



MÉTODOS PARA EVALUAR LA RESPUESTA INMUNE

**GUILLERMO
TERÁN
ANGEL**

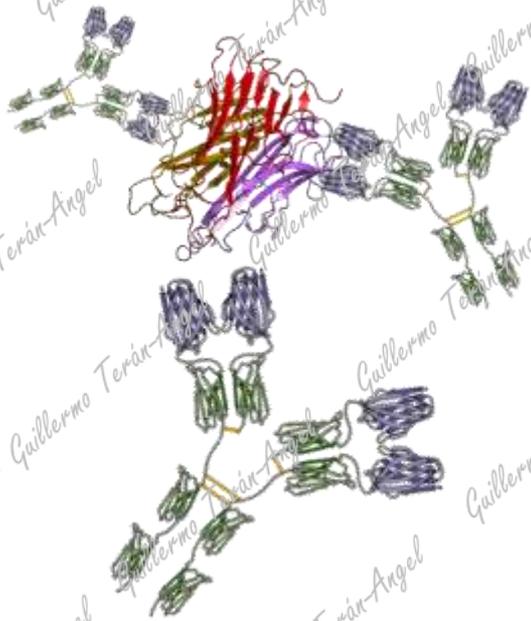
**IDIC
ULA**

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



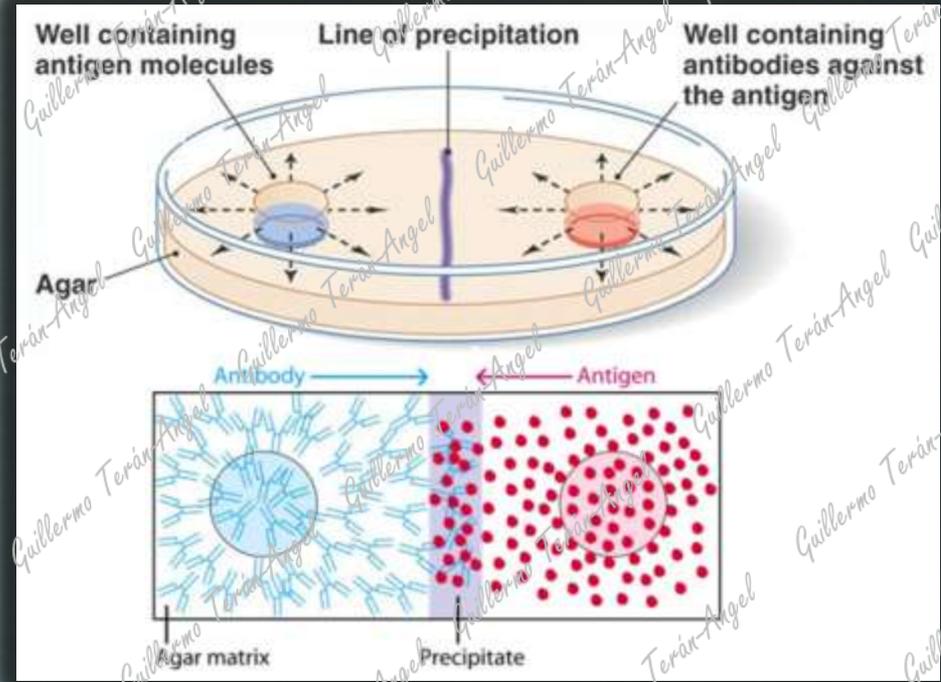
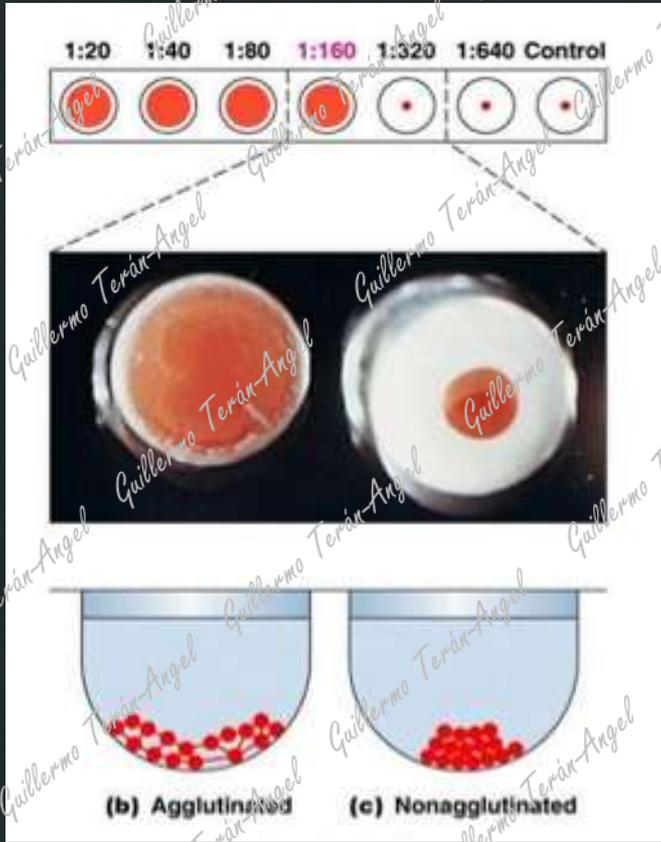
**Formación de
inmunocomplejos
(antígeno/anticuerpo)**

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 inmunoenzimático (EIA):**
El marcador es una **enzima**.
- **Fluoroimmunoanálisis:**
El marcador es una partícula **fluorescente**.
- **Precipitación**
- **Aglutinación**

RAPIDITO: LOS NO MARCADOS

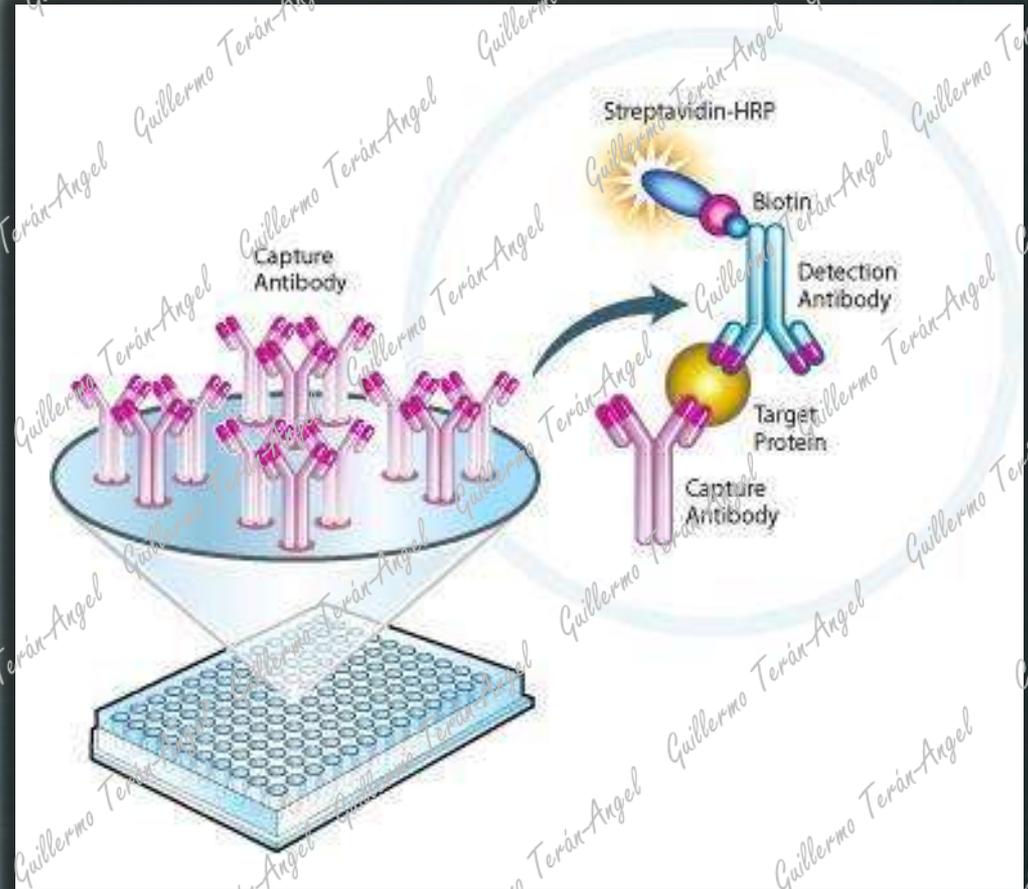


ANÁLISIS INMUNOENZIMÁTICO

Ensayo de Inmunoabsorción 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

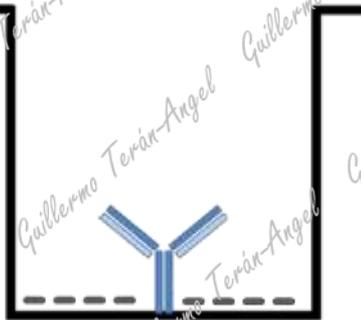


Reacción enzimática

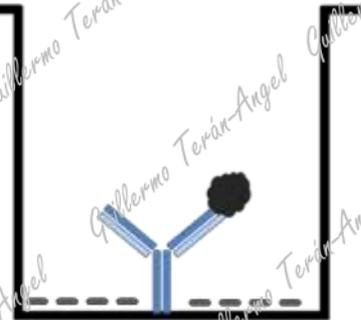


Lectura

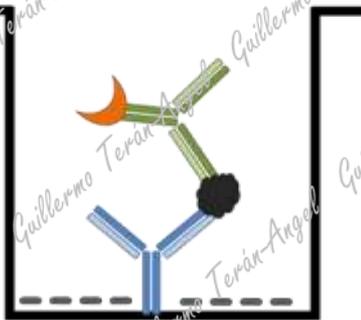
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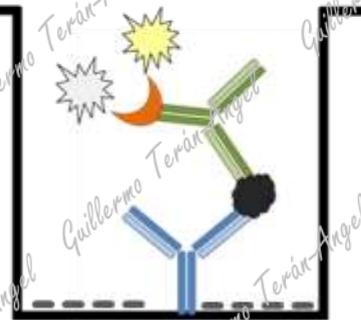
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3



