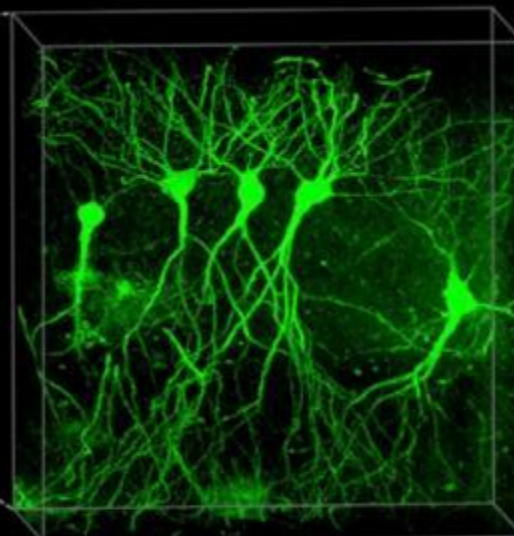
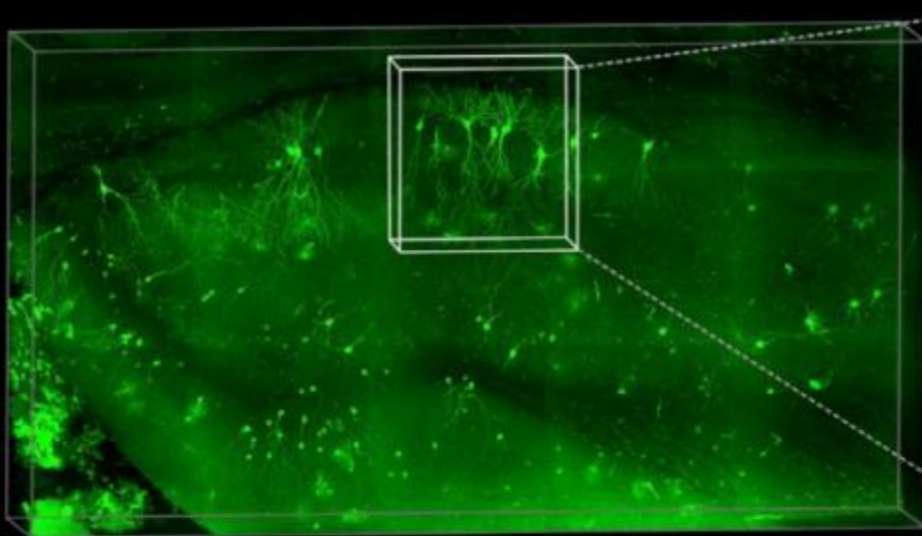


MÉTODOS PARA EVALUAR LA RESPUESTA INMUNE



**GUILLERMO
TERÁN
ANGEL**

**IDIC
ULA**

TEMARIO

- Métodos para evaluar la respuesta inmune humoral
- Métodos para evaluar la respuesta celular
- **PERO PRIMERO**
 - Ensayo inmunoenzimático (ELISA)
 - Inmunofluorescencia
 - Citometría de flujo
 - Métodos bioquímicos y moleculares: PAGE, WB, PCR, reacción en cadena de la polimerasa convencional y en tiempo real

INMUNOENSAYO



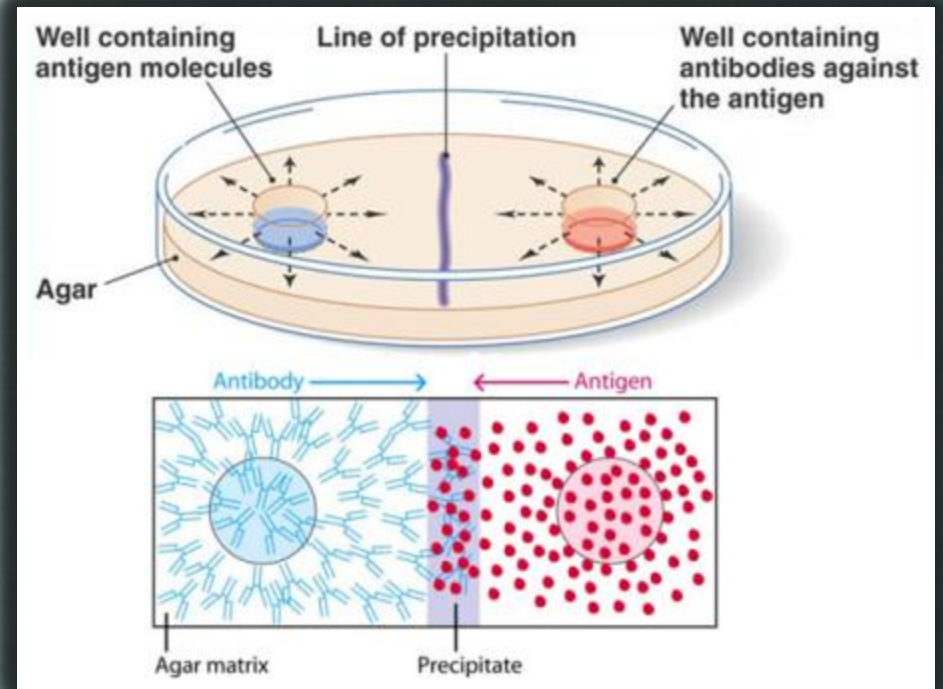
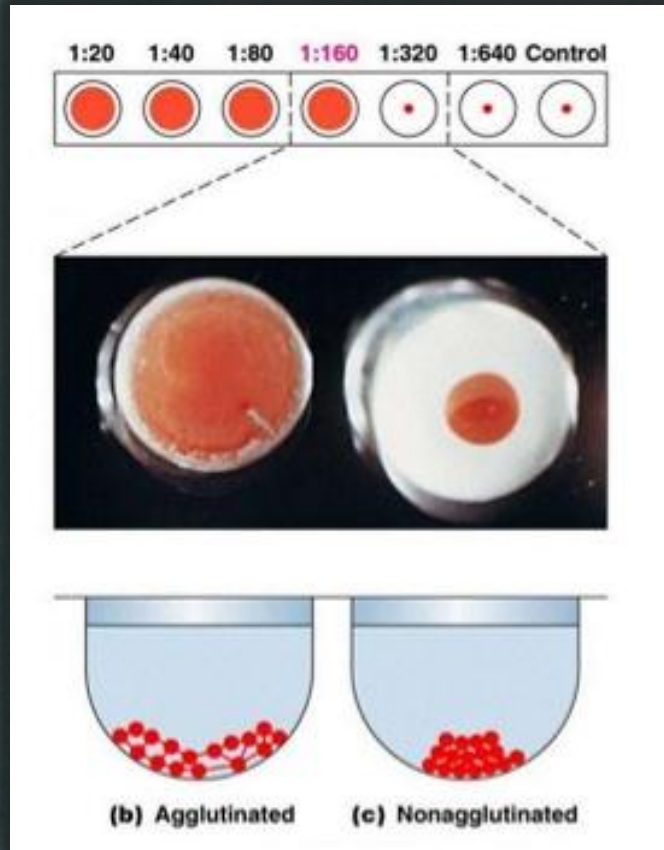
**Formación de
inmunocomplejos
(antígeno/anticuerpo)**

Marcados:
Conjugados a moléculas
que emiten señales
detectables

No marcados:
Son medidos por dispersión
de luz o por visualización
directa

- Radioinmunoensayo (RIA):
El marcador es un isótopo **radioactivo**.
 - Ensayos inmunoquimioluminiscente:
El marcador es una sustancia **quimioluminiscente**.
 - **Análisis inmunoenzimáticos** (EIA):
El marcador es una **enzima**.
 - Fluoroimmunoanálisis:
El marcador es una partícula **fluorescente**.
- Precipitación
 - Aglutinación

RAPIDITO: LOS NO MARCADOS

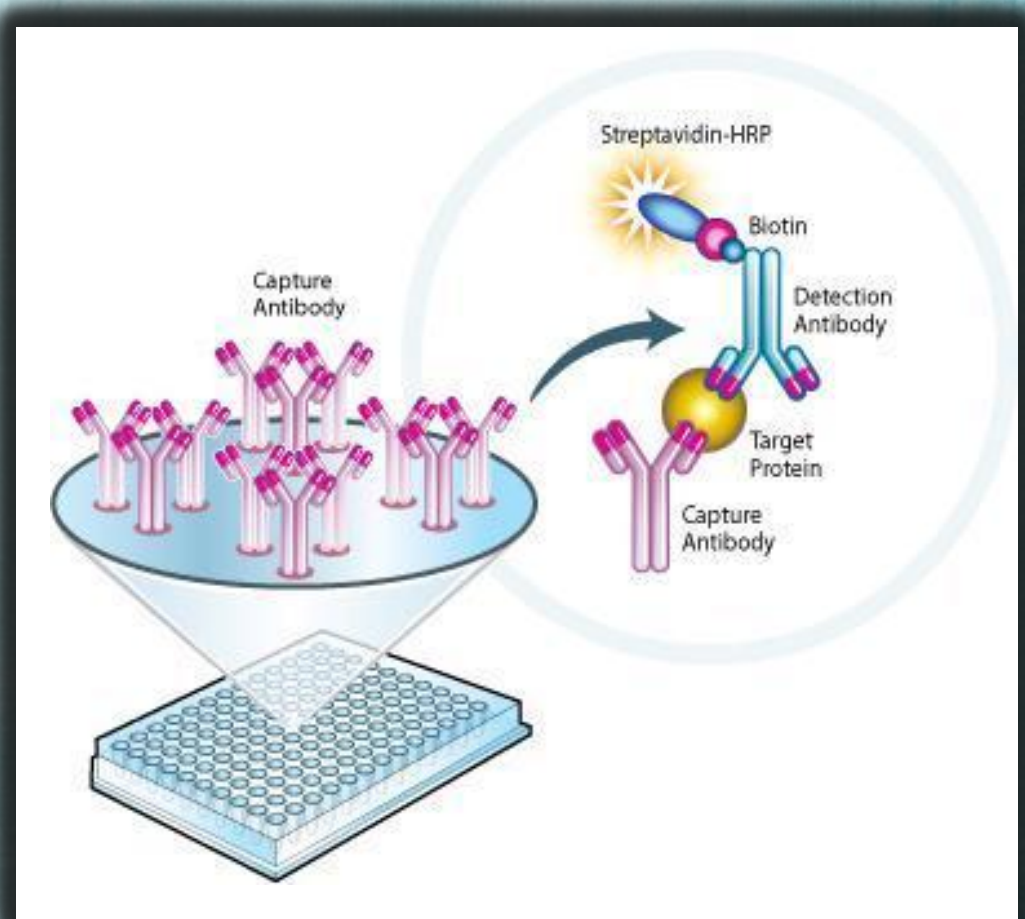


ANÁLISIS INMUNOENZIMÁTICO

Ensayo de Inmunoadsorción Ligado a Enzima

Determina presencia de anticuerpos o antígenos en la muestra de un paciente

El basamento de la técnica radica en la formación de inmunocomplejos que se encuentren fijos o adsorbidos a una superficie, y una reacción enzimática como sistema de revelado



PASOS GENERALES DEL ELISA

Sensibilización



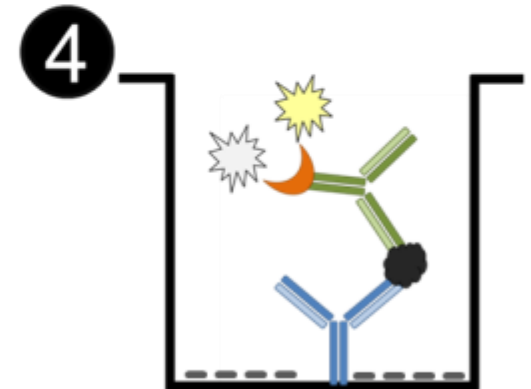
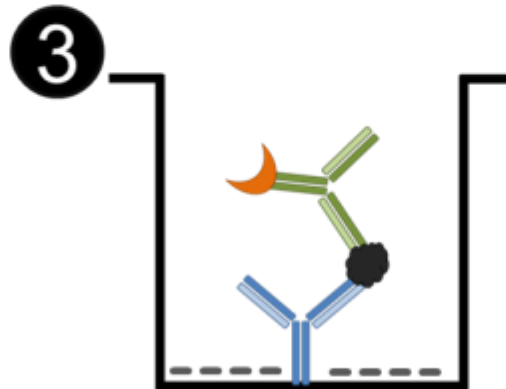
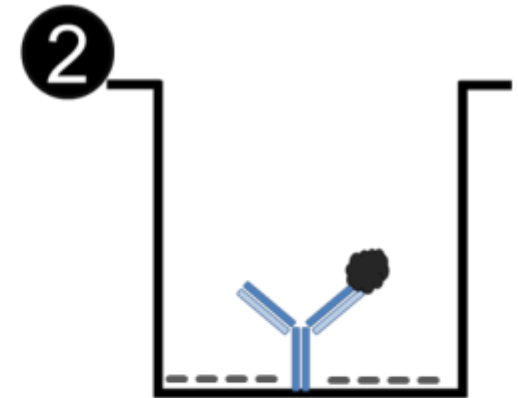
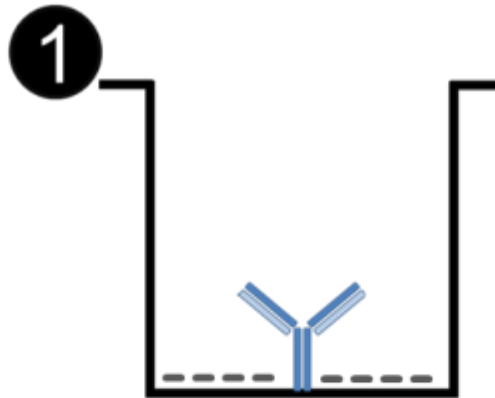
Formación de Inmunocomplejos



Reacción enzimática



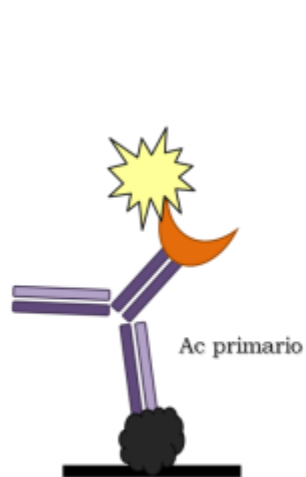
Lectura



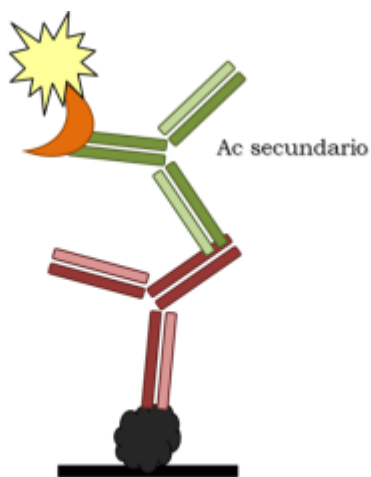
 Bloqueos

 Lavados

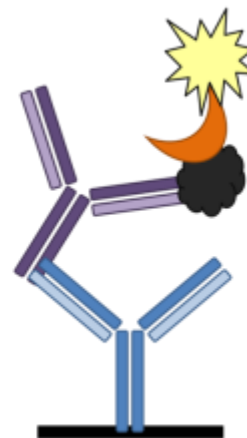
TIPOS O VARIANTES DEL ELISA



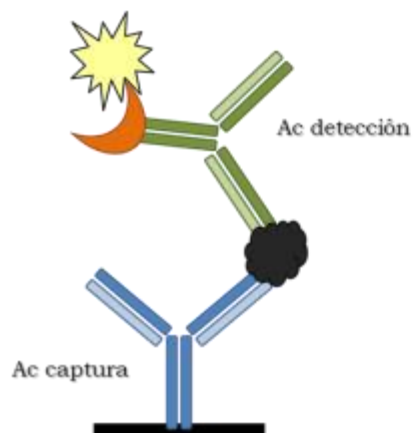
Directo



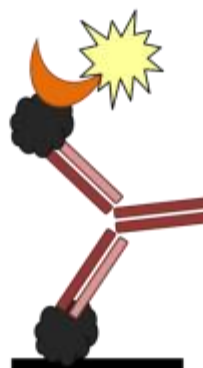
Indirecto



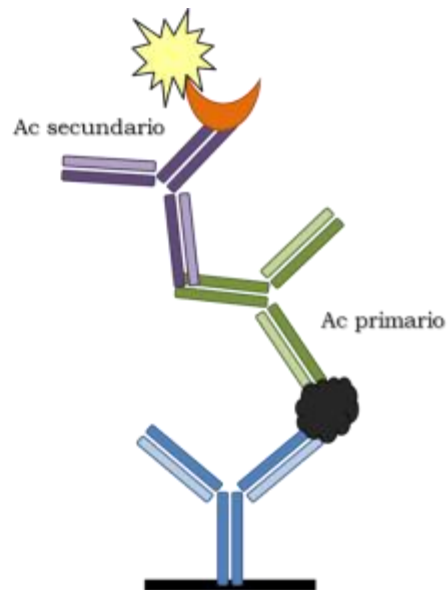
?????



de Ab



de Ag



Indirecto

ELISA DE PLASMONES

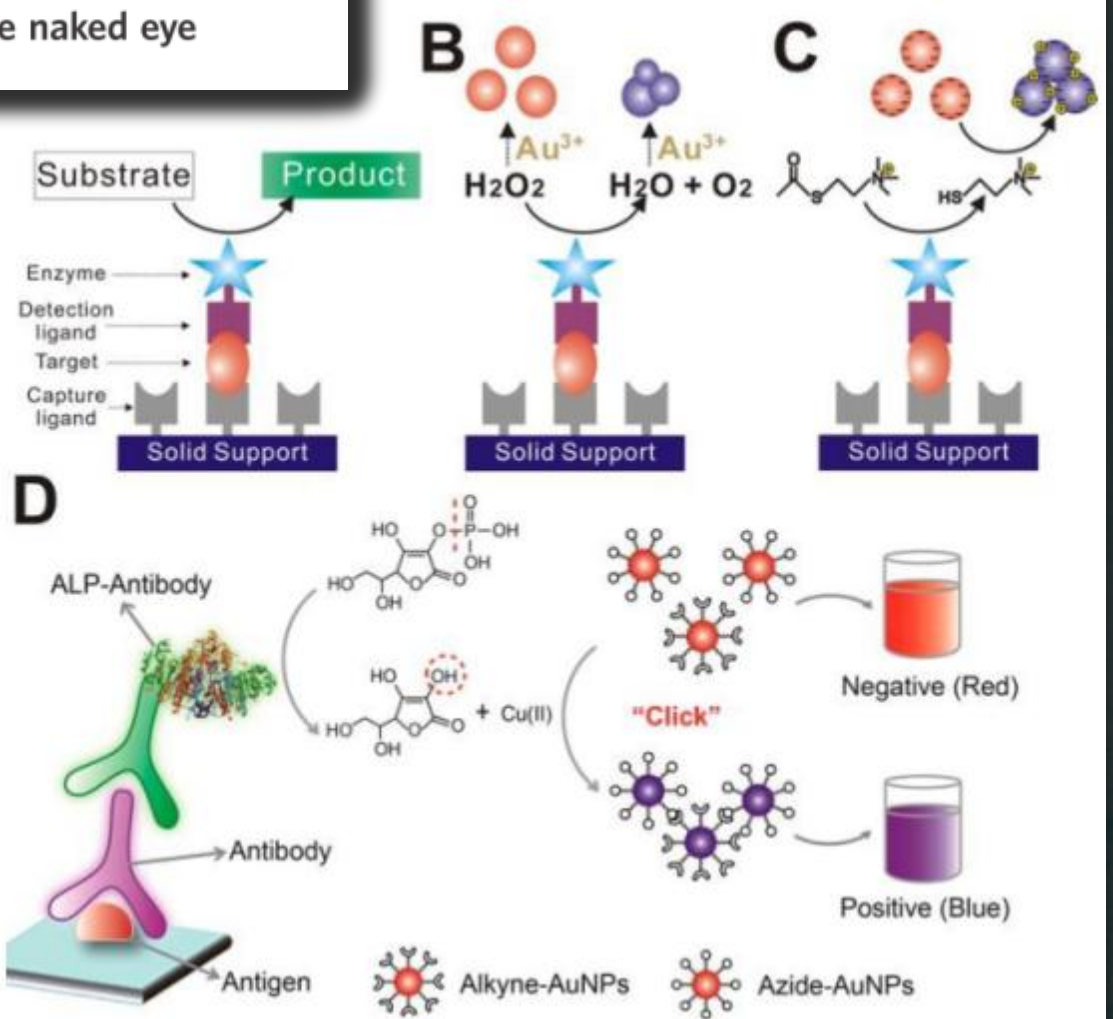
nature
nanotechnology

LETTERS

PUBLISHED ONLINE: 28 OCTOBER 2012 | DOI: 10.1038/NNANO.2012.186

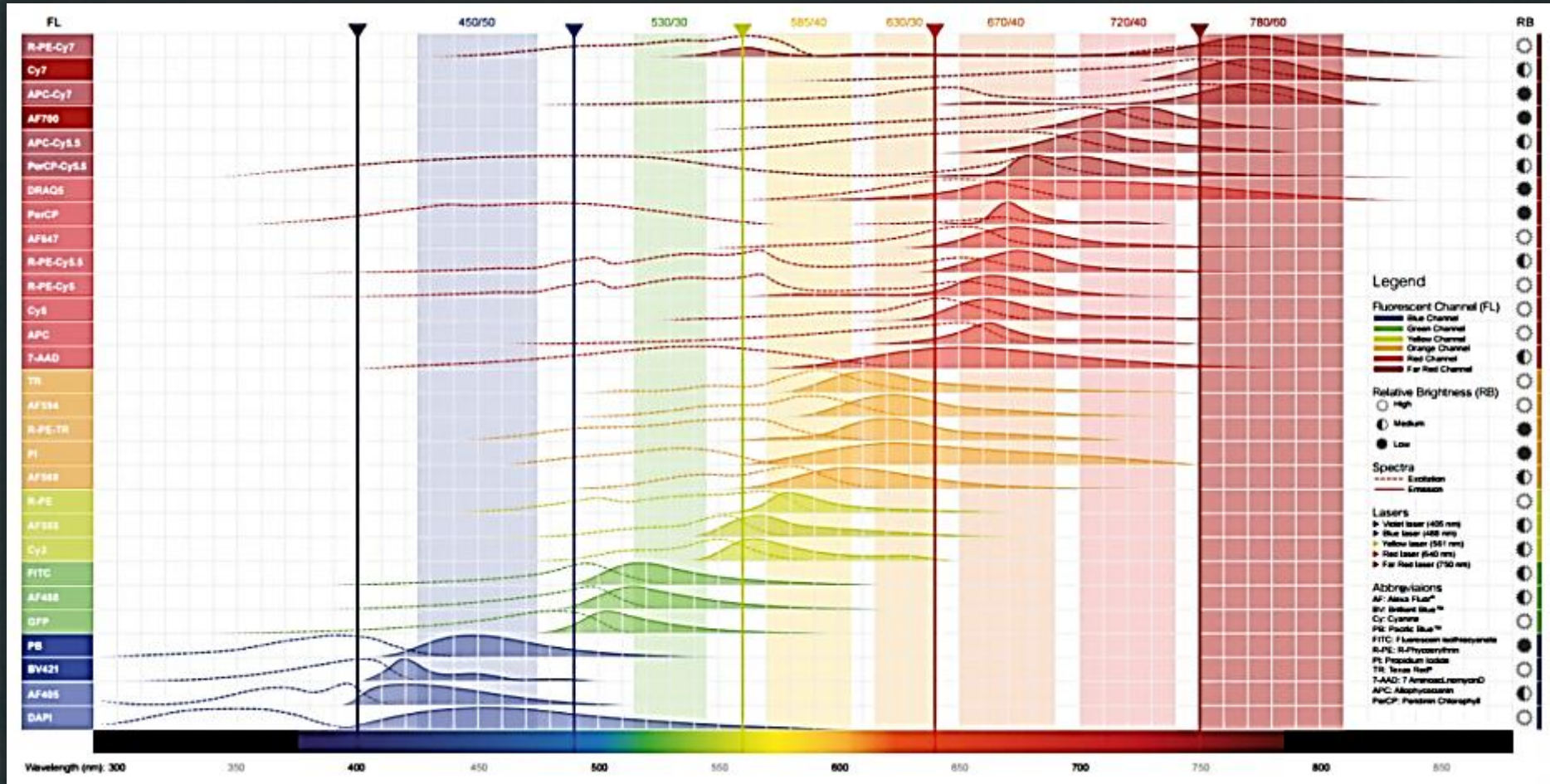
Plasmonic ELISA for the ultrasensitive detection of disease biomarkers with the naked eye

