Ferramentas imunologicas

Alessandra Pontillo

Posso manipular a imunologia??

ou seja

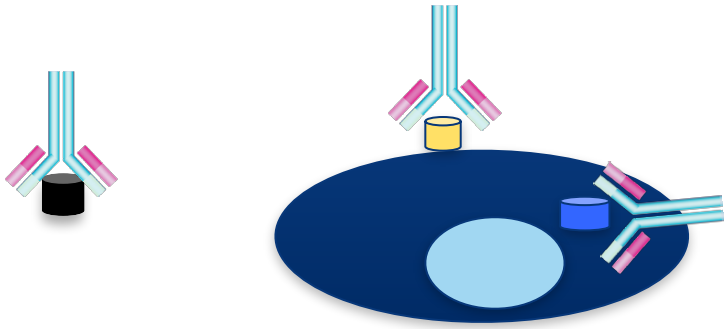
Posso utilizar ferramentas da imuno para algo de "utilidade pratica"?

Anticorpos

uma ferramenta altamente especifica

Elevada especificidade dos AC = PERFEITOS PARA

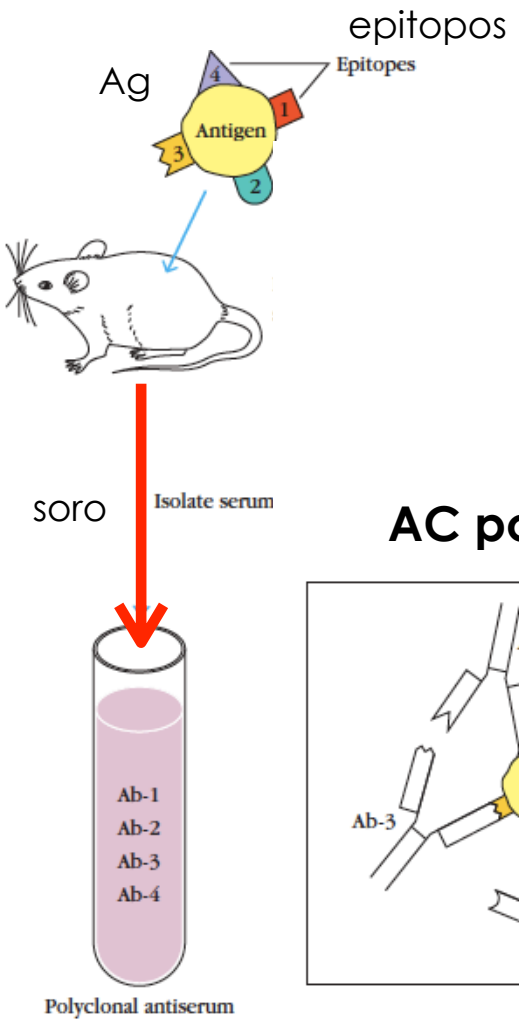
- Identificar
- Separar/Purificar
- Quantificar
- Caraterizar/Observar
- "veicular" outras moléculas contra um alvo



Pesquisa
Diagnostico
Terapia

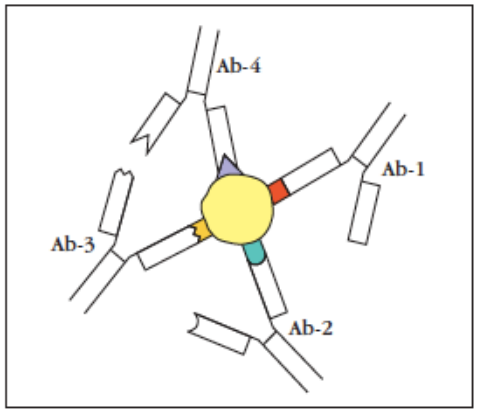
Anticorpos

Produção



soro policlonal

AC policlonais



varias células B ativadas pelo Ag (clones diferentes) produziram vários tipos de AC (contra os diferentes epitopos)

nas seguintes imunizações com o mesmo Ag, posso obter AC com maior afinidade (media)!

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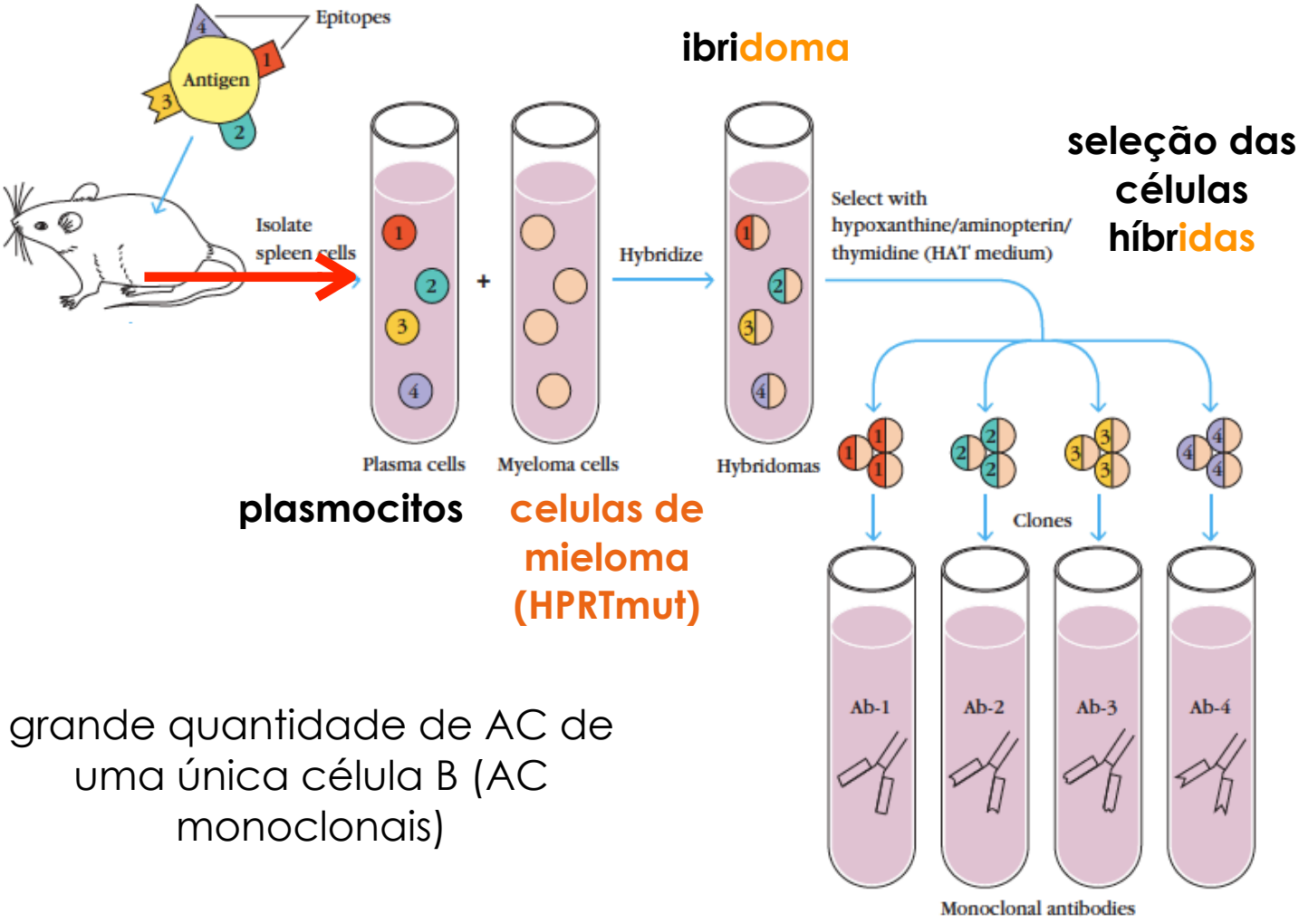
Anticorpos

Produção

Georges Köhler and Cesar Milstein (1975), Nobel prize 1984



ibridoma

seleção das células híbridas

plasmocitos

celulas de mieloma (HPRTmut)

grande quantidade de AC de uma única célula B (AC monoclonais)

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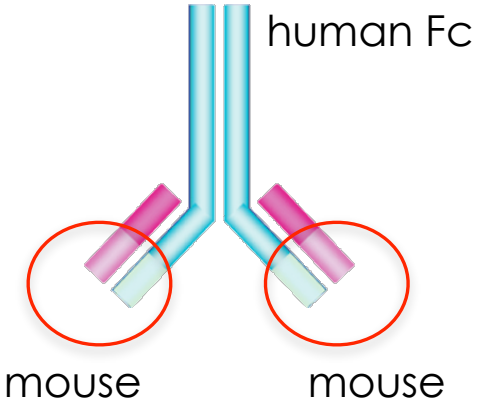
Modificação de mAC

manipulo o mAC

- coloco a região de ligação específica do mAC do animal em uma estrutura mAC humana (AC quimera)
- conjugo uma toxina a Fc do mAC



minimizo reação contra AC non próprios

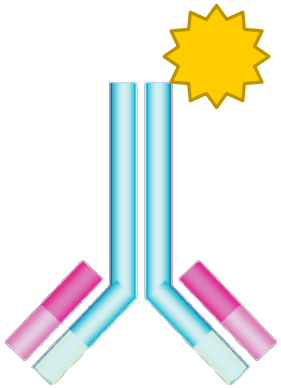
medio a lise da cella reconhecida

Anticorpos

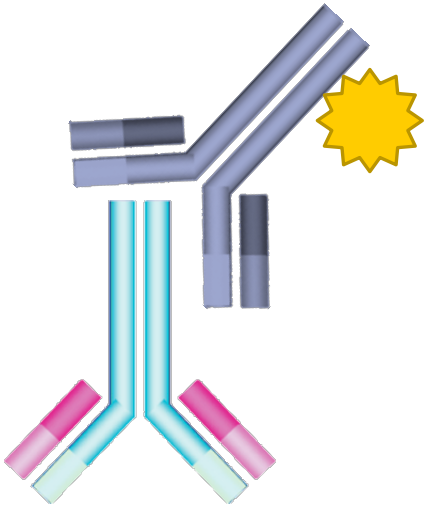
Modificação de mAC

ligo de modo covalente o mAC (Fc) com

- marcador: radio-isotopo, enzima (biotina, peroxidase), fluorocromo
- partículas inorgánicas ou sintéticas (resina, magnete, ouro)



Ac primario marcado



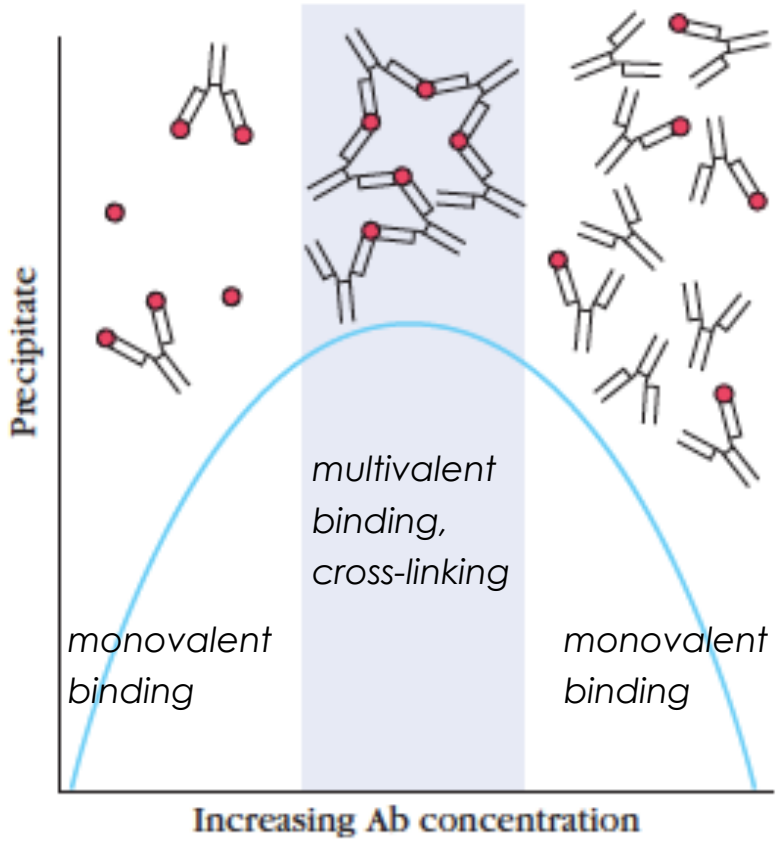
Ac primario NAO marcado
Ac segundario anti-Fc marcado

Imuno-precipitação

quando AC e Ag (solúvel) são misturados (1:1)

- a natureza bi- ou multivalente dos AC permite que um único AC ligue mais de 1 Ag
- se o Ag é multivalente pode ligar mais AC

o resultado é a formação de complexos que tendem a precipitar



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Imuno-precipitação

Tecnica que utiliza um Ac especifico para isolar um Ag proteico numa mistura de proteinas

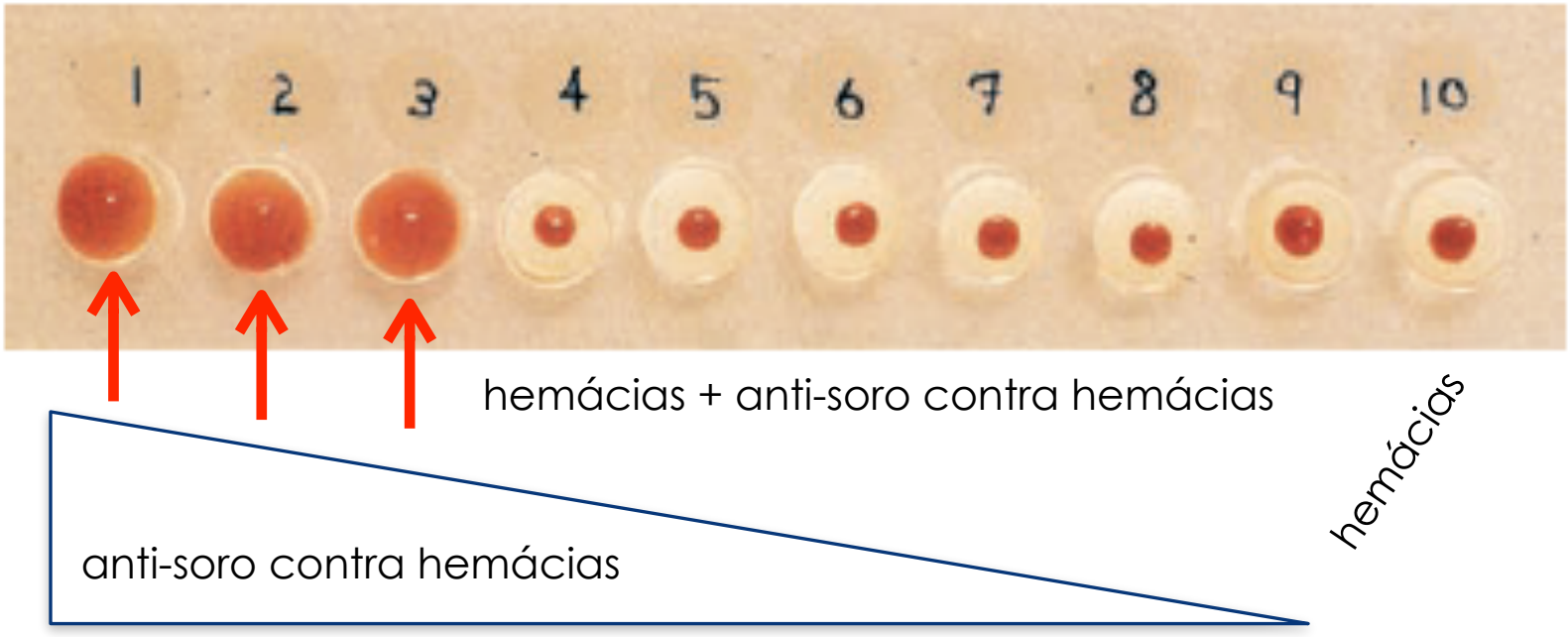
1. Ac preso quimicamente a uma particula de fase solida (i.e. agarose) è incubado com a mistura de proteinas
2. As proteinas que nao se ligam ao Ac sao eliminadas por lavagem
3. Os Ag especificos sao soltos (eluidos) do Ac pela alteraçao do pH ou de outras condiçoes do solvente que reduzam a afinidade de ligaçao