4

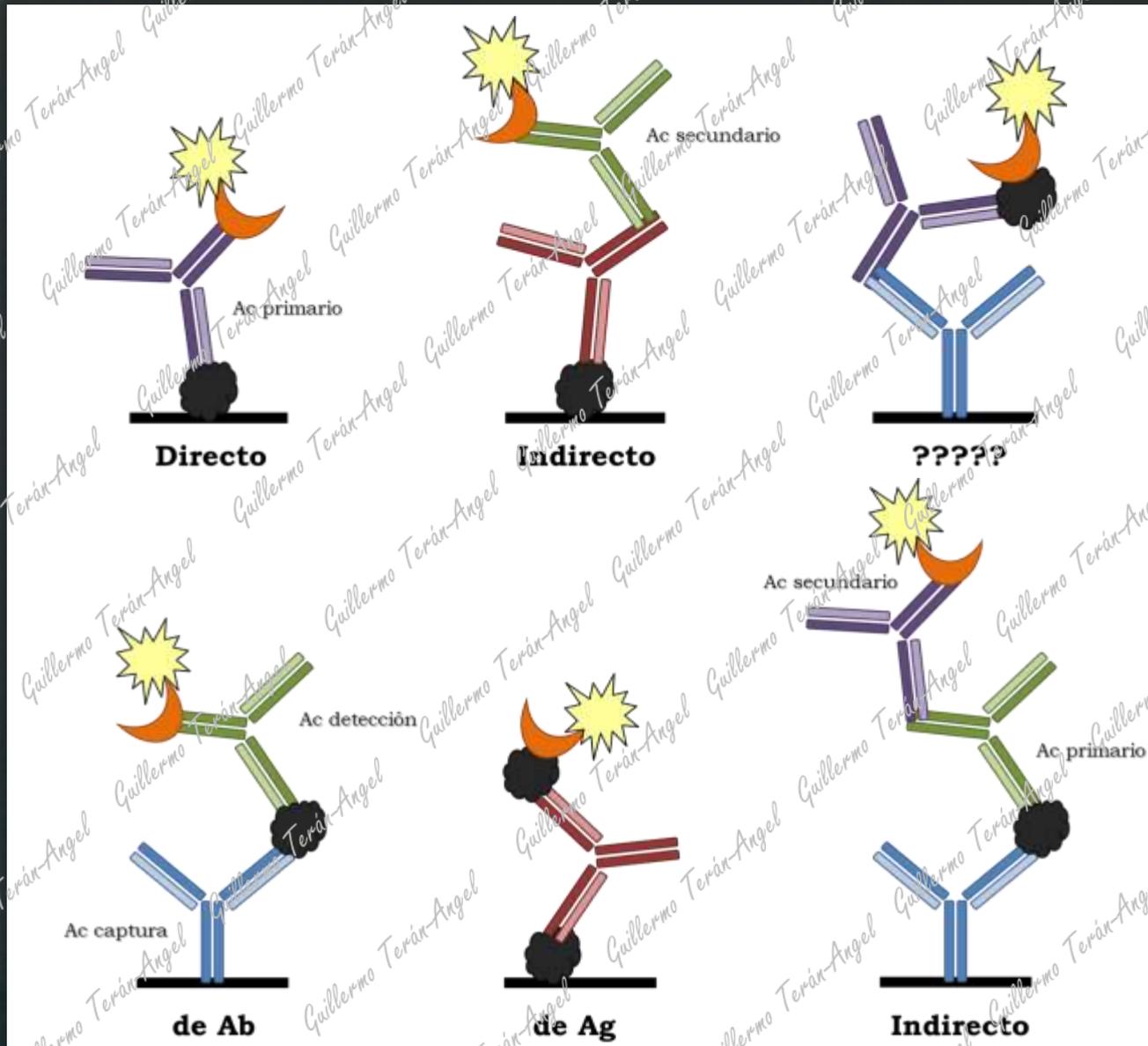


Bloqueos

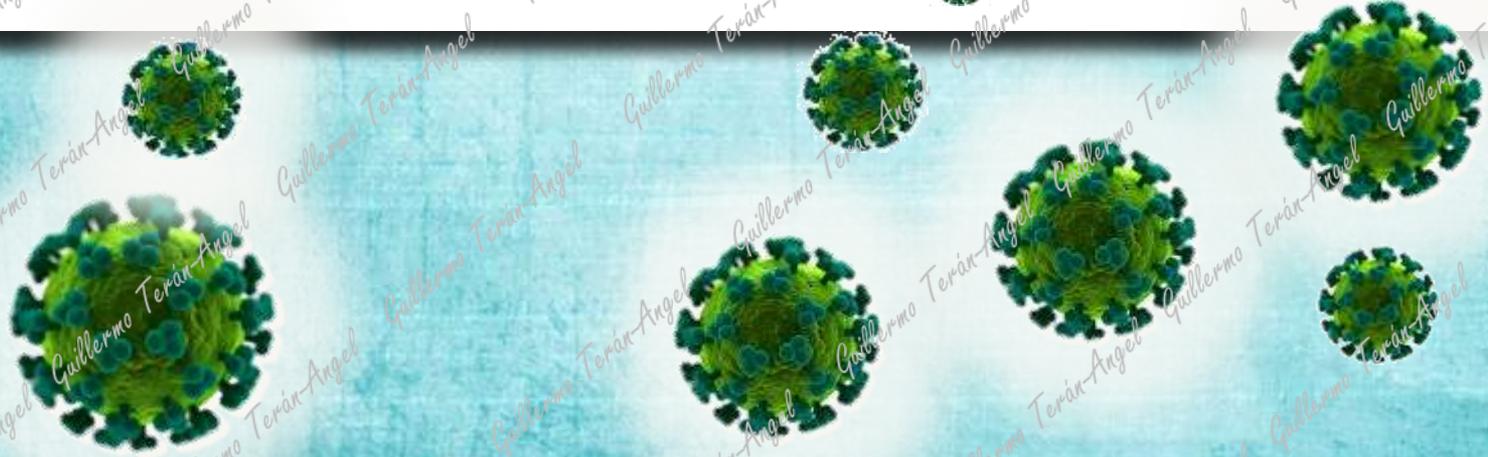
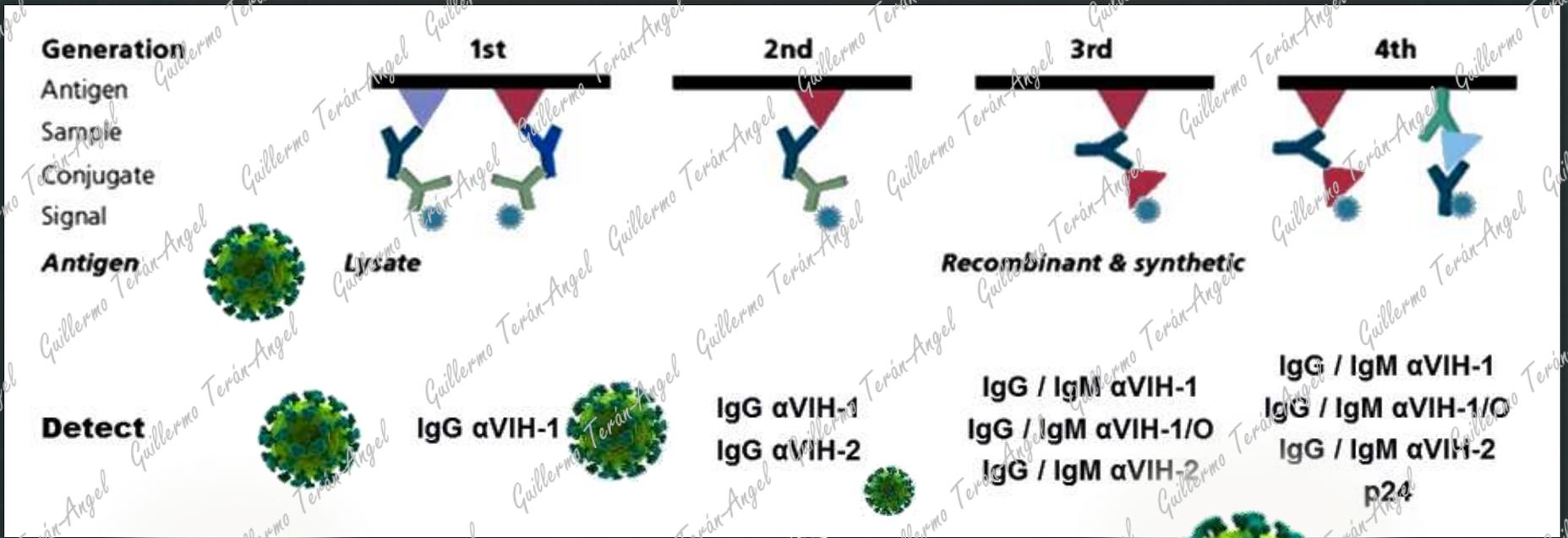


Lavados

TIPOS O VARIANTES DEL ELISA

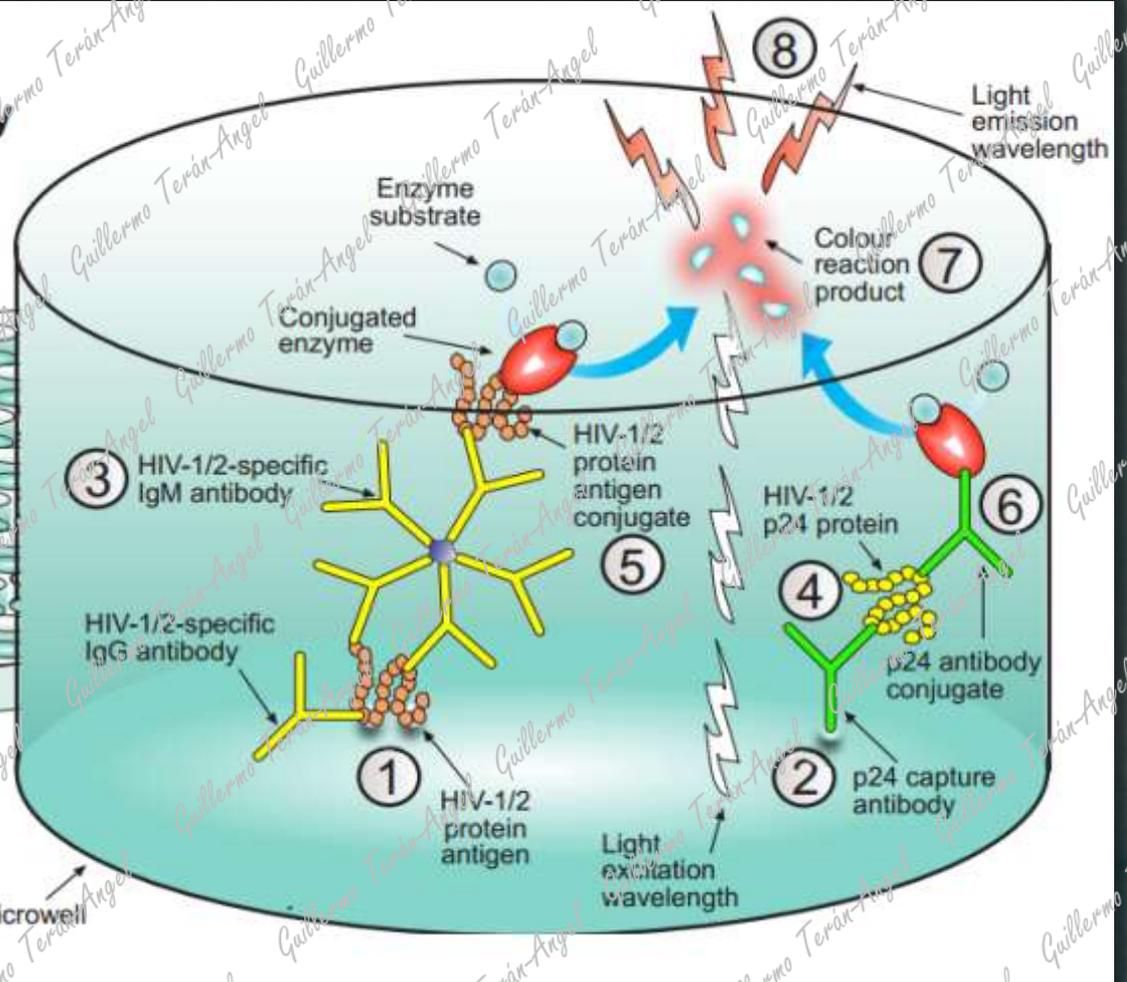
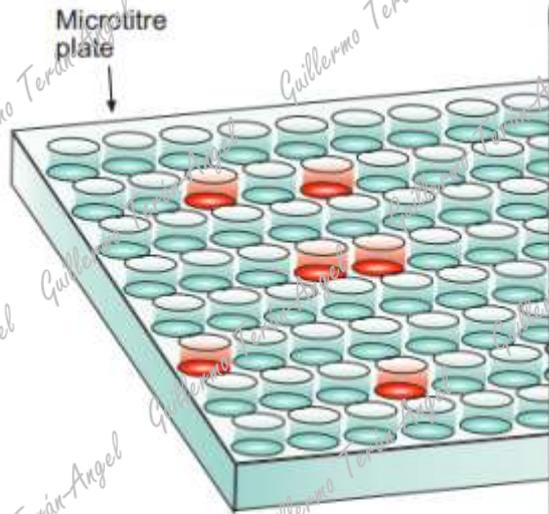