Roberto de la Rica* and Molly M. Stevens*



Analyst, 2016,141, 3473-3481

FLUOROCROMOS



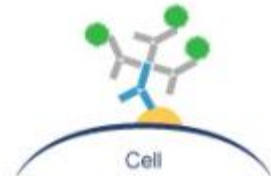
INMUNOFLUORESCENCIA



Direct

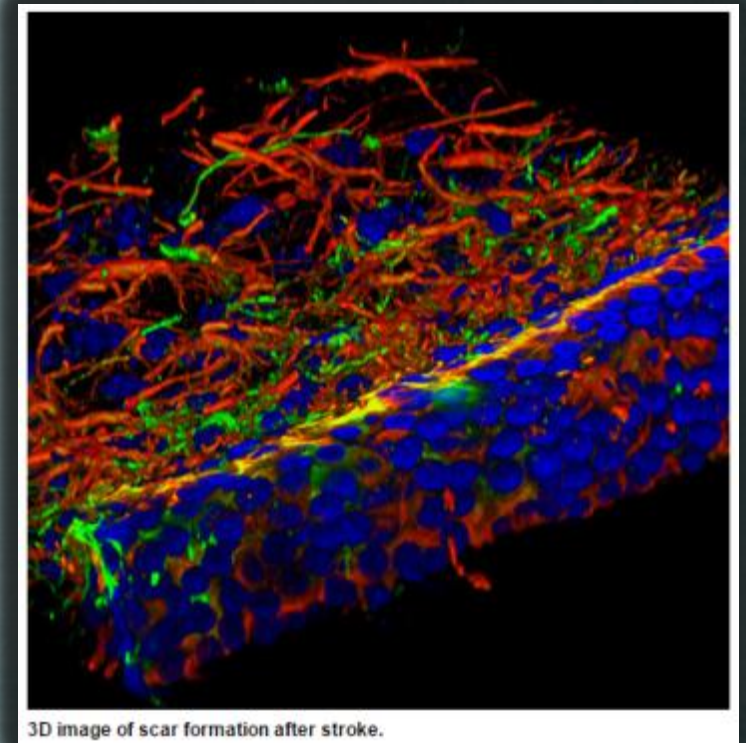
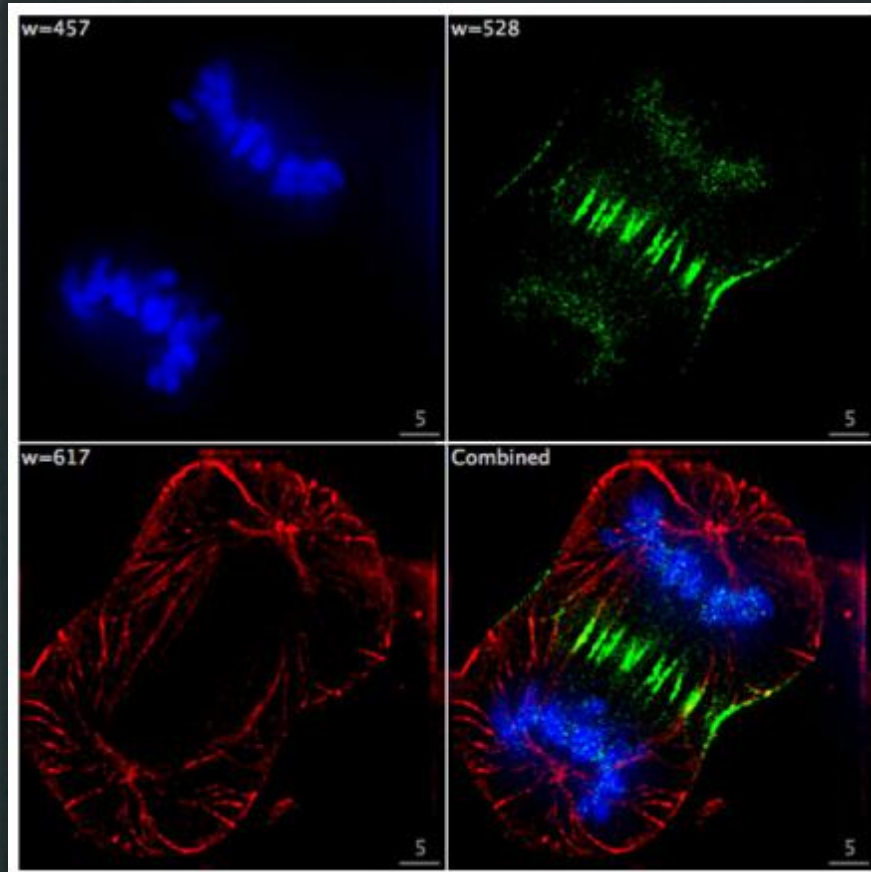


Indirect



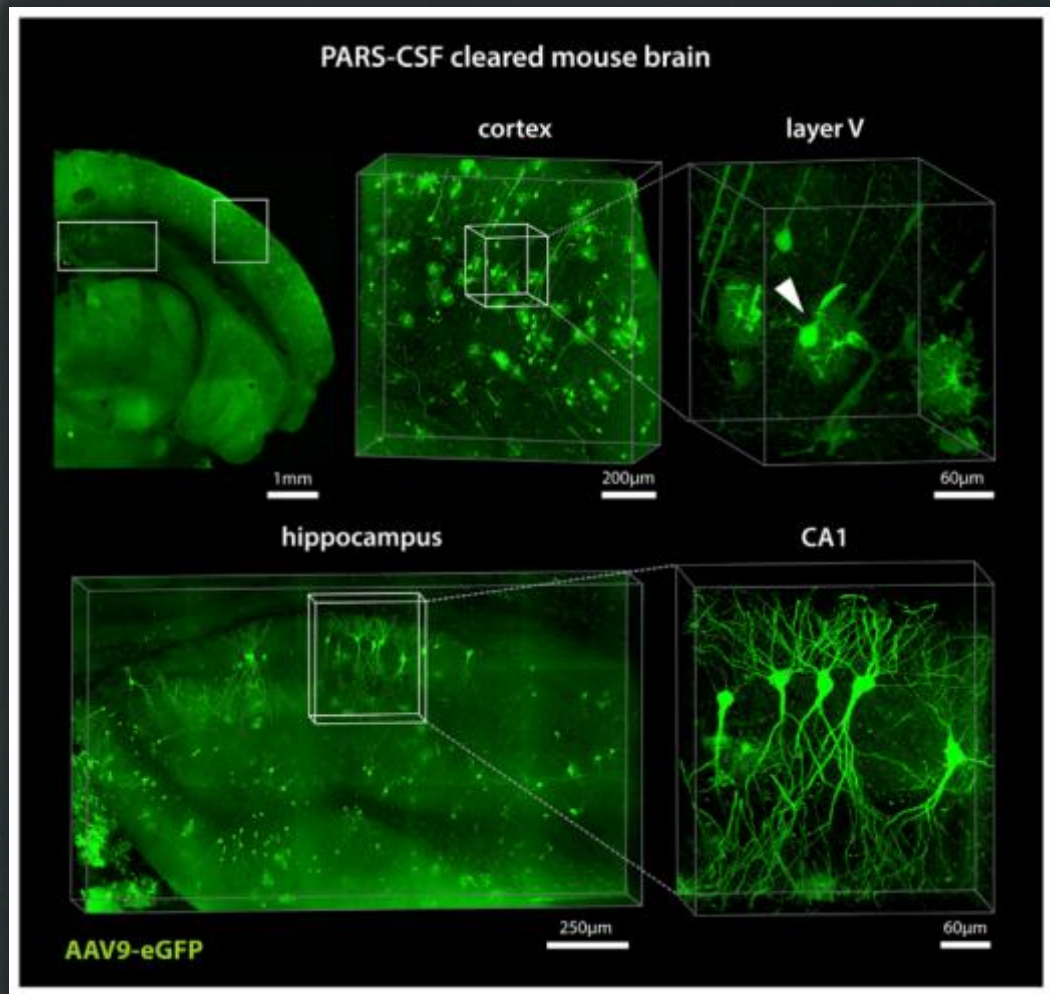
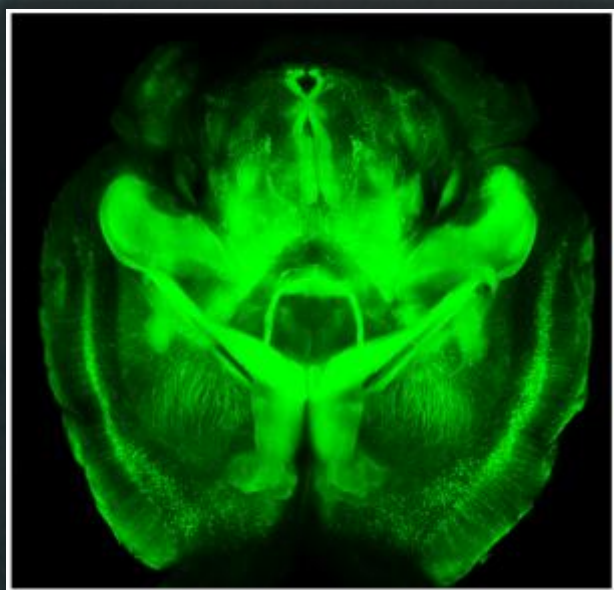
- Antigen
- Primary Antibody
- Secondary Antibody
- Fluorophore

MICROSCOPIA DE FLUORESCENCIA

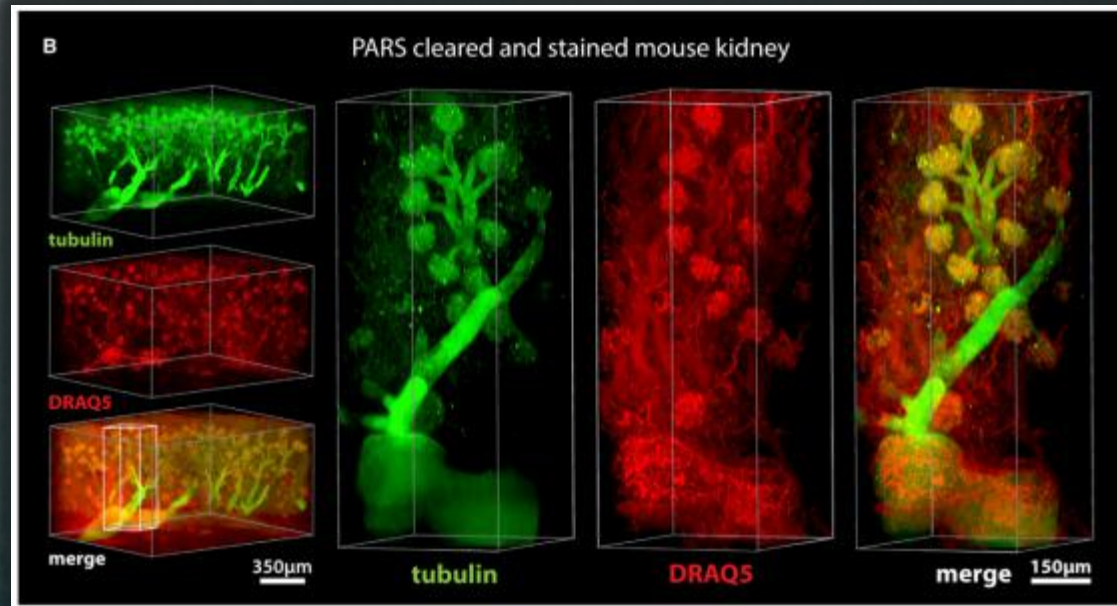
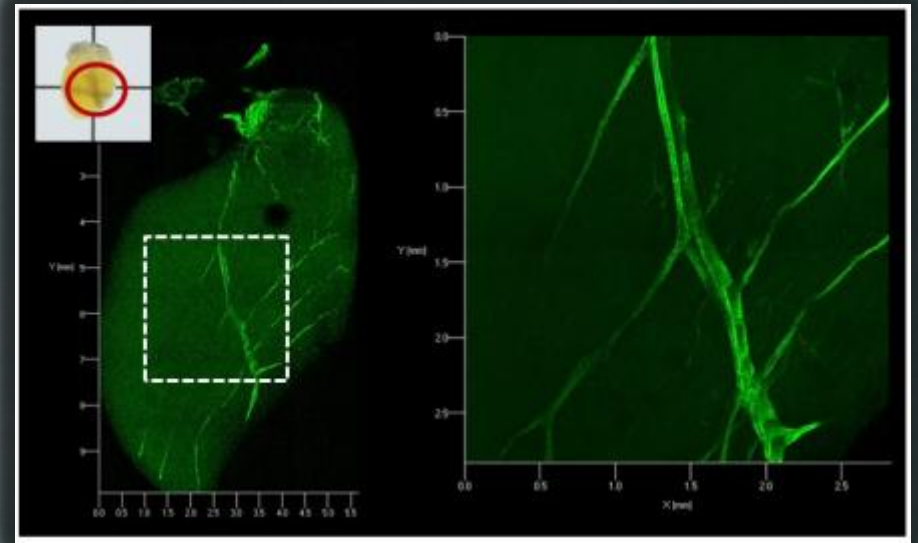
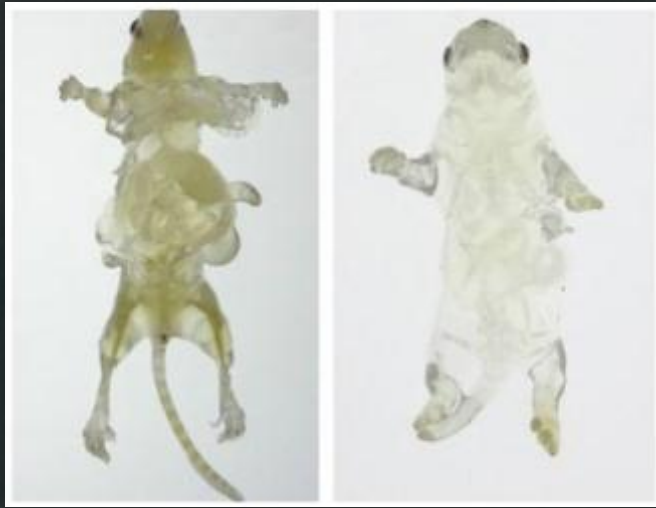


3D image of scar formation after stroke.

CLARITY

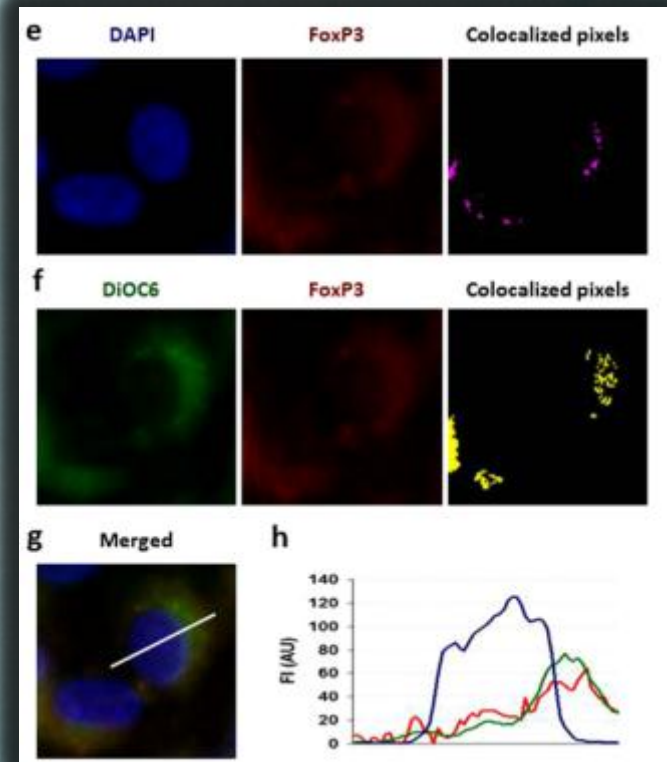
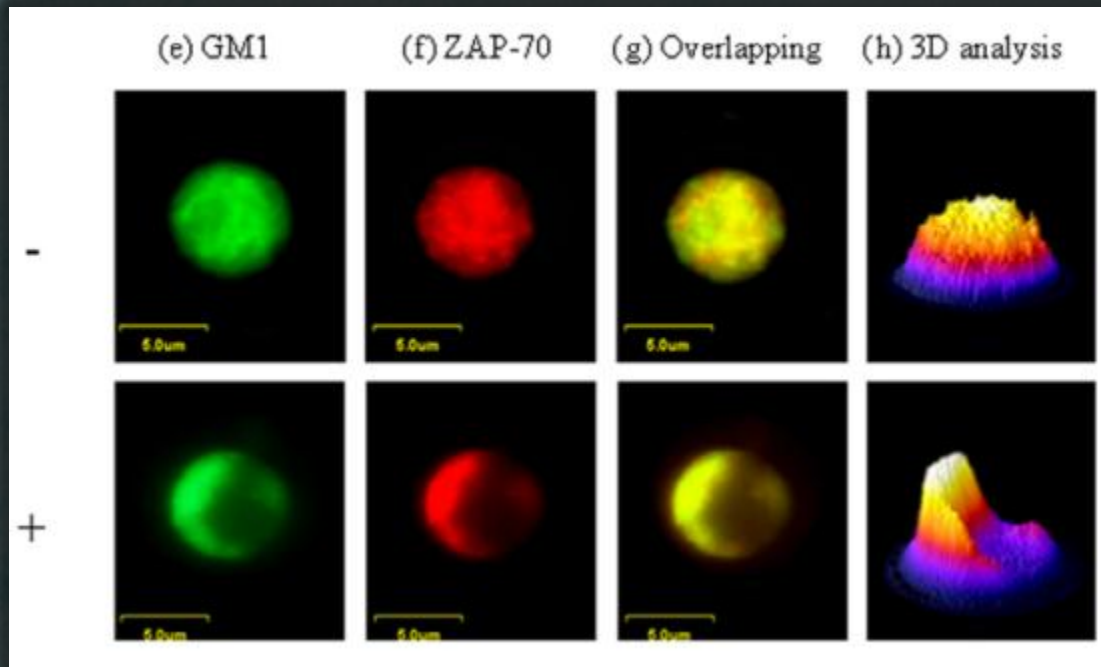


CLARITY

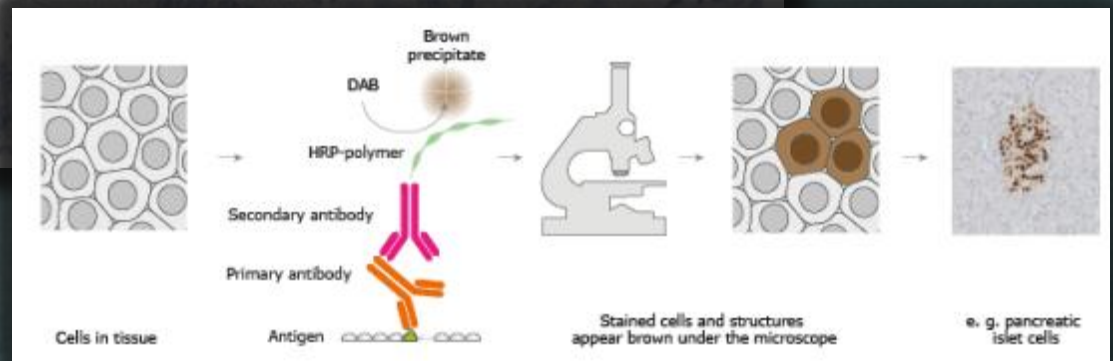
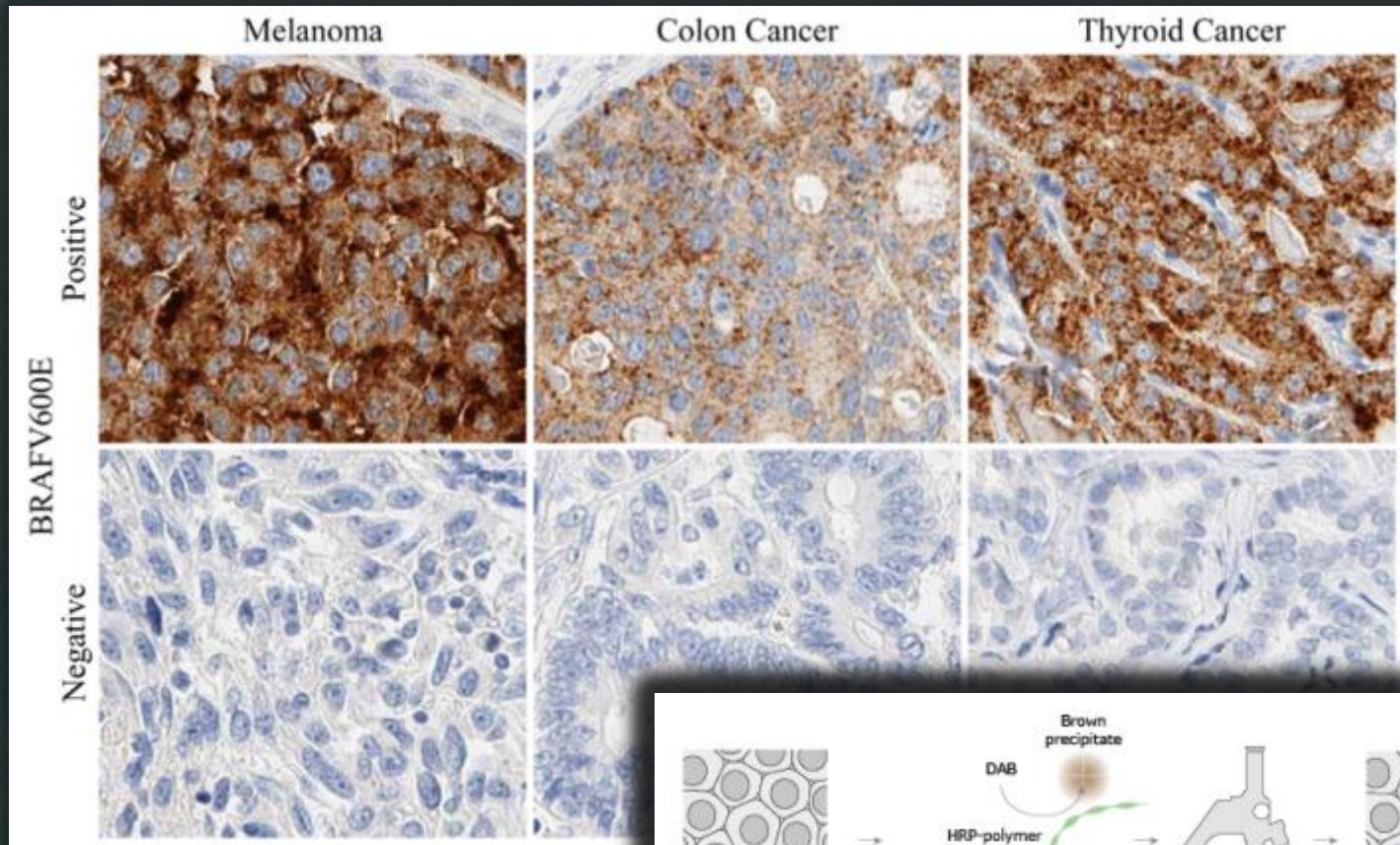


INMUNOFLUORESCENCIA

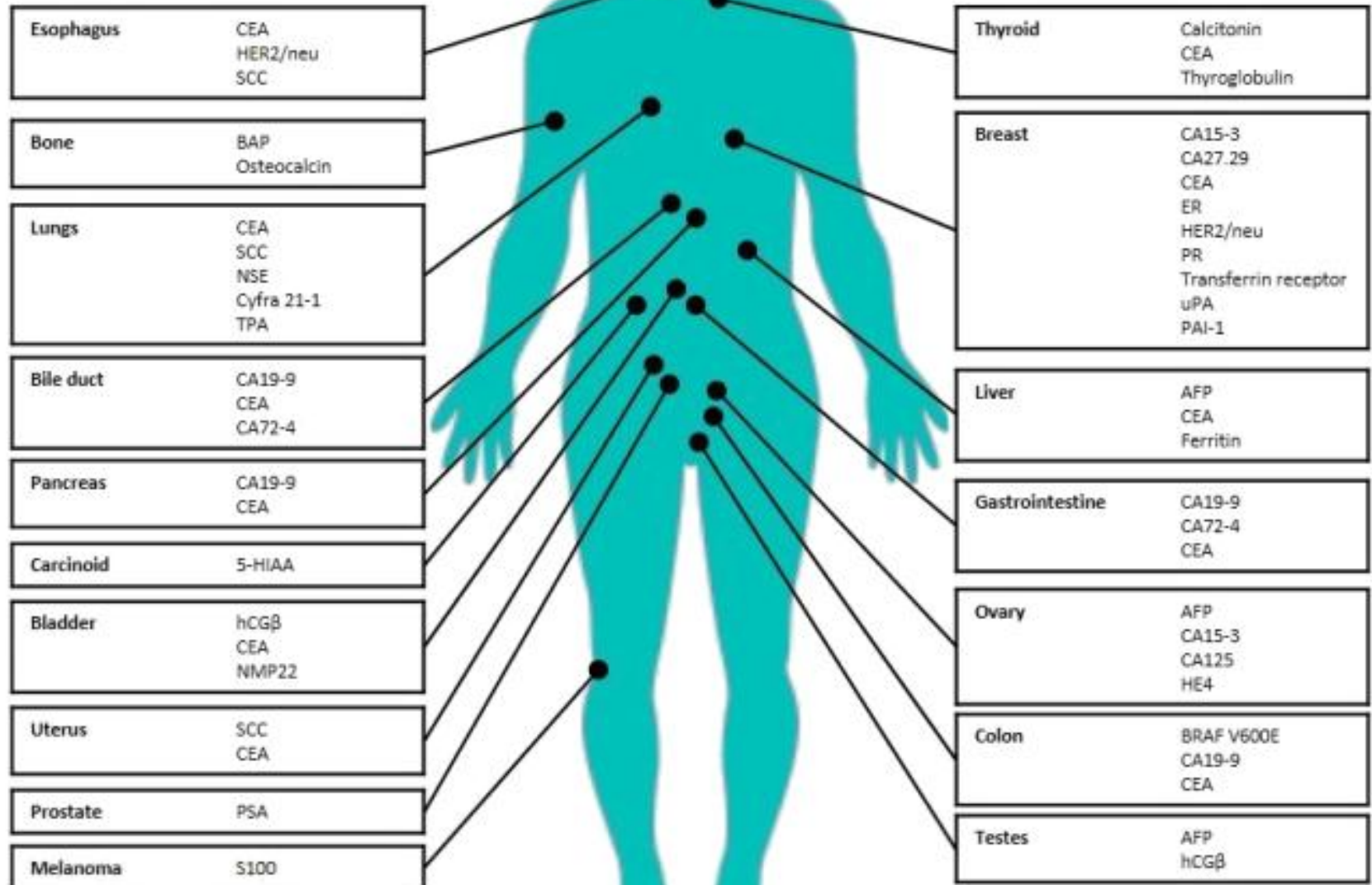
Aprovechando software ante las carencias