TECNICAS com AC

Aglutinação

quando AC bi- ou multivalentes ligam bacteria ou outros Ag multivalentes se formam complexos de Ac/Ag grandes e visíveis *a olho nu (é uma imuno precipitação que leva a formação de complexos maiores)*

Hemo-aglutinação



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uso clinico

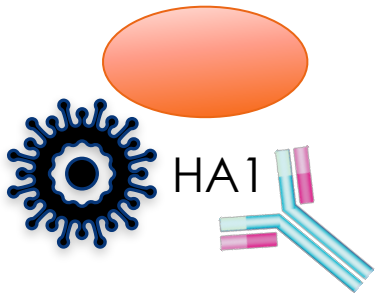
Hemo-aglutinação

tipagem AB0

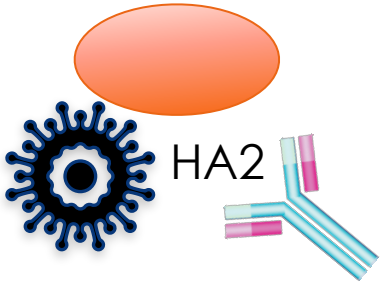
hemácias vem misturadas com antisera anti-A ou anti-B

deteção de virus e AC anti-virus

hemácias vem misturadas com antisera de paciente com virus com *aglutininas* (exemplo: influenza virus)



AC_1



AC_2

TECNICAS com AC

Sensibilidade dos testes

TABLE 20-1 Sensitivity of various immunoassays

Assay	Sensitivity* (μg antibody/ml)
Precipitation reaction in fluids	20–200
Precipitation reaction in gels	
Ouchterlony double immunodiffusion	20–200
Agglutination reactions	
Direct	0.3
Agglutination inhibition	0.006–0.06
Radioimmunoassay (RIA)	0.0006–0.006
Enzyme-linked immunosorbent assay (ELISA)	~0.0001–0.01
ELISA using chemiluminescence	~0.00001–0.01†
Immunofluorescence	1.0
Flow cytometry	0.006–0.06

*The sensitivity depends on the affinity of the antibody used for the assay as well as the epitope density and distribution on the antigen.

† Note that the sensitivity of chemiluminescence-based ELISA assays can be made to match that of RIA.

Source: Updated and adapted from N. R. Rose et al., eds., 1997, *Manual of Clinical Laboratory Immunology,* 5th ed., Washington, DC: American Society for Microbiology.

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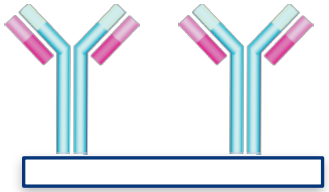
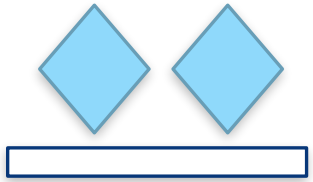
TECNICAS com AC

Ensaios com moléculas ligadas a superficies solidas



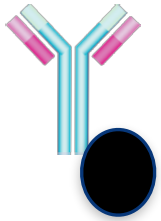
AC ligado a suporte solido (placa de reação, lamina)

detecta Ag



Ag ligado a suporte solido (placa de reação, lamina)

detecta AC

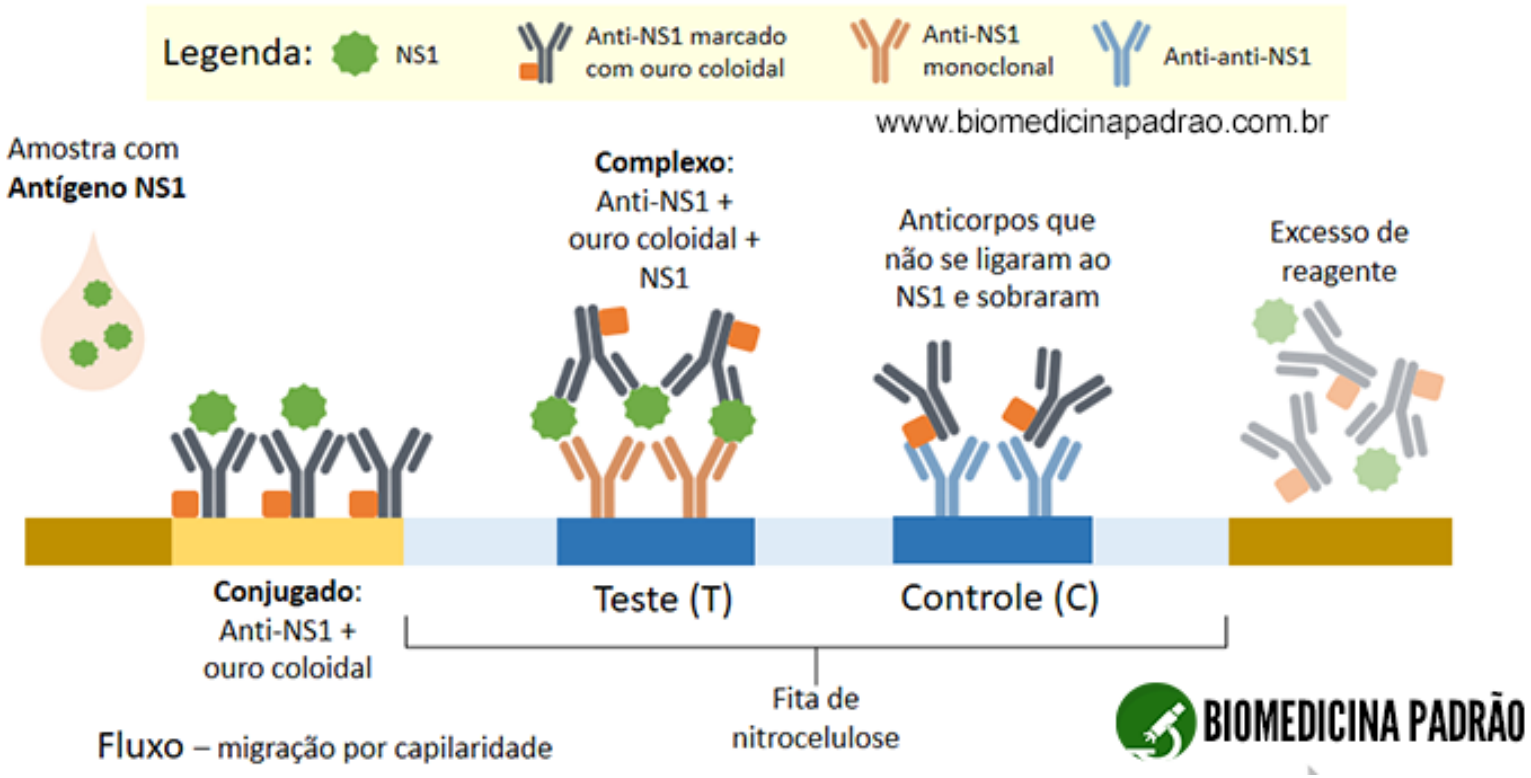


AC com esfera (beads) inorgânica/sintetica

detecta Ag

TECNICAS com AC

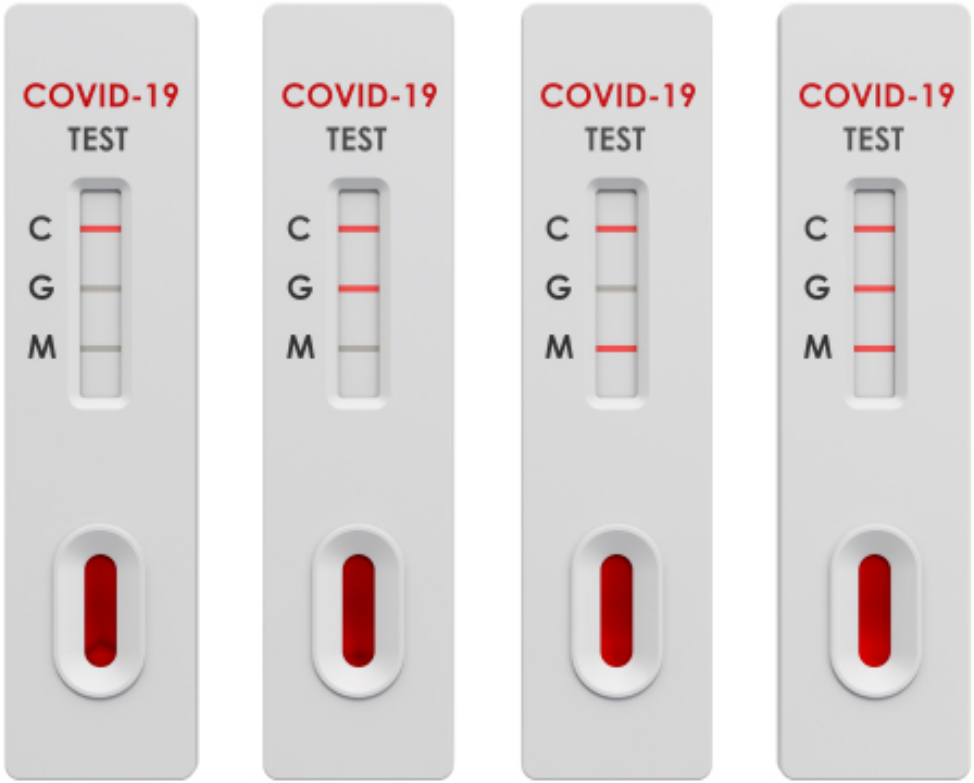
Imunocromatografia



uso clinico

Teste rapido

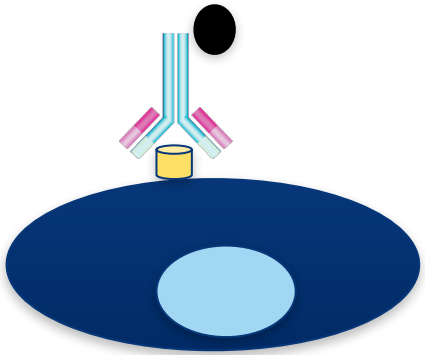
Ag virais são fixados em um suporte sólido