EL PROTOTIPO: ELISA PARA VIH

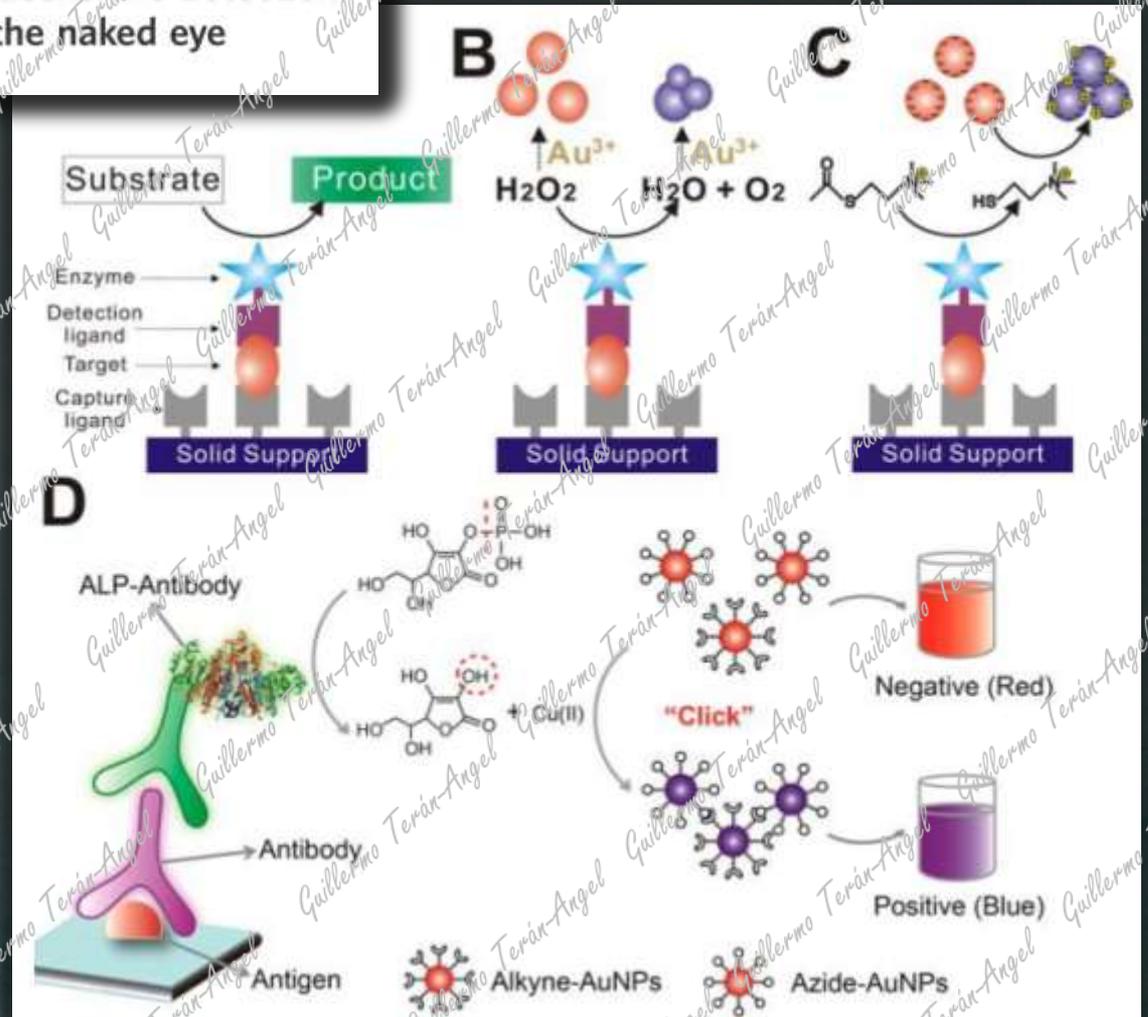
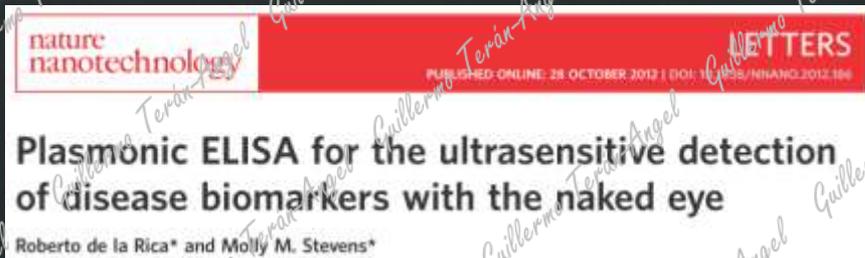


ELISA "DUAL"

4th generation HIV antibody and p24 ELISA (Combo)

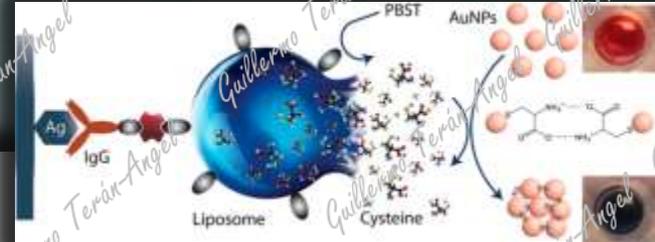
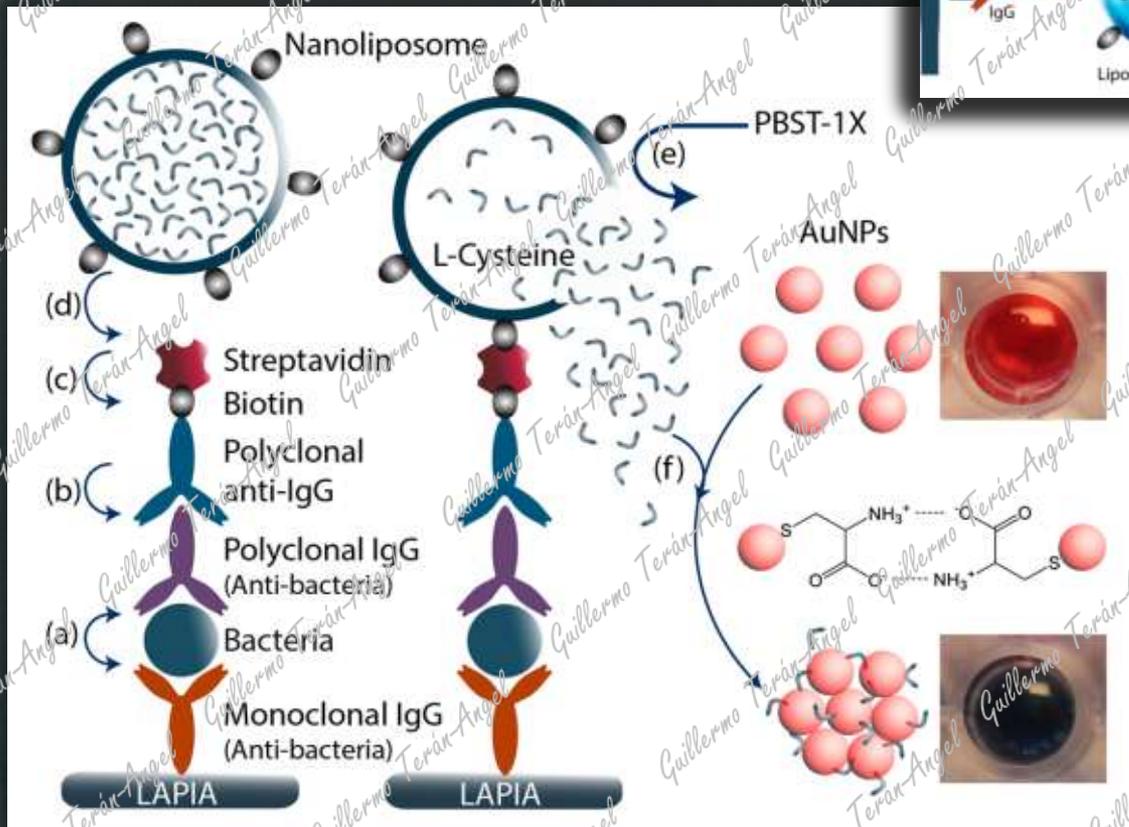


ELISA DE PLASMONES

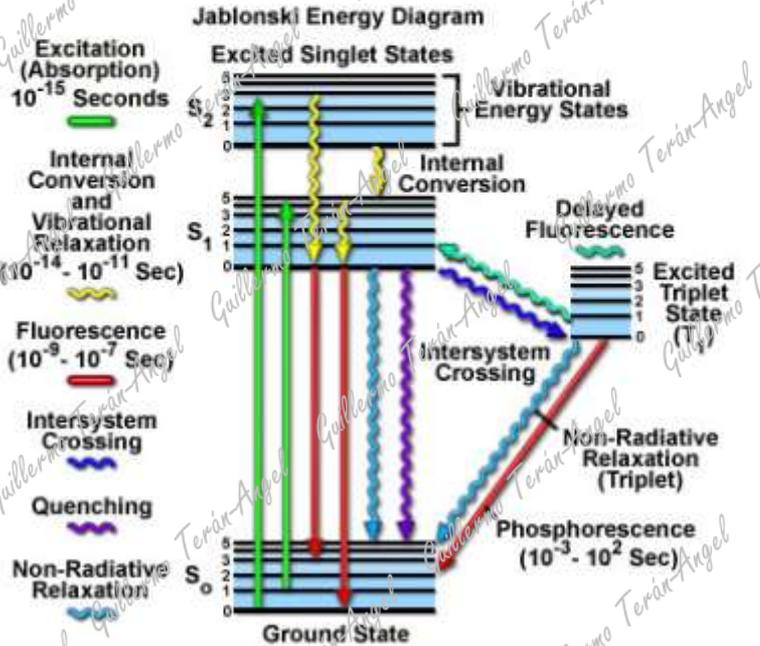
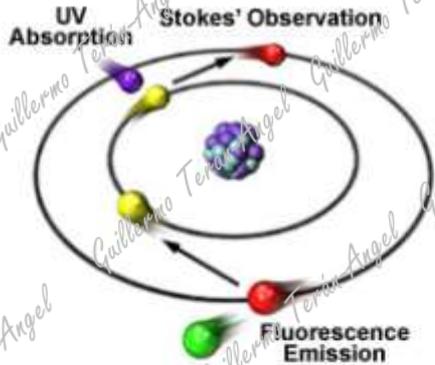