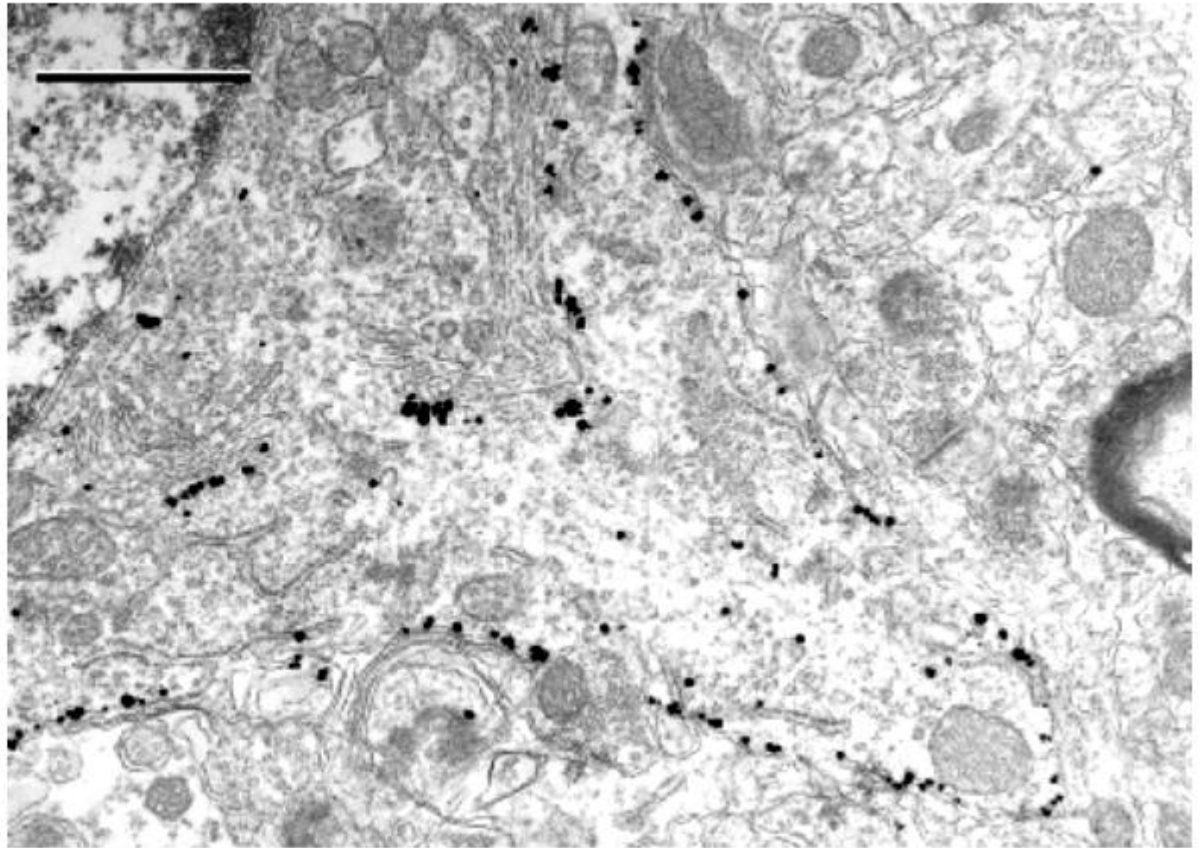
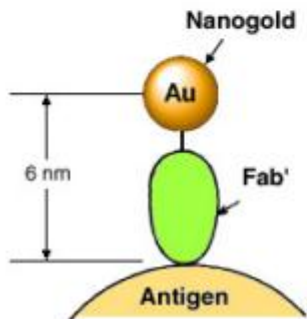
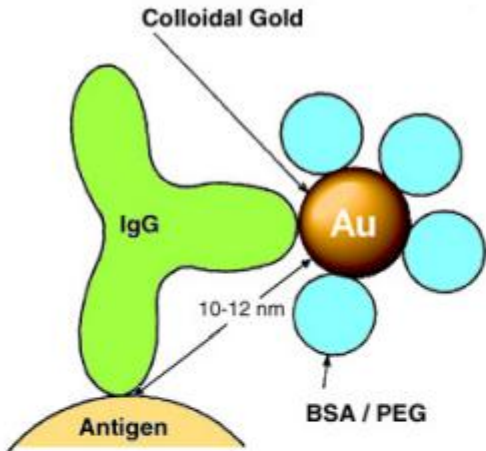
UN PARÉNTESIS: INMUNOHISTOQUÍMICA



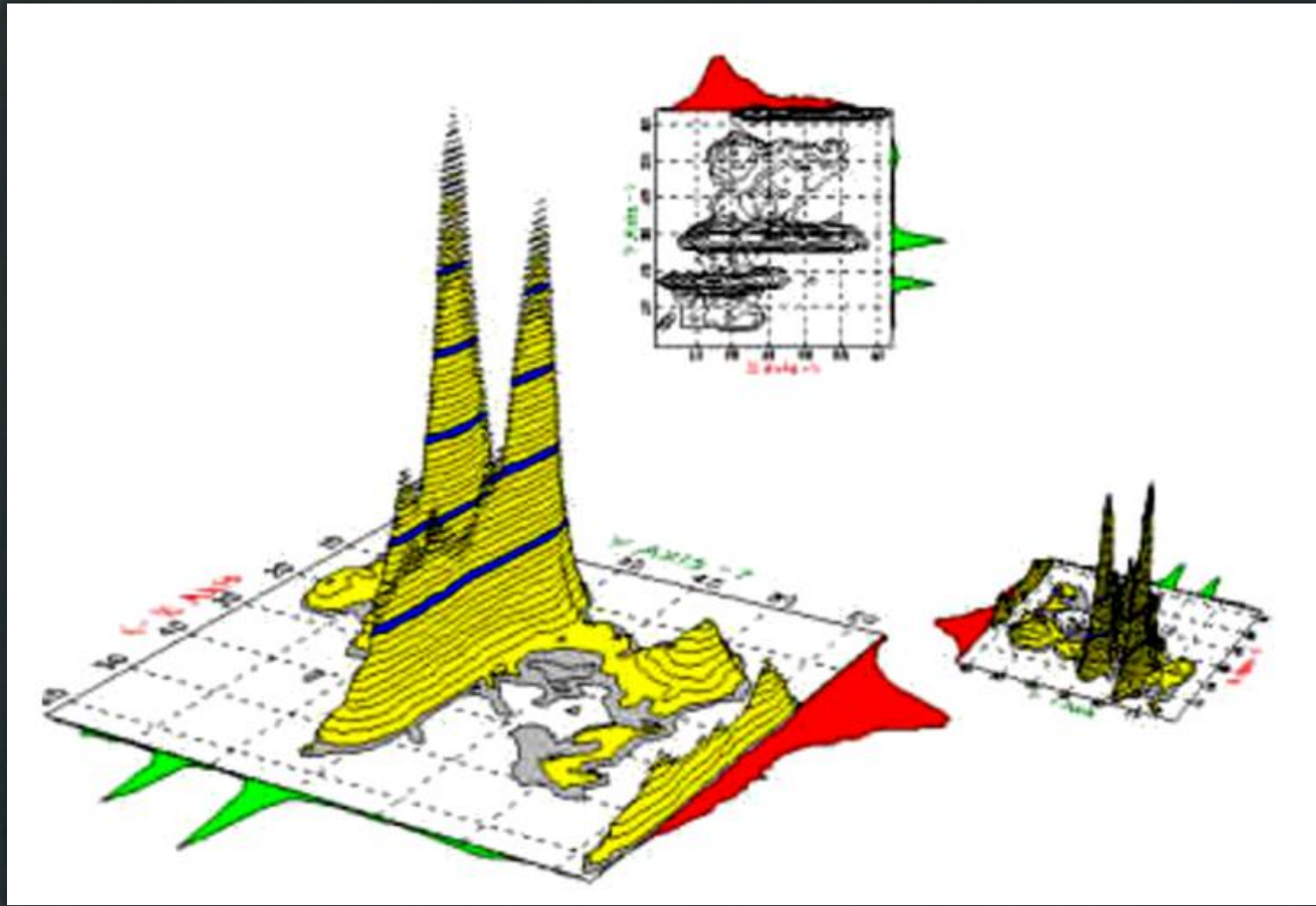
MARCADORES



OTRO PARÉNTESIS: TEM



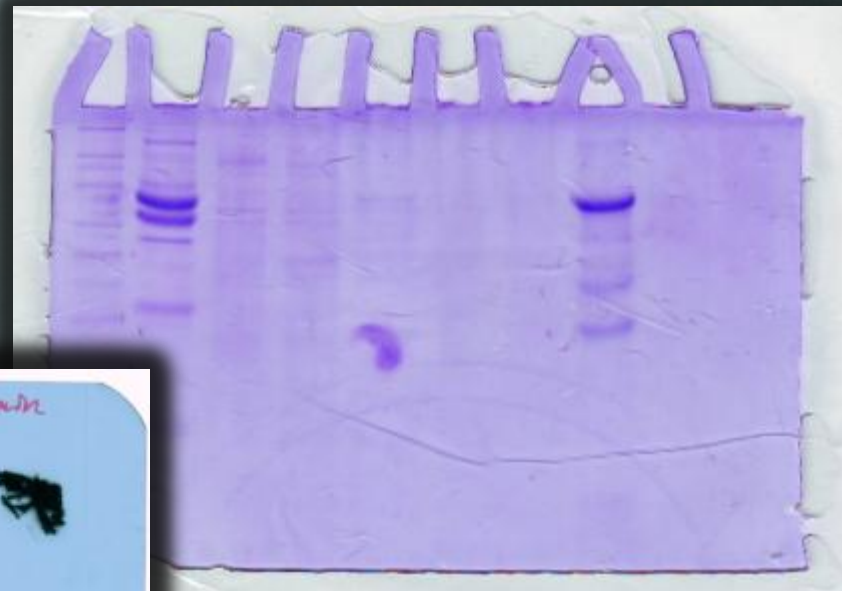
Y LA CITOMETRÍA DE FLUJO



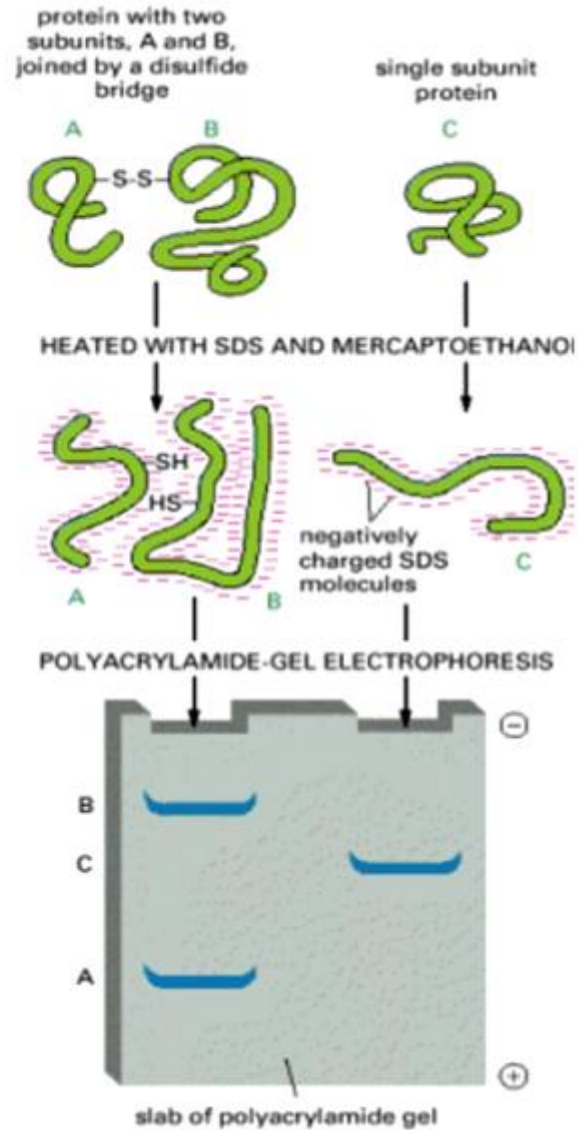
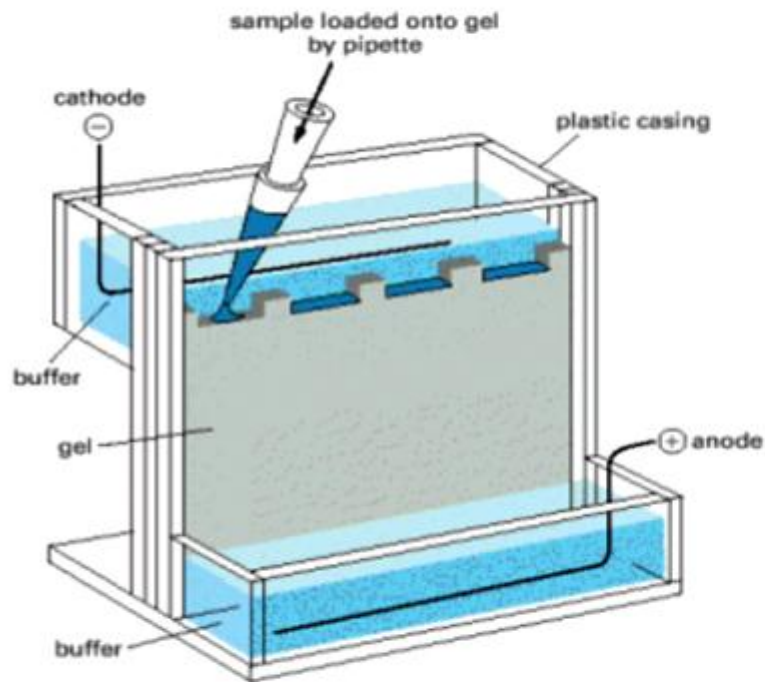
CITOMETRÍA DE FLUJO

<https://www.youtube.com/watch?v=gEdZvuDrWo4>

TÉCNICAS BIOQUÍMICAS Y MOLECULARES

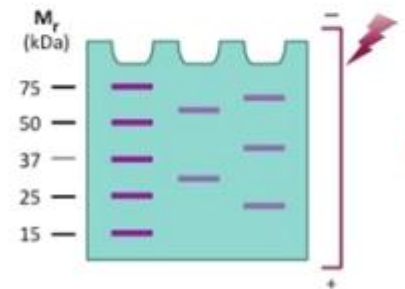
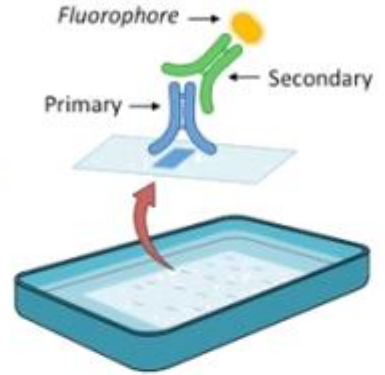
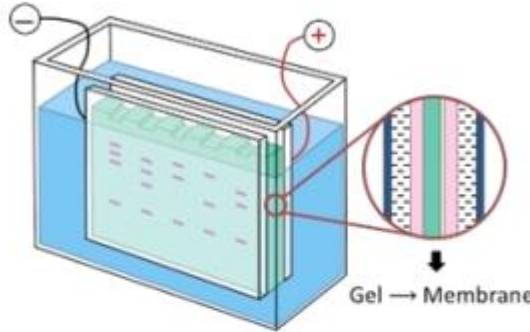
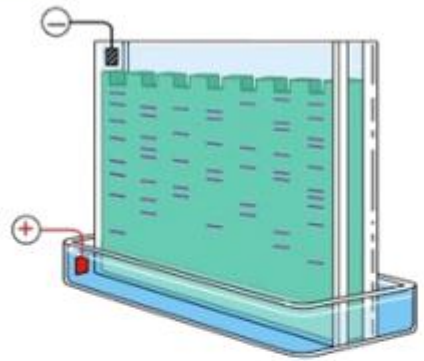


ELECTROFORESIS DE PROTEÍNAS

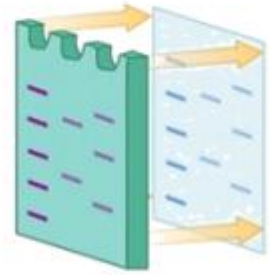


WESTERN BLOT

- 1 Gel Electrophoresis**
- 2 Gel Transfer**
- 3 Antibody Staining**



Electric charge separates proteins according to size

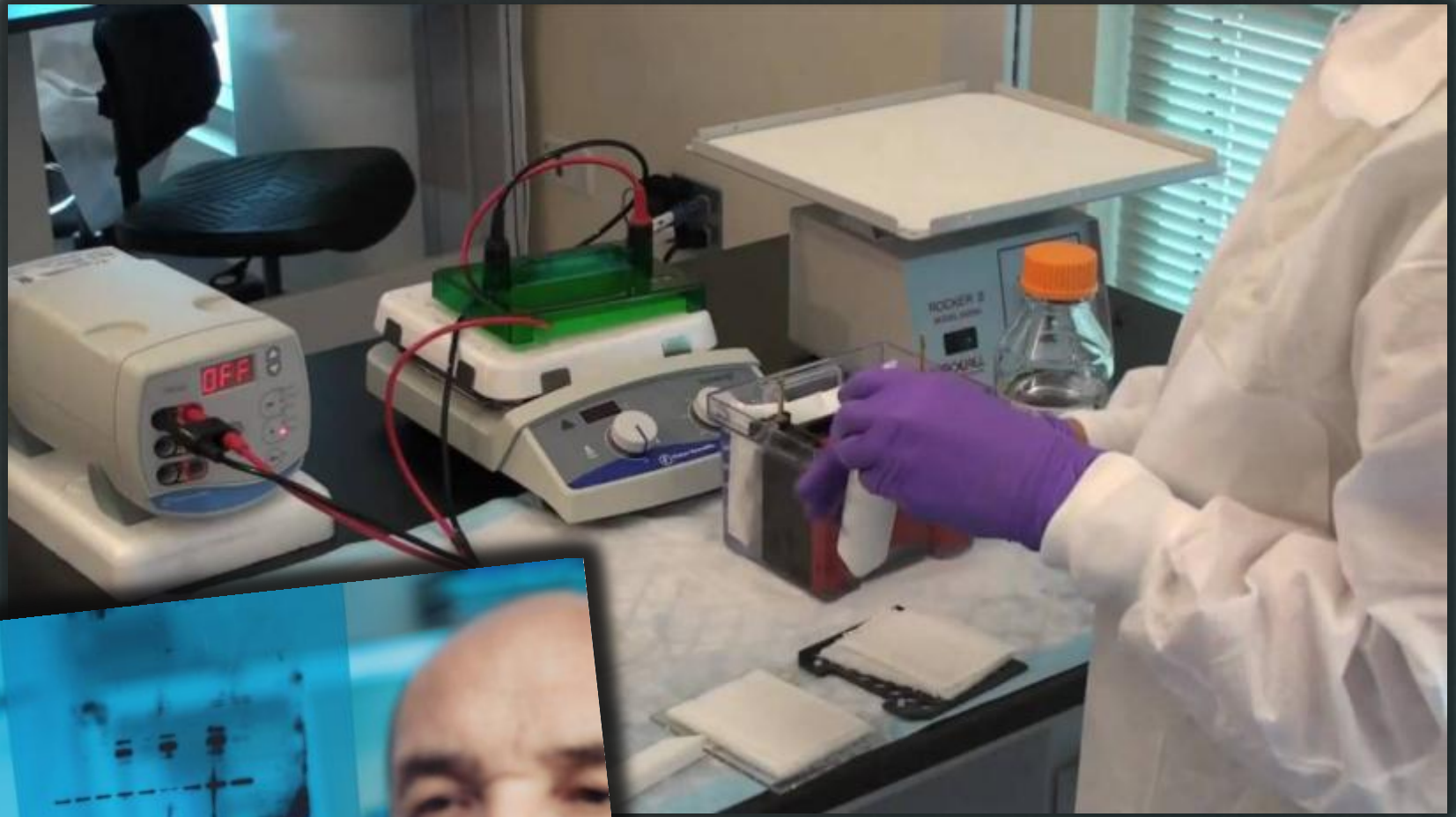


Proteins transferred to a solid support membrane

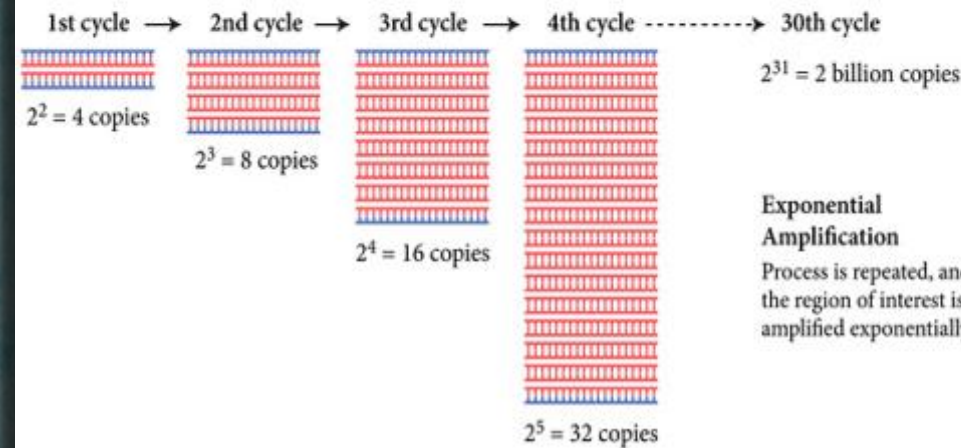
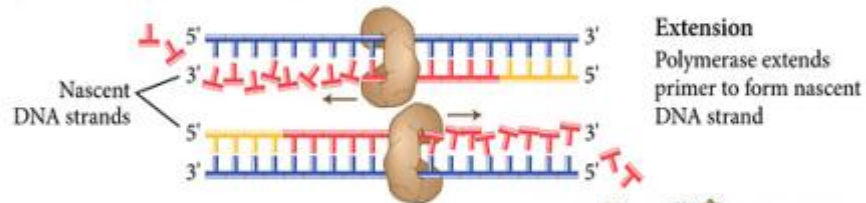
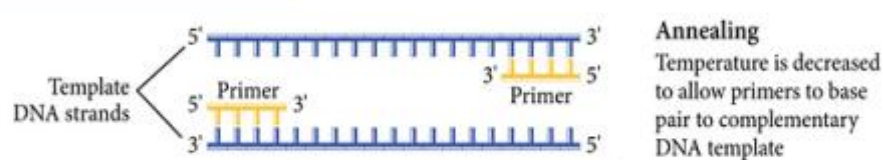
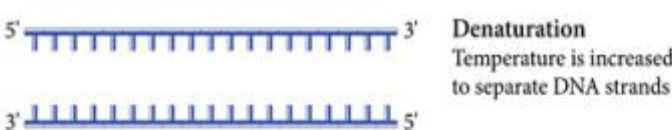


Proteins stained with specific antibodies for visualisation

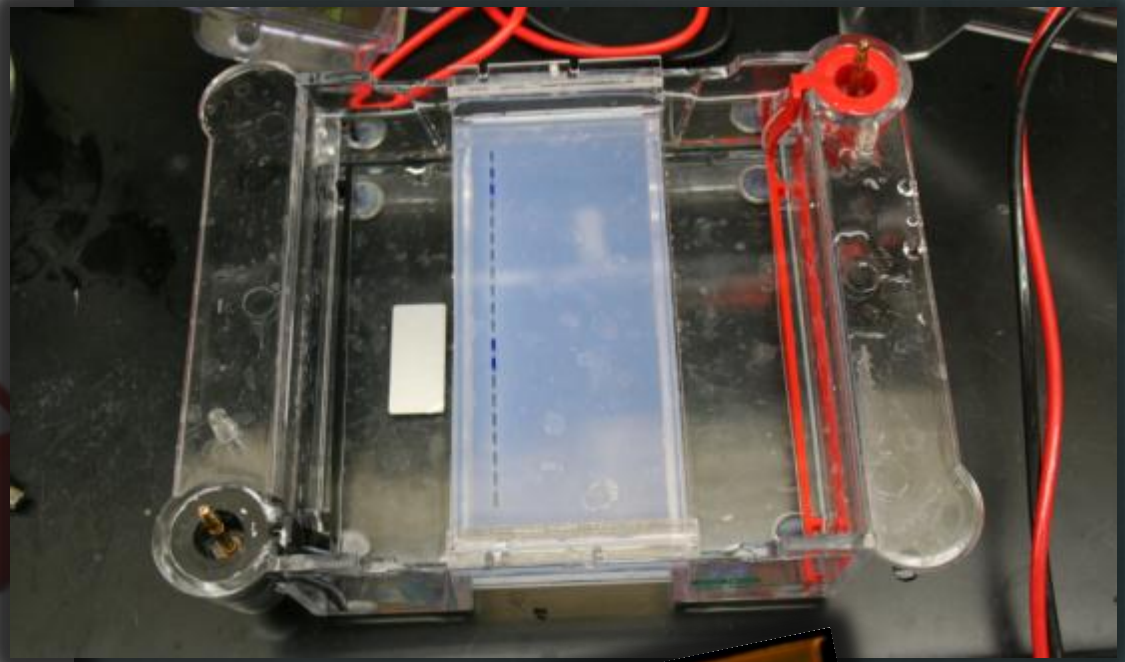
WESTERN BLOT



PCR (NO PROTEÍNA C REACTIVA...)