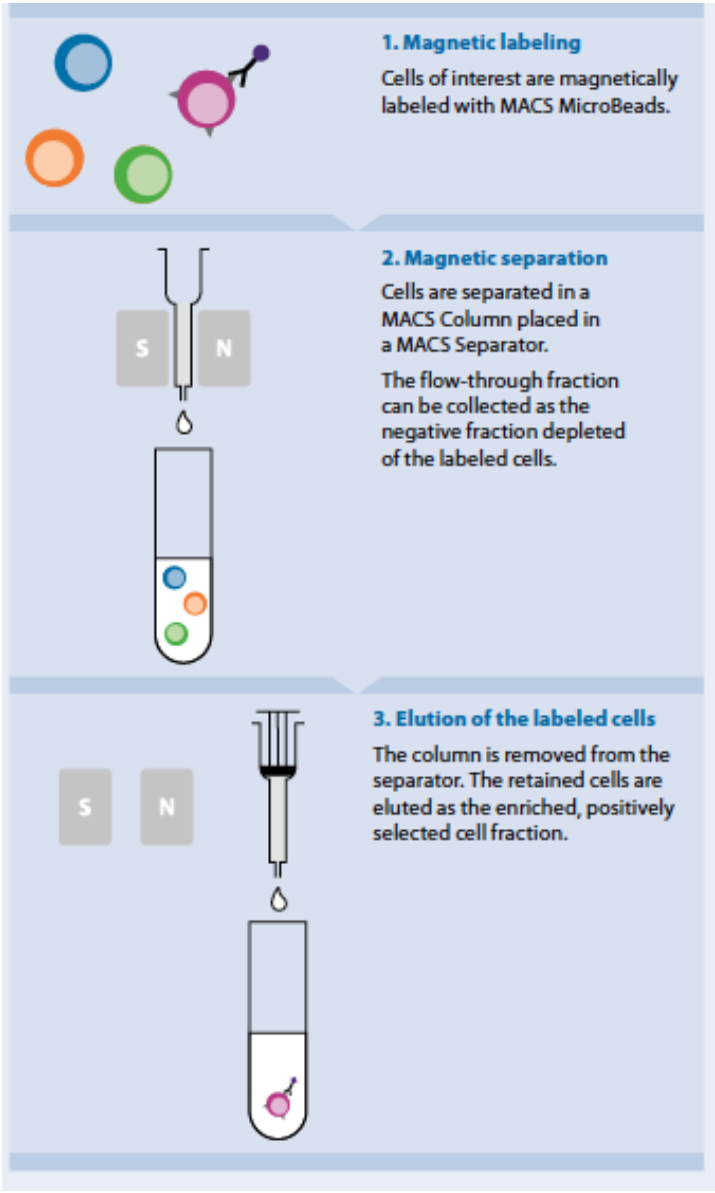


https://www.igenomix.com.br

TECNICAS com AC

Separação de células



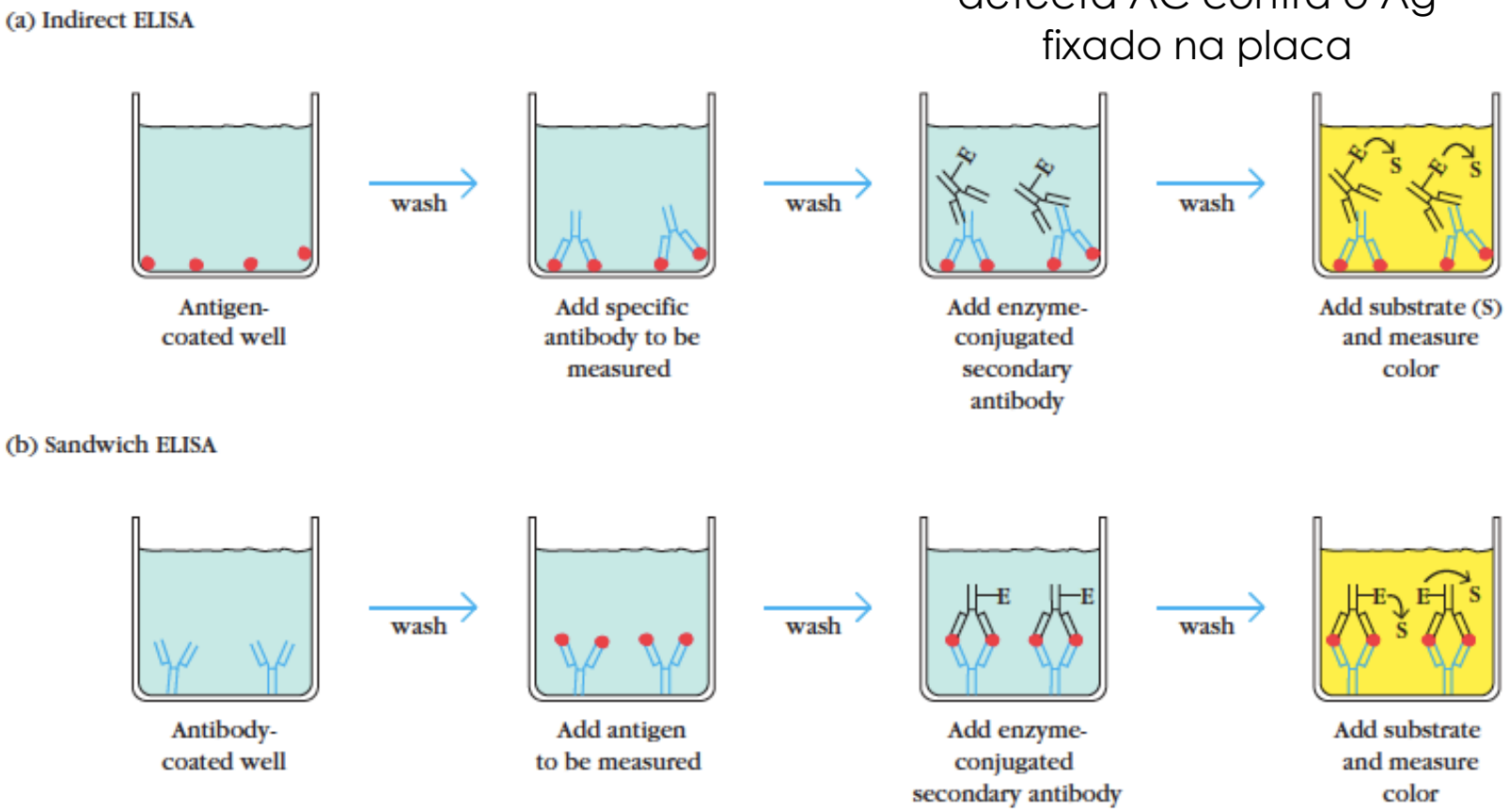


TECNICAS com AC

ELISA

Enzyme Linked Immuno-Sorbent Assay

detecta AC contra o Ag fixado na placa



detecta Ag reconhecidos pelo AC fixado na placa e 2°

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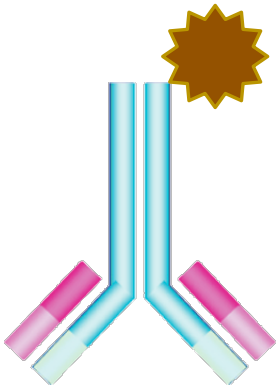
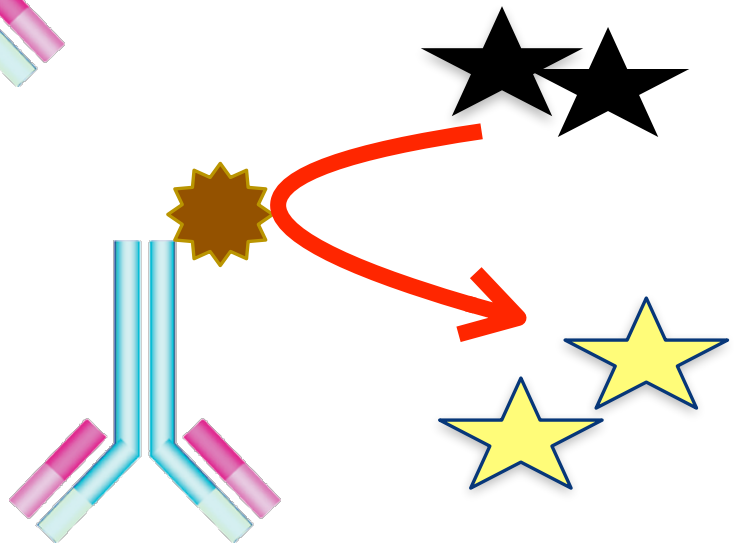
TECNICAS com AC

ELISA

como visualizo a reação?



o AC 2º é conjugado com um enzima
- fosfatase alcalina (PA)
- peroxidase de rafano (horseradish) (HRP)



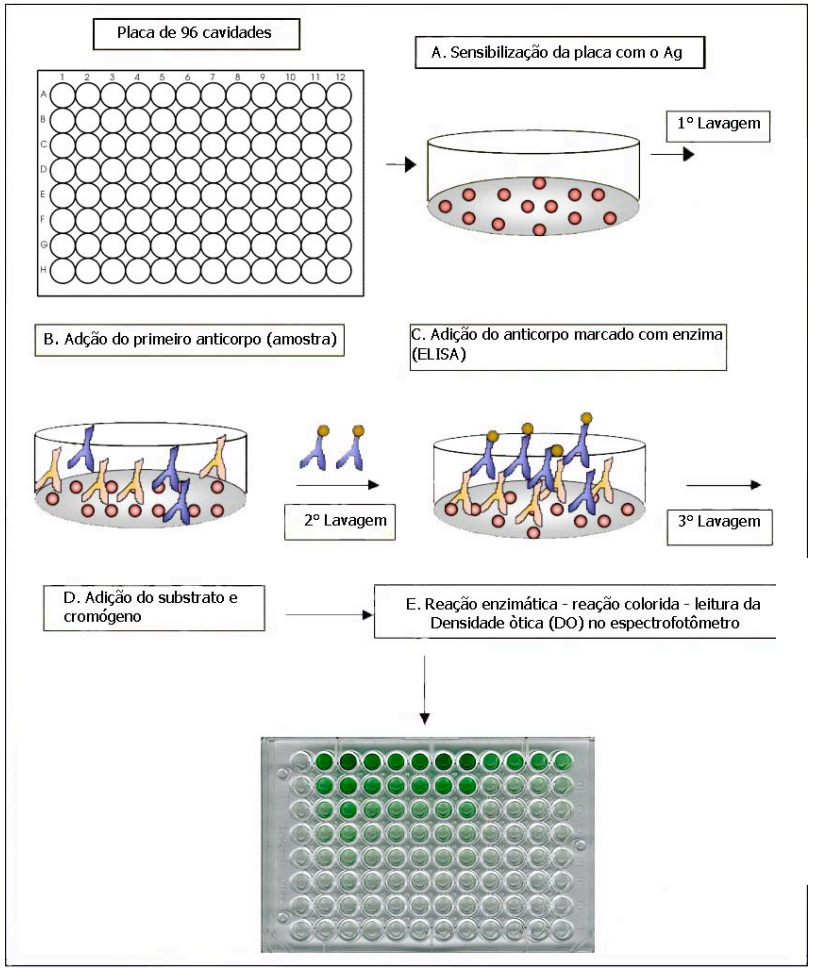
substrato do enzima
= cromogeno
pNPP (PA)
TMB (HRP)

muda cor

TECNICAS com AC

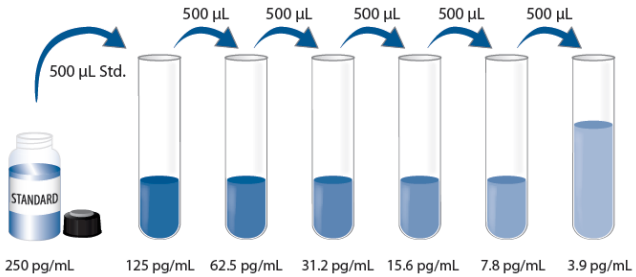
ELISA

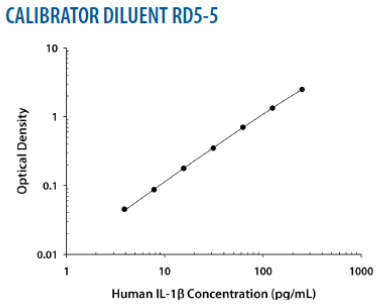
como quantifico a reação?



Curva padrao (referencia)

- Molécula de concentração conhecida
- Diluições seriais
- Curve fit
- Extrapolação dos dados nao conhecidos



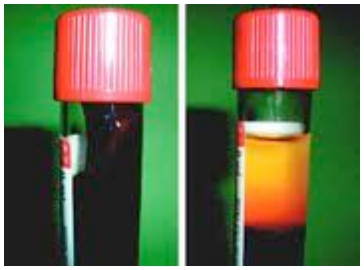


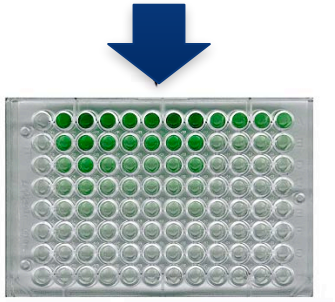
(pg/mL)	O.D.	Average	Corrected
0	0.027 0.023	0.025	—
3.9	0.071 0.070	0.070	0.045
7.8	0.115 0.109	0.112	0.087
15.6	0.206 0.197	0.202	0.177
31.2	0.374 0.373	0.374	0.349
62.5	0.733 0.715	0.724	0.699
125	1.366 1.349	1.358	1.333
250	2.495 2.517	2.506	2.481

uso clinico e in lab

ELISA

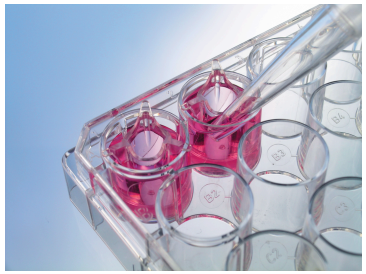
Clinica

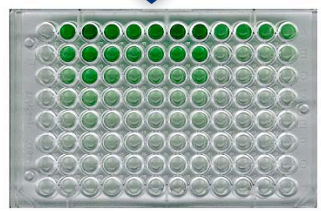




Dosagem no soro
- de Ac
- de proteinas

Pesquisa





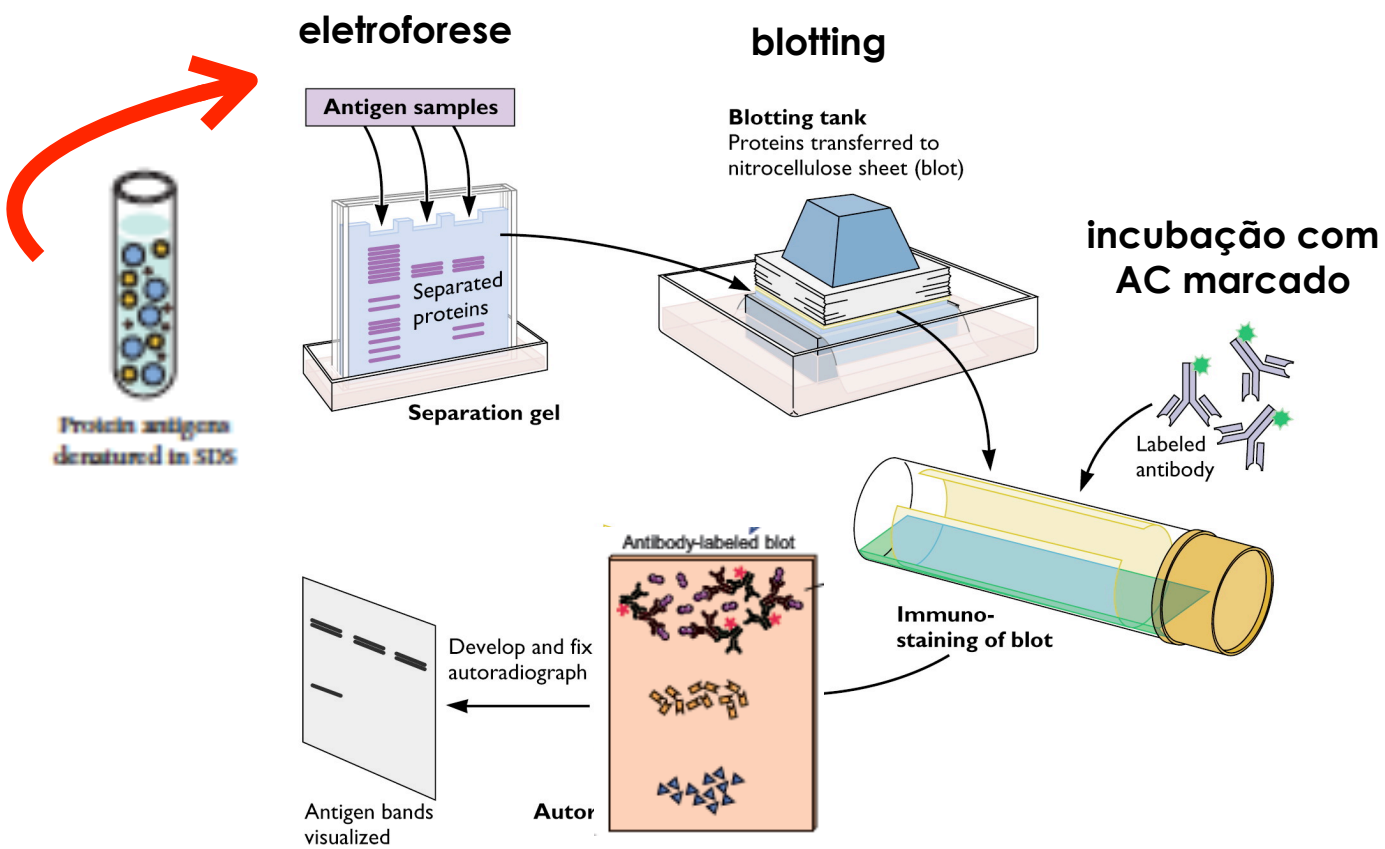
Dosagem no sobrenadante
- de moleculas secretadas/liberadas (ex: citocinas; NO; ROI)

Dosagem no lisado celular
- de proteinas

TECNICAS com AC

WESTERN BLOT

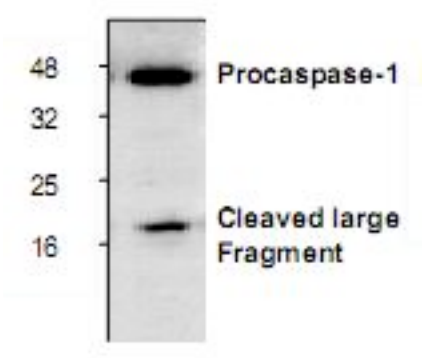
identifica uma proteína alvo e determina uma primeira quantificação/caraterizaçao (PM) dentro de uma mistura