Analyst, 2016,141, 3473–3481

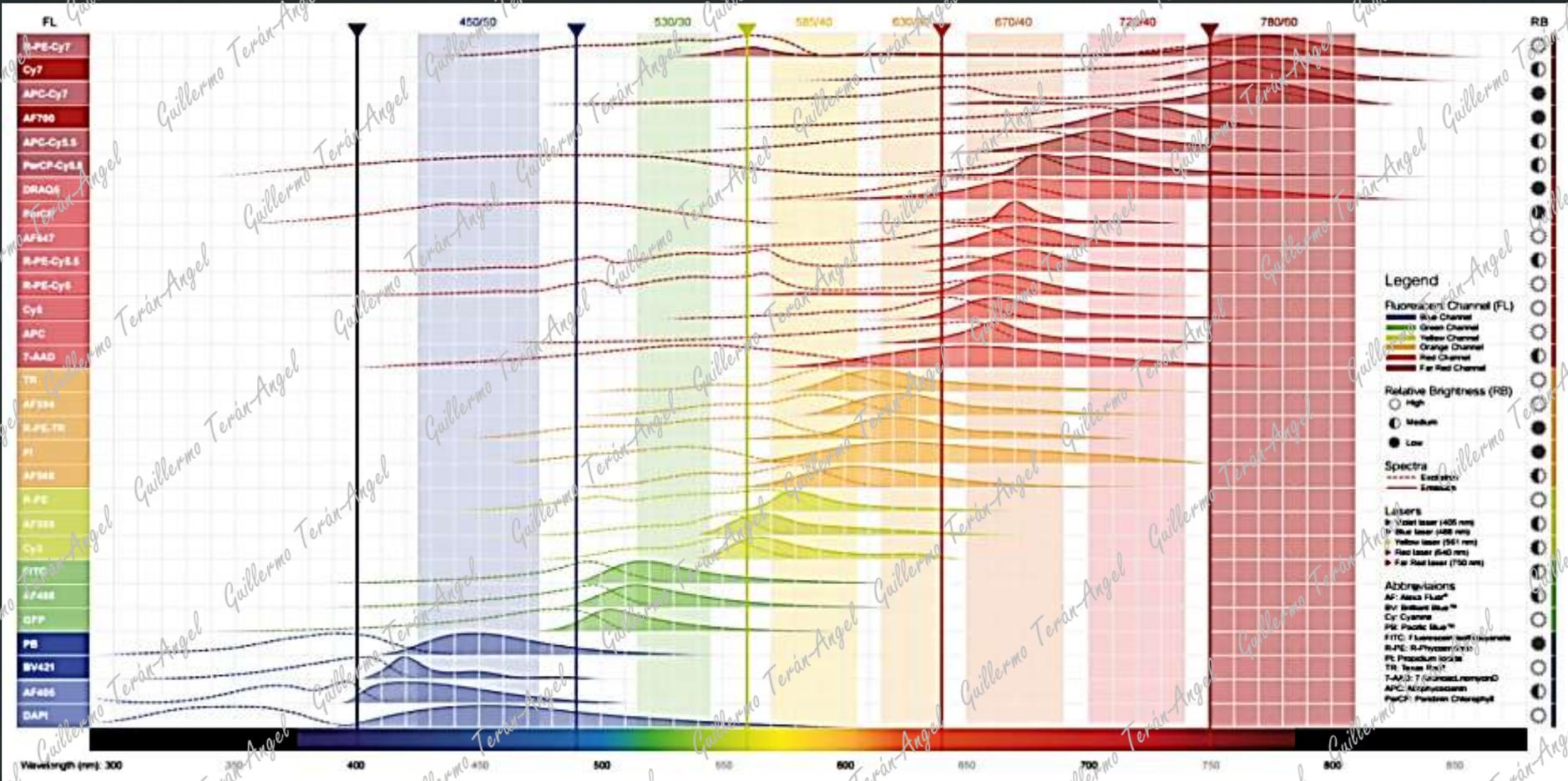
ELISA DE PLASMONES Y LIPOSOMAS



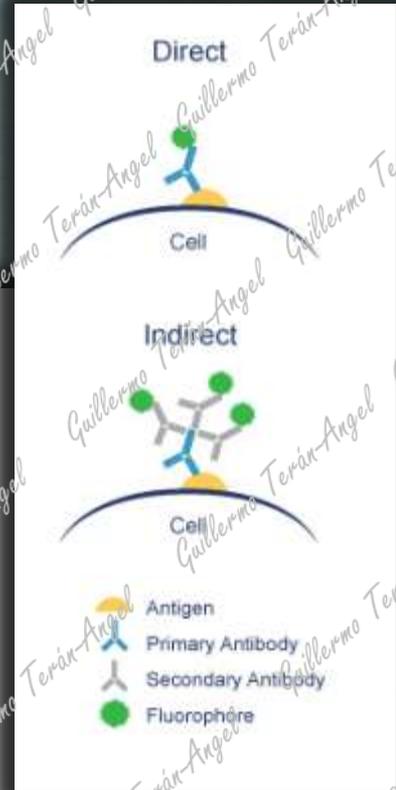
FLUORESCENCIA



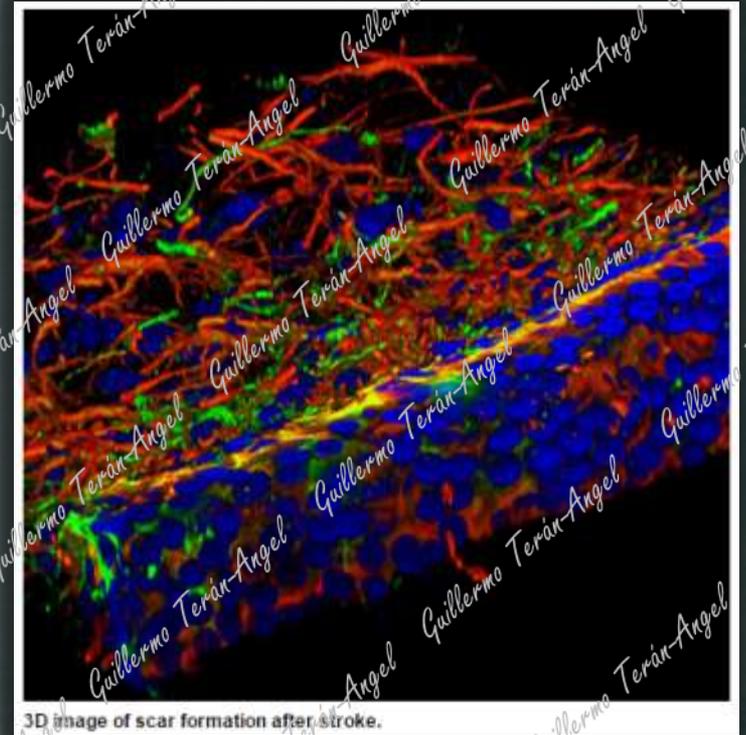
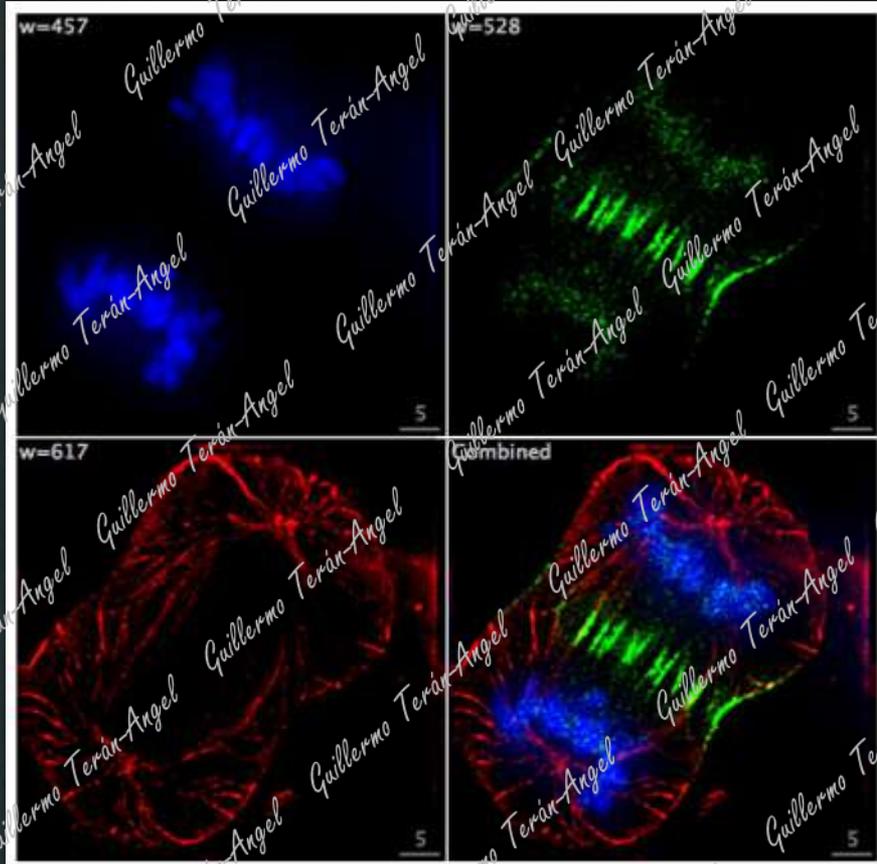
FLUOROCROMOS



INMUNOFLORESCENCIA



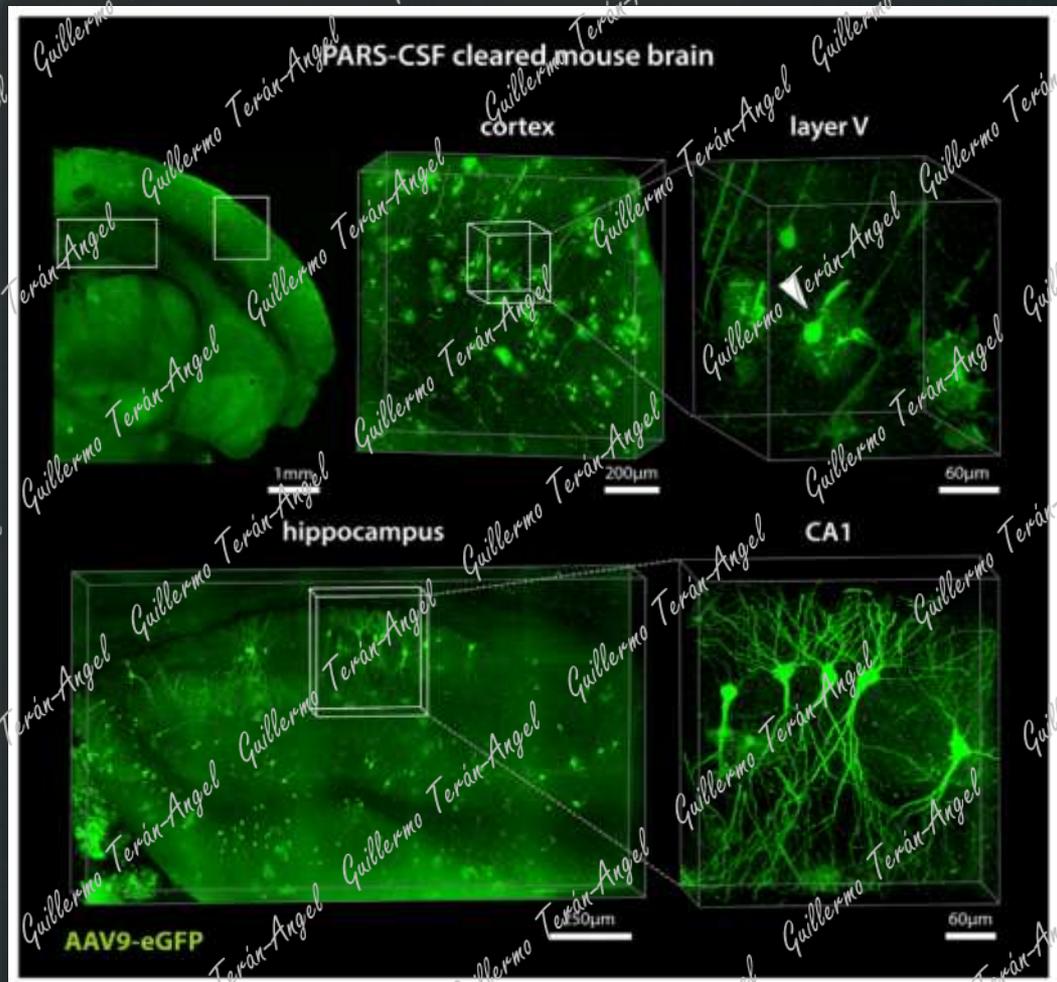
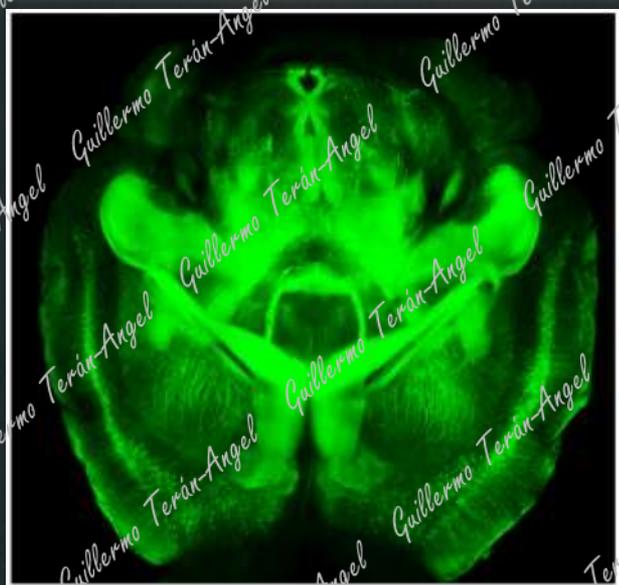
MICROSCOPIA DE FLUORESCENCIA