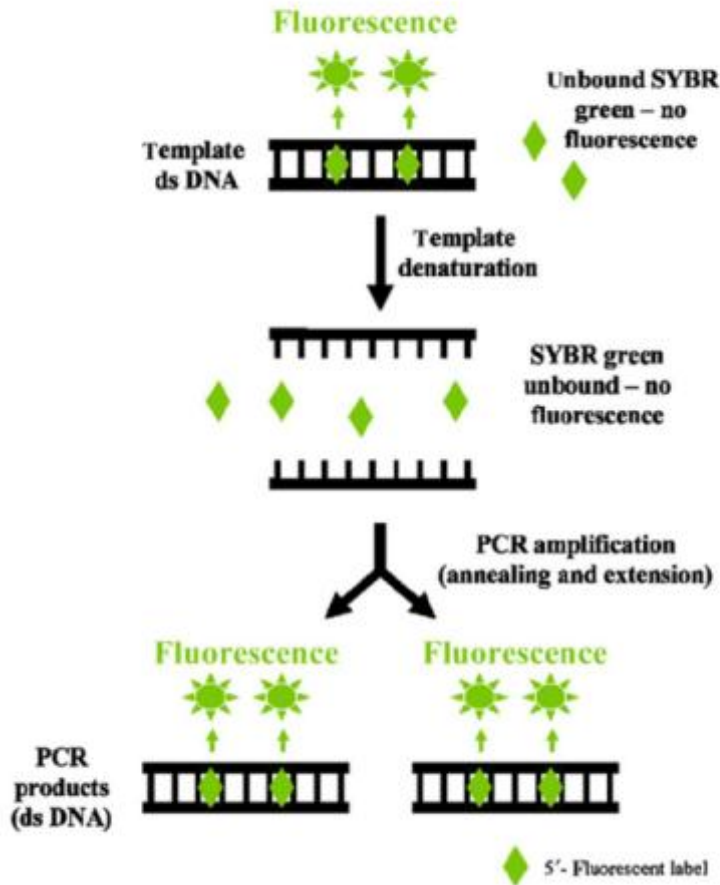


REACCIÓN EN CADENA DE LA POLIMERASA



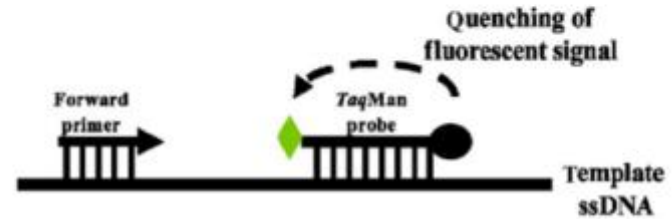
PCR EN TIEMPO REAL

SYBR green assays

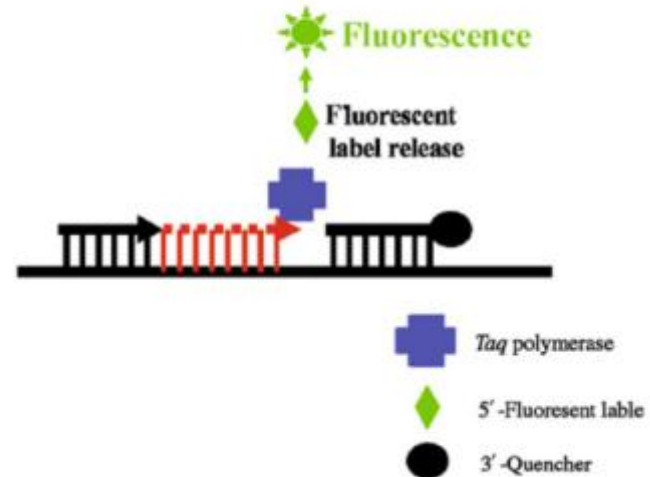


TaqMan (5' nuclease) assays

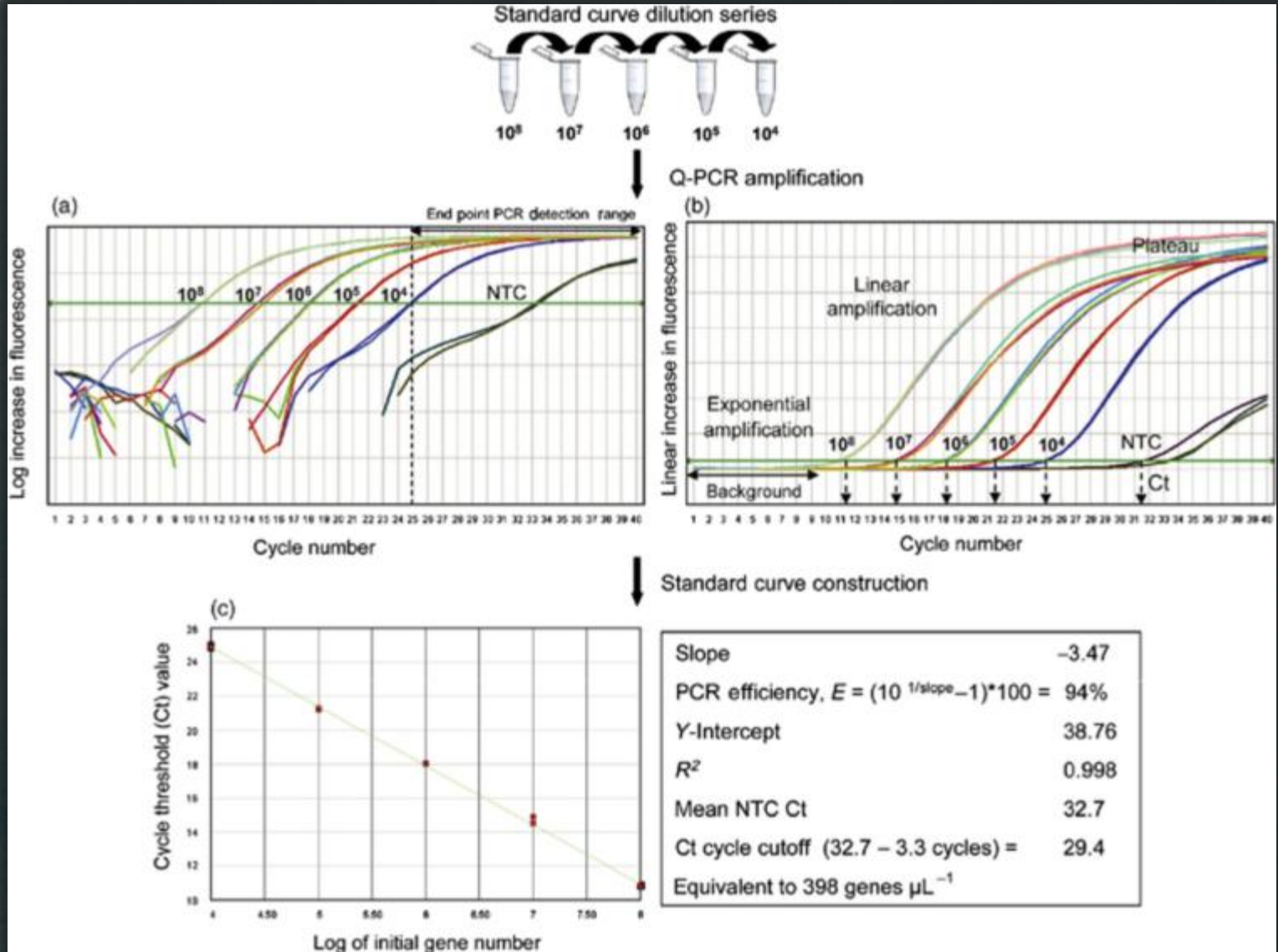
1. Primer and probe annealing



2. Extension and cleavage of fluorescent label

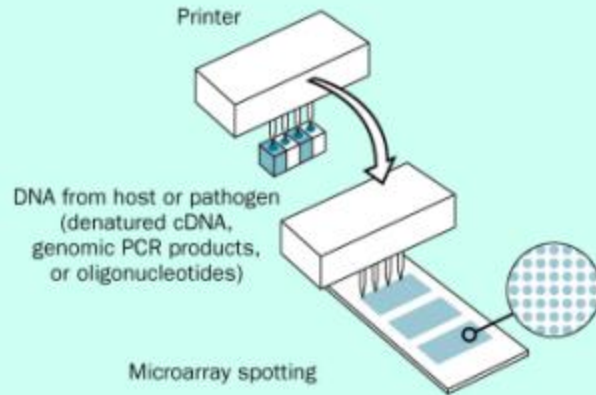


¡CUANTIFICABLE!

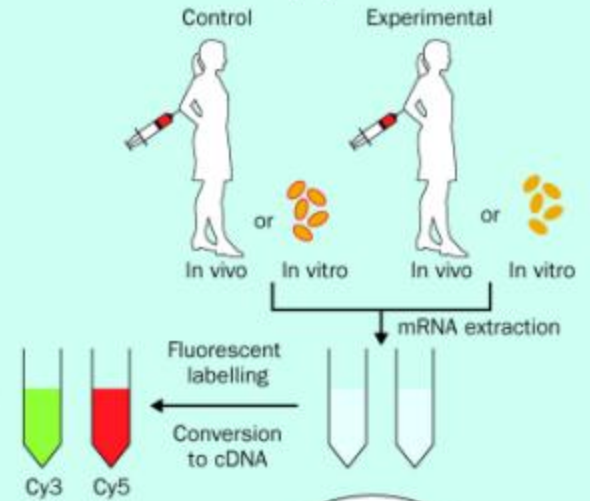


CHIPS DE ADN

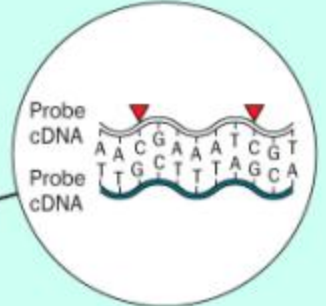
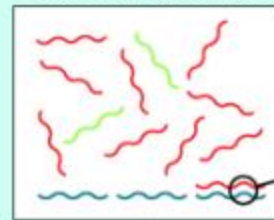
Target preparation



Probe preparation



Competitive hybridisation



Scanning

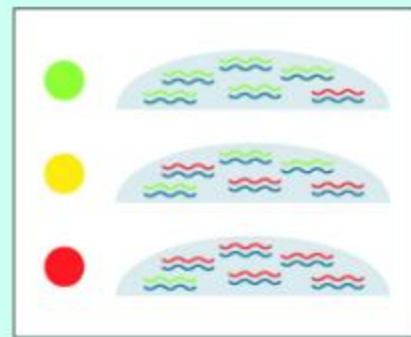
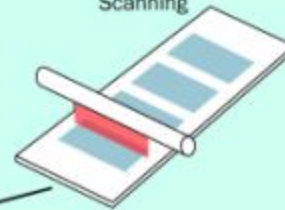
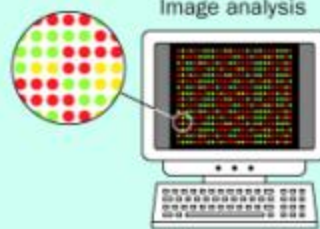


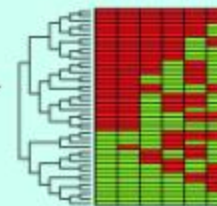
Image analysis



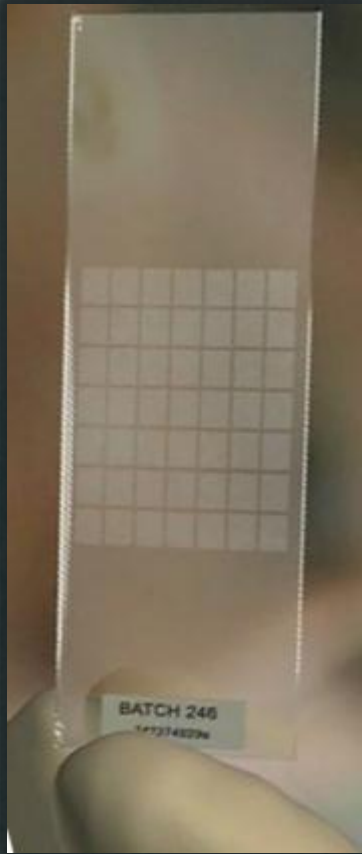
Data normalisation



Data analysis



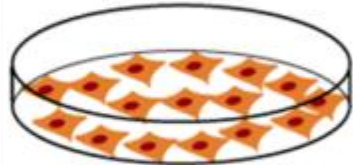
Data interpretation



CHIPS DE ADN

Oligonucleotide microarray

Cells of person 1/condition 1



RNA isolation

mRNA

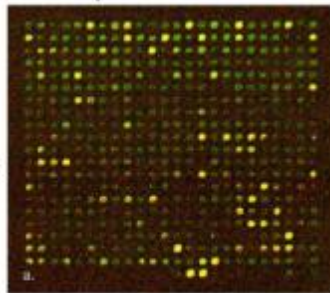
Reverse transcriptase labeling

cDNA

"Green Fluorescent" Targets

Hybridize to microarray

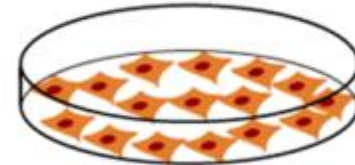
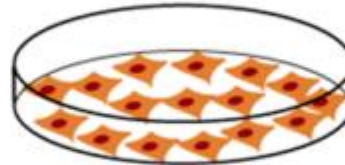
Microarray with short ssDNA spanning the entire genome



cDNA microarray

Cancer cells

Normal cells



RNA isolation

mRNA

Reverse transcriptase labeling

cDNA

"Red Fluorescent" Targets

mRNA

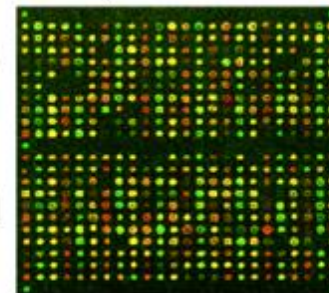
cDNA

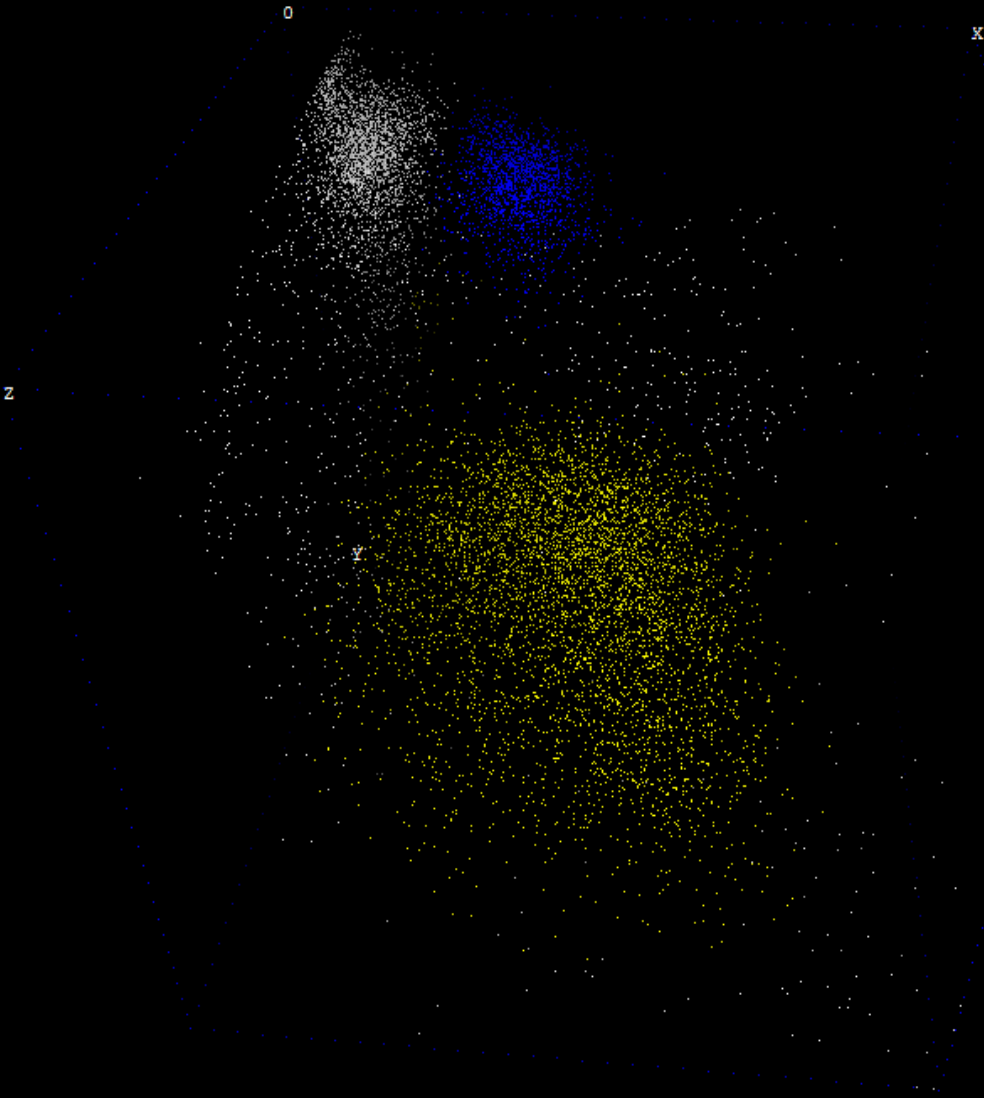
"Green Fluorescent" Targets

Combine targets

Hybridize to microarray

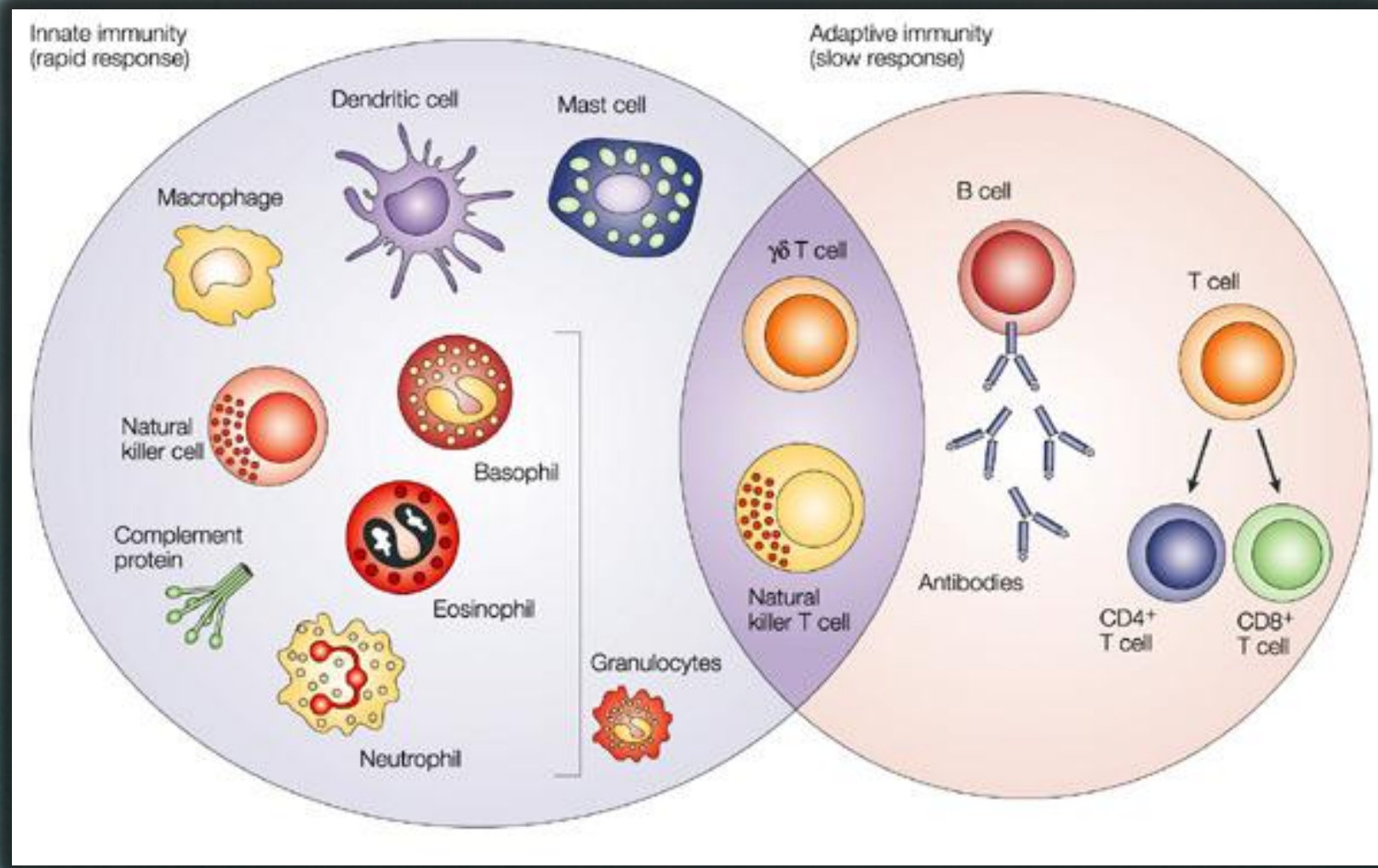
Microarray with long cDNA covering the transcriptional activity of the cell type



