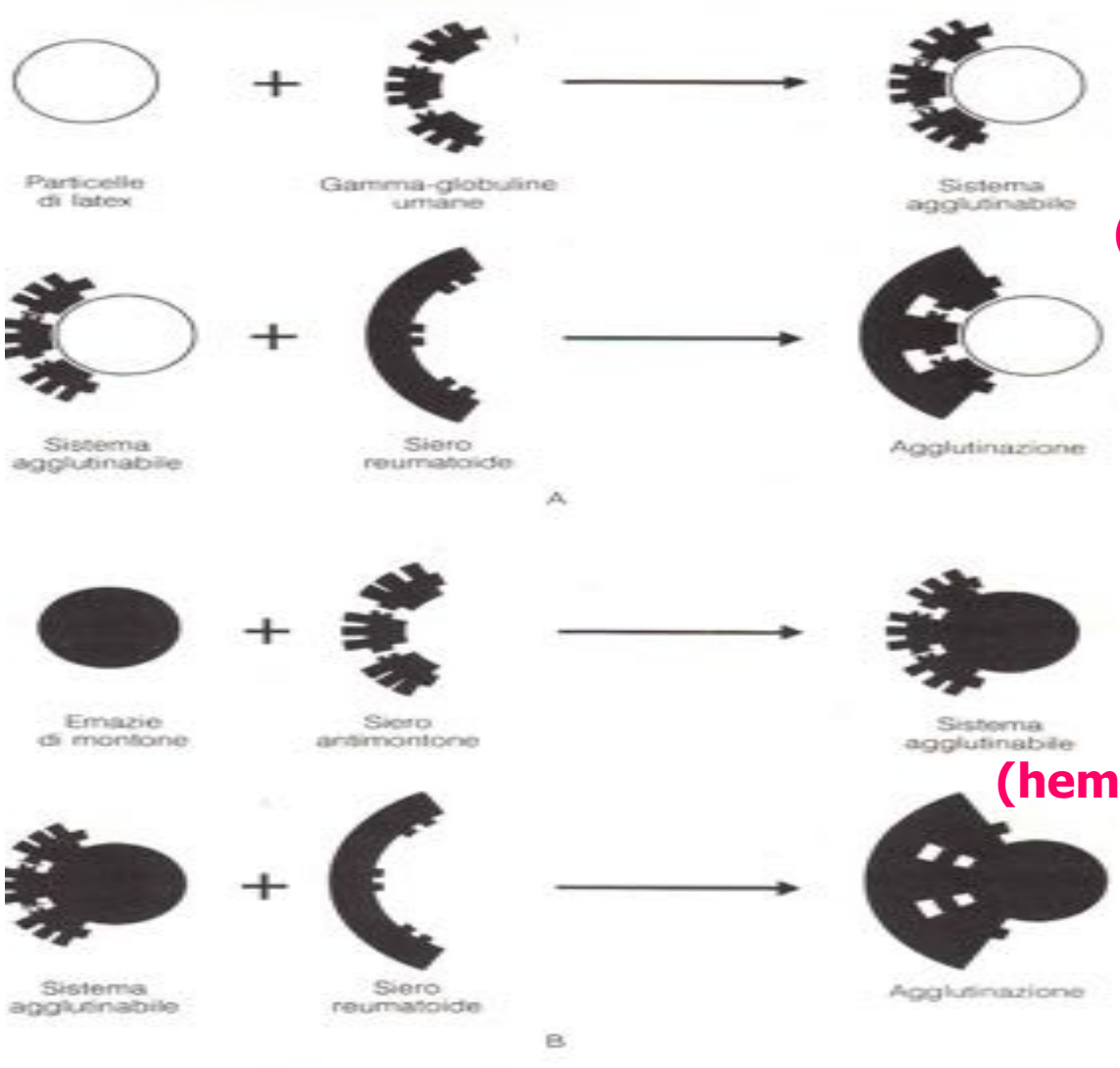
- Autoantibody directed against the Fc component of human and animal IgG.
- More frequently are IgM, but also IgG, IgA or IgE.
- The **RF** is produced by plasma cells in the lymph nodes, spleen, and in the synovial membrane.