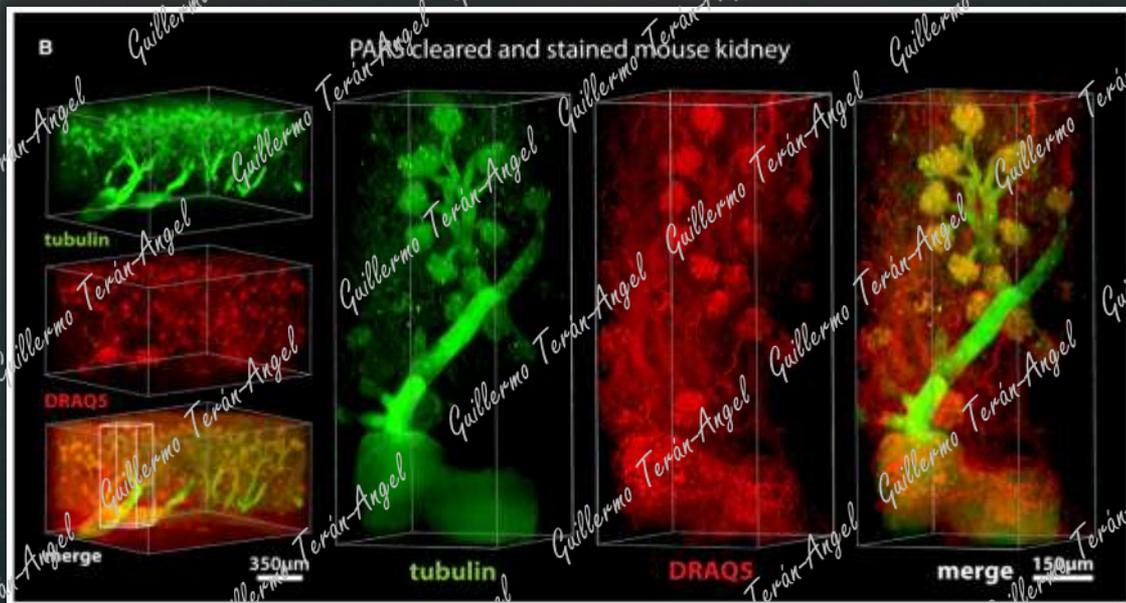
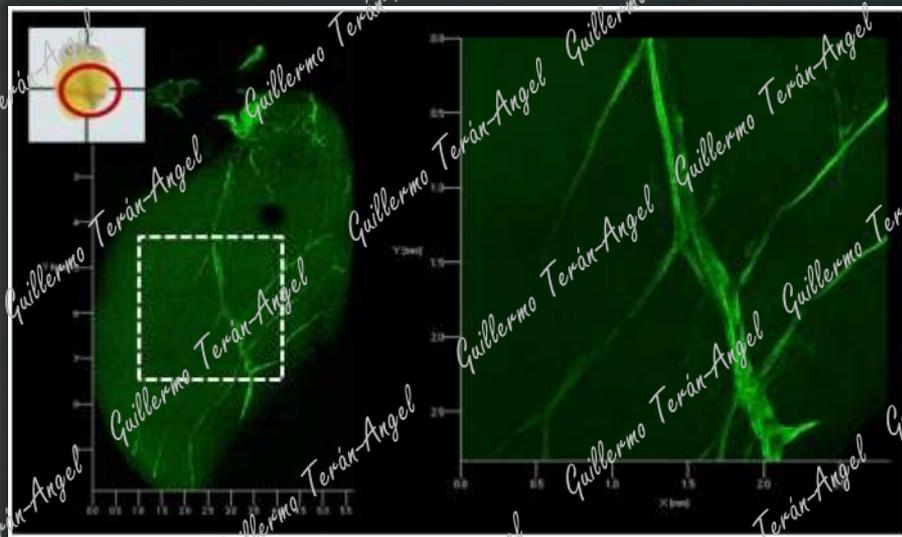
EPIFLUORESCENCIA VERSUS CONFOCAL



CLARITY

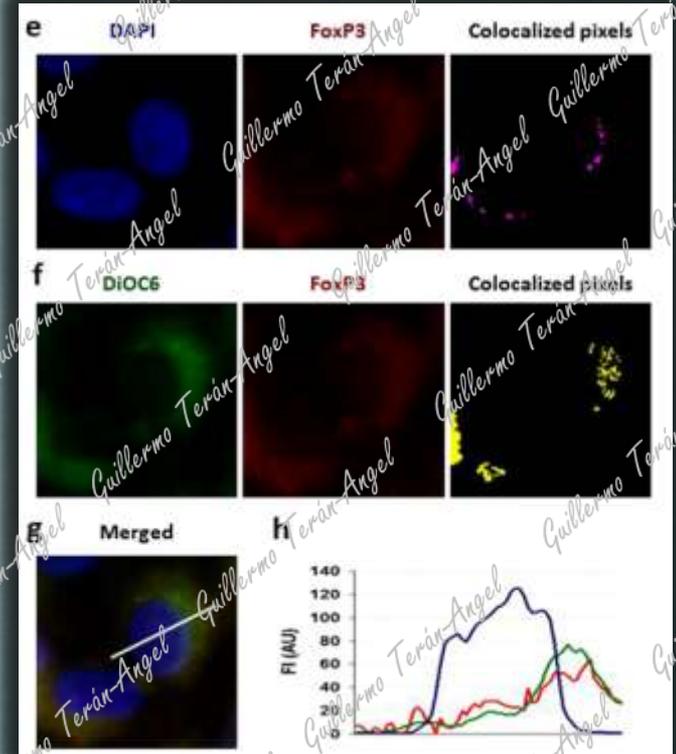
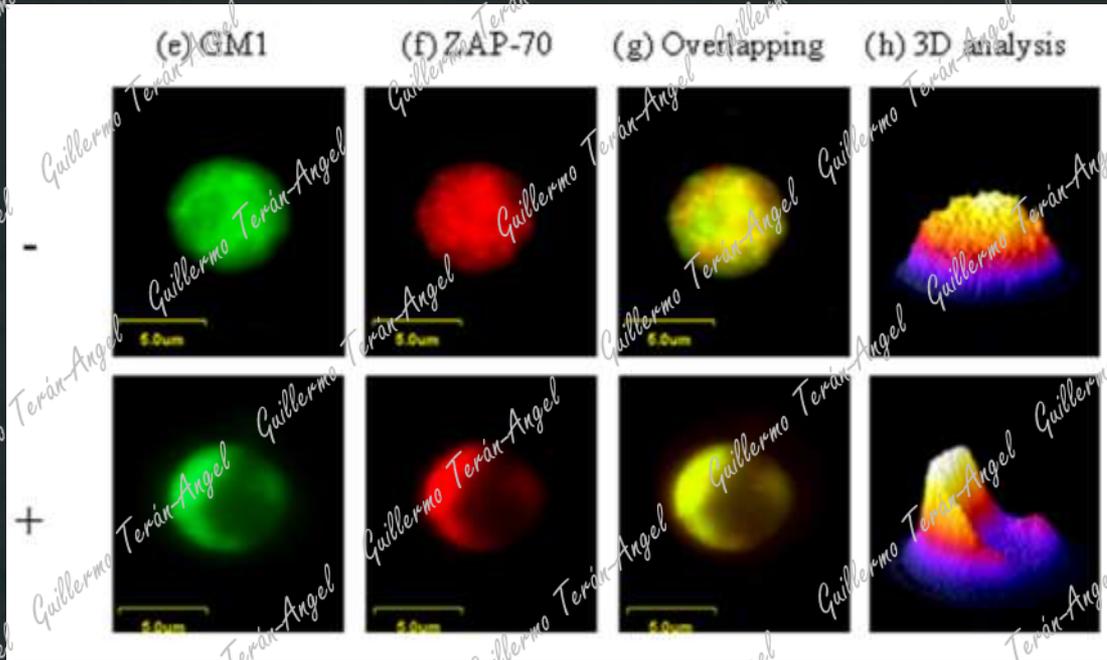


CLARITY

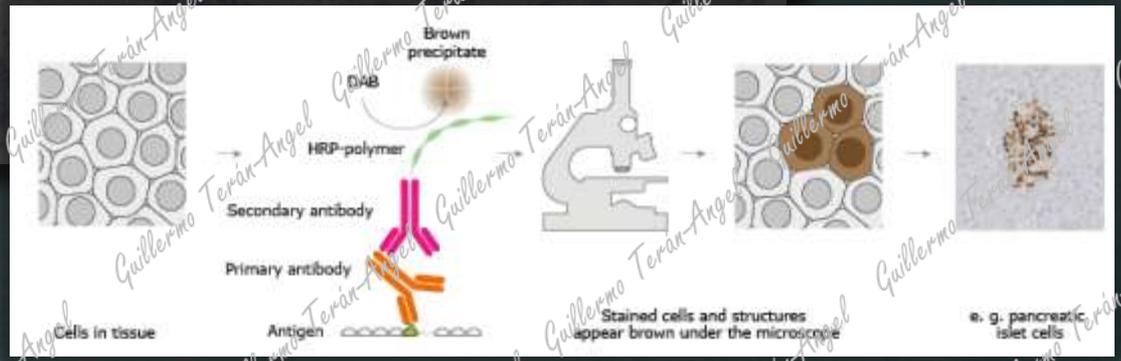
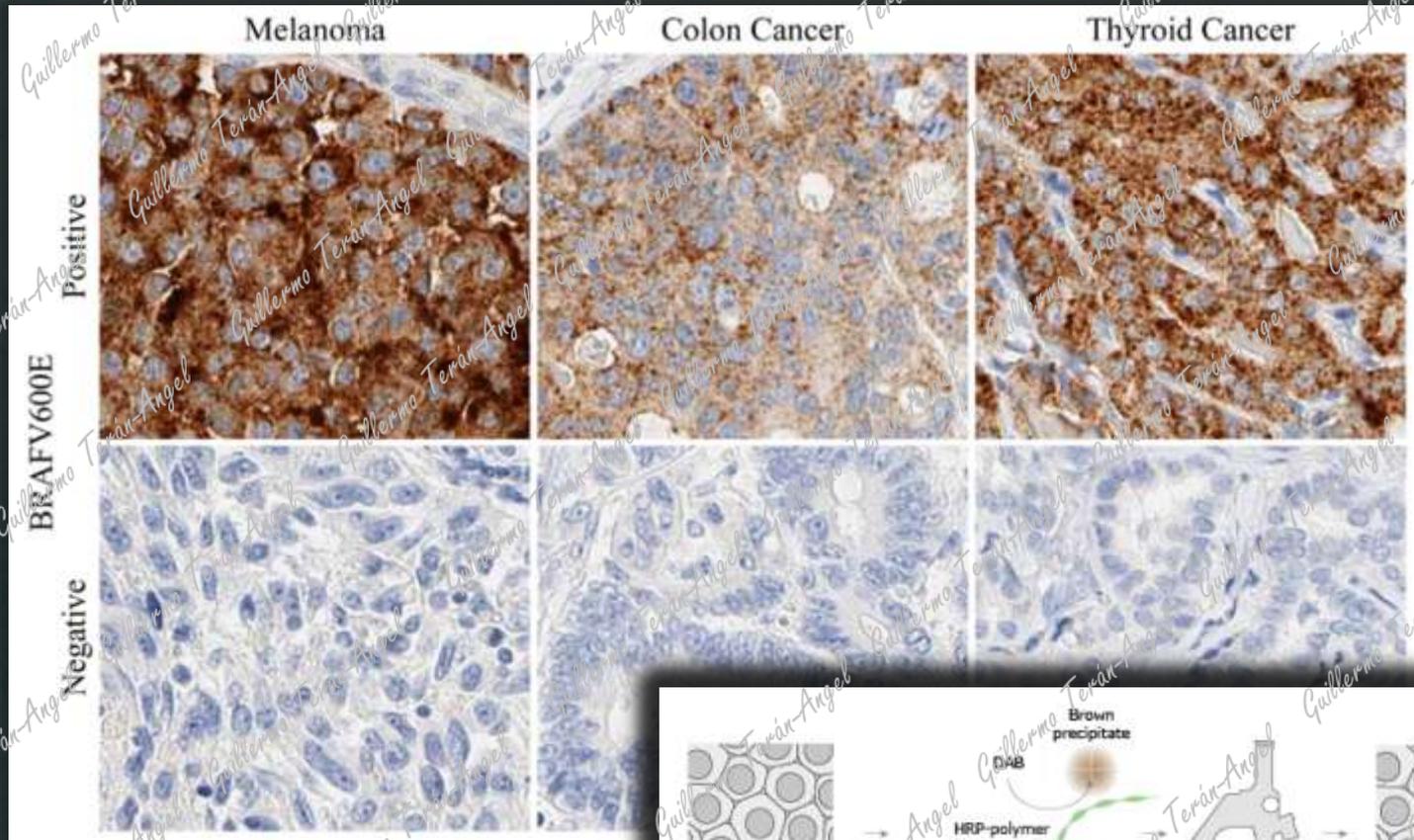