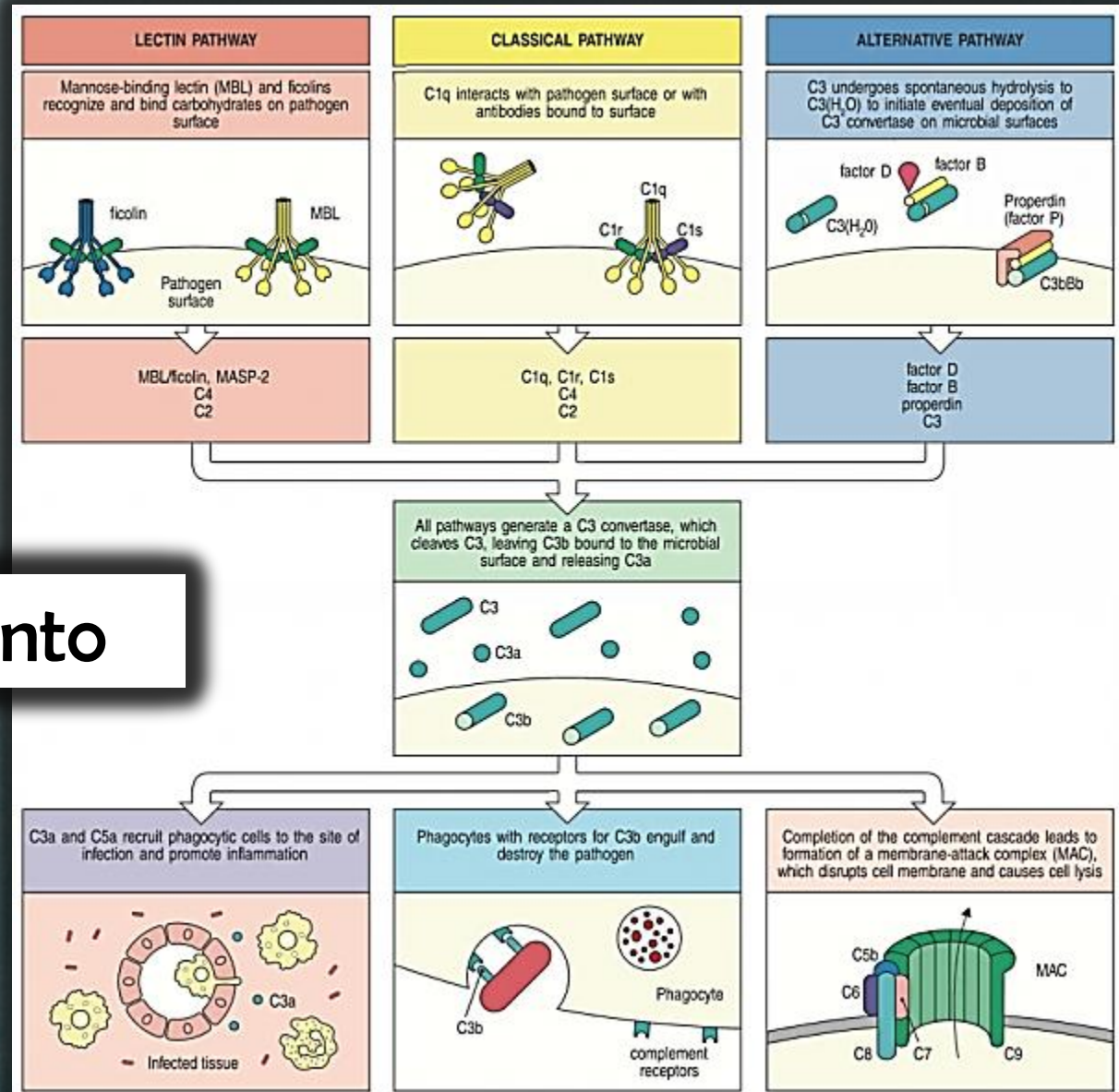


**AHORA SI: LA CLASE
MÉTODOS PARA EVALUAR LA RESPUESTA
INMUNE HUMORAL Y CELULAR**

EL OBJETO DE ESTUDIO

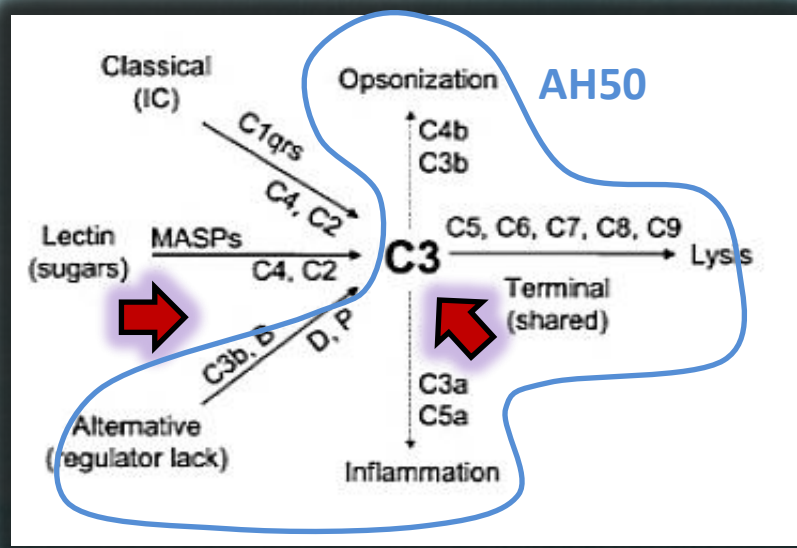
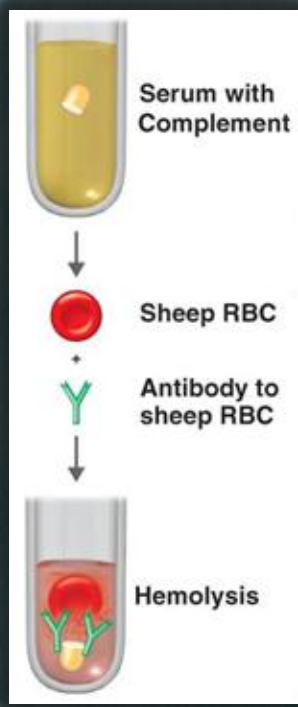
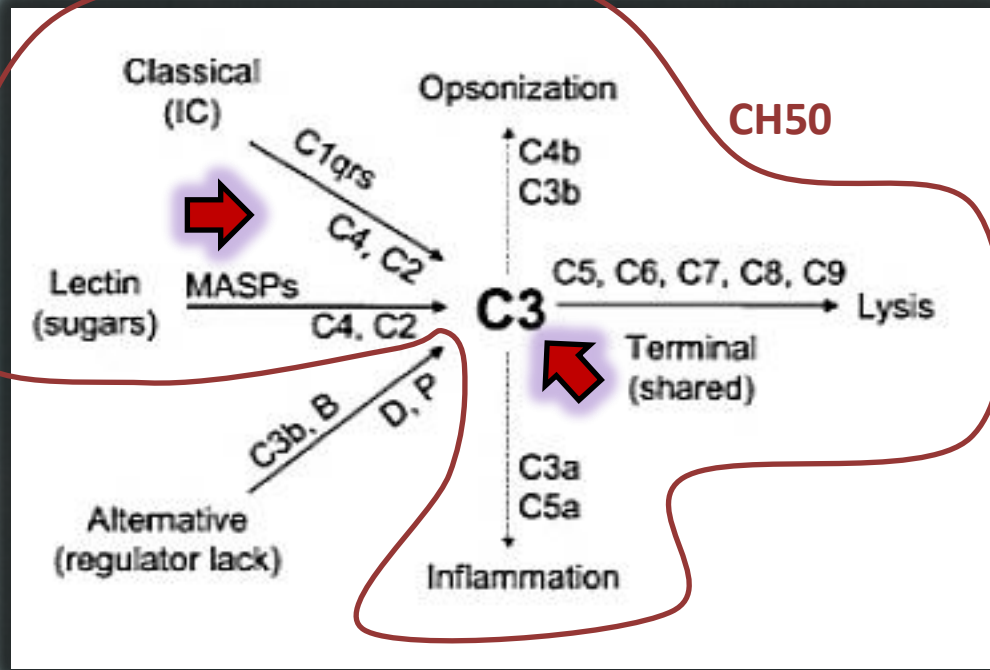


RESPUESTA HUMORAL

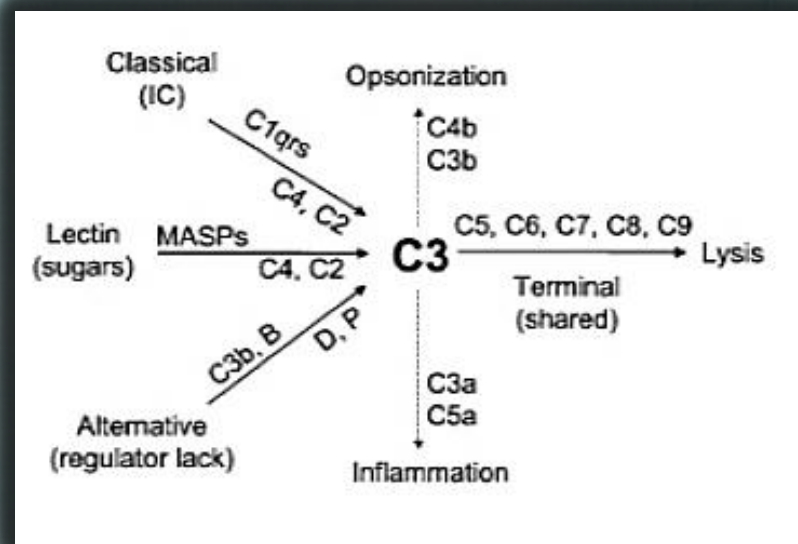
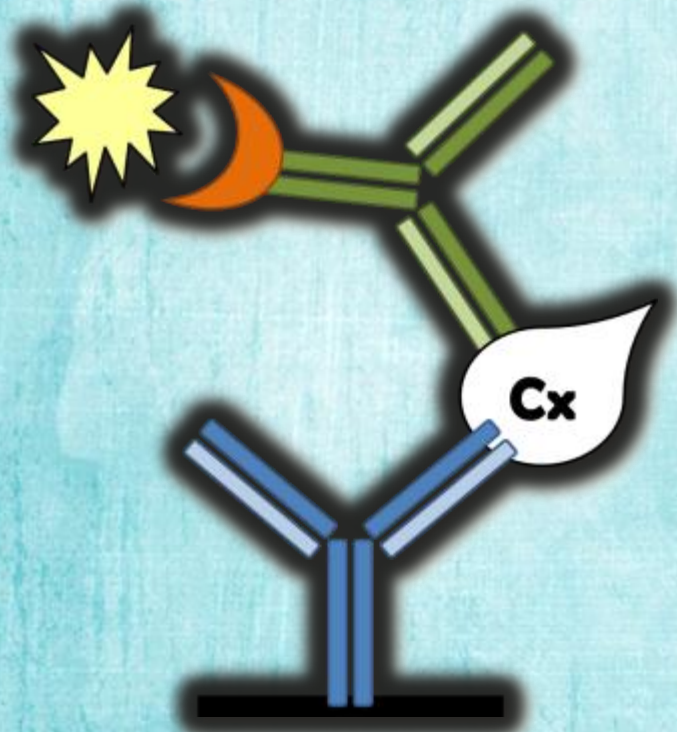


Complemento

EVALUACIÓN DEL COMPLEMENTO



EVALUACIÓN DEL COMPLEMENTO



A/CH50 75 a 160 U/ml
C1 Inhibidor 16 a 33 mg/ dl
C3 Hombres 88 a 252 mg/ dl
C3 Mujeres 88 a 206 mg/ dl
C4 Hombres 12 a 72 mg/ dl
C4 Mujeres 13 a 75 mg/ dl

CH50=0 & AH50=normal ► Clásica (upst)

CH50=normal & AH50=0 ► Alterna (upst)

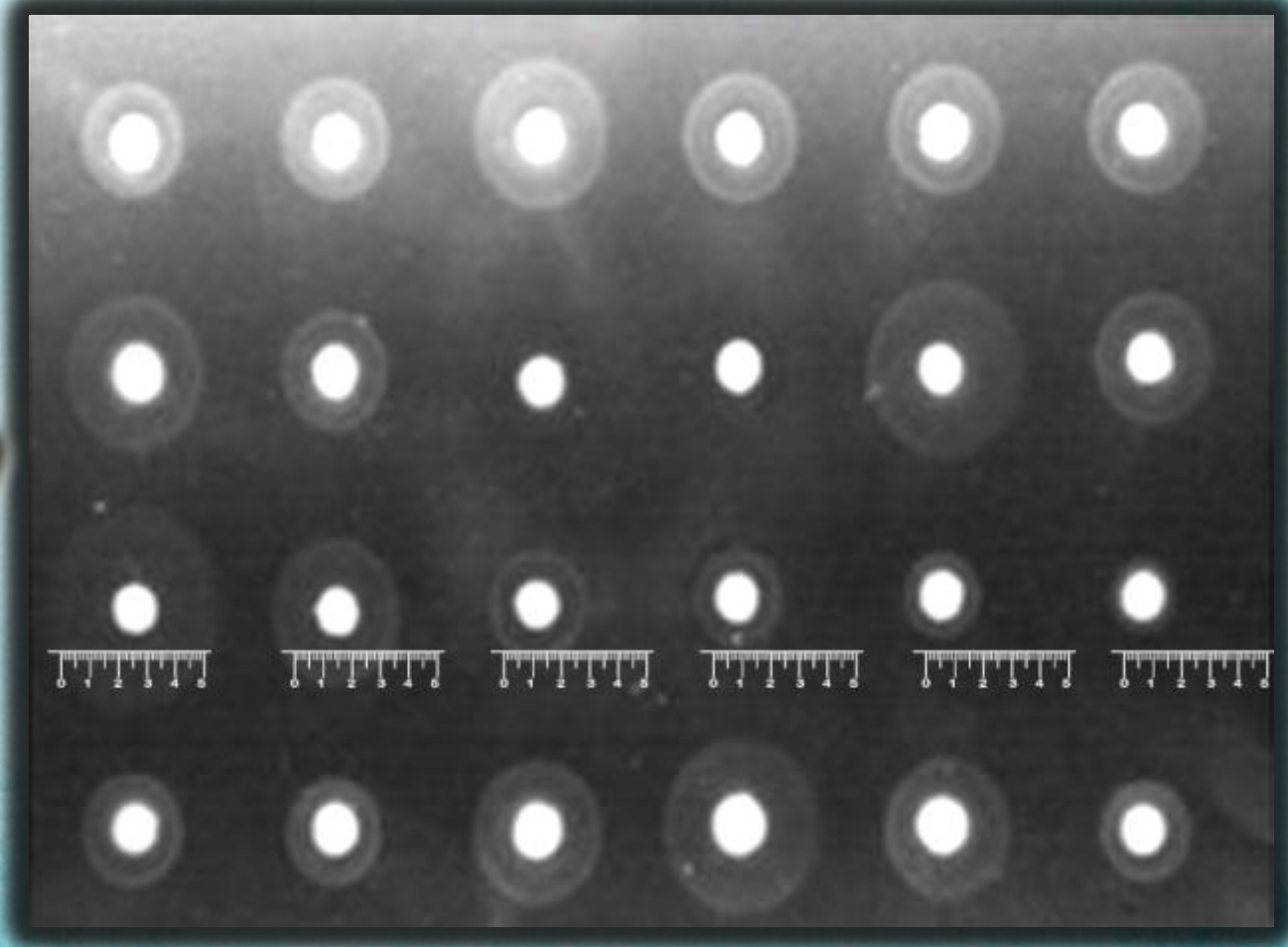
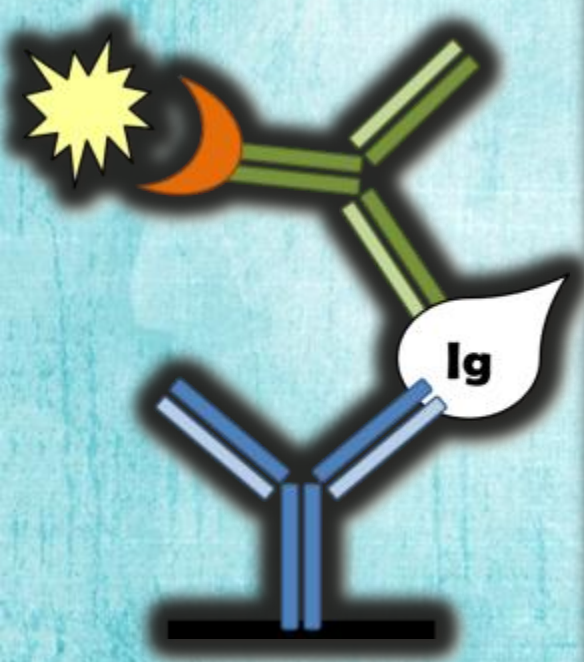
CH50=0 & AH50=0 ► Común

INMUNOGLOBULINAS

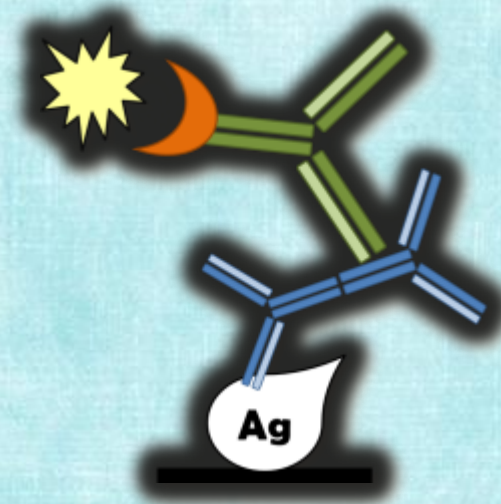
Property	IgG	IgA	IgM	IgD	IgE
Usual molecular form	Monomer	Monomer, dimer	Pentamer, hexamer	Monomer	Monomer
Other chains	None	J chain, SC	J chain	None	None
Subclasses	G1, G2, G3, G4	A1, A2	None	None	None
Heavy chain allotypes	Gm (=30)	No A1, A2m (2)	None	None	None
Molecular mass, kDa	150	160, 400	950, 1150	175	190
Serum level in average adult, mg/mL	9.5–12.5	1.5–2.6	0.7–1.7	0.04	0.0003
Percentage of total serum Ig	75–85	7–15	5–10	0.3	0.019
Serum half-life, days	23	6	5	3	2.5
Synthesis rate, mg/kg per day	33	65	7	0.4	0.016
Antibody valence	2	2, 4	10, 12	2	2
Classical complement activation	+(G1, 2?, 3)	-	++	-	-
Alternate complement activation	+(G4)	+	-	+	-
Binding cells via Fc	Macrophages, neutrophils, large granular lymphocytes	Lymphocytes	Lymphocytes	None	Mast cells, basophils, B cells
Biologic properties	Placental transfer, secondary Ab for most antipathogen responses	Secretory immunoglobulin	Primary Ab responses	Marker for mature B cells	Allergy, antiparasite responses

Source: After L. Carayannopoulos, JD Capra, in WE Paul (ed): *Fundamental Immunology*, 3rd ed. New York, Raven, 1993; with permission.

CUANTIFICACIÓN DE IG



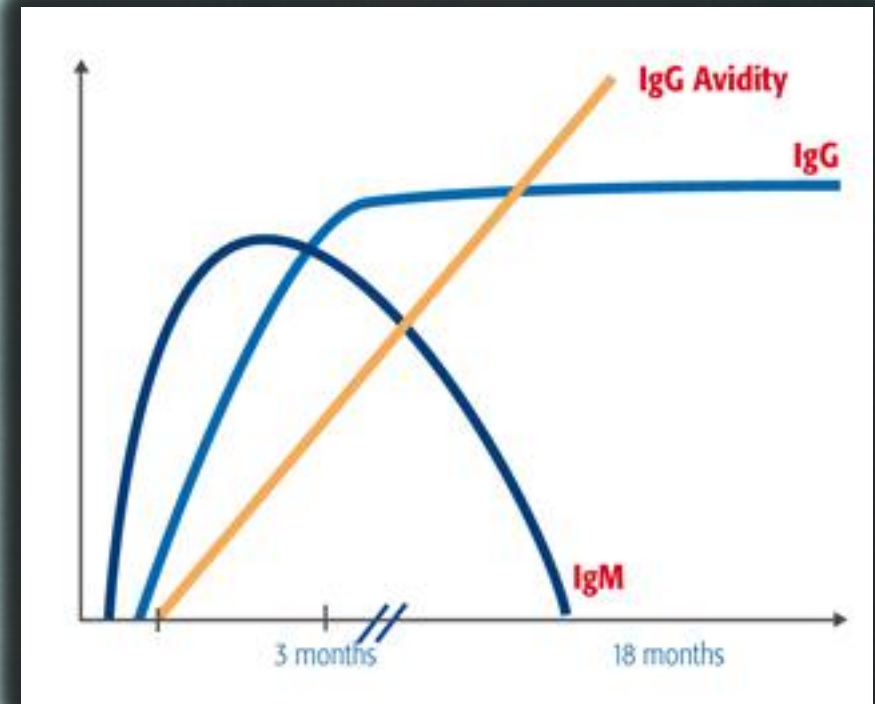
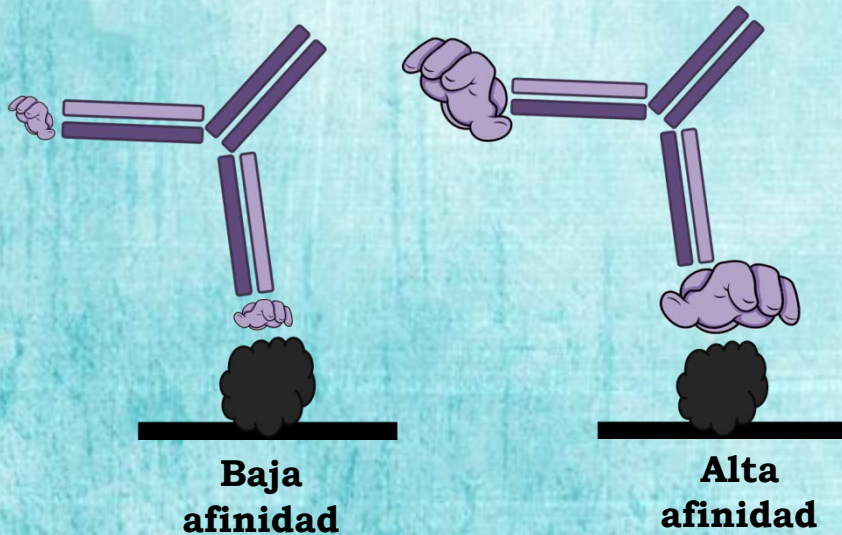
IG ESPECÍFICAS



AVIDEZ Y AFINIDAD

Afinidad: fuerza de unión de un anticuerpo con un antígeno monovalente.

Avidéz: fuerza de unión de un antisuero con los antígenos multivalente. Se incrementa con el tiempo de la estimulación antigénica, depende de la afinidad y de las valencias.



Infección por *Toxoplasma gondii*

LA PREGUNTA CLÁSICA

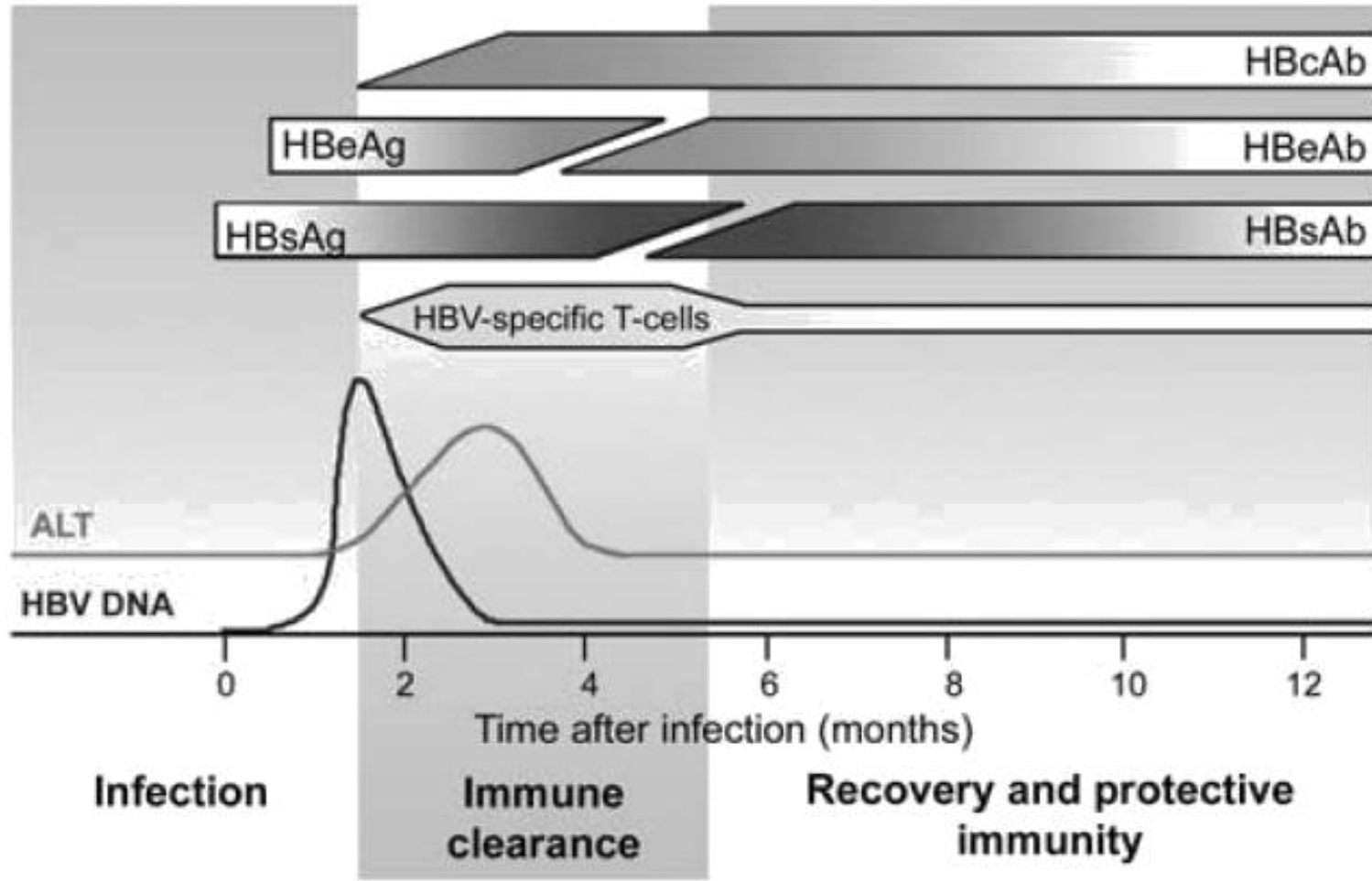


Dra.: Me salió positiva la prueba de Hepatitis B, el Anti antígeno de superficie. Me voy a morir?