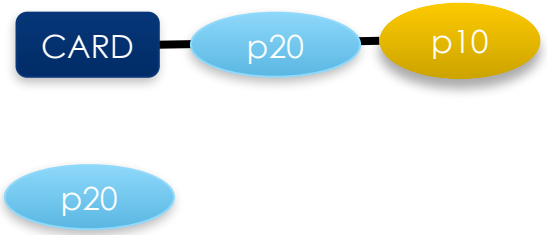
TECNICAS com AC

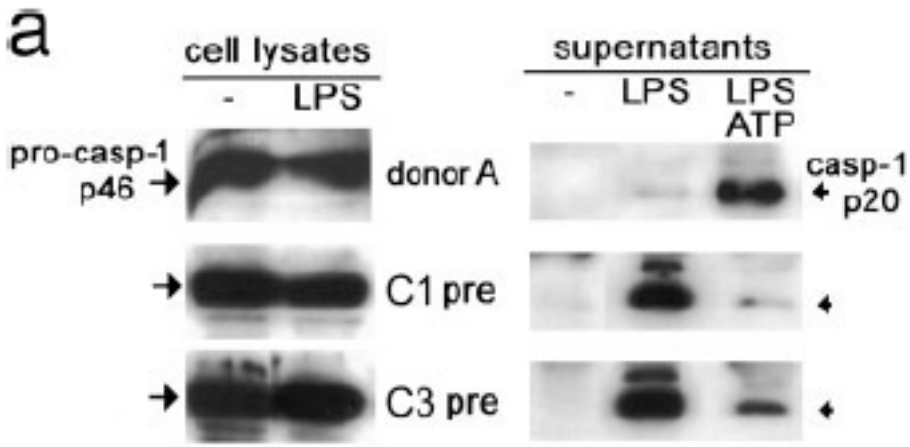
WESTERN BLOT

Exemplo



Pro-caspase-1 (46 Kda)



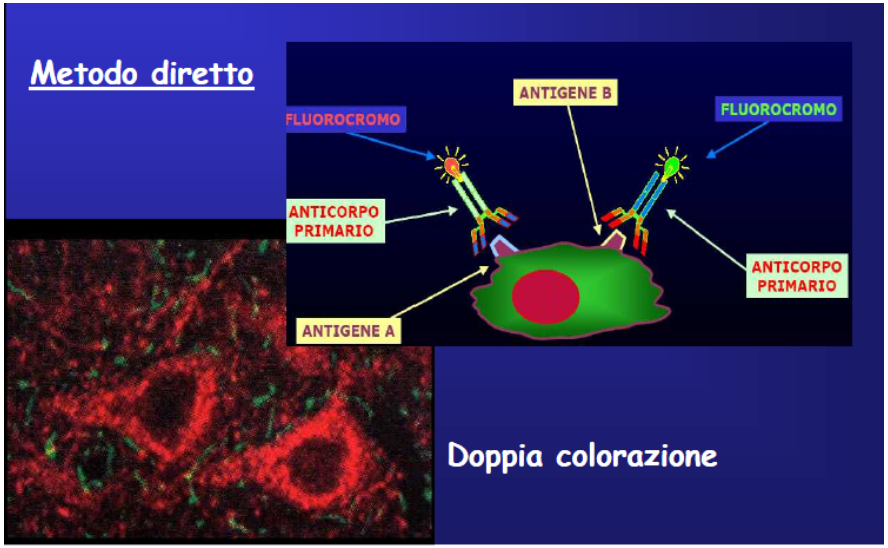


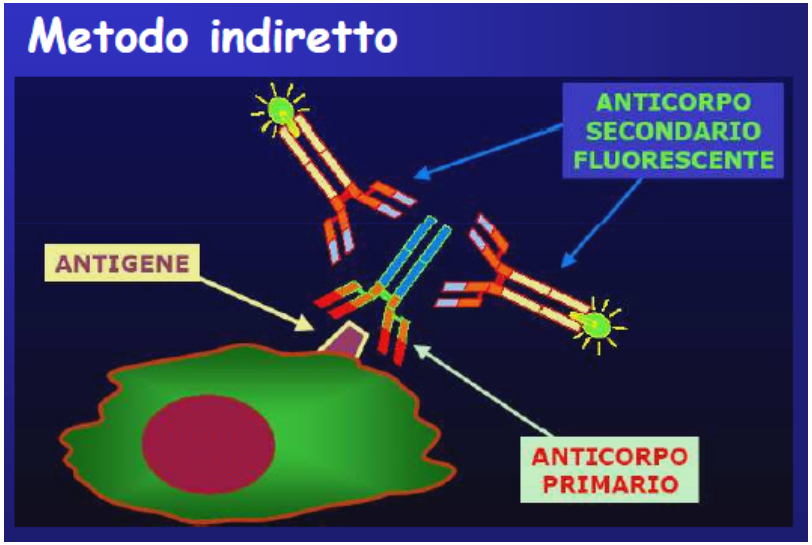
TECNICAS com AC

VISUALIZACAO DE CELULAS/TECIDOS

AC pode ser conjugado com

- Enzima: HRP, PA —> imuno-histoquimica e microscopia optica
- Ouro —> microscopia electronica
- Fluorocromo: isotiocianato de fluoresceina (FITC); ficoeritrina (PE); tetrametil rodamina —> imuno-fluoresciencia





TECNICAS com AC

IMUNO HISTOQUIMICA

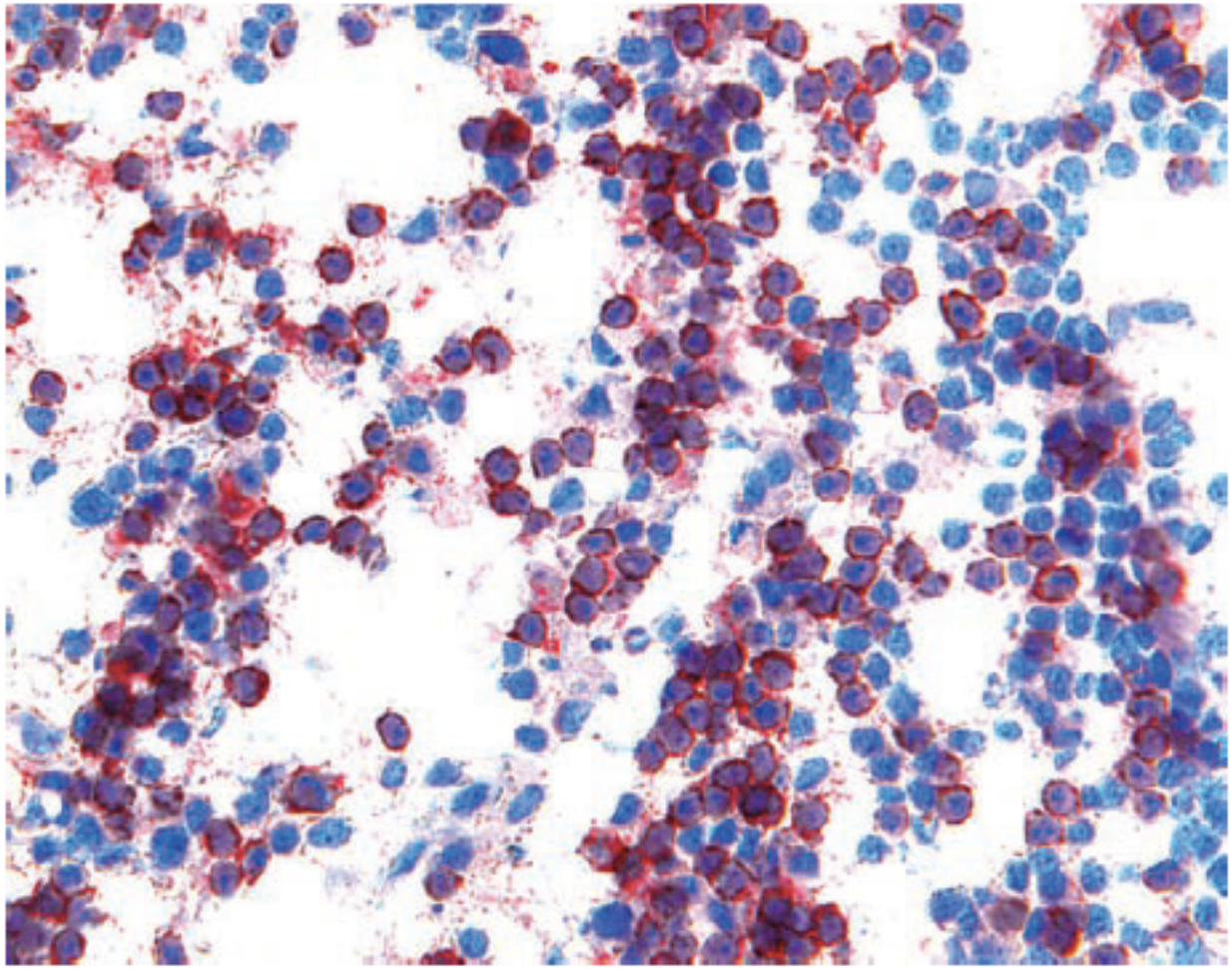


FIGURE 20-14 Immunohistochemical staining of lymph node. Human lymph node material was embedded in paraffi n, fi xed, and stained with antibody to CD4 (red stained cells). The lymph node was then counter-stained with hematoxylin (blue). [Courtesy R&D Systems, Inc., Minneapolis, MN, USA.]

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IMUNO ELETROMICROGRAFIA

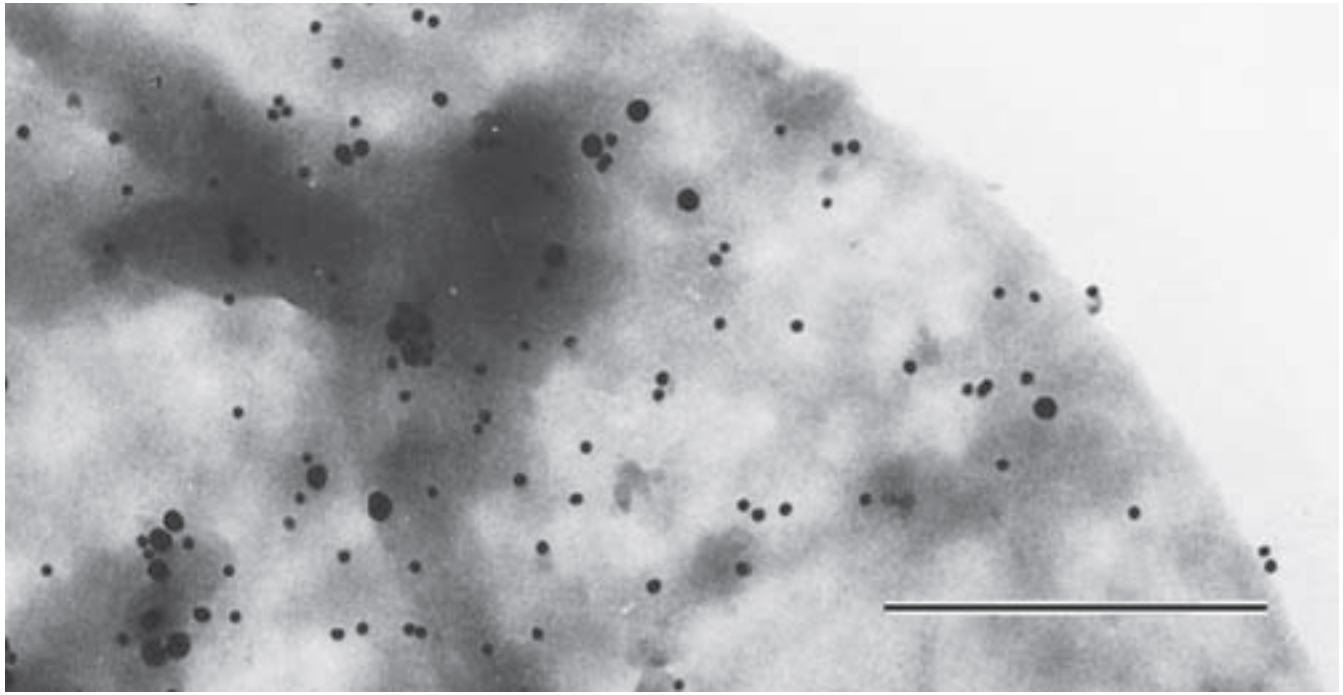


FIGURE 20-15 An immunoelectronmicrograph of the surface of a B-cell lymphoma was stained with two antibodies: one against class II MHC molecules labeled with 30-nm gold particles and another against MHC class I molecules labeled with 15-nm gold particles. The density of class I molecules exceeds that of class II on this cell. Bar 500 nm. [From A. Jenei et al., 1997, PNAS 94:7269–7274; courtesy of A. Jenei and S. Damjanovich, University Medical School of Debrecen, Hungary.

IMUNOFLUORESCIENCIA

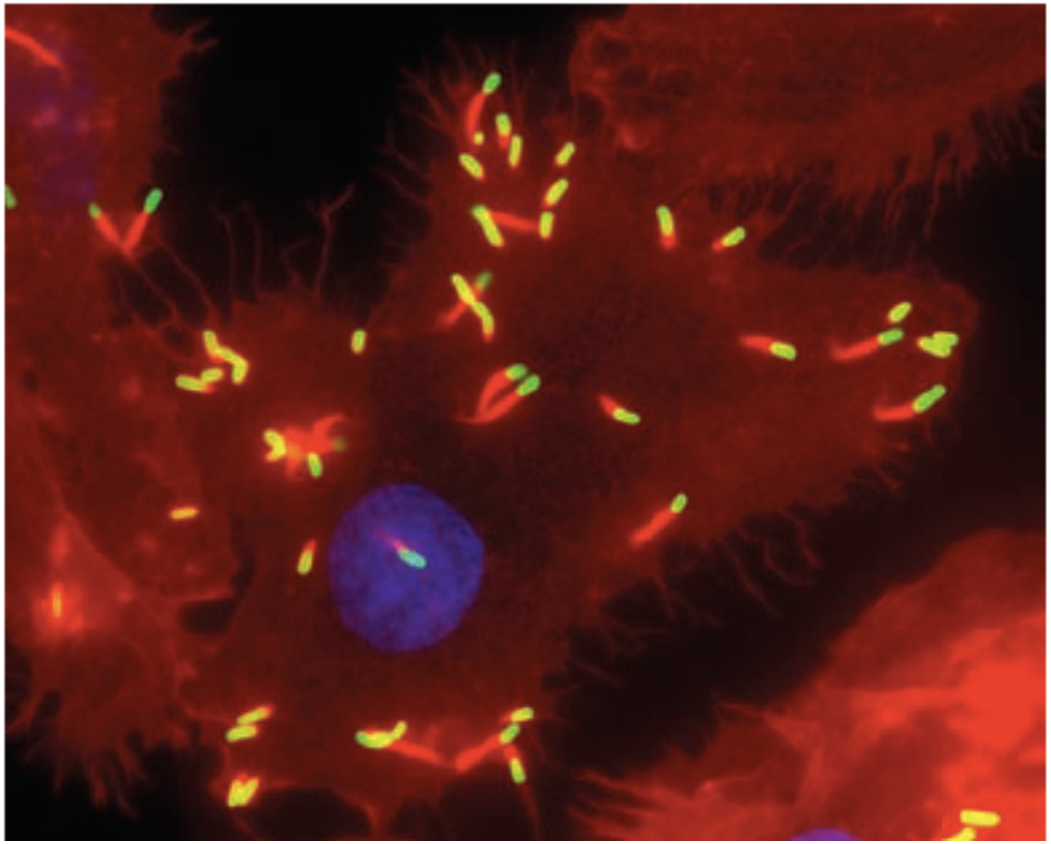
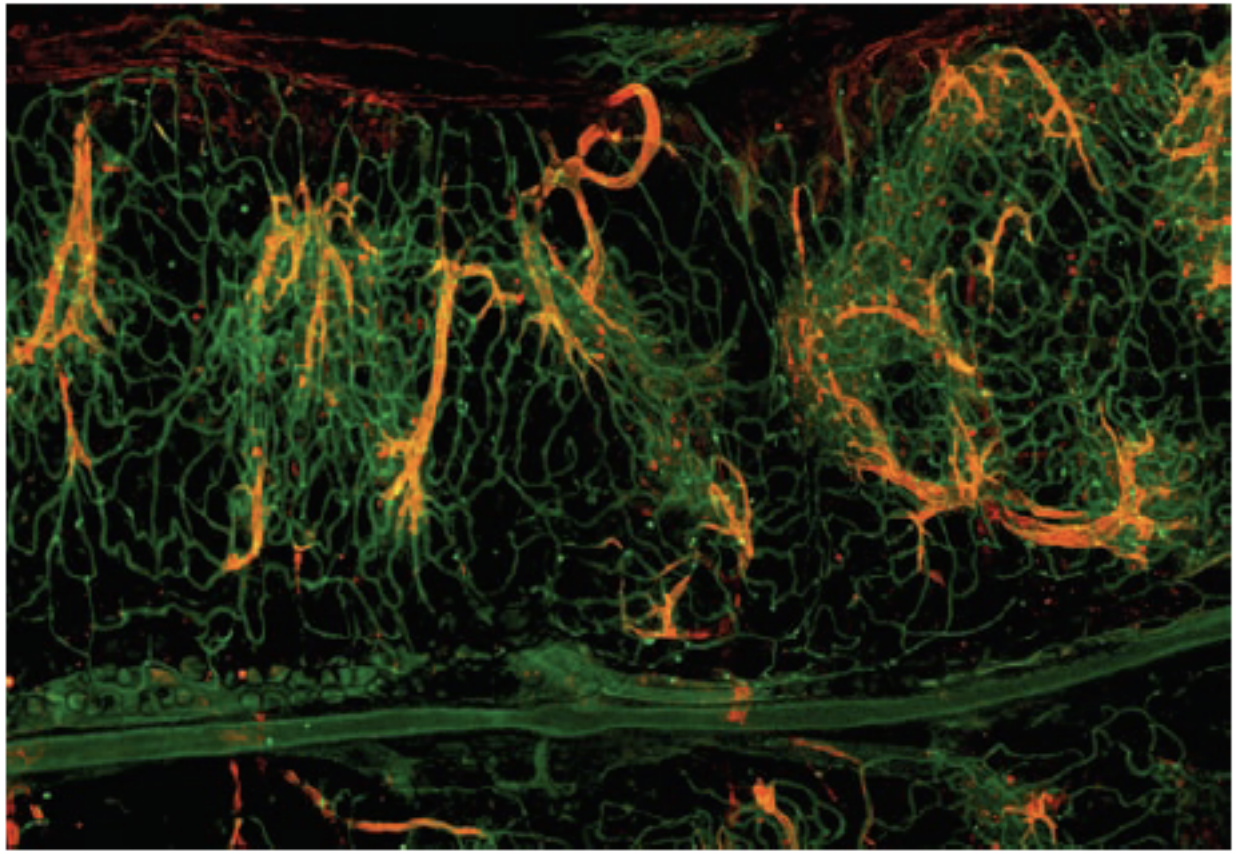