RHEUMATOID FACTOR PROPERTIES

	RHEUMATOID ARTHRITIS	OTHER DISEASES
TITER	high	low
REACTIVITY WITH HUMAN and ANIMAL IgG	frequent	infrequent
ISOTYPES	IgM, IgG, IgA	main IgM
PRODUCTION SITE	synovial membrane and other extravascular sites	unknown

- **RF** is the ONLY SEROLOGICAL INDICATOR OF DISEASE INCLUDED IN THE ACR CRITERIA
- The HIGH TITLE correlates with a more severe disease, extra-articular manifestations and rheumatoid nodules



RA test/ LATEX

(agglutination – human IgG)

WAALER-ROSE

(hemoagglutination – rabbit IgG)

Rheumatoid Factor

METHOD	SENSITIVITY	SPECIFICITY
Waaler-Rose	50%	90%
Latex (RA test)	75%	75%
Nephelometry	82%	96%
RIA	82%	93%
ELISA	85%	94%

DISEASE ASSOCIATED with POSITIVE **FR**

RHEUMATIC DISEASES	(%)
Artrite reumatoide	*75*
Sindrome di Sjögren	90
LES	30
Sclerodermia	20
Polimiosite	20
Connettivite mista	25
Artrite cronica giovanile	15

OTHER	(%)
Crioglobulinemia	70
Macrogl. di Waldenstrom	30
Plasmocitoma	30
Epatiti croniche	25
Fibrosi polmonare	25
Sarcoidosi	10
Silicosi	5
Trapianto renale	5

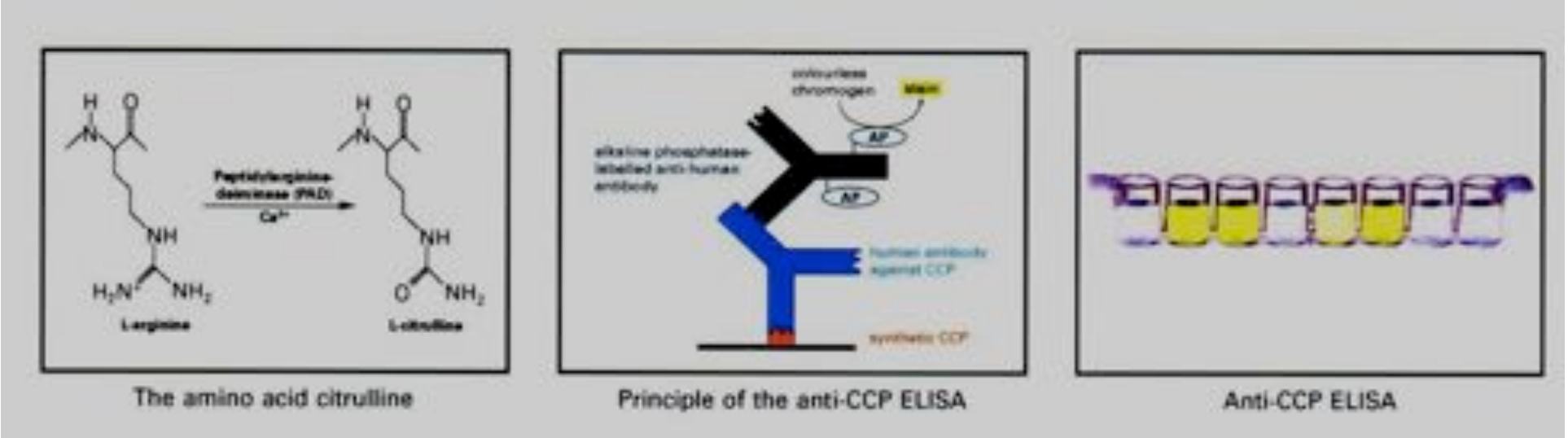
INFECTIOUS DISEASES	(%)
Endocardite batt. subacuta	40
Epatite virale	25
Lebbra	25
Tubercolosi	15
Sifilide	10
Brucellosi	5
Mononucleosi	5
Salmonellosi	5
Malaria	5
Influenza	5
Tripanosomiasi	5
Leishmaniosi	5

HEALTHY SUBJECTS (%)	<5
SUBJECTS >60 yrs (%)	15

ANTIBODY anti-CCP (cyclic citrullinated peptides)

Numerous nuclear or cytoplasmic proteins undergo post-transcriptional modifications during apoptosis such as the citrullination of arginine residues. This event may be responsible for the induction of an autoreactive response, in relation to an insufficient clearance of the apoptotic cells or to a delay in the completion of the cell death program. Citrullinated protein fragments would thus be presented to the immune system by stimulating a specific antibody response.

ELISA to detect Antibody anti-CCP in Rheumatoid Arthritis specific diagnosis



The anti-CCP ELISA has high specificity for rheumatoid arthritis (up to 95% in all studies) and adequate sensitivity (41-68%) and allows to detect the anti-CCP antibodies in 35% of sera negative for rheumatoid factors.