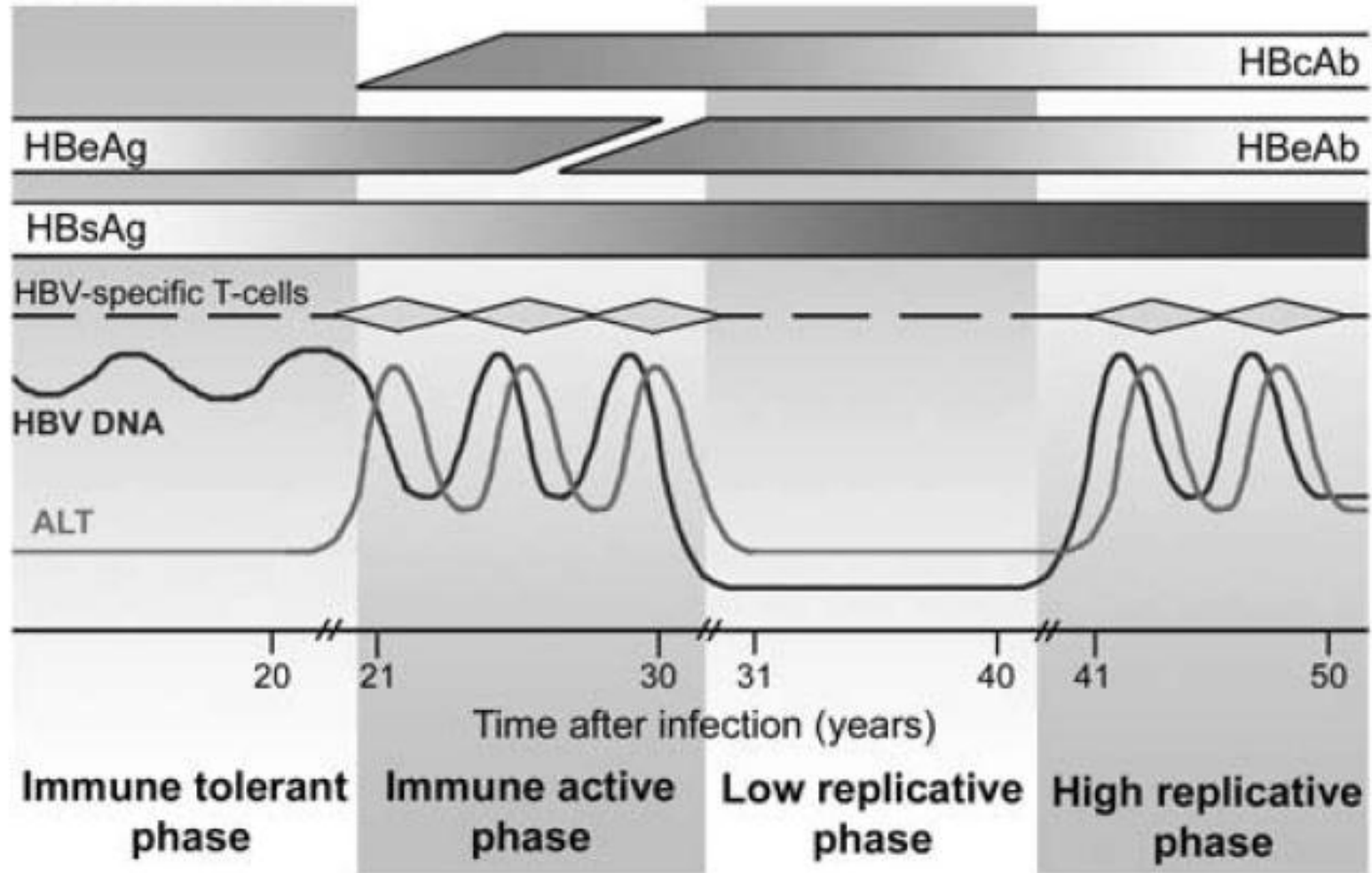
INFECCIÓN AGUDA

Acute HBV



INFECCIÓN CRÓNICA

Chronic HBV



UN "RESUMEN"

Marcadores diagnósticos para la infección por virus de la hepatitis B							Interpretación
HBsAg	HBsAb	IgG-HBcAb	IgM-HBcAb	HBeAg	HBeAb	ADN	
Respuesta Favorable							
-	+	-	-	-	-	-	Vacunado
+	-	-	+/-	+/-	+/-	+	Infección aguda periodo de ventana
+	-	+	+	+	-	+	Infección aguda temprana
+	-	+	+	-	+	-	Infección aguda en resolución
-	+	+	-	+/-	+/-	-	Fase de recuperación
-	+	+	-	-	-	-	Curado
Respuesta Desfavorable							
+	-	+	-	+	-	+++	Infección crónica de alta infectividad
+	-	+	-	-	+	+/-	Infección crónica de baja infectividad
-	-	+/-	-	-	-	+/-	Infección oculta

LA PREGUNTA CLÁSICA



Dra.: He dado positivo a 15 elisas de VIH de 4ta generación en labs privados diferentes, pero en el laboratorio del programa doy negativo a 20 westernblot. Me voy a morir?



POLÉMICAS CONFIRMATORIAS

Inmunoblot VERSUS Elisa 4^{ta} generación

Is Western blot alone sufficient to confirm a reactive result of a fourth-generation HIV screening assay?

SWISS MED WKLY 2006;136:672-673 · www.smw.ch

*Marinko Dobeč^a, Adrian Naegeli^b,
Kurt Furrer^a, Franz Kaeppli^a*

^a medica, medizinische Laboratorien
Dr. F. Kaeppli, Zürich, Switzerland

^b Medical practice Dr. A. Naegeli,
Zürich, Switzerland

Collection place	Collection date	HIV 4 th generation Antigen/Antibody test Combo AxSYM, Abbott*	Immunoblot Inno-Lia HIV-Confirmation, Innogenetics	Cobas Amplicor HIV-1 Monitor Test Version 1.5, Roche Diagnostics
Practitioner A	September 23, 2004	4.33	no antibodies detectable	no material available

* cut off 1.0

** retrospectively performed on June 14, 2005



El laboratorista debe tener claridad del alcance diagnóstico de la pruebas que realiza, Y DEBE INFORMAR AL PACIENTE

POLÉMICAS CONFIRMATORIAS

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Practitioner A	September 23, 2004	4.33	no antibodies detectable	no material available
Practitioner A	September 24, 2004	4.32	not done	384,000 copies / mL**

* cut off 1.0

** retrospectively performed on June 14, 2005



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Collection place	Collection date	HIV 4 th generation Antigen/Antibody test Combo AxSYM, Abbott*	Immunoblot Inno-Lia HIV-Confirmation, Innogenetics	Cobas Amplicor HIV-1 Monitor Test Version 1.5, Roche Diagnostics
Practitioner A	September 23, 2004	4.33	no antibodies detectable	no material available
Practitioner A	September 24, 2004	4.32	not done	384,000 copies / mL**
Practitioner B	June 14, 2005	17.66	positive (p17, p24, p31, gp41, gp120)	33,100 copies / mL

* cut off 1.0

** retrospectively performed on June 14, 2005



El laboratorista debe tener claridad del alcance diagnóstico de la pruebas que realiza, Y DEBE INFORMAR AL PACIENTE

EL PROTOTIPO: ELISA PARA VIH

Generation

Antigen
Sample
Conjugate
Signal

Antigen

Detect

1st

Lysate

2nd

IgG α VIH-1
IgG α VIH-2

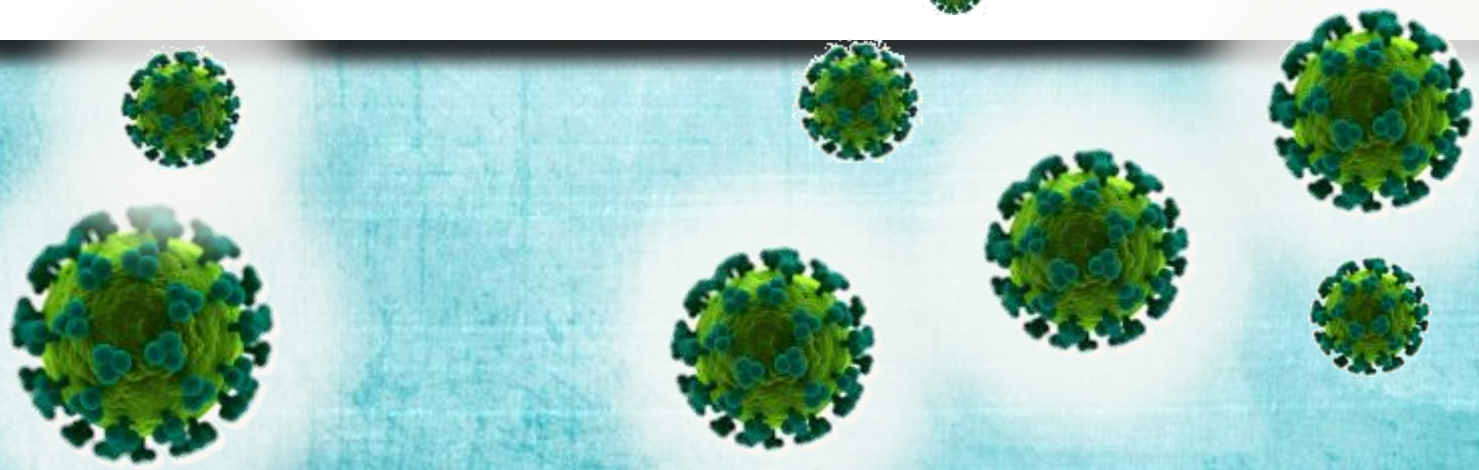
3rd

Recombinant & synthetic

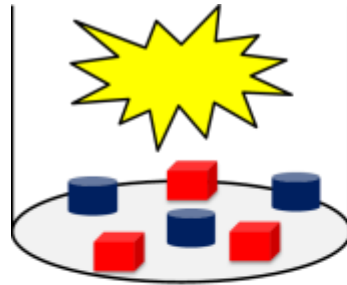
IgG / IgM α VIH-1
IgG / IgM α VIH-1/O
IgG / IgM α VIH-2

4th

IgG / IgM α VIH-1
IgG / IgM α VIH-1/O
IgG / IgM α VIH-2
p24

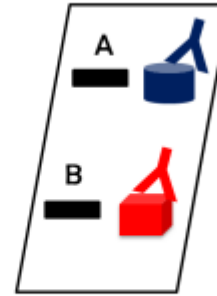


DIAGNÓSTICO

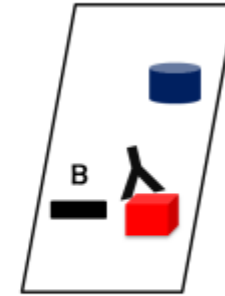


Ensayos en tiritas

Ensayos en suspensión



POSITIVO crónico o agudo



REACCIÓN CRUZADA



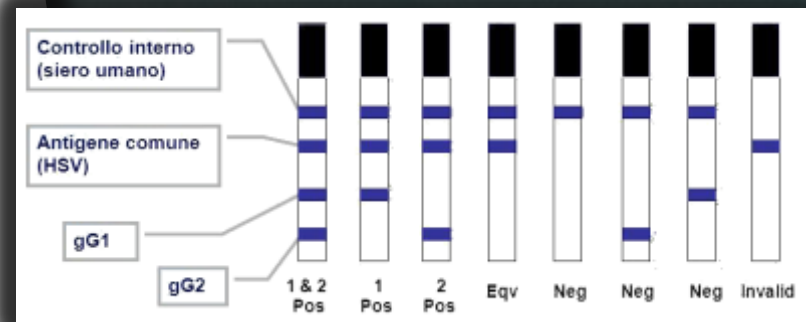
POSITIVO crónico



POSITIVO falso / reacción cruzada



POSITIVO agudo



Y EN RECIÉN NACIDOS??????



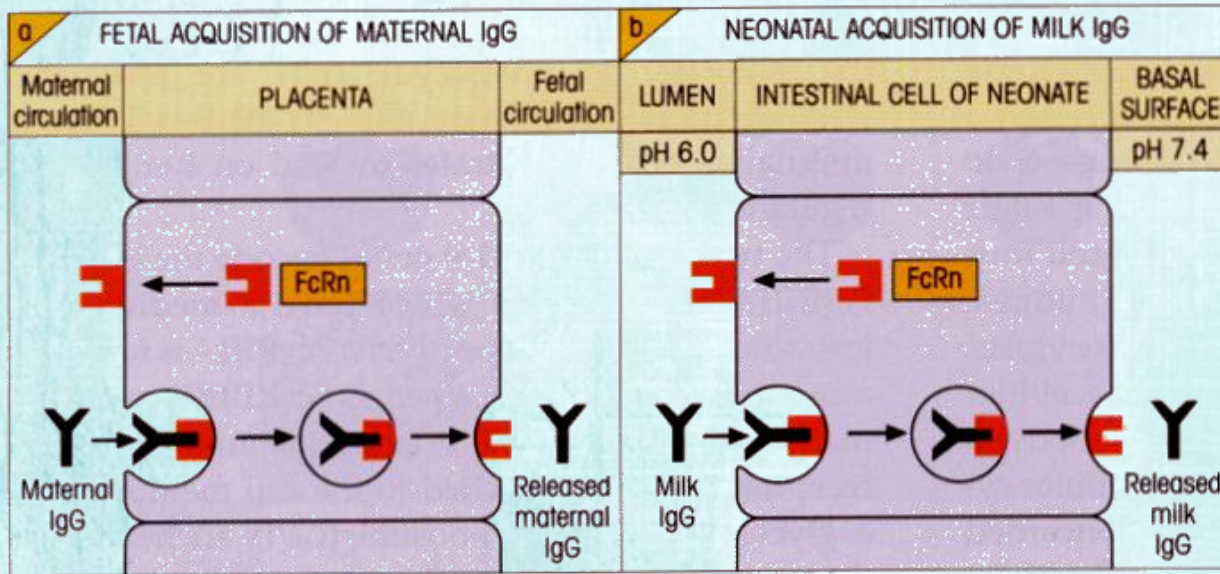
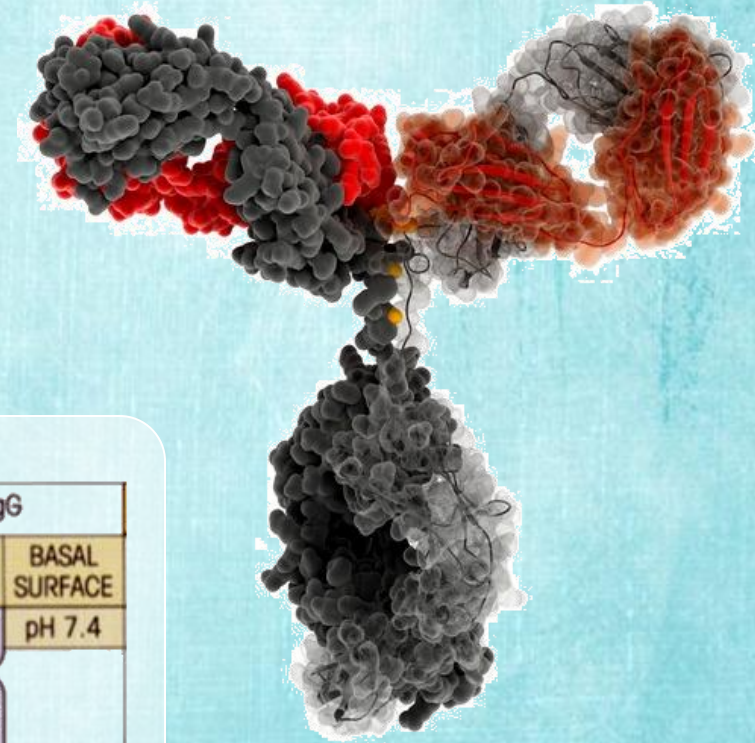
Es lo mismo pero
diferente!!!!