INMUNOFLUORESCENCIA

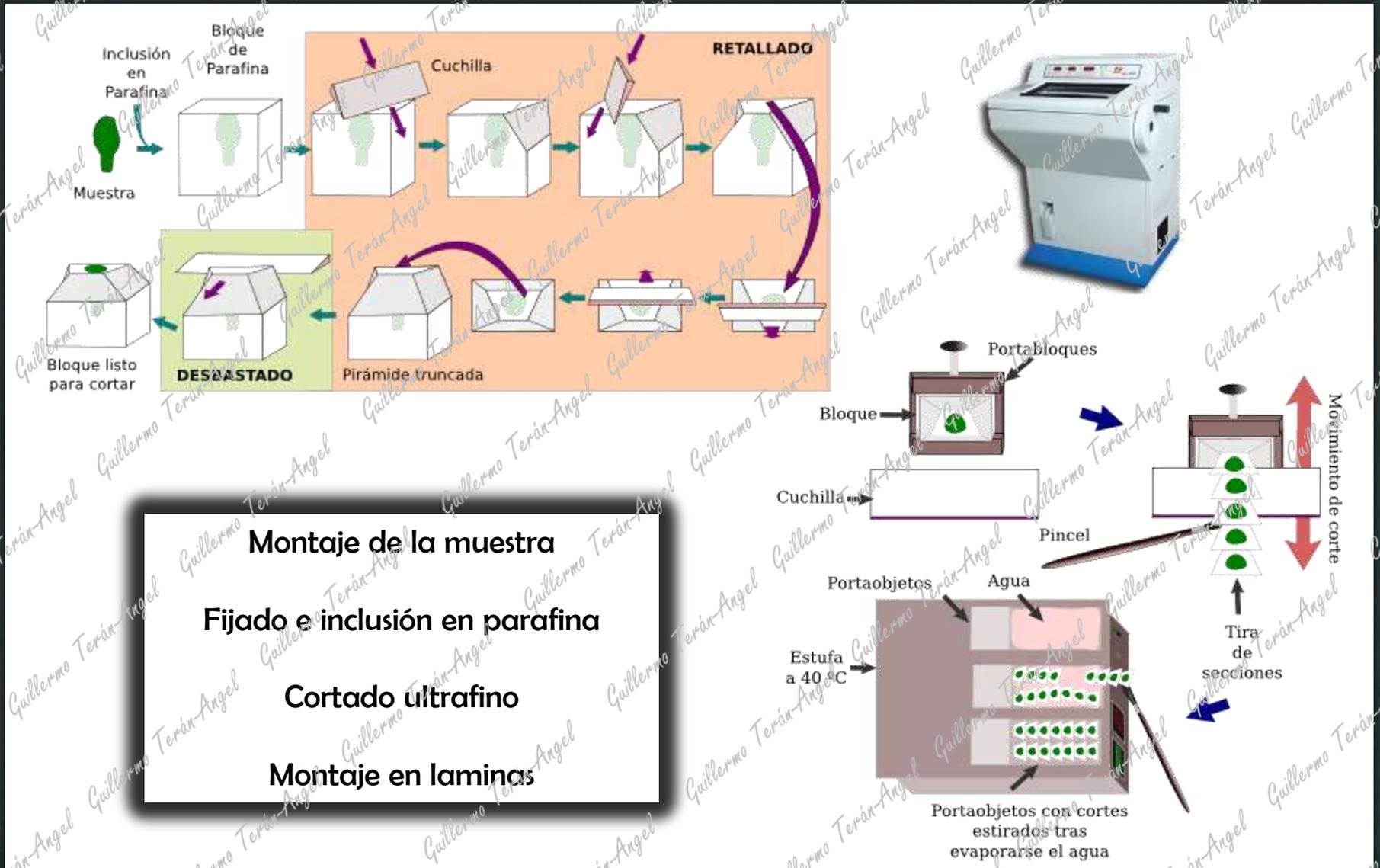
Aprovechando software ante las carencias