FIGURE 20-16 Fluorescently-labeled cells and the passage of light through a fl uorescence microscope. (a) Fluorescence microscopic image of a human dendritic cell infected with engineered Listeria monocytogenes. The green fl uorescence is generated by Alexa fl uor 488-conjugated phalloidin (which binds actin fi laments). Red fl uorescence derives from red fl uorescent protein expressed by the bacterium. DNA is stained with DAPI (blue).

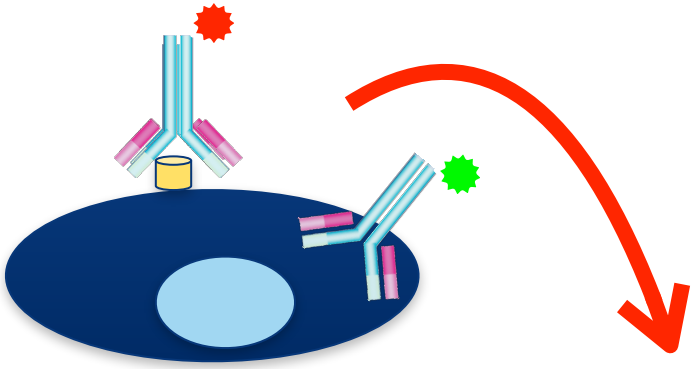
IMUNOFLUORESCIENCIA



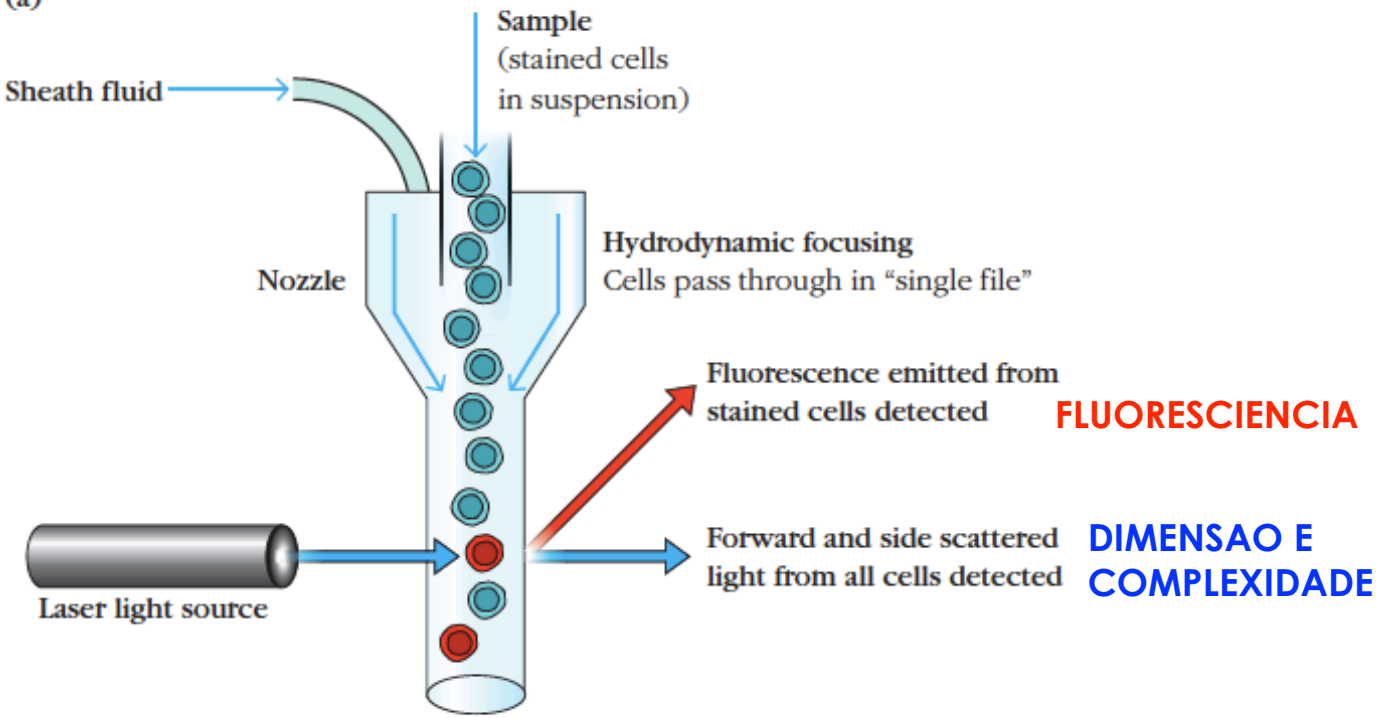
Confocal image of a mouse omentum. The omentum was stained with green dye-conjugatedantibodies specifi c for CD31, which stains all endothelial vessels in the omentum. A second, red dye-conjugated antibody was then added specifi c for the molecule DARC, that is present only on the endothelial cells of the post-capillary end venules (the vessels from which extravasation of immune cells can occur). The red and green images were acquired separately and merged. Vessels staining both red and green appear yellow in this image. [(b) Courtesy Aude Thiriot and Ulrich von Andrian, Harvard Medical School.]

TECNICAS com AC

CITOMETRIA DE FLUXO



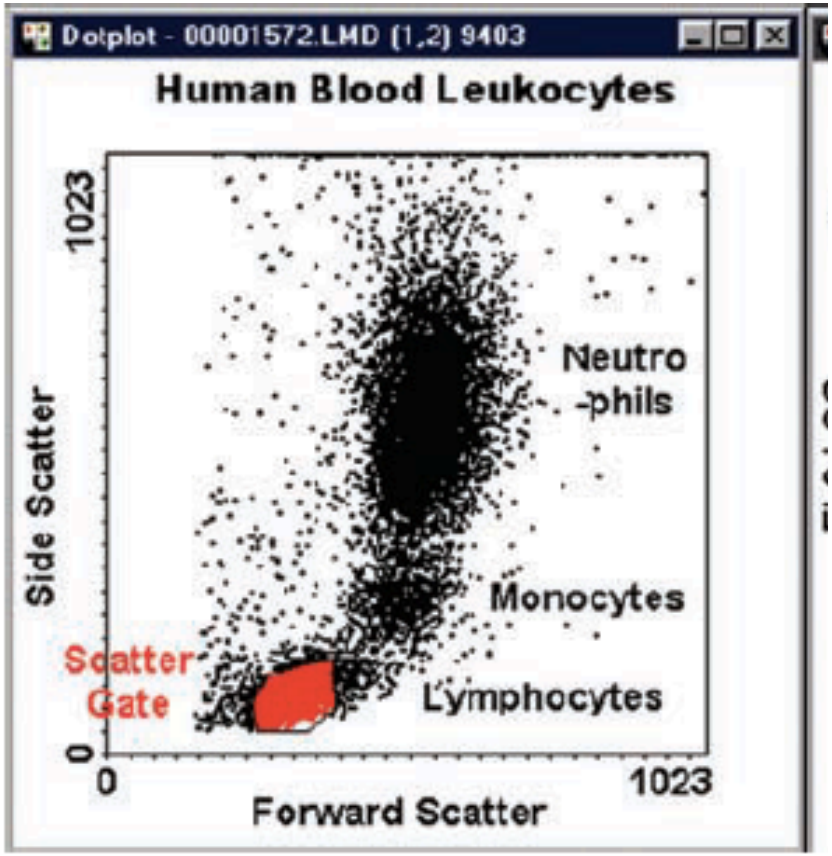
(a)



CITOMETRIA DE FLUXO

Que população celular?

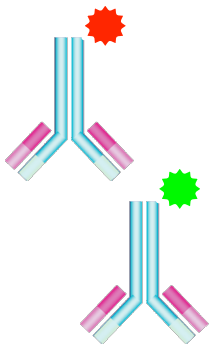
Dimensão e complexidade



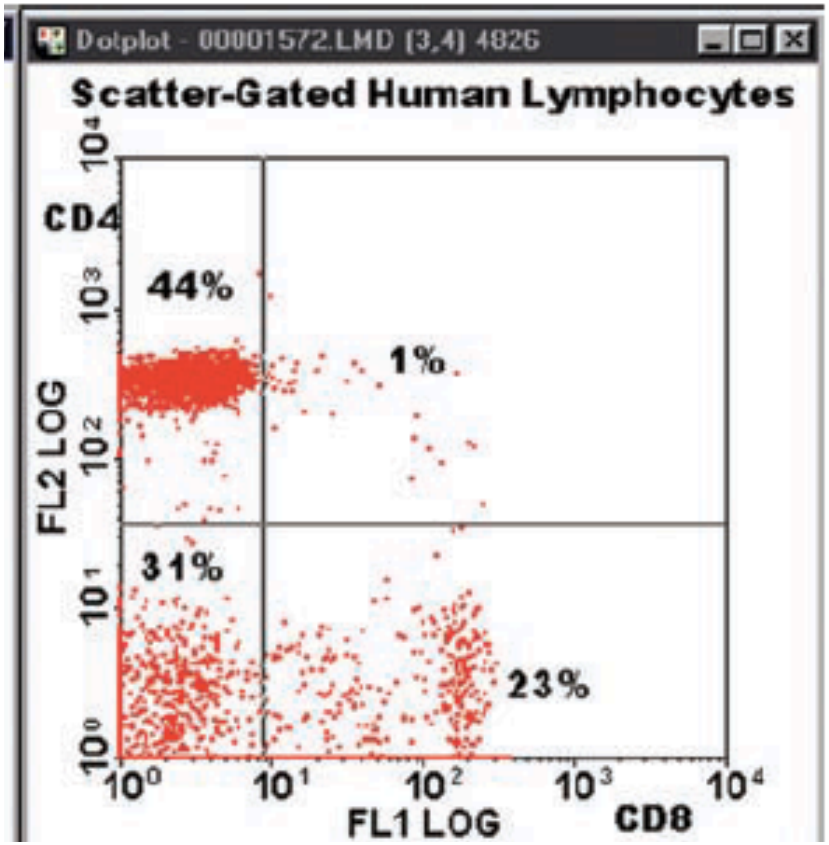
CITOMETRIA DE FLUXO

Que população celular?

Célula especifica

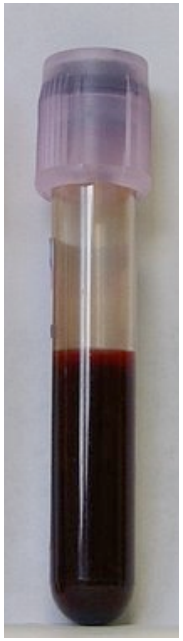


anti-CD4
anti-CD8

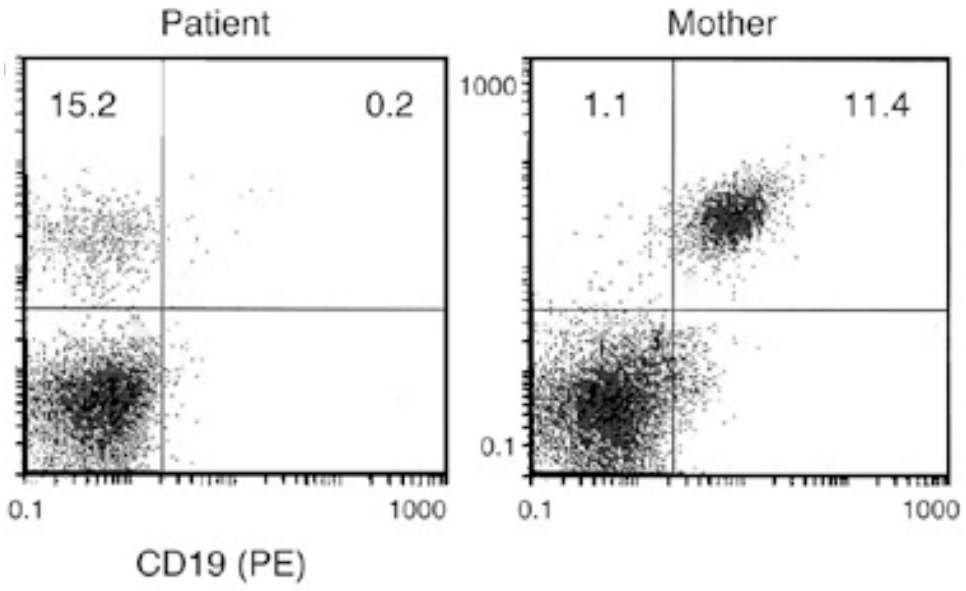


CITOMETRIA DE FLUXO

Paciente com deficiencia células B



anti-CD19



TECNICAS com AC

Sensibilidade dos testes

TABLE 20-1 Sensitivity of various immunoassays

Assay	Sensitivity* (μg antibody/ml)
Precipitation reaction in fluids	20–200
Precipitation reaction in gels	
Ouchterlony double immunodiffusion	20–200
Agglutination reactions	
Direct	0.3
Agglutination inhibition	0.006–0.06
Radioimmunoassay (RIA)	0.0006–0.006
Enzyme-linked immunosorbent assay (ELISA)	~0.0001–0.01
ELISA using chemiluminescence	~0.00001–0.01†
Immunofluorescence	1.0
Flow cytometry	0.006–0.06

*The sensitivity depends on the affinity of the antibody used for the assay as well as the epitope density and distribution on the antigen.