DIAGNÓSTICO EN NEONATOS

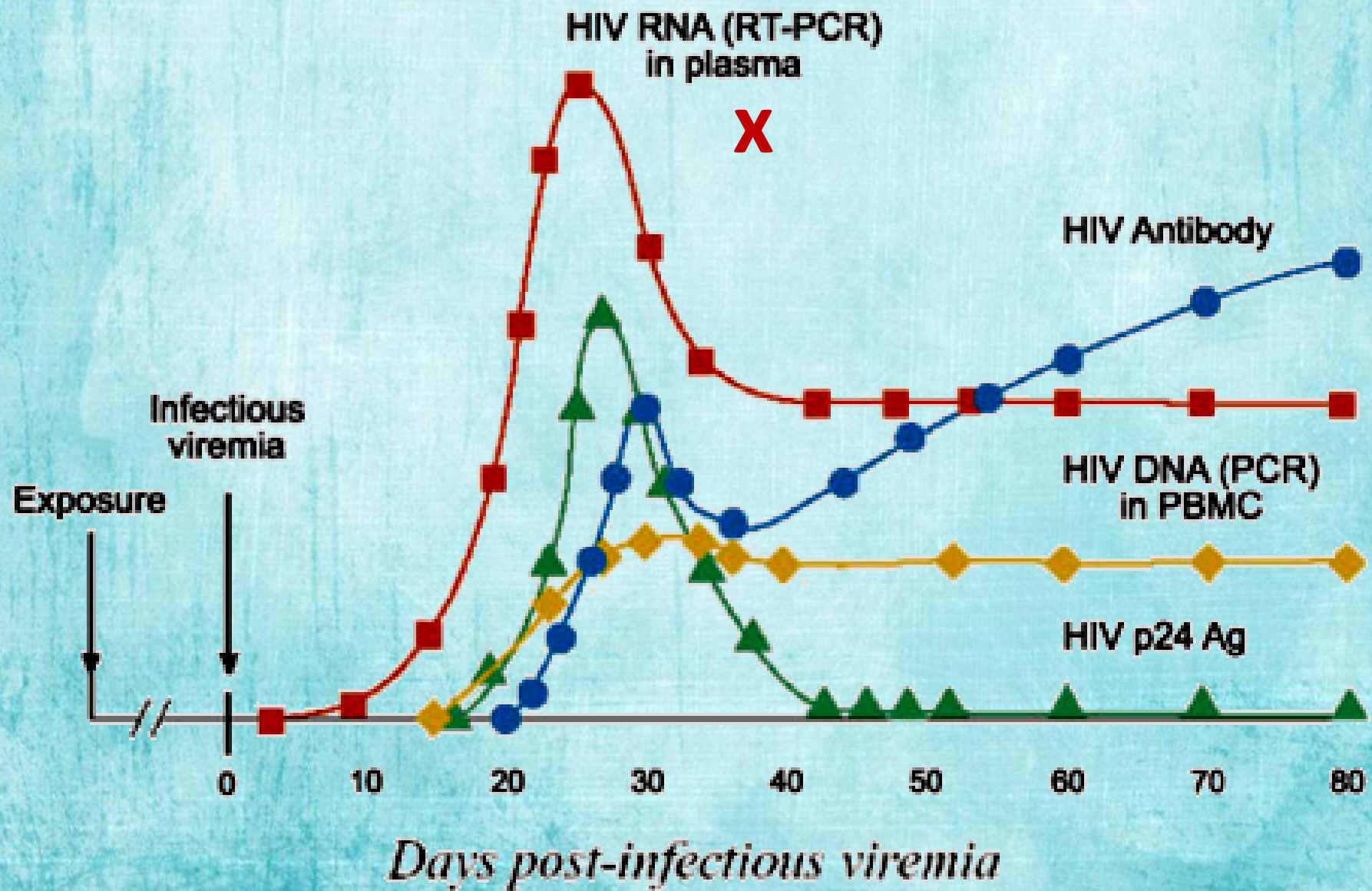


Las técnicas de inmunoensayo no son aplicables pues resultarán positivas

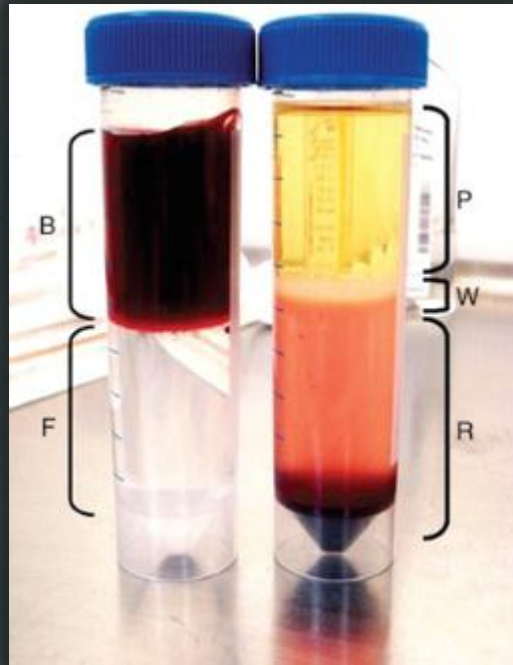


El diagnóstico se basa en la determinación de ADN proviral

DIAGNÓSTICO VIH



RESPUESTA CELULAR



Separando células

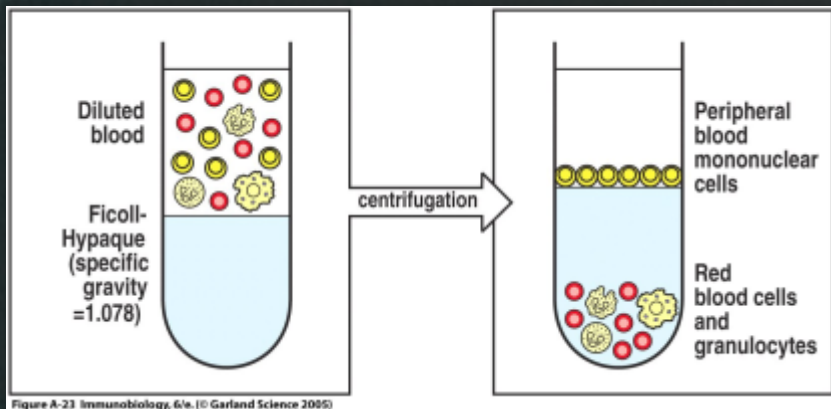
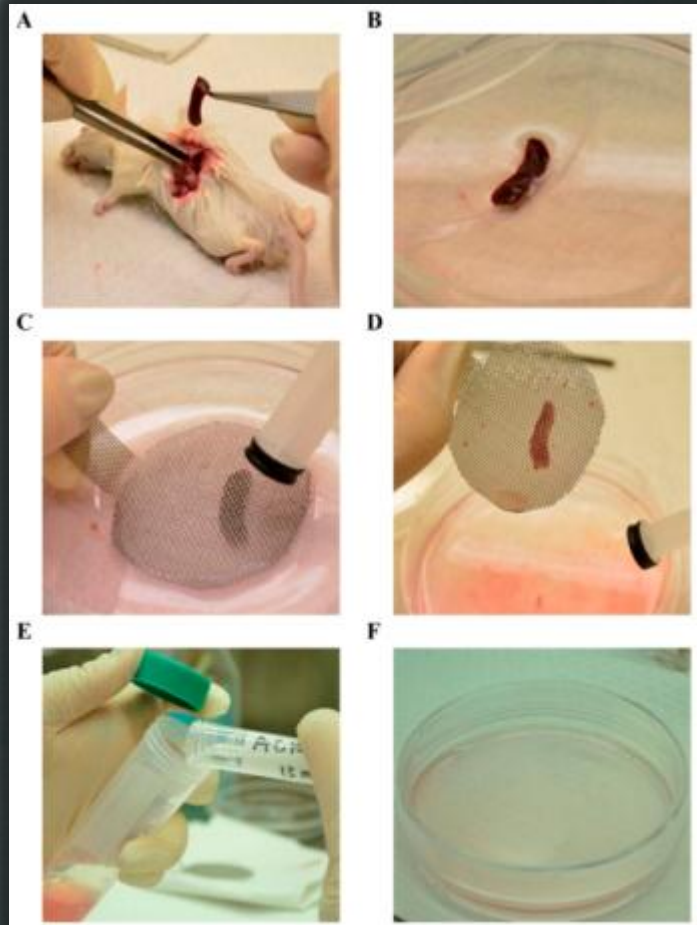
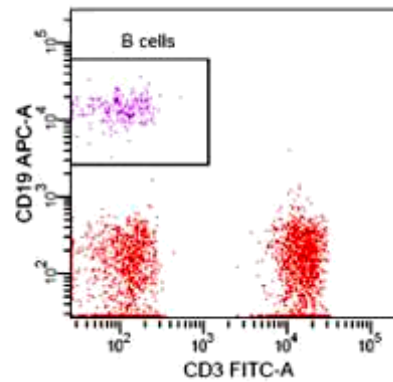
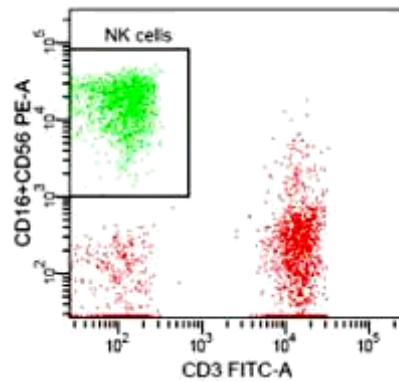
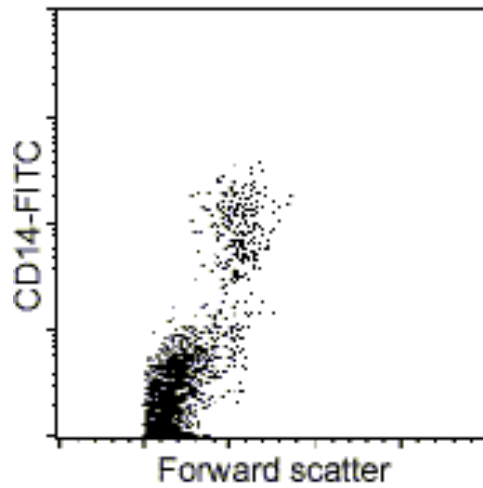
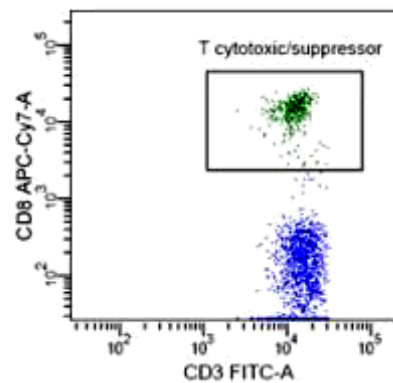
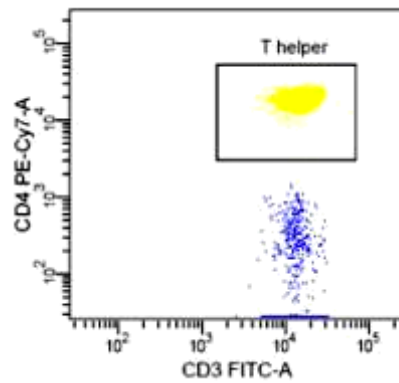
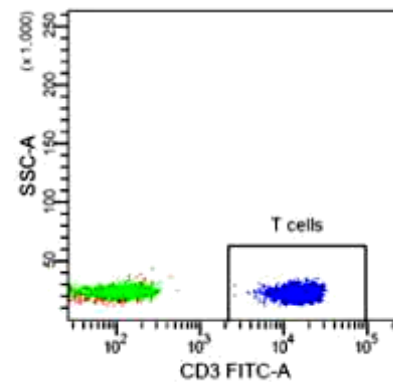
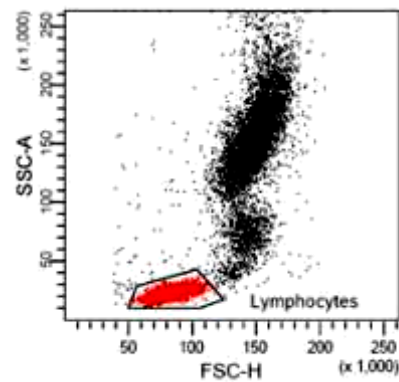
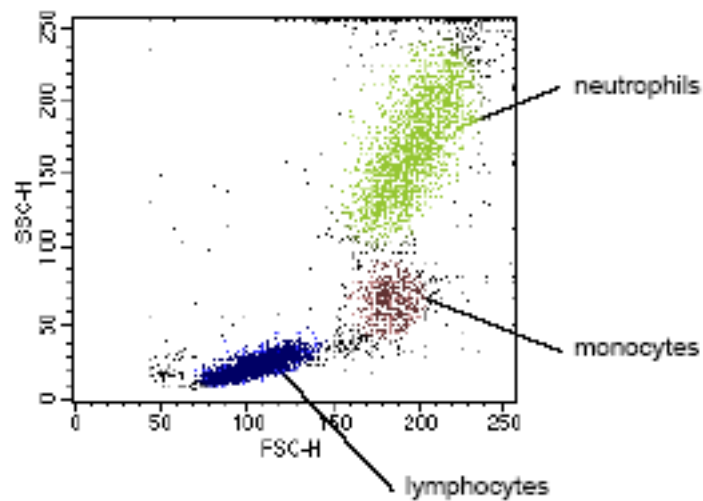
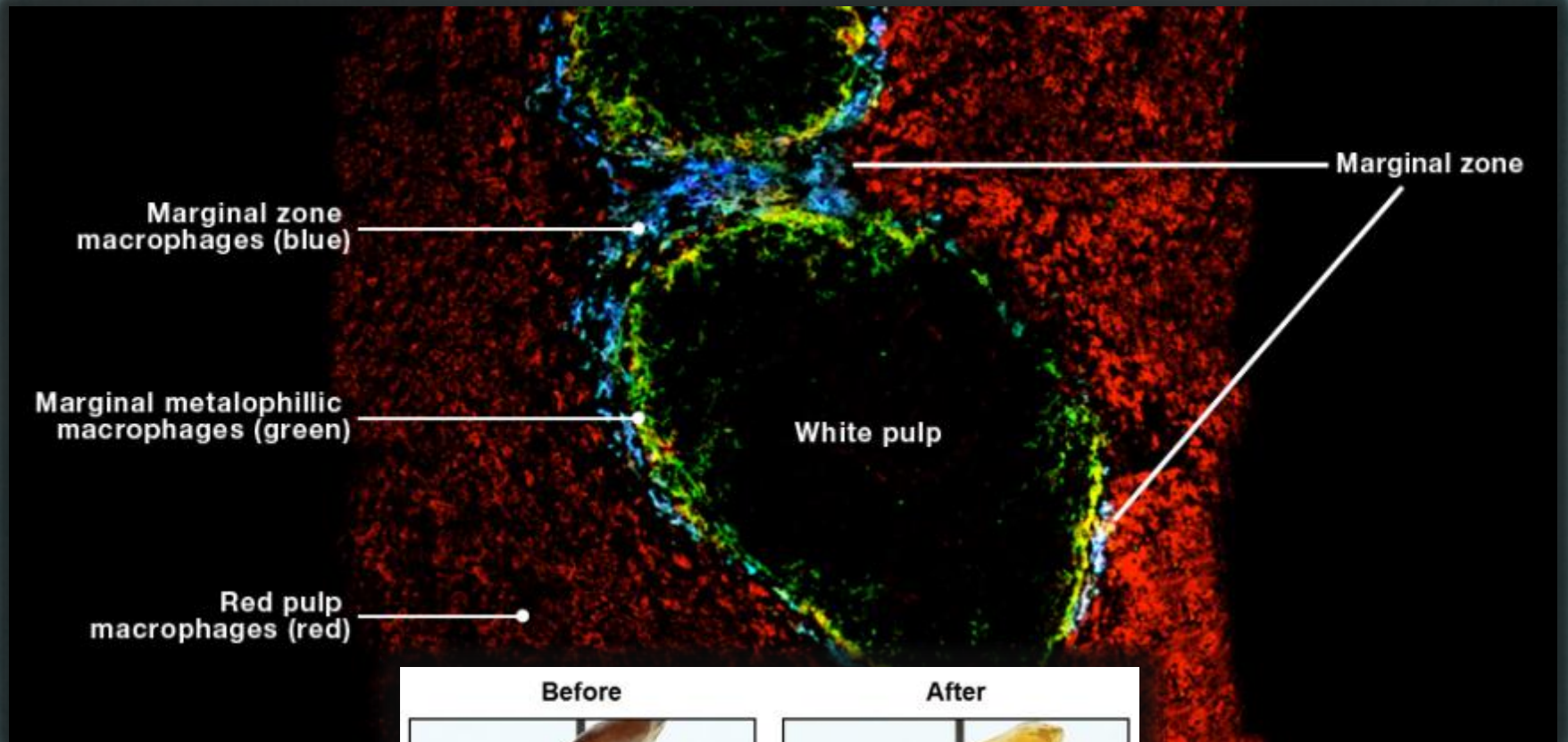


Figure A-23 Immunobiology, 6/e. © Garland Science 2005

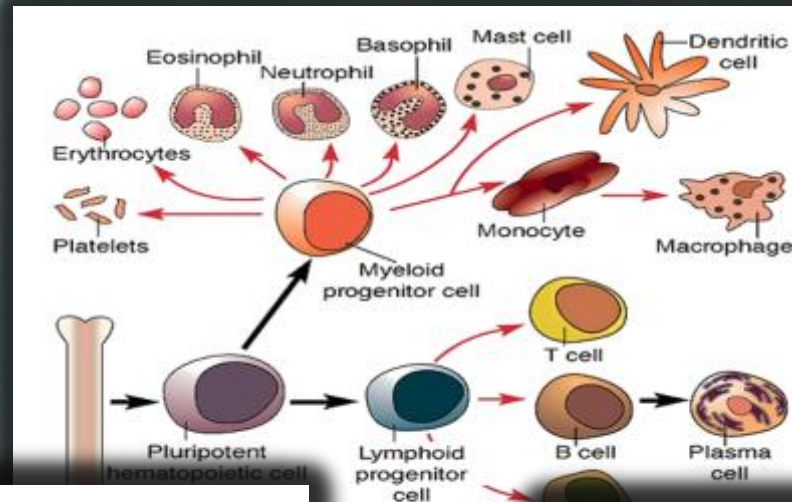
LAS CÉLULAS BLANCAS



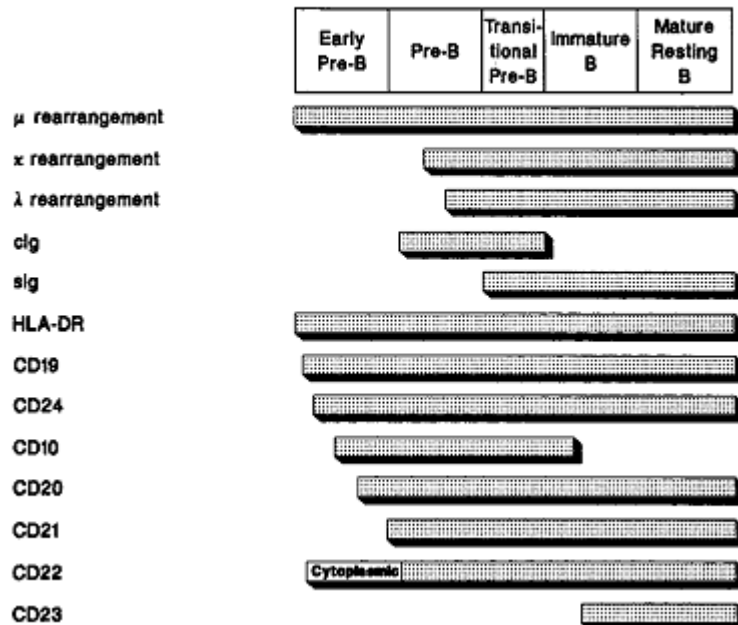
IN SITU



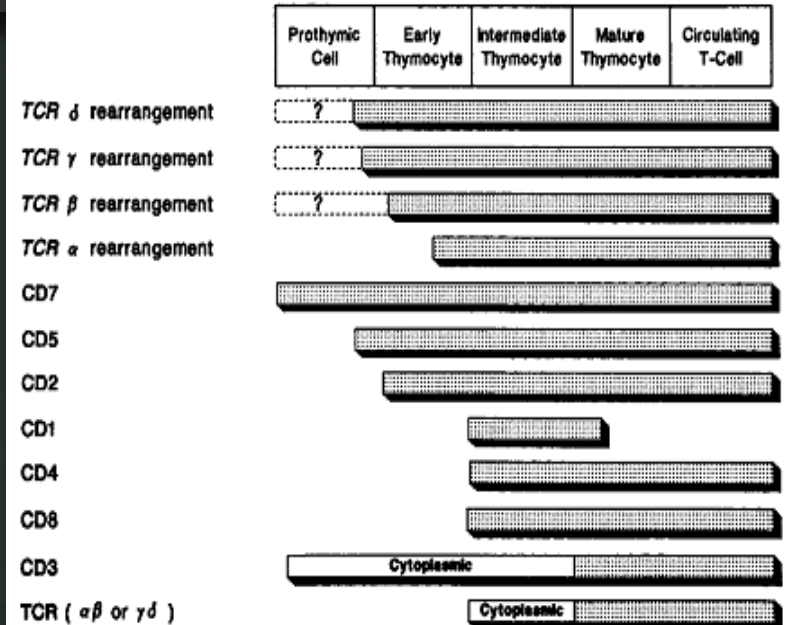
EVALUANDO EL ORIGEN



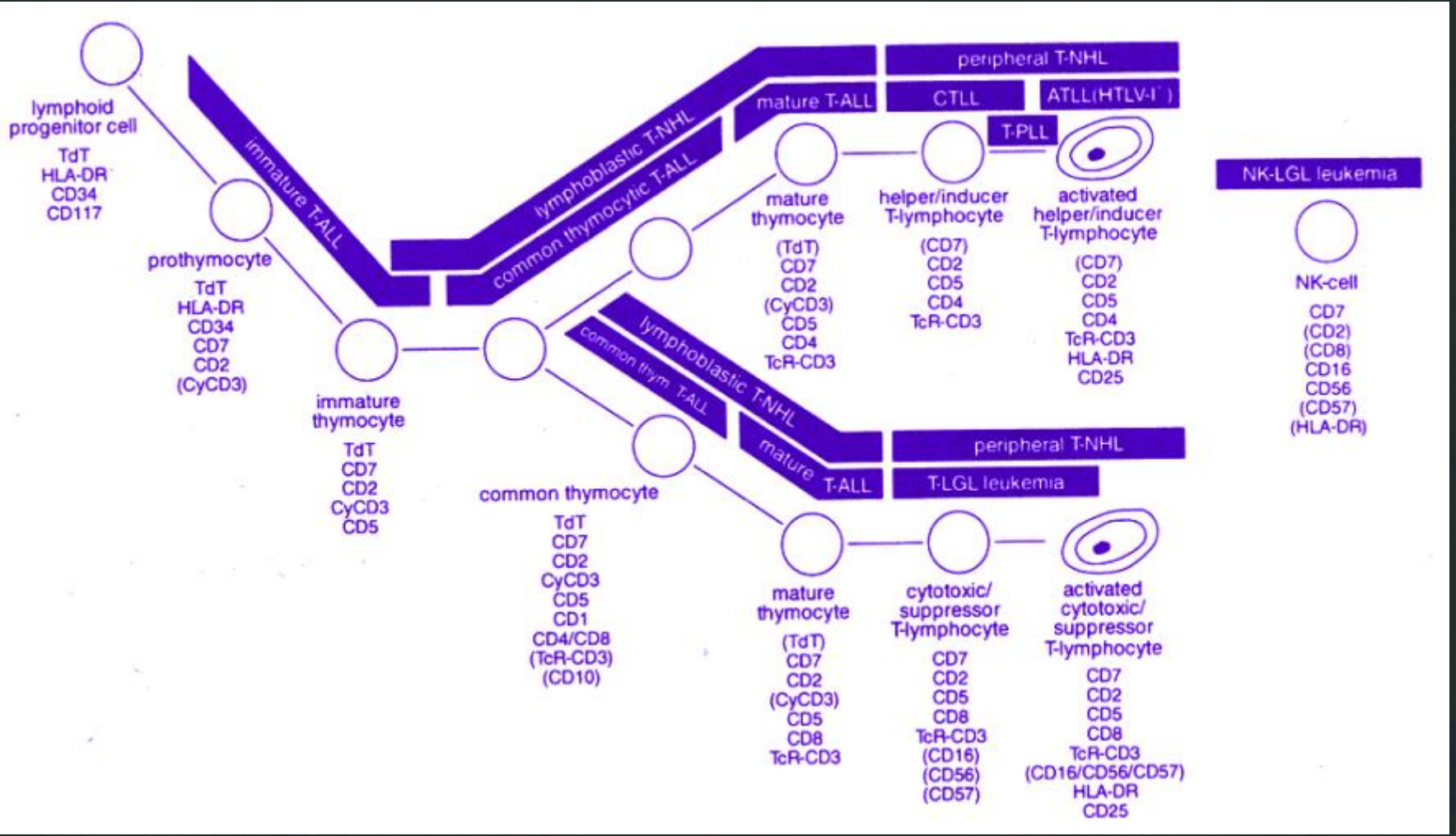
B-Cell Ontogeny



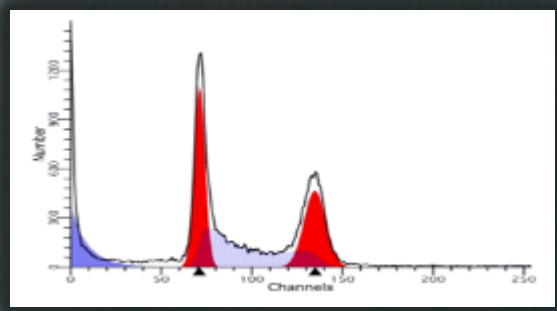
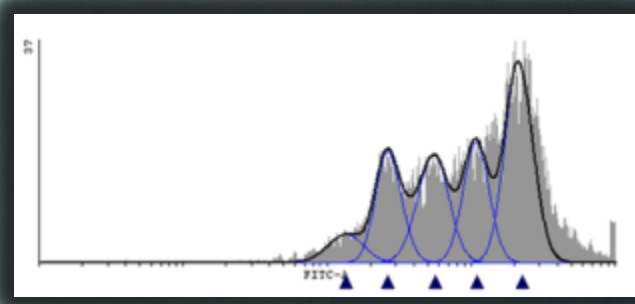
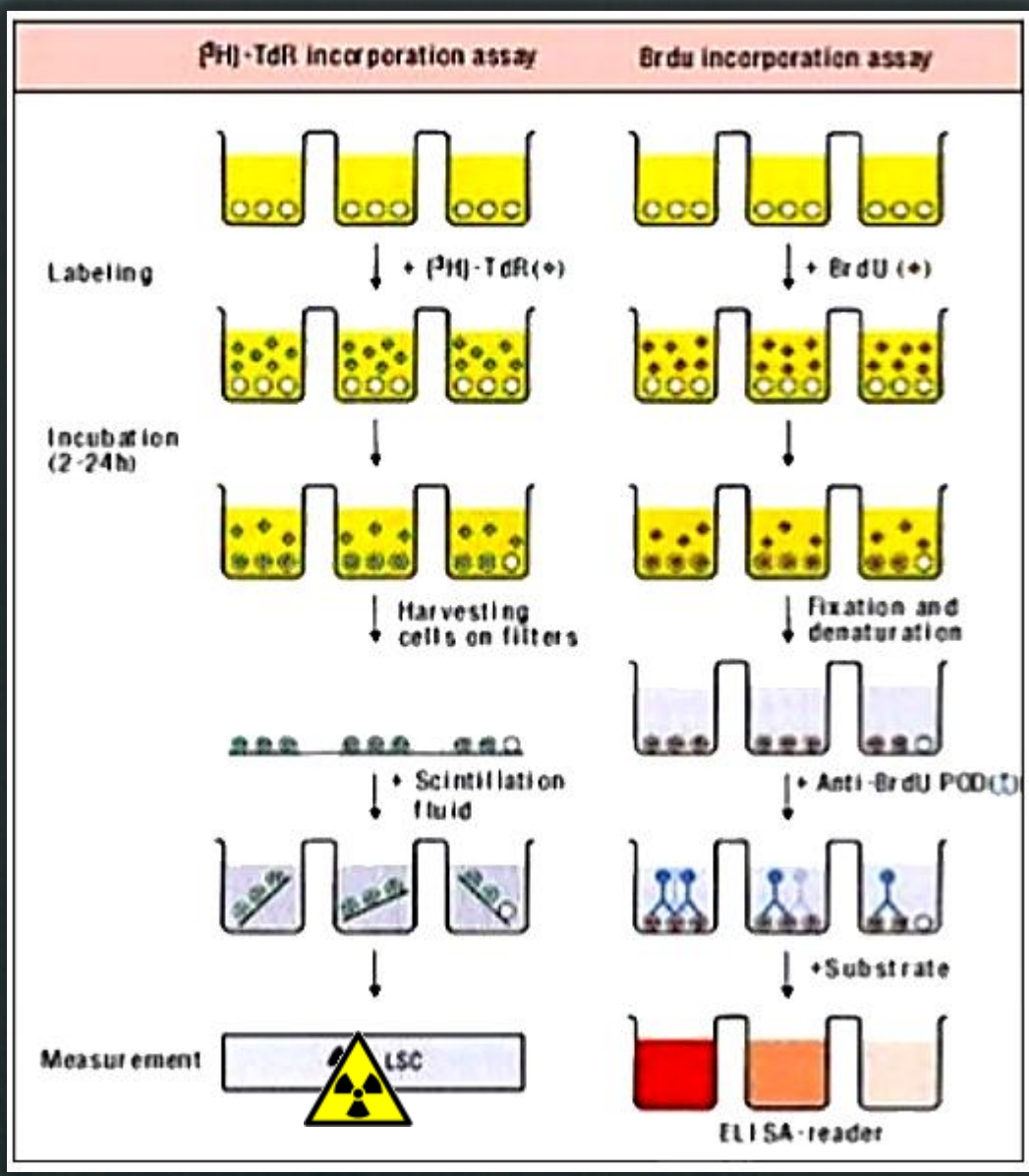
T-Cell Ontogeny



LEUCEMIAS (DE T)

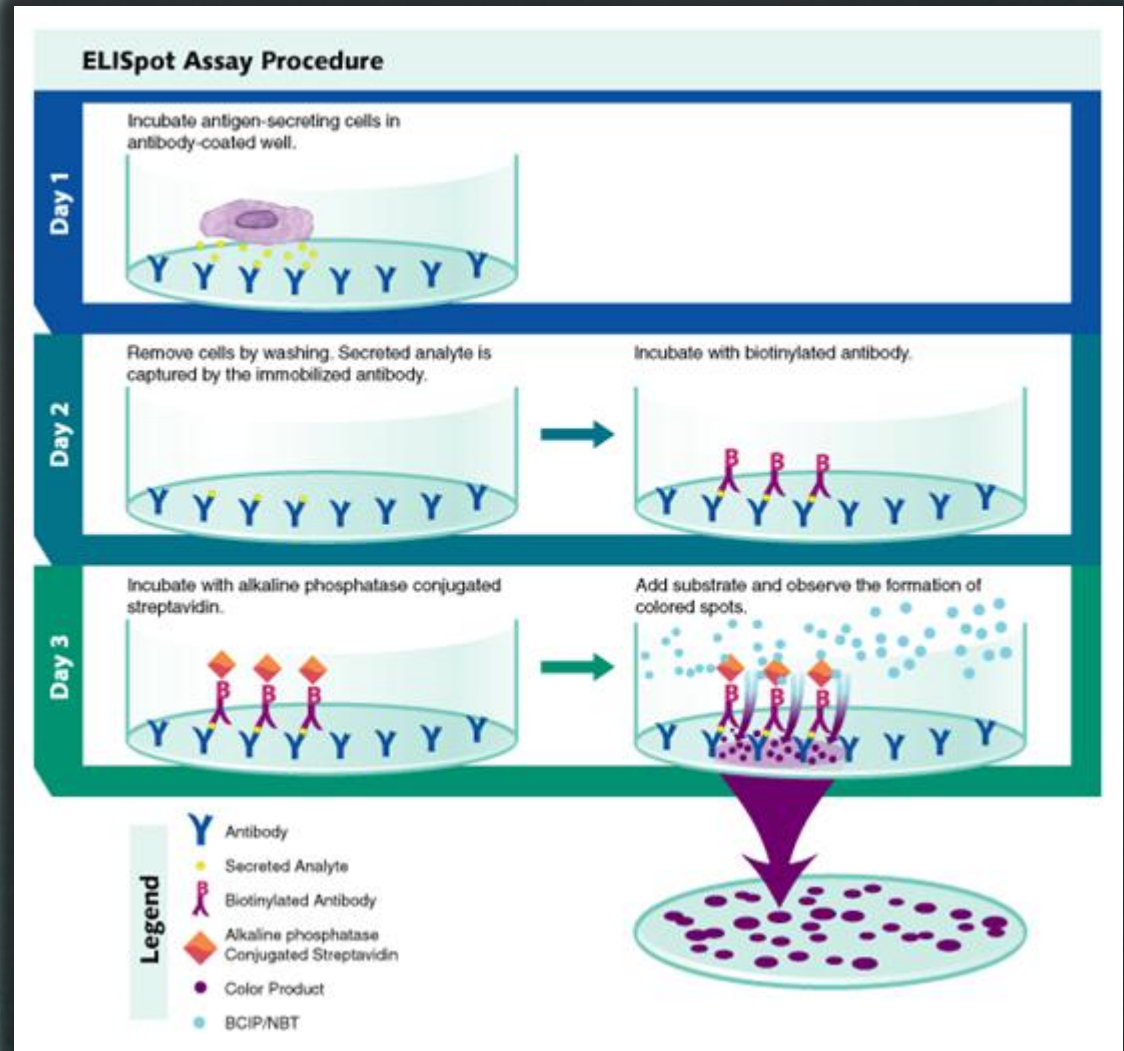
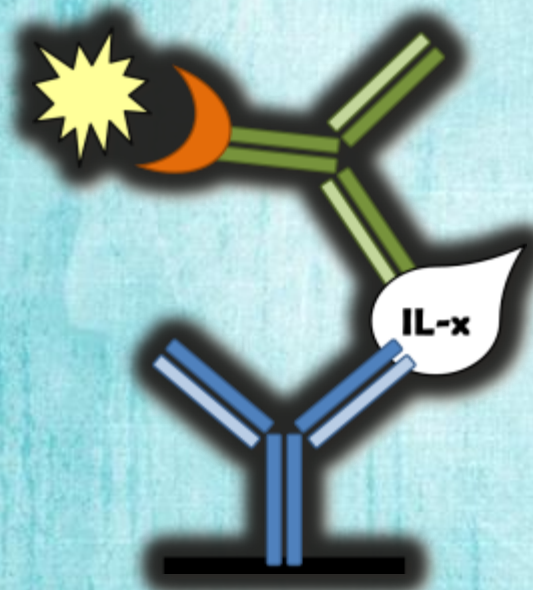