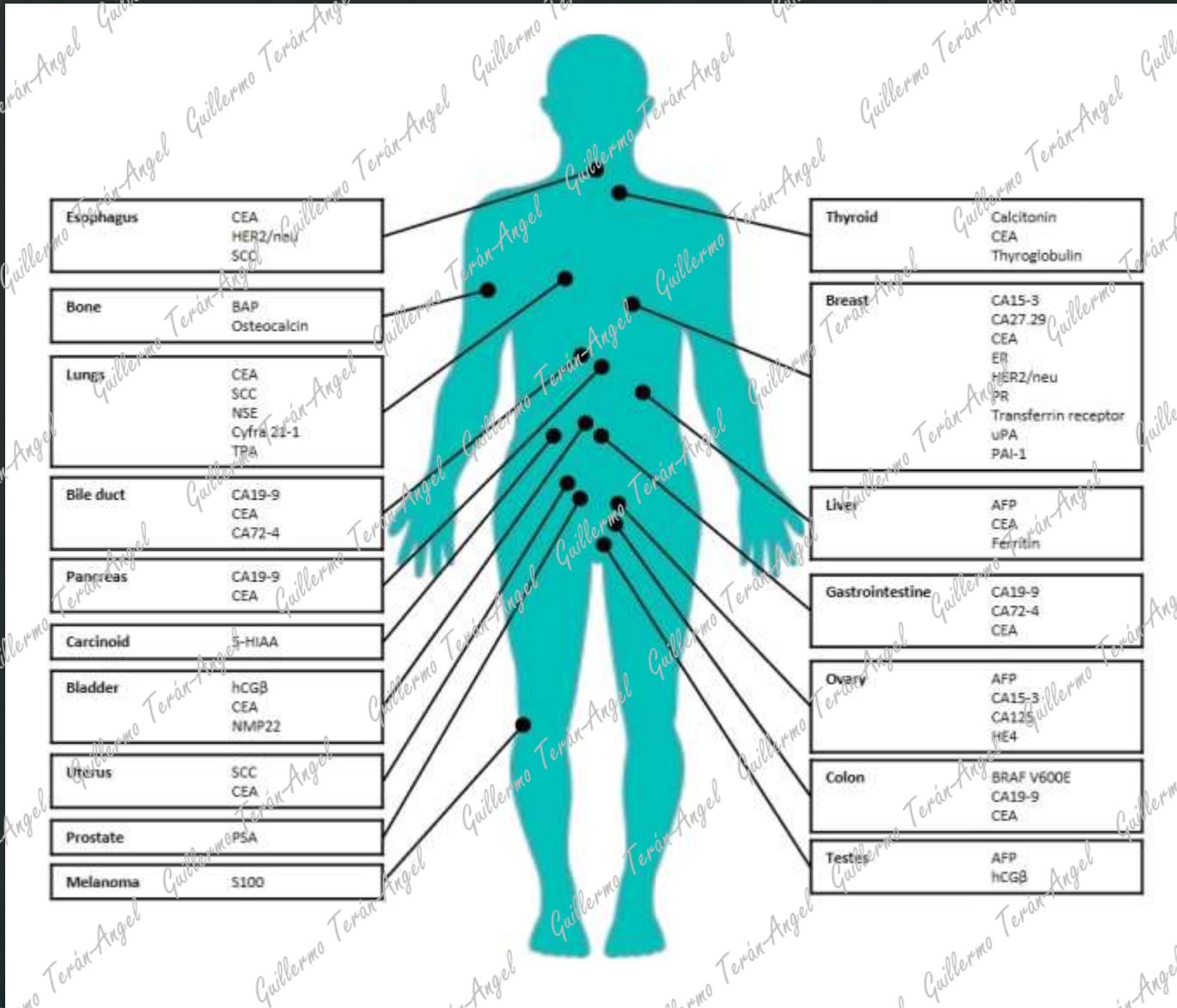
UN PARENTESIS: INMUNOHISTOQUÍMICA



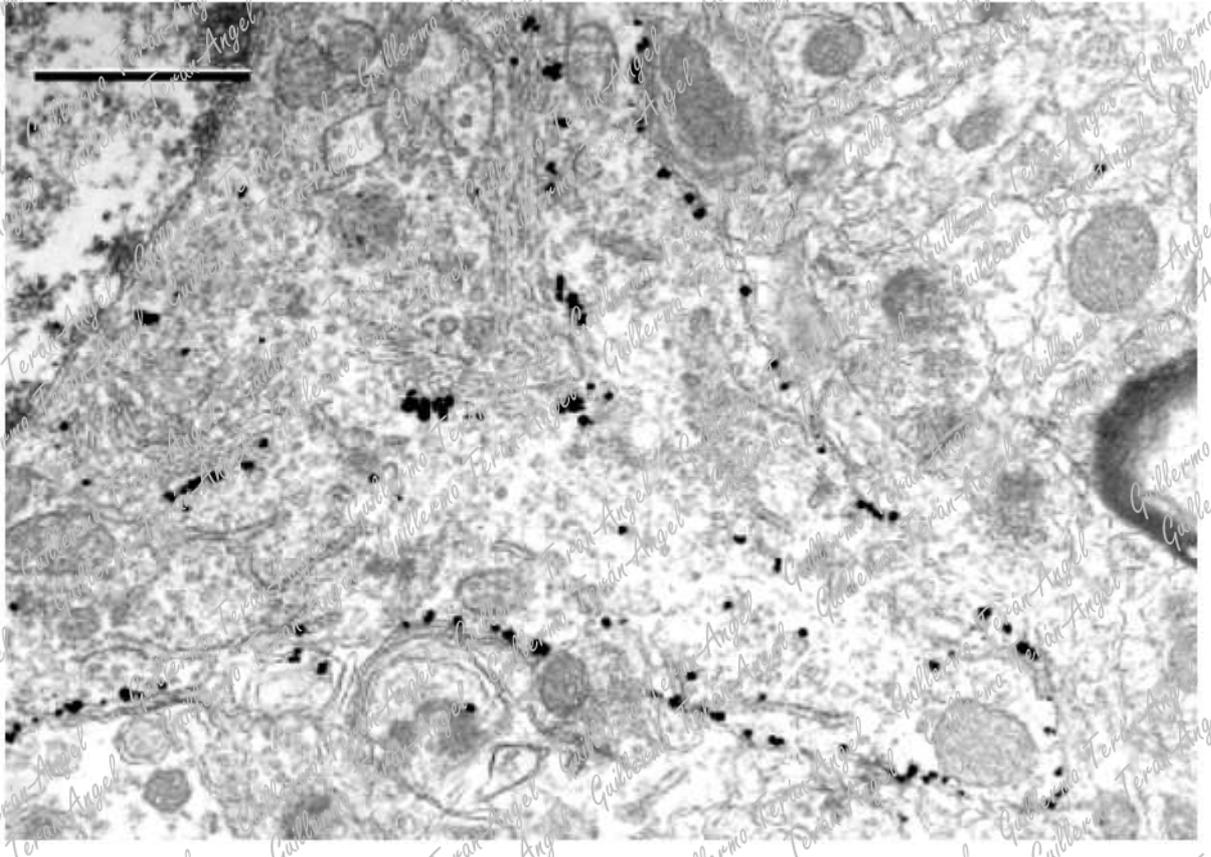
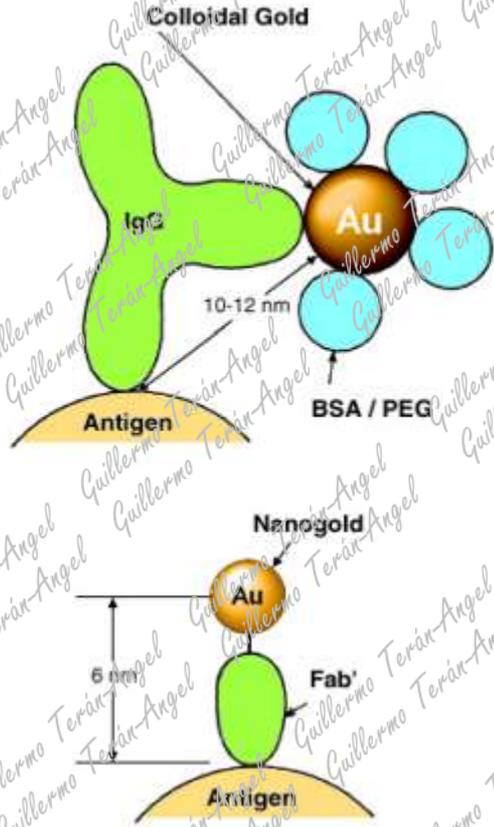
INMUNOHISTOQUÍMICA, BIOPSIAS



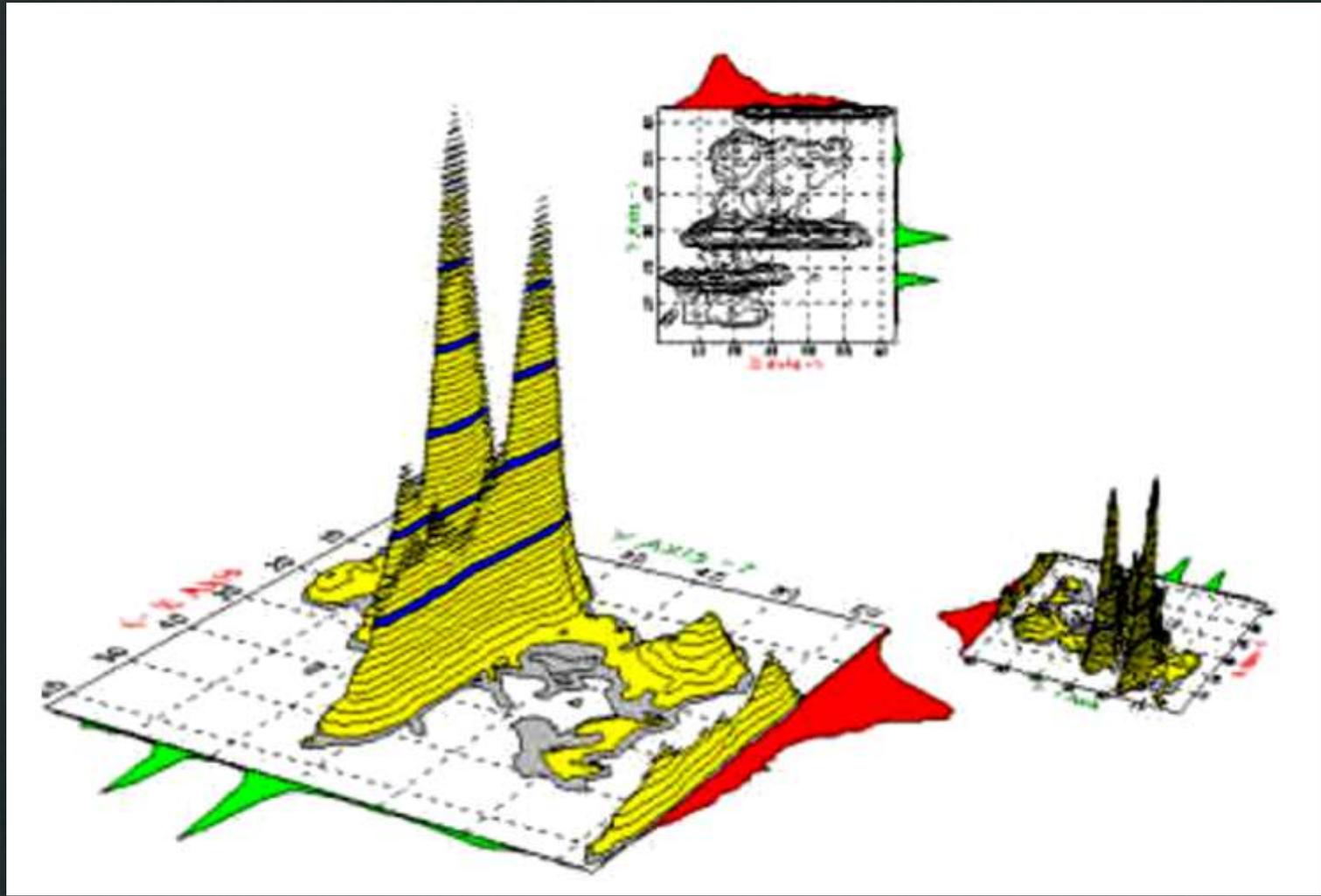
ALGUNOS MARCADORES



OTRO PARÉNTESIS: TEM



Y LA CITOMETRÍA DE FLUJO



CITOMETRÍA DE FLUJO

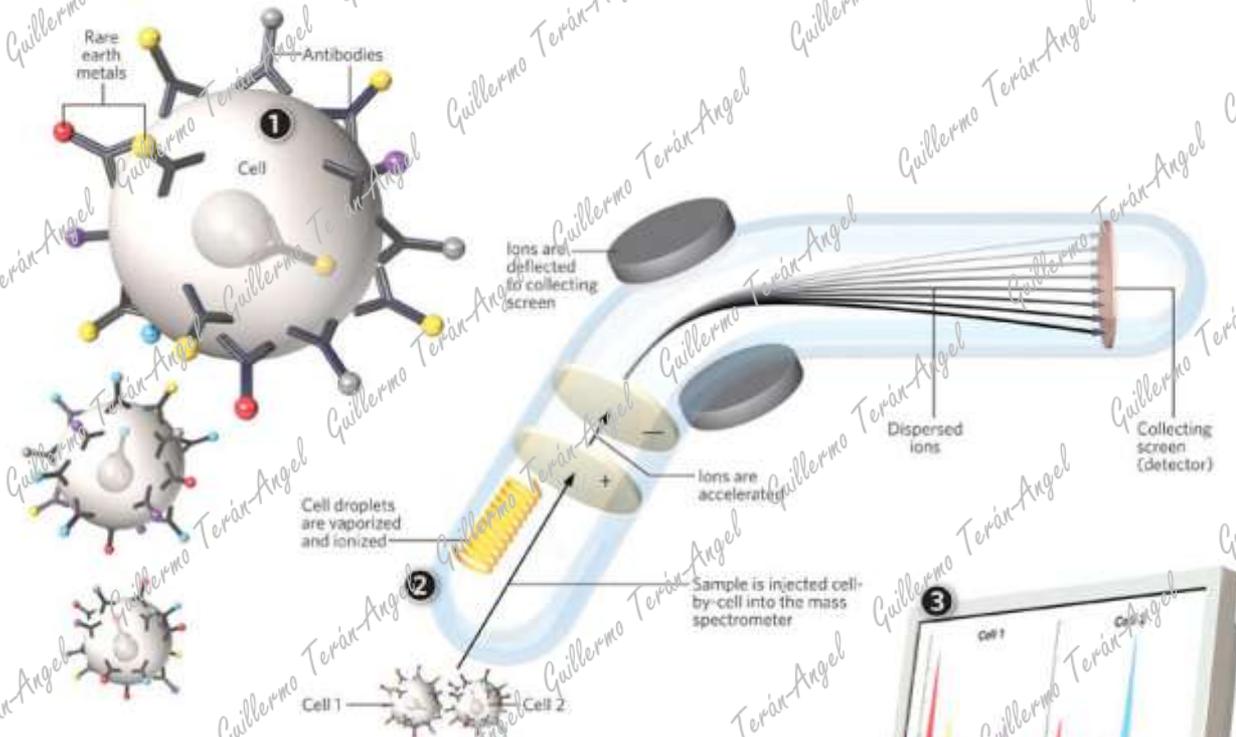
<https://www.youtube.com/watch?v=gEdZvuDrWb4>

CYTOFF : ADIÓS COMPENSACIÓN

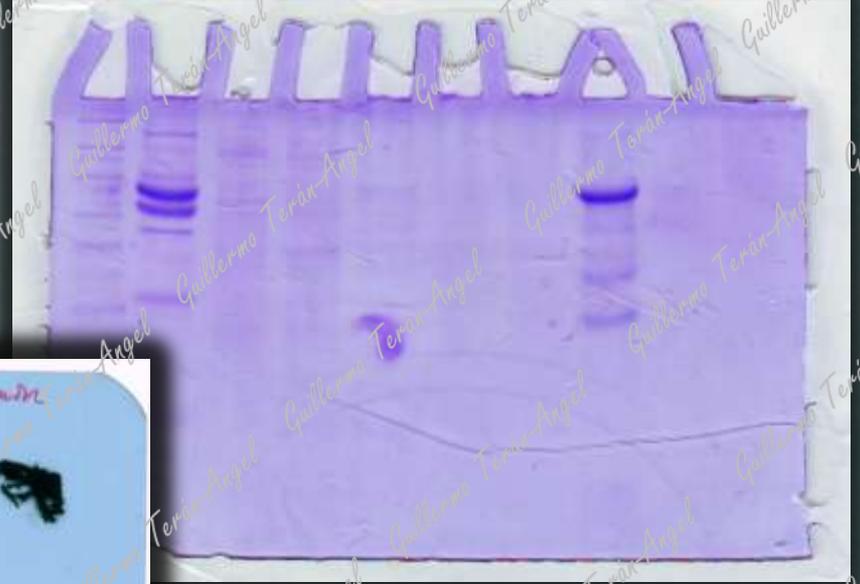
1 Permeabilized cells are mixed with 30-100 different antibodies, each labeled with a different rare earth metal.

2 The cells are sprayed, as single-cell droplets into an argon plasma hotter than 5,000°C, which vaporizes each cell and strips electrons from the metals to generate ions. The metal ions are accelerated in an electrostatic field and a deflector separates as they fly towards a detector at different speeds depending on their mass. The mass spectrometer is precise enough to distinguish isotopes that differ by a single atomic mass unit, making it possible to measure up to 100 different parameters in a single experiment.

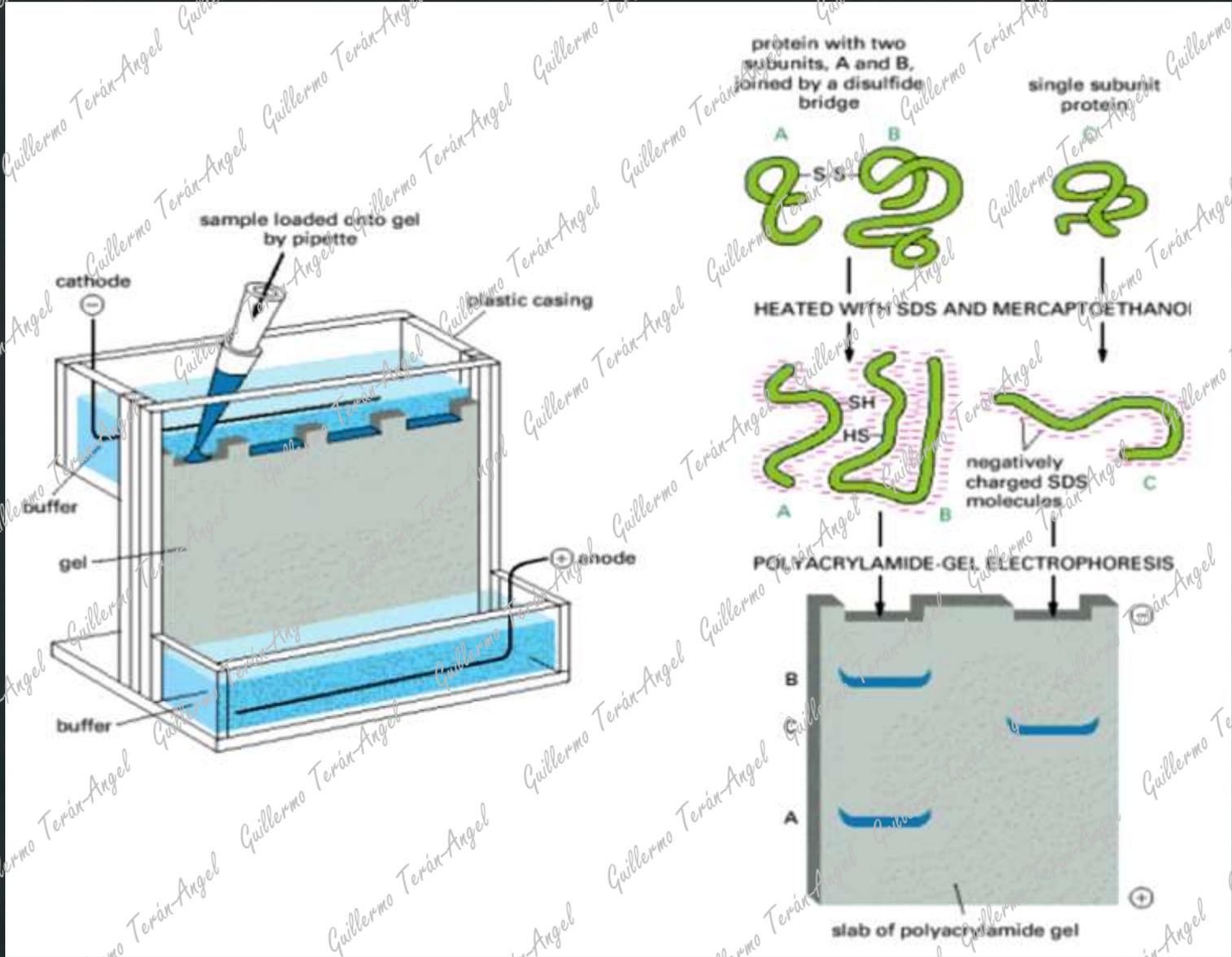
3 The detector counts the arriving ions, quantifying how much of each metal—and therefore of its tagged antibody's target—was on or in each cell. Analyzing single cells with so many parameters has allowed Nolan and coworkers to study changes in phosphorylation in any given cell type.



TÉCNICAS BIOQUÍMICAS Y MOLECULARES



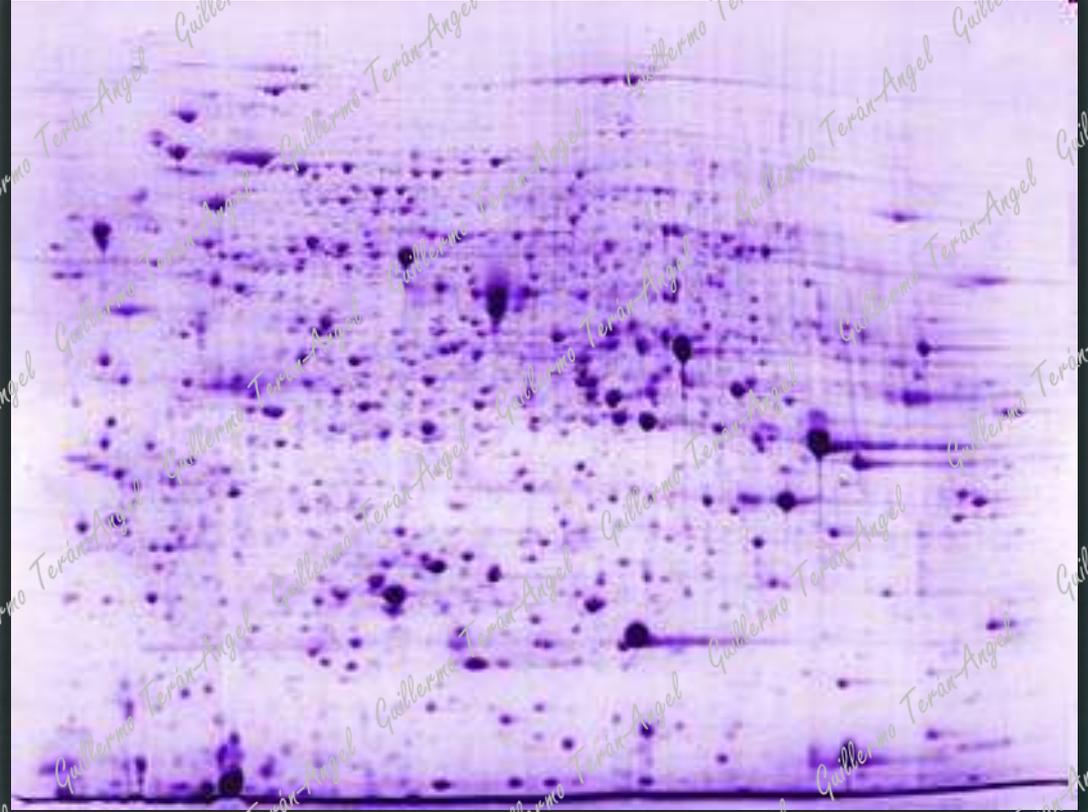
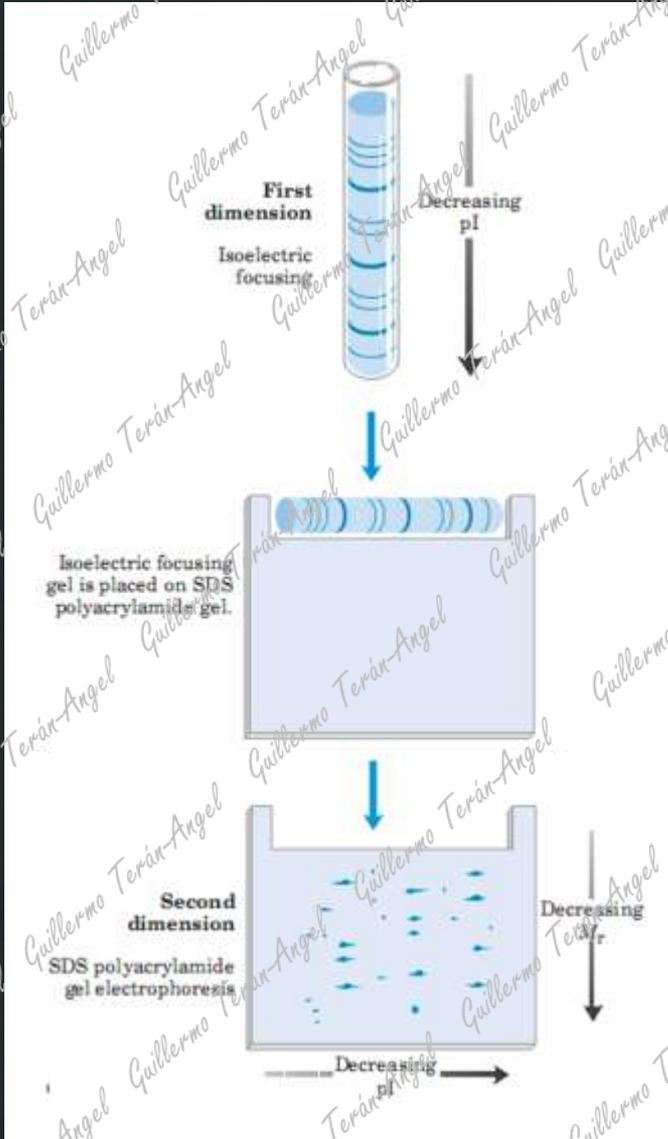
ELECTROPHORESIS DE PROTEÍNAS



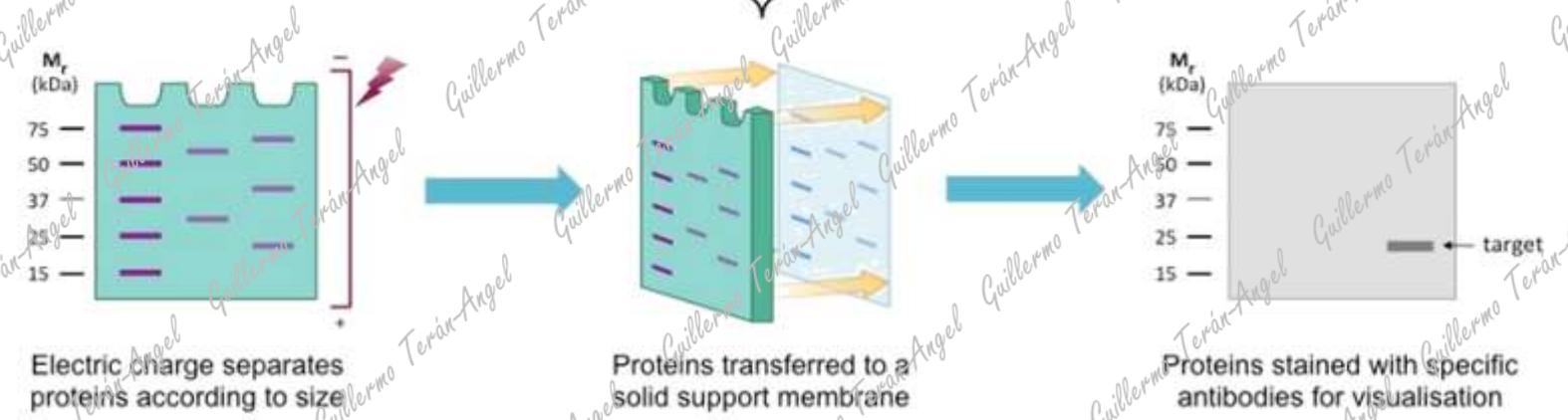