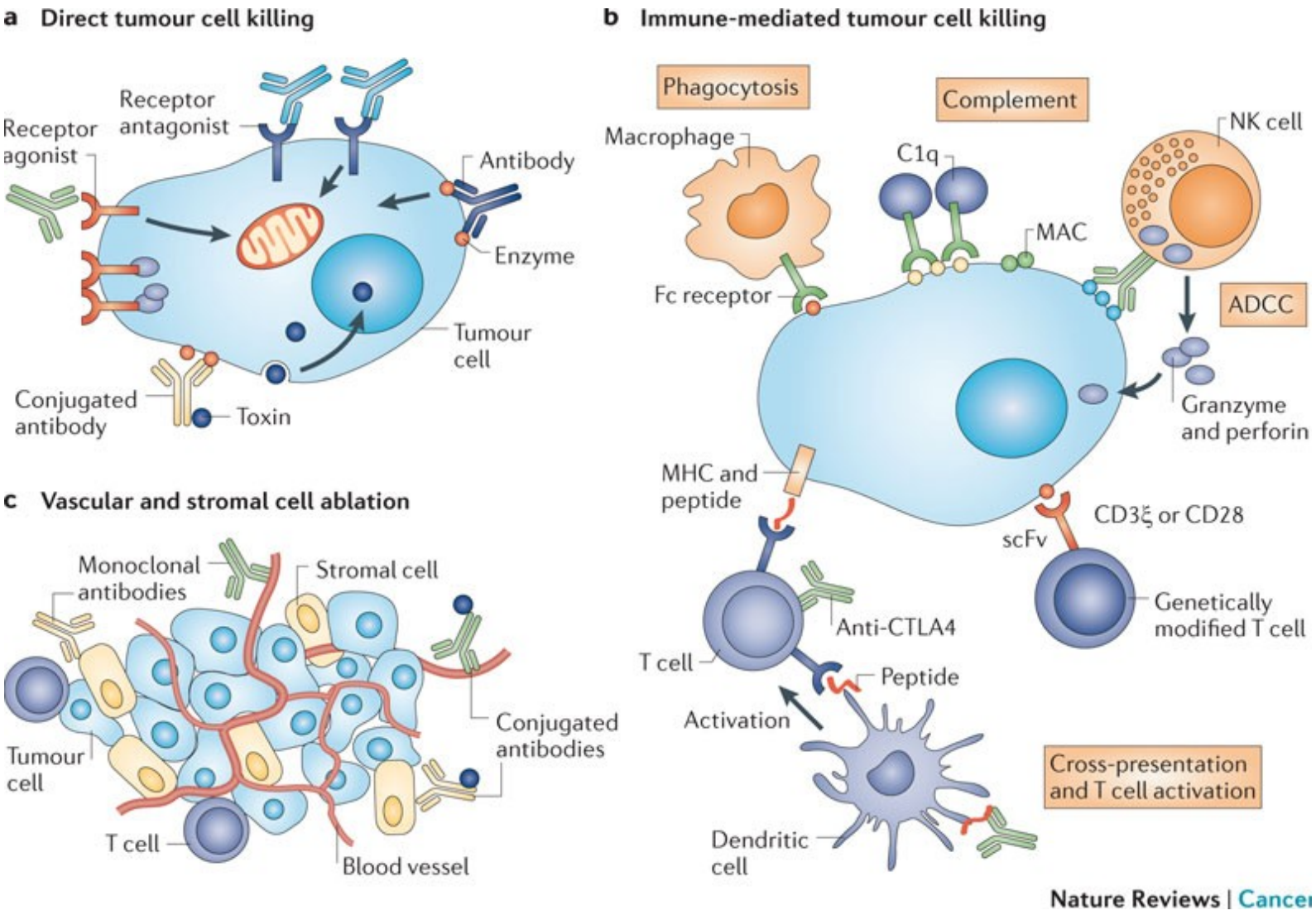
† Note that the sensitivity of chemiluminescence-based ELISA assays can be made to match that of RIA.

Source: Updated and adapted from N. R. Rose et al., eds., 1997, *Manual of Clinical Laboratory Immunology,* 5th ed., Washington, DC: American Society for Microbiology.

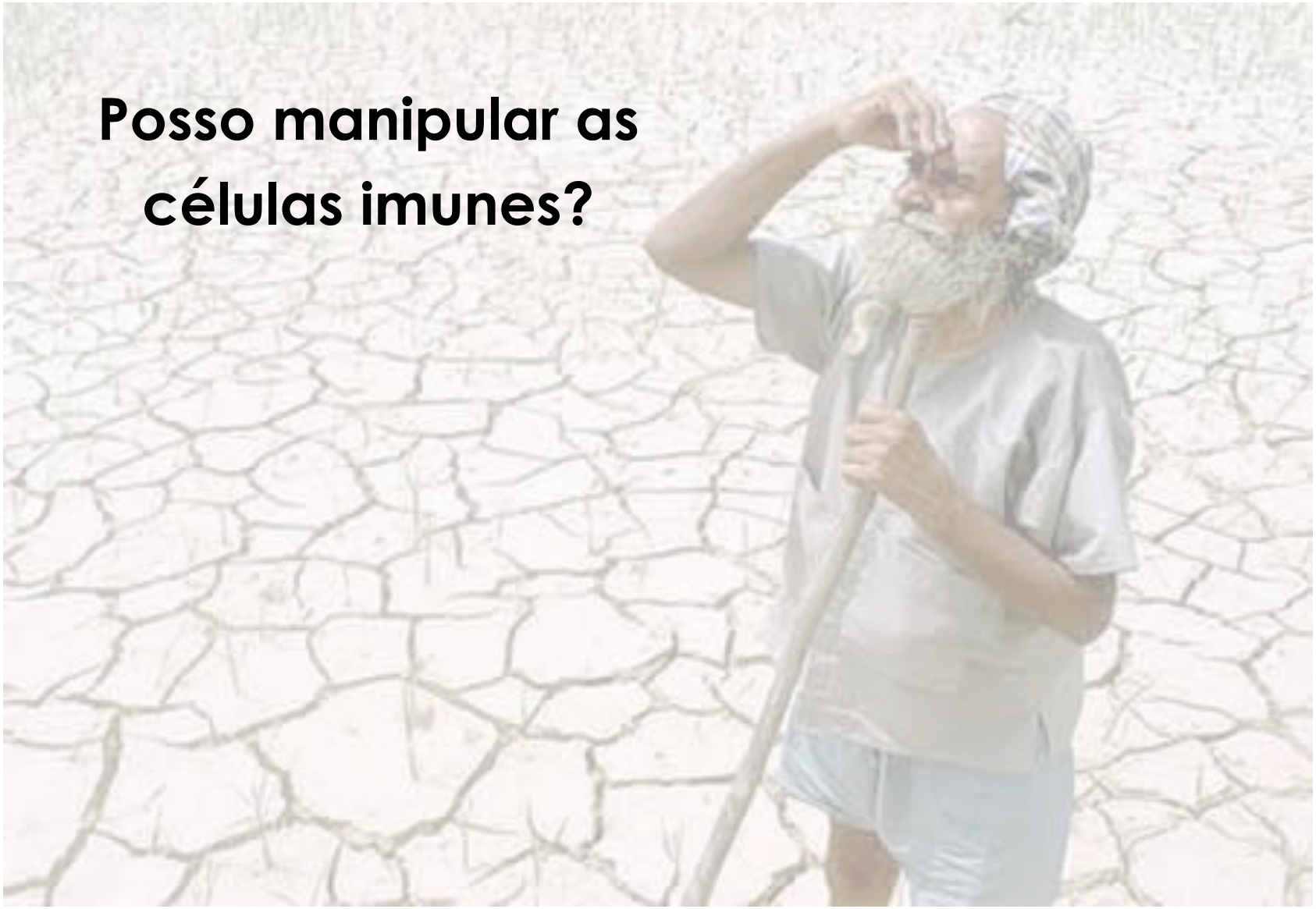
Kuby, 7th 2013

uso clinico

AC contra cancer



Posso manipular as células imunes?



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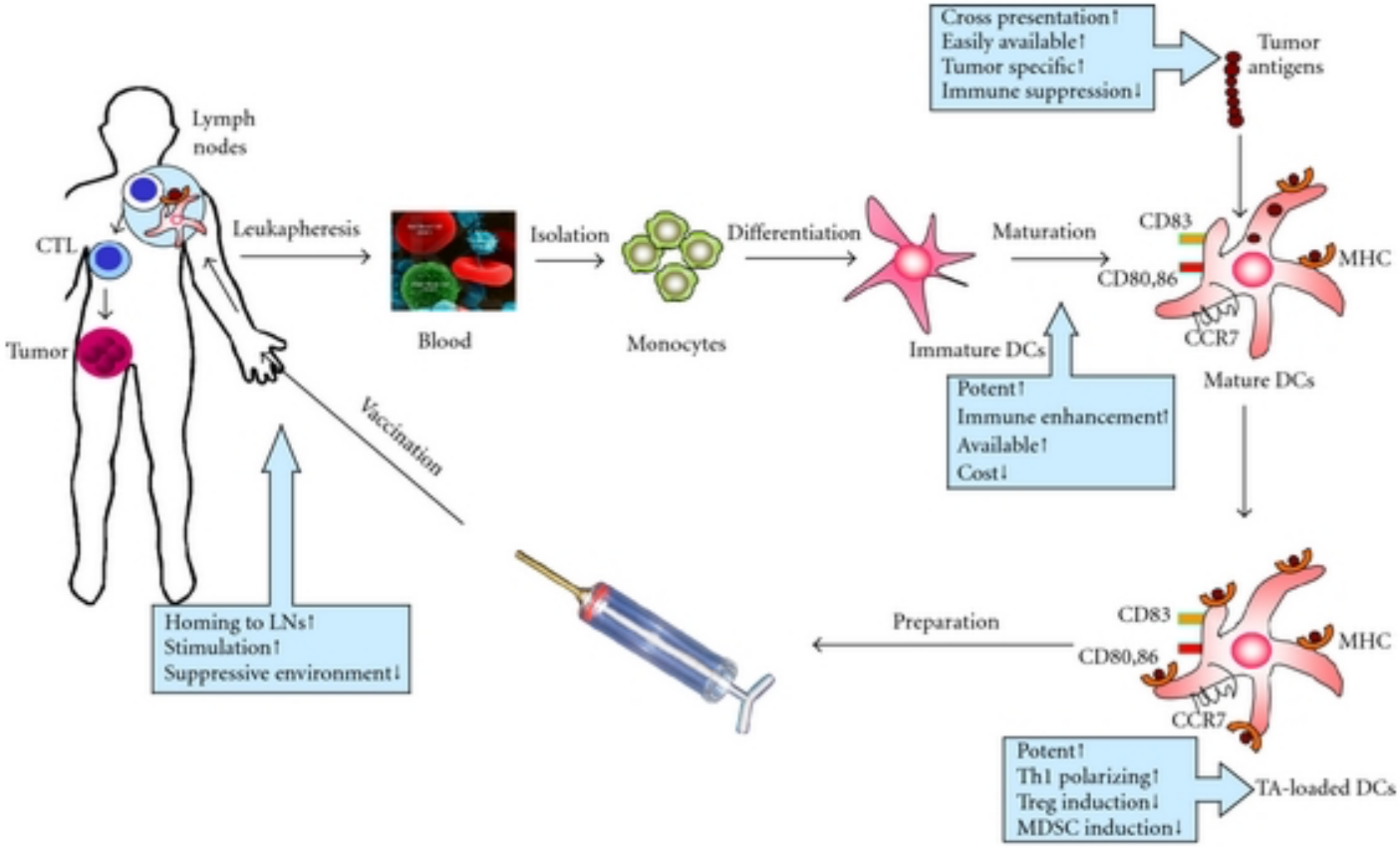
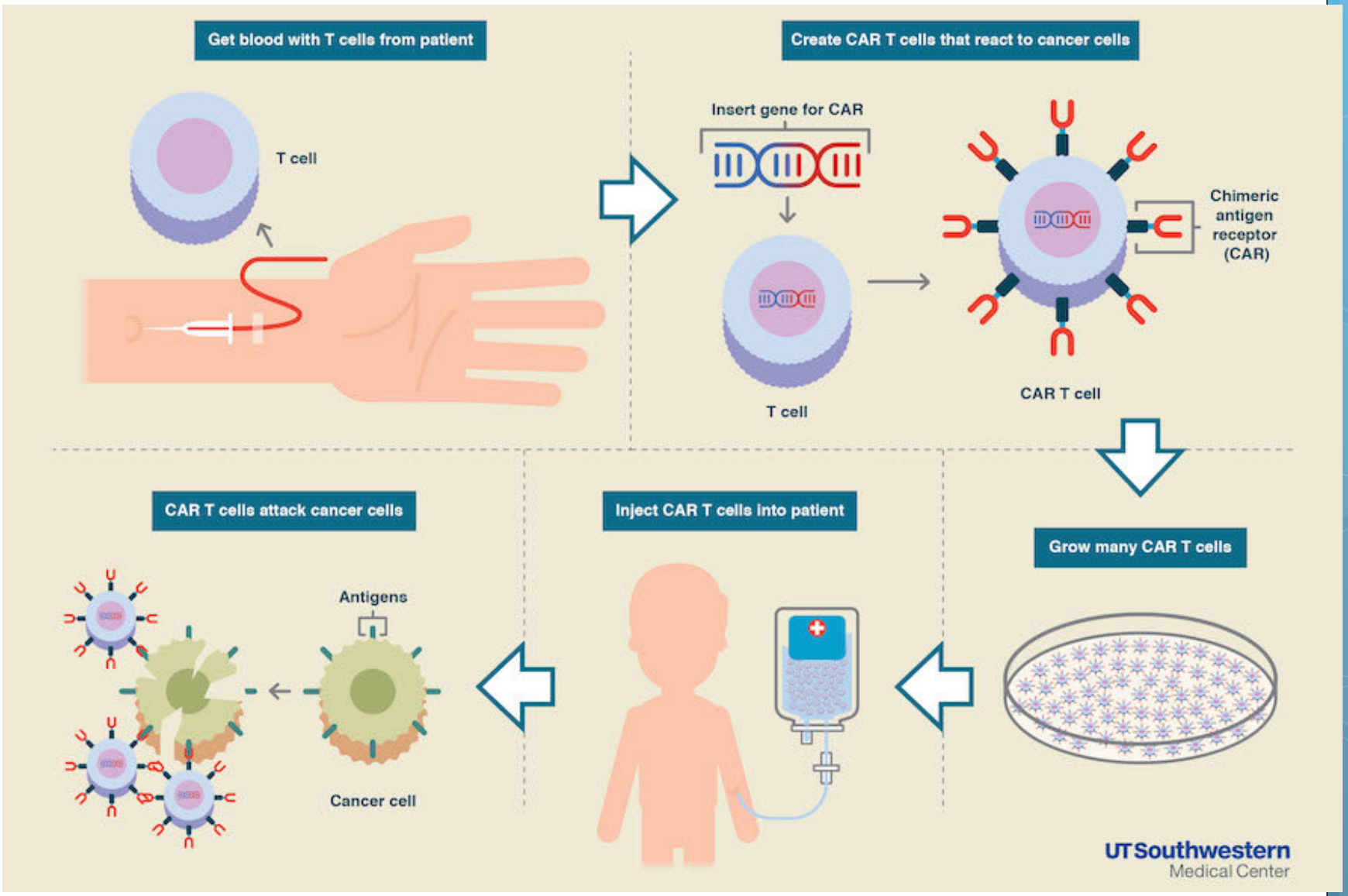
DC como imunoterapia

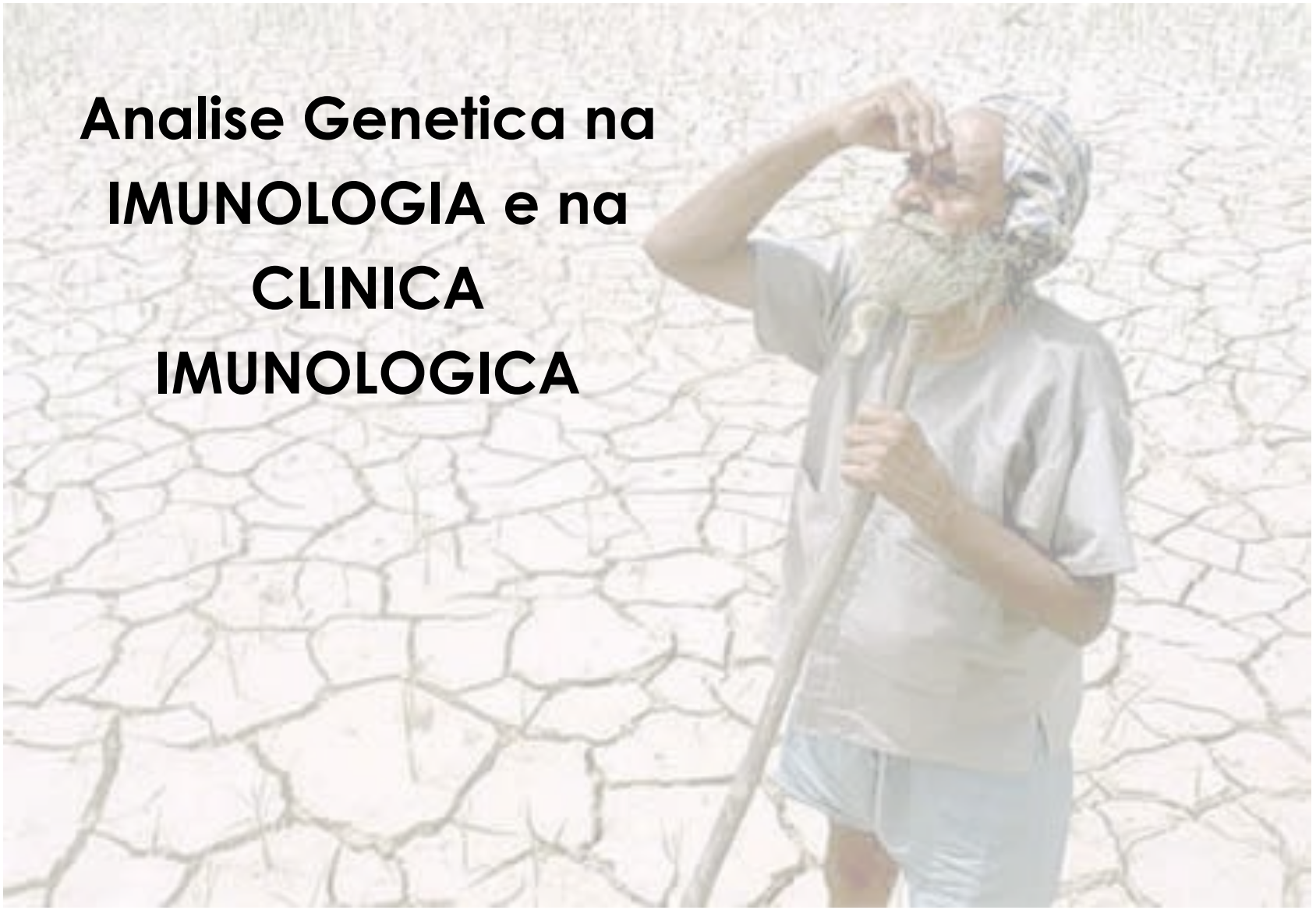


uso clinico

Celulas T contra cancer



Analise Genetica na IMUNOLOGIA e na CLINICA IMUNOLOGICA

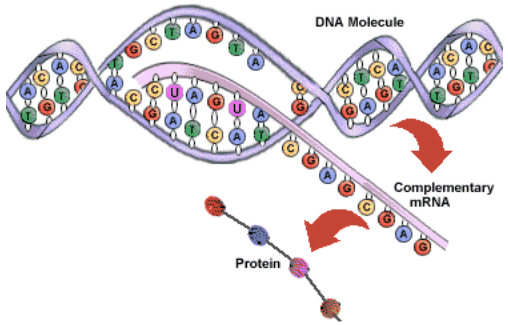


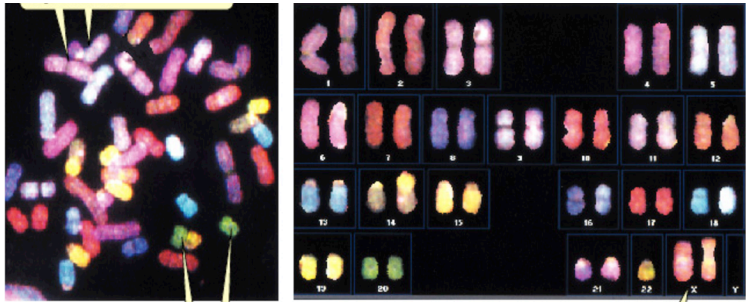
ANALISE GENETICA

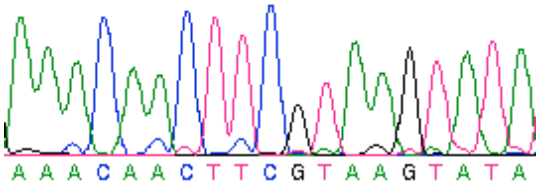
Biologia Molecular – Genética Medica

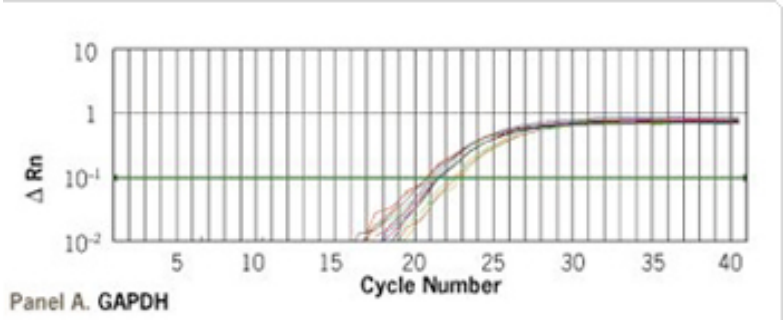
✓ *Analise do material genetico*

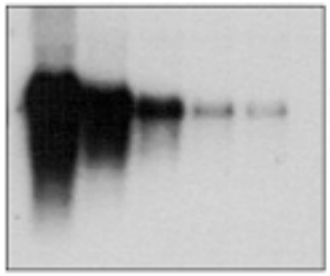
- Cromossomos/cariotipo
- DNA
- RNA







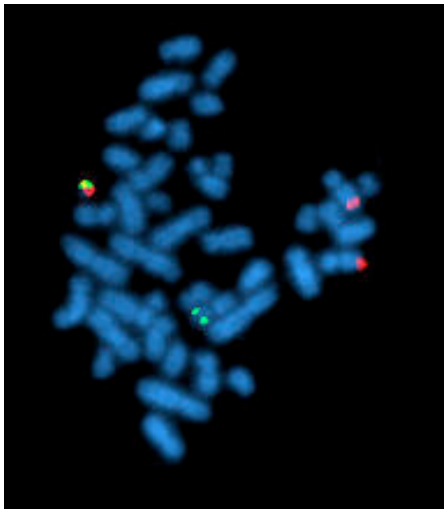




ANALISE GENETICA

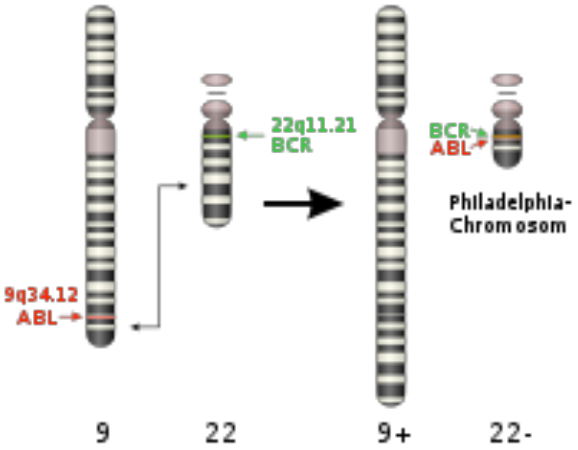
✓ *Analise do material genetico - cariotipo*

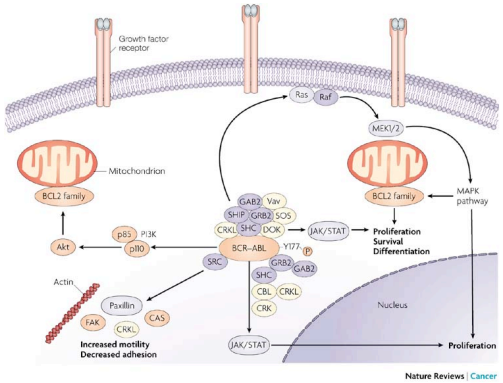
Leucemia mielóide crônica (ou LMC)



Translocação entre o cromossomo 9 e 22; t(9;22).
Cromossomo Filadélfia (Ph1).
Ocorre a fusão de dois genes nos cromossomos 9 e 22, chamados respectivamente de abl e bcr.

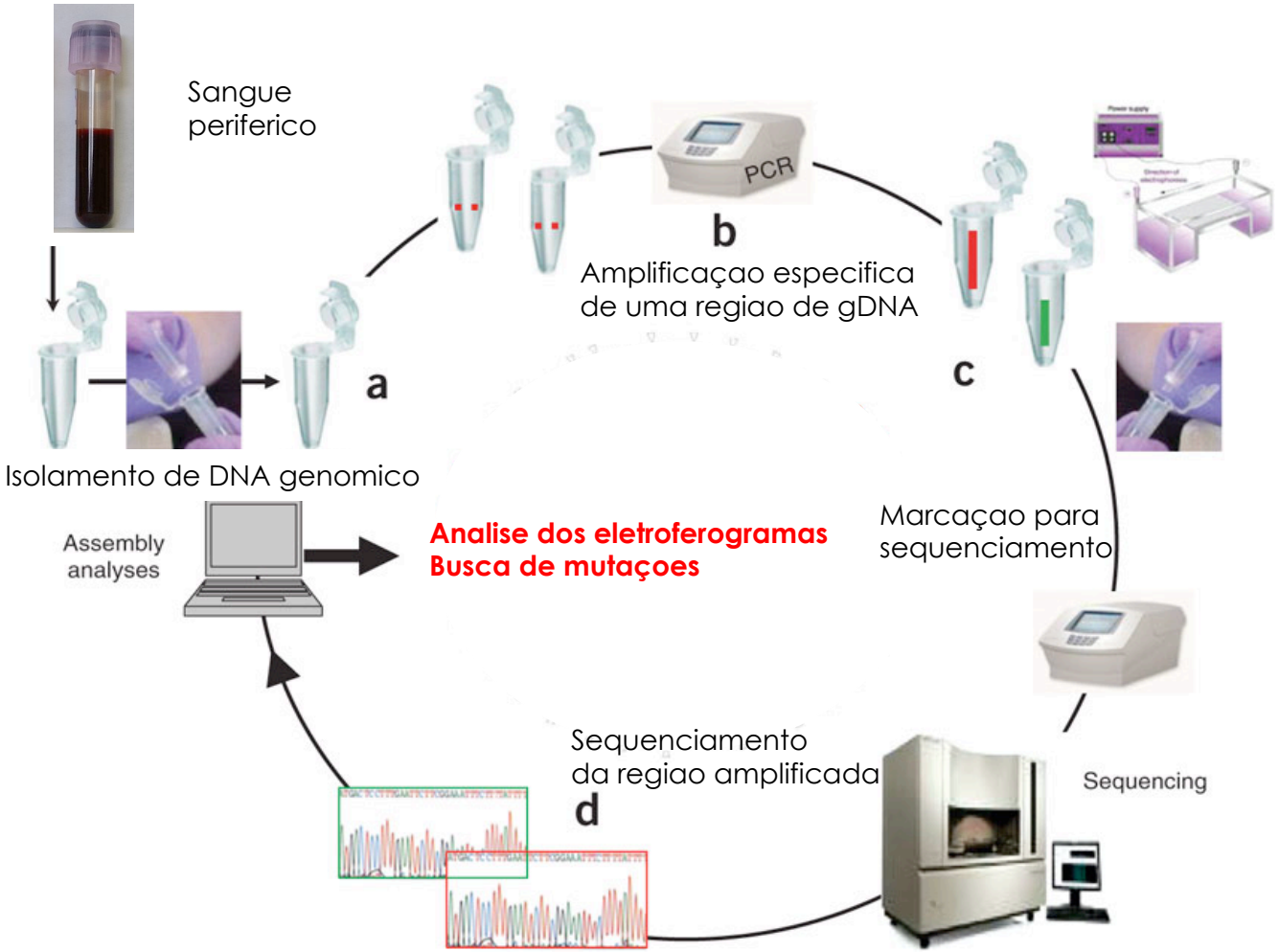
Proteina de fusao è uma quinase iper ativa que stimula a proliferaçao leucemica dos mieloblastos





ANALISE GENETICA

✓ *Analise do material genetico – sequencia do DNA*



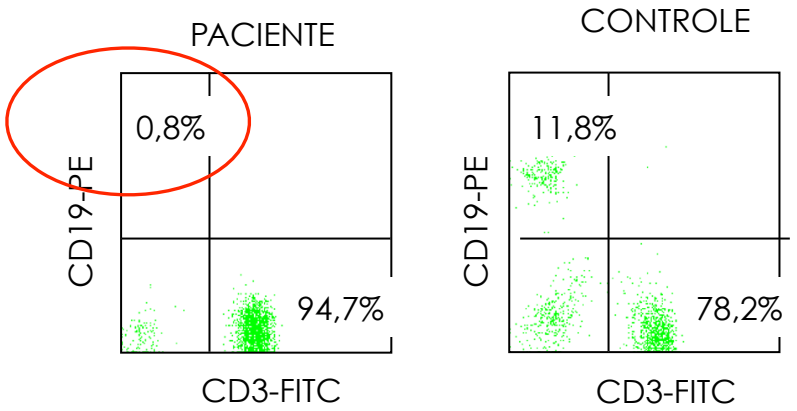
ANALISE GENETICA

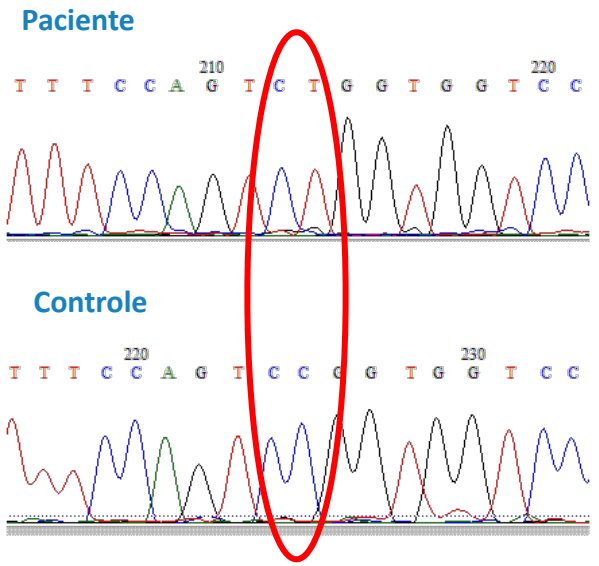
Agamaglobulinemia ligada ao X

Paciente 3: JV, 2 anos, masculino

Ausência de linfócitos B e agamaglobulinemia

Leucócitos totais	
Linfócitos	72.0
Linfócitos T	94.7
Linfócitos T CD4	67.4
Linfócitos T CD8	25.1
Linfócitos B CD19	**0.8**
Linfócitos T CD56	1.3





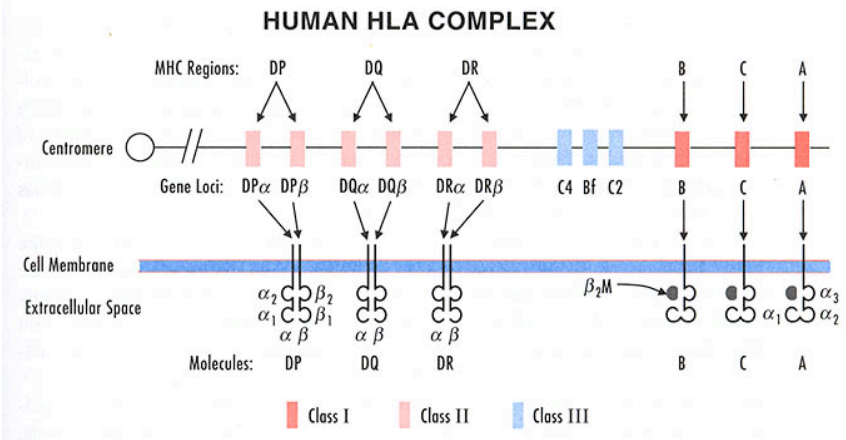
Btk

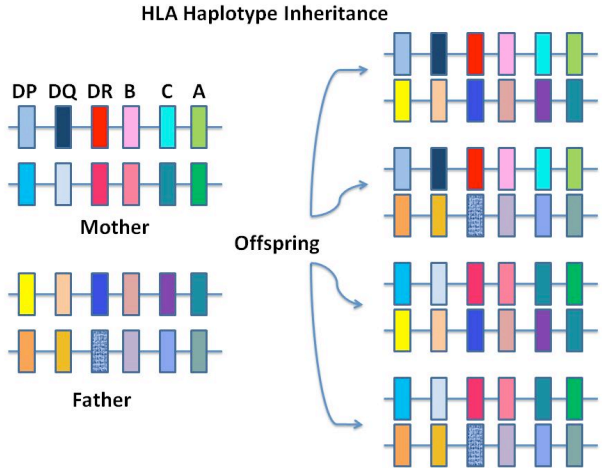
cromossomo X

Xq21.3 – Xq22

ANALISE GENETICA

✓ *Analise do material genetico – analise de HLA*





Predisposiçao para autoimunidade

Disease	Associated HLA allele	Relative risk*
Ankylosing spondylitis	*B27*	90
Goodpasture's syndrome	*DR2*	16
Gluten-sensitive enteropathy	*DR3*	12
Hereditary hemochromatosis	*A3*	9.3
	B14	2.3
	A3/B14	90
Insulin-dependent diabetes mellitus	*DR4/DR3*	20
Multiple sclerosis	*DR2*	5
Myasthenia gravis	*DR3*	10
Narcolepsy	*DR2*	130
Reactive arthritis (*Yersinia, Salmonella, Gonococcus*)	*B27*	18
Reiter's syndrome	*B27*	37
Rheumatoid arthritis	*DR4*	10
Sjogren's syndrome	*Dw3*	6
Systemic lupus erythematosus	*DR3*	5

Transplante

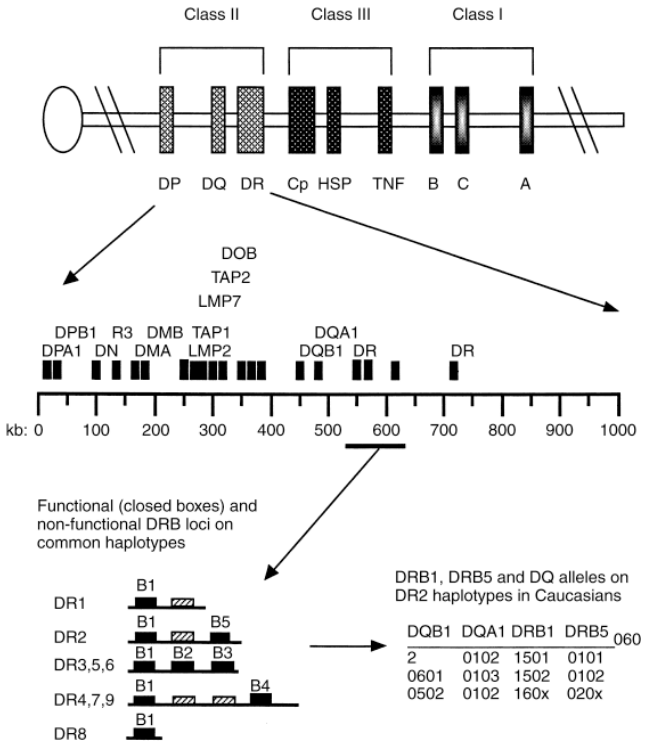
Tipo di trapianto	Necessità di immunosoppressione	Incidenza di GvHD
autologo	no	no
allogenico da gemello monoovulare	no	no
allogenico da fratello HLA identico	+	+
allogenico da HLA identico non correlato	++	++
allogenico da HLA parzialmente compatibile	+++	+++
da sangue cordonale	+	±

ANALISE GENETICA

✓ *Analise do material genetico – SSP-PCR para analise de HLA*

SSP-PCR (Sequence Specific Primers):

- Amplificato comune
- Amplificato specifico (gruppo specifico/ allele specifico)



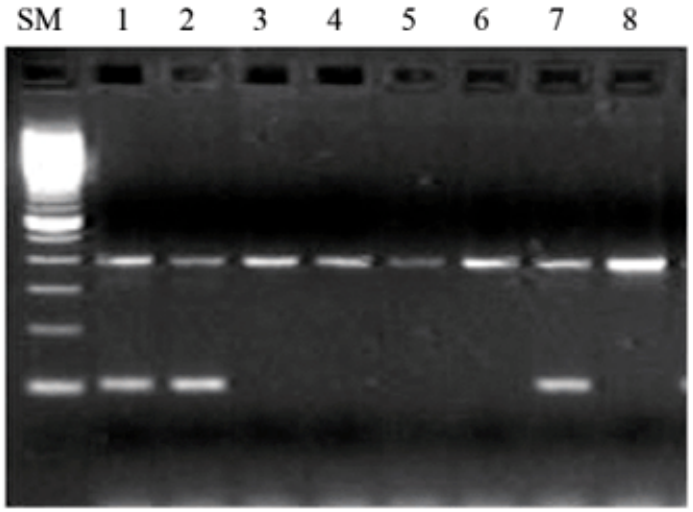
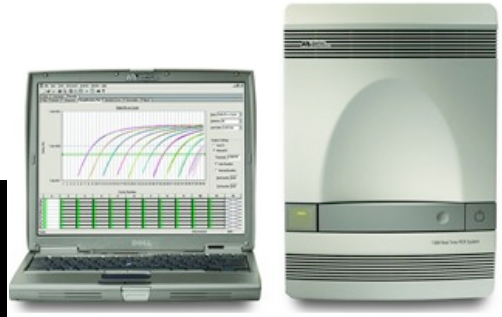


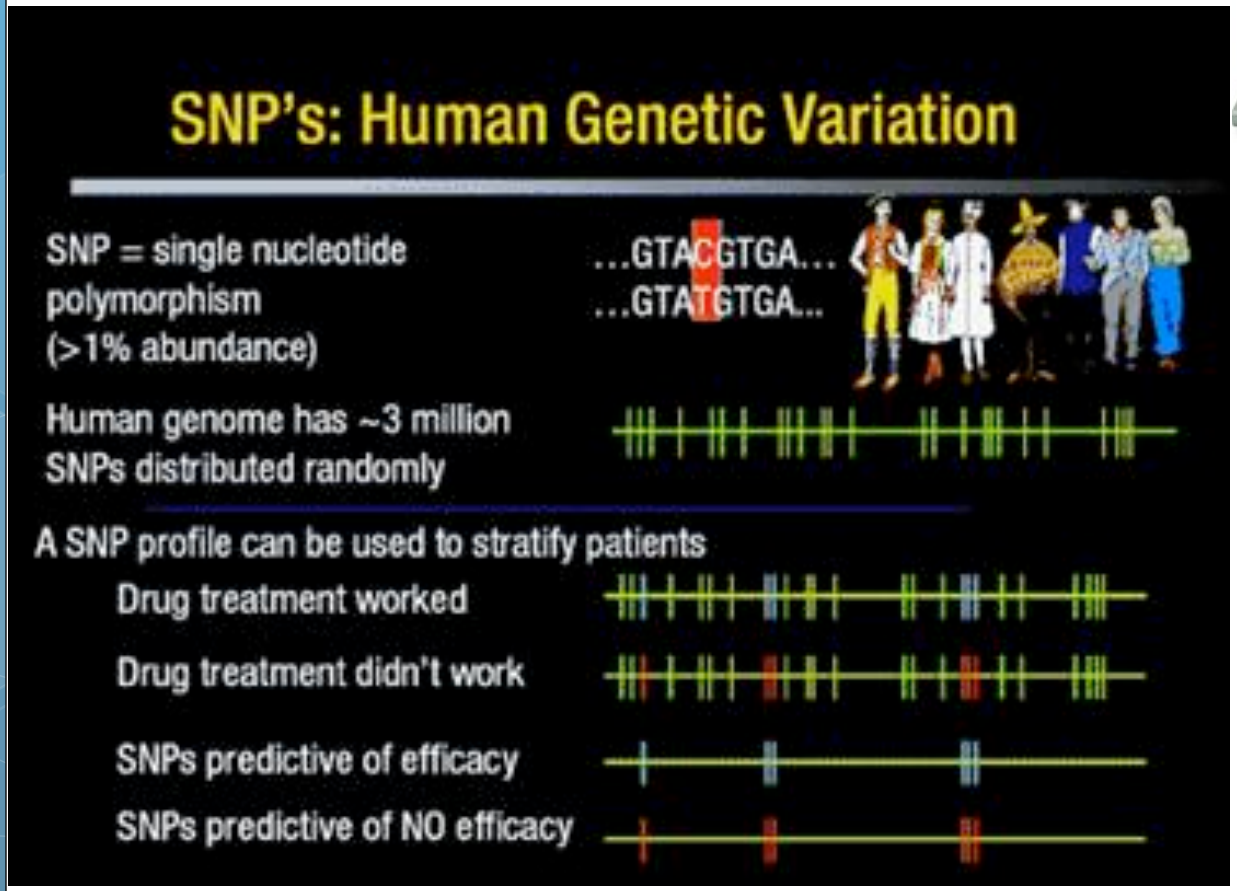
Figure 3 - Representative result of HLA-A*01 genotyping. Lane 1, 2 and 7 show a positive band, indicative of the HLA-A*01 allele.

HLA-A*01

ANALISE GENETICA

- ✓ *Analise do material genetico – analise de polimorfismos*

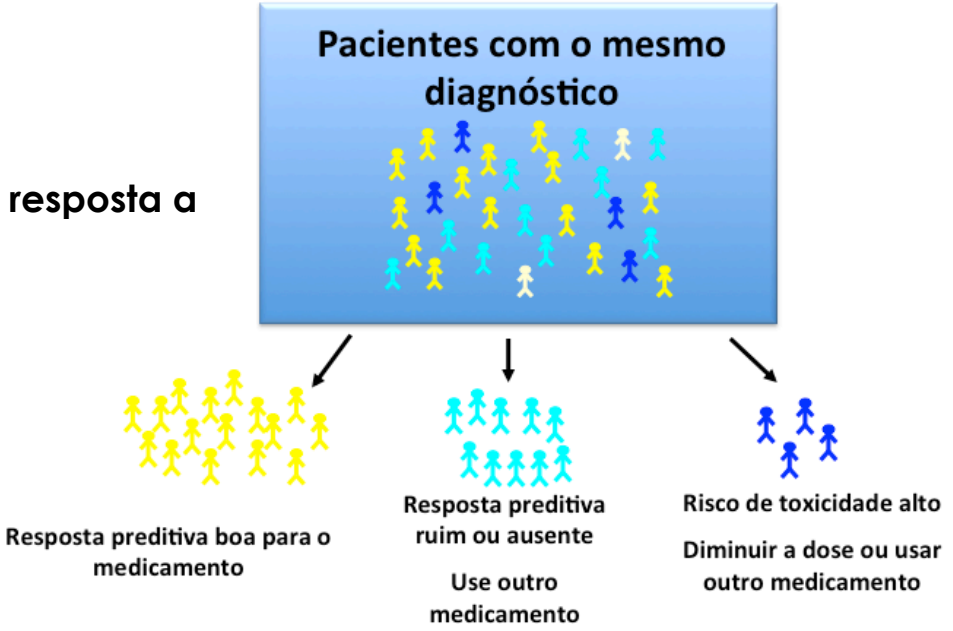




ANALISE GENETICA

- *Farmacogenetica*

Variabilidade individual na resposta a medicamentos



Disease	Drug Class	Rate of poor response
Asthma	Beta-agonists	40-75%
Hypertension	Various	30%
Solid Cancers	Various	70%
Depression	SSRIs, tricyclics	20-40%
Diabetes	Sulfonylureas, others	50%
Arthritis	NSAIDs, COX-2 inhibitors	30-60%
Schizophrenia	Various	25-75%

ANALISE GENETICA

- ✓ *Farmacogenetica*

  Thiopurine S-methyltransferase (TPMT)

  Terapia anti-cancer para leucemia linfoide acuta em crianças