EVALUANDO LA PROLIFERACIÓN

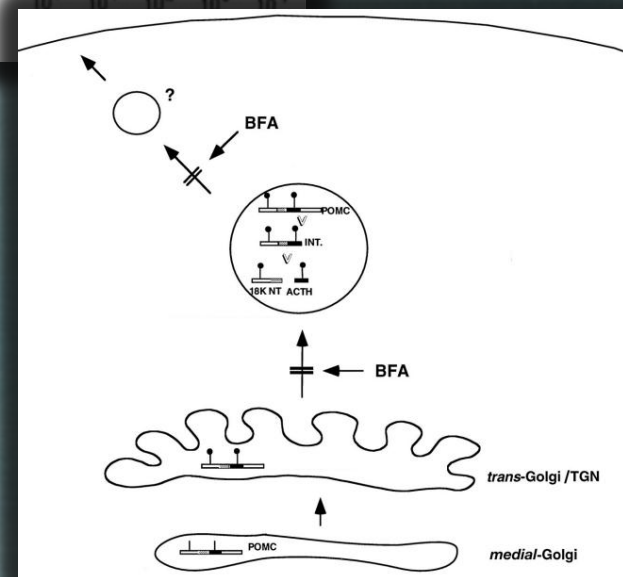
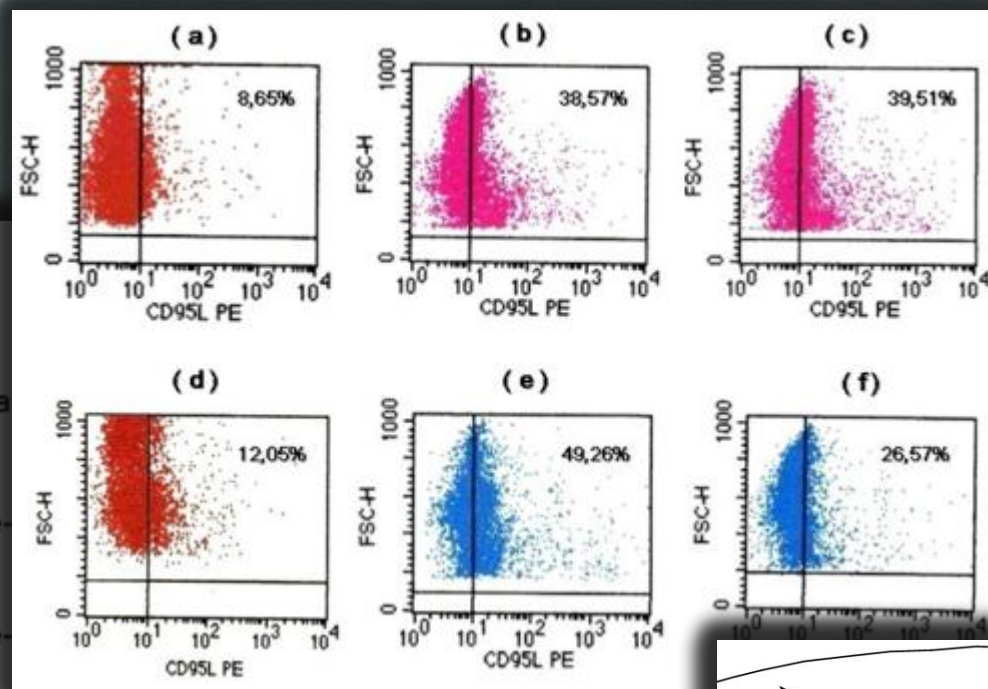
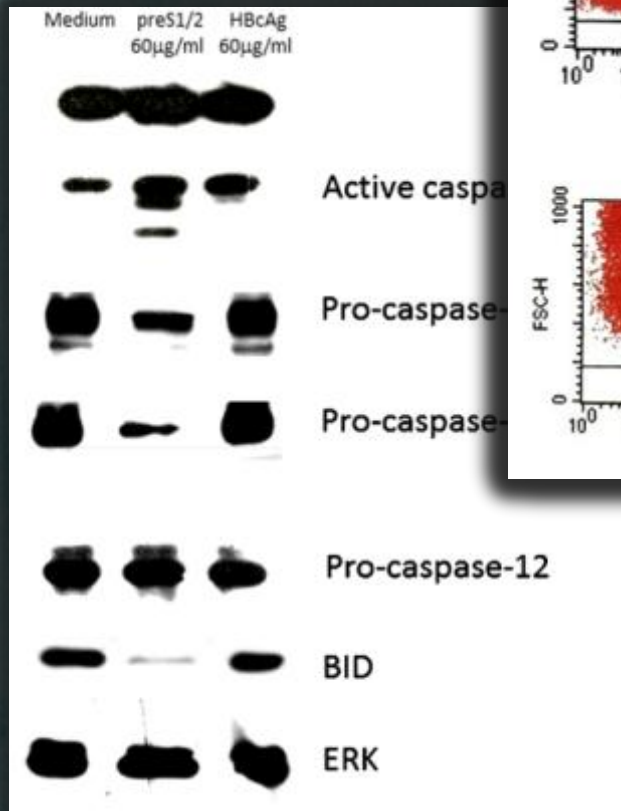


TIA
BrdU
CFSE
PI

PRODUCCIÓN DE CITOCINAS

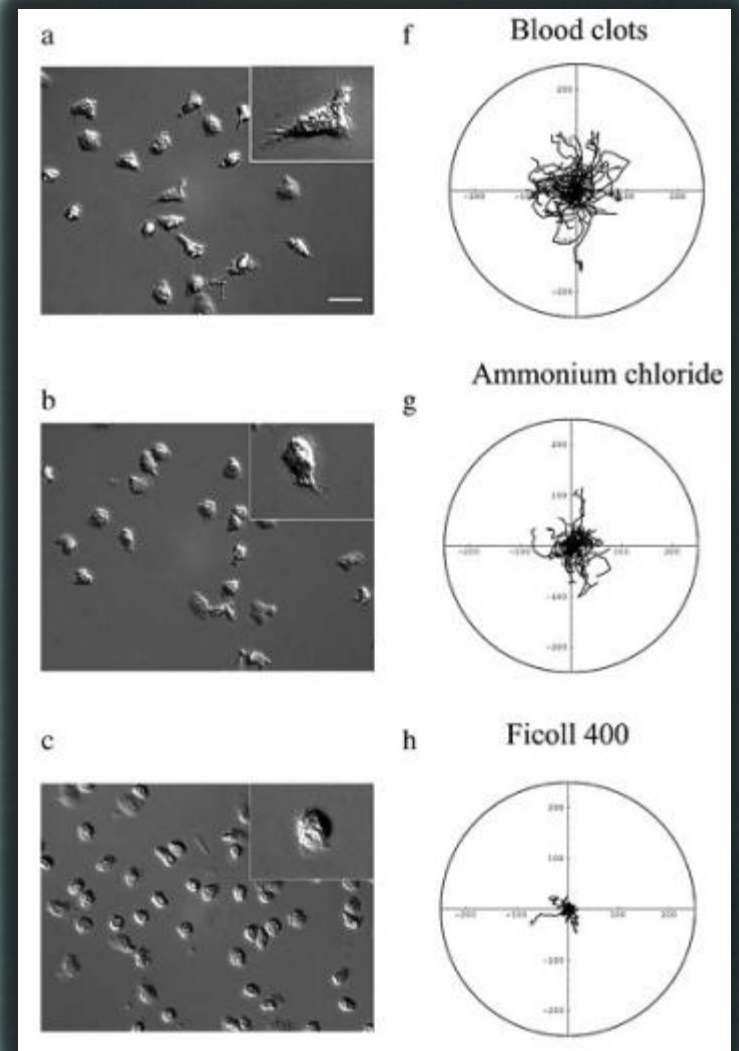
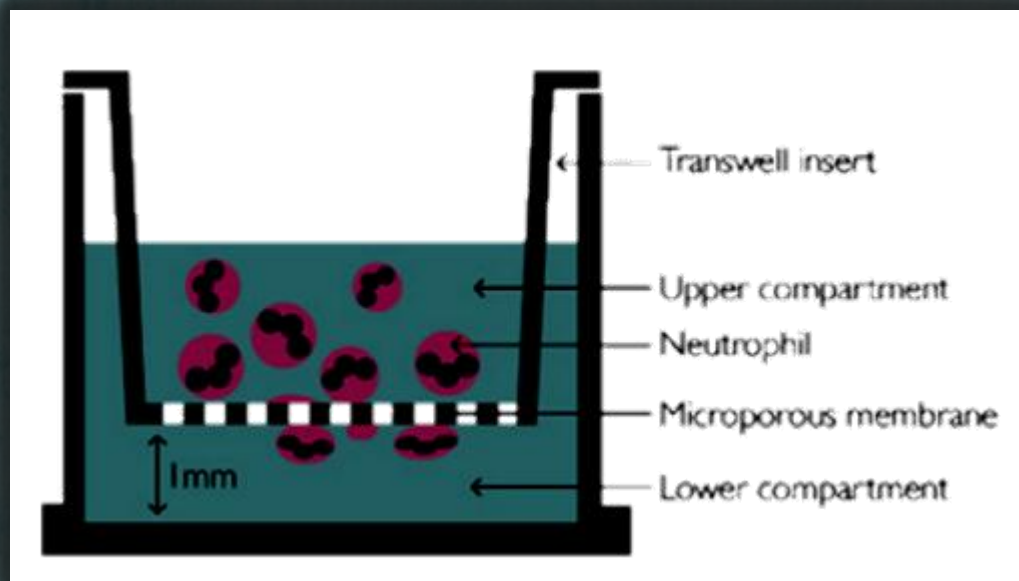


MOLÉCULAS INTRA Y EXTRA

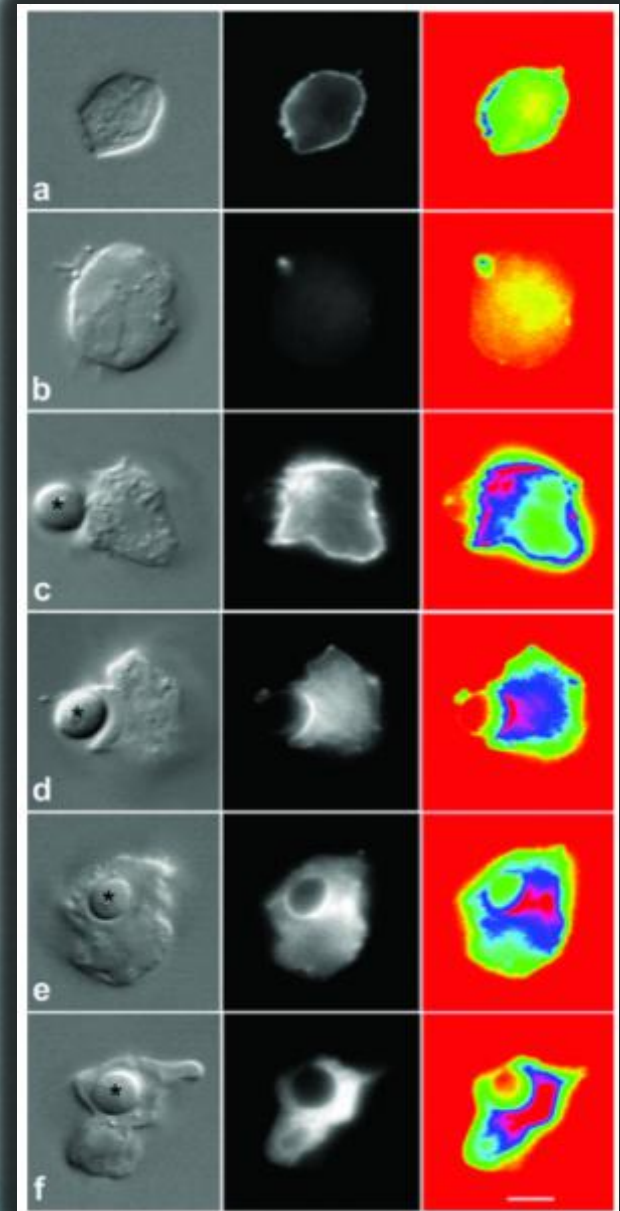
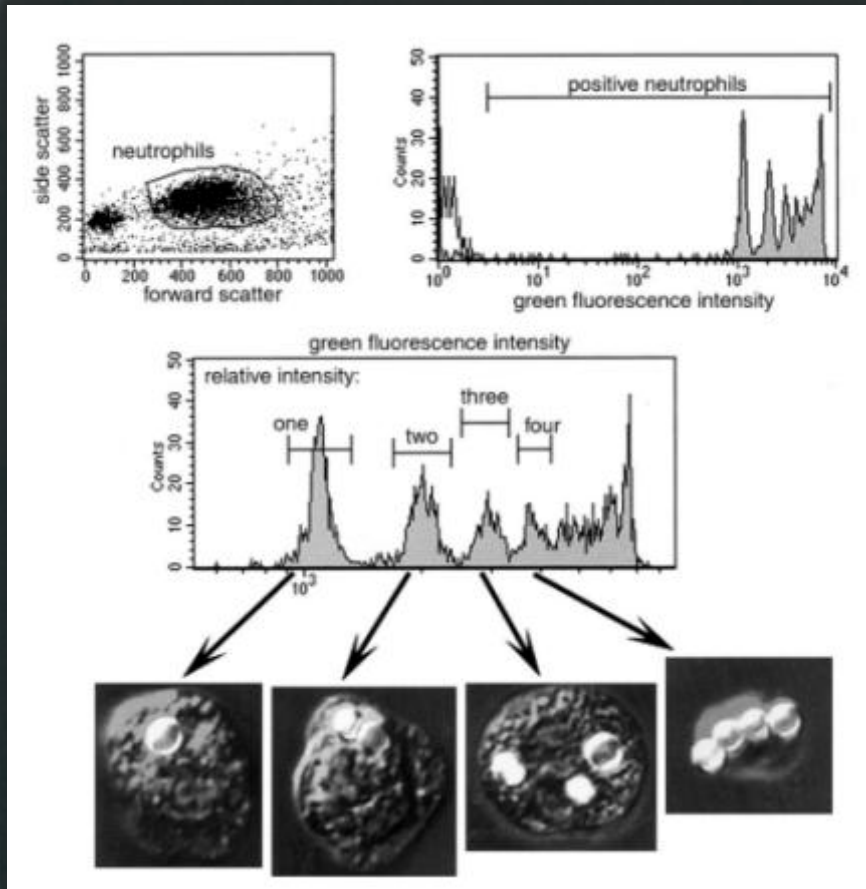


Journal of Medical Virology 86:496–504 (2014),
Guillermo Terán Angel ULA 2001

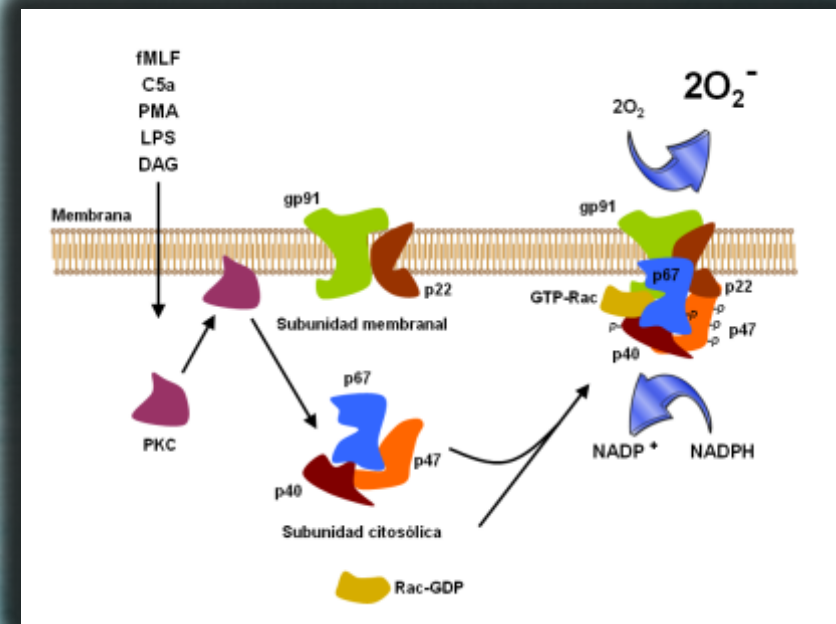
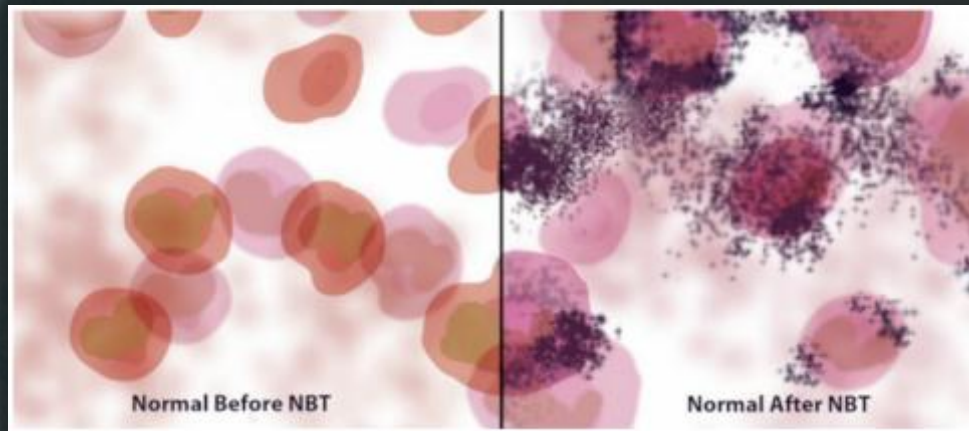
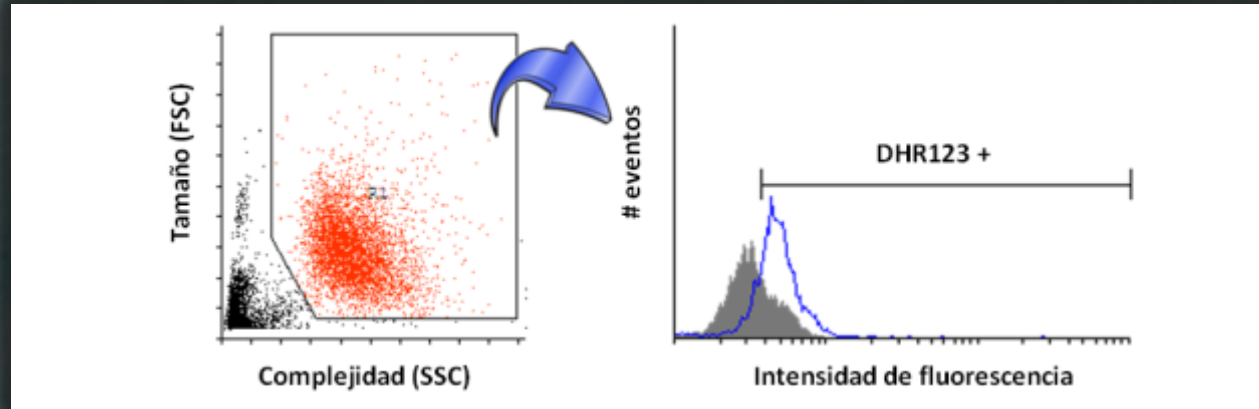
MOVILIDAD Y QUIMIOTAXIS



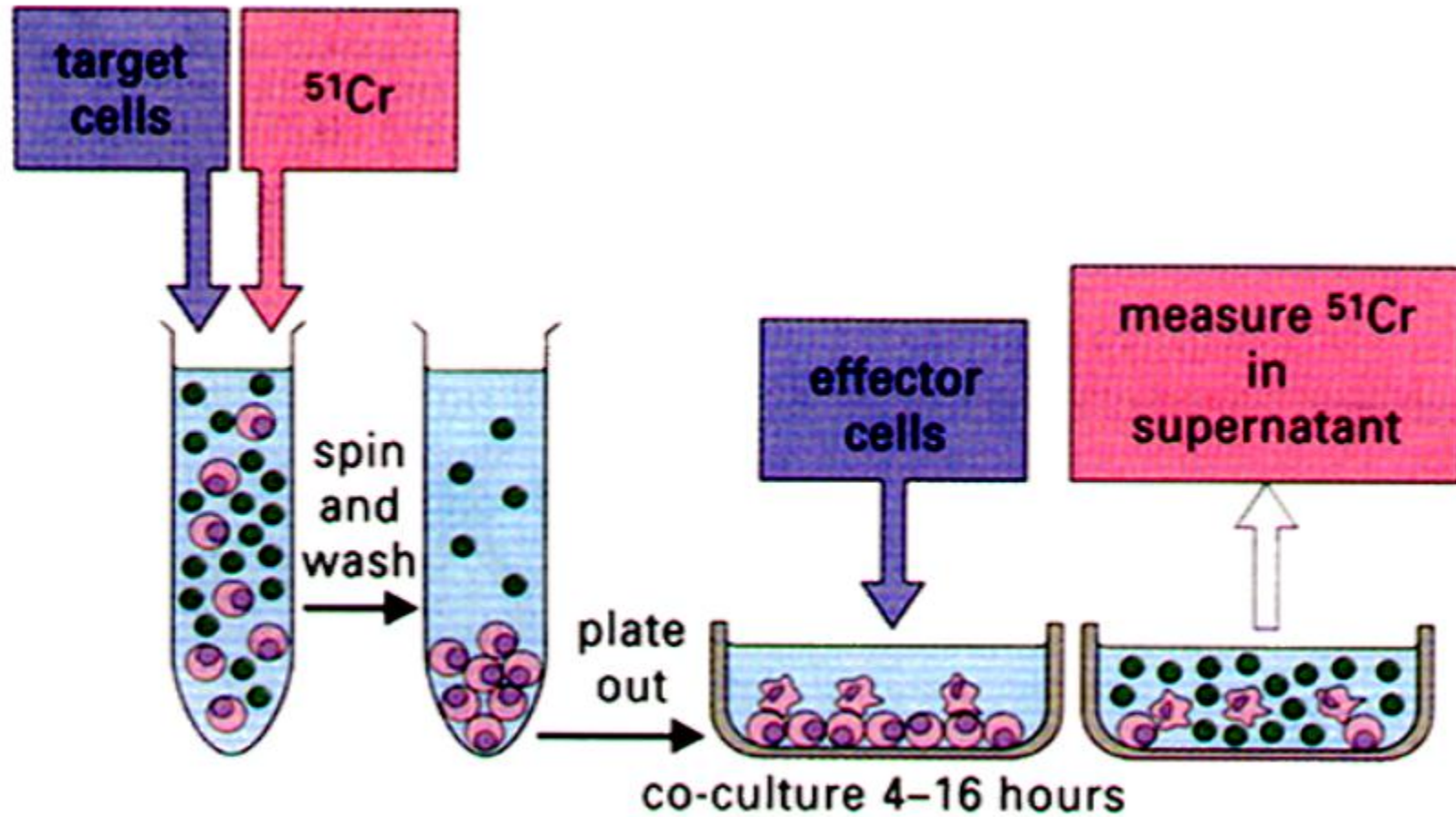
EVALUACIÓN DE CÉLULAS FAGOCÍTICAS



ESTRÉS OXIDATIVO



CÉLULAS CITOTÓXICAS



¡Pregunten Ahora o Callen Para Siempre!



Guillermo Teran-Angel
guillermondi@gmail.com
<http://guillermo.vv.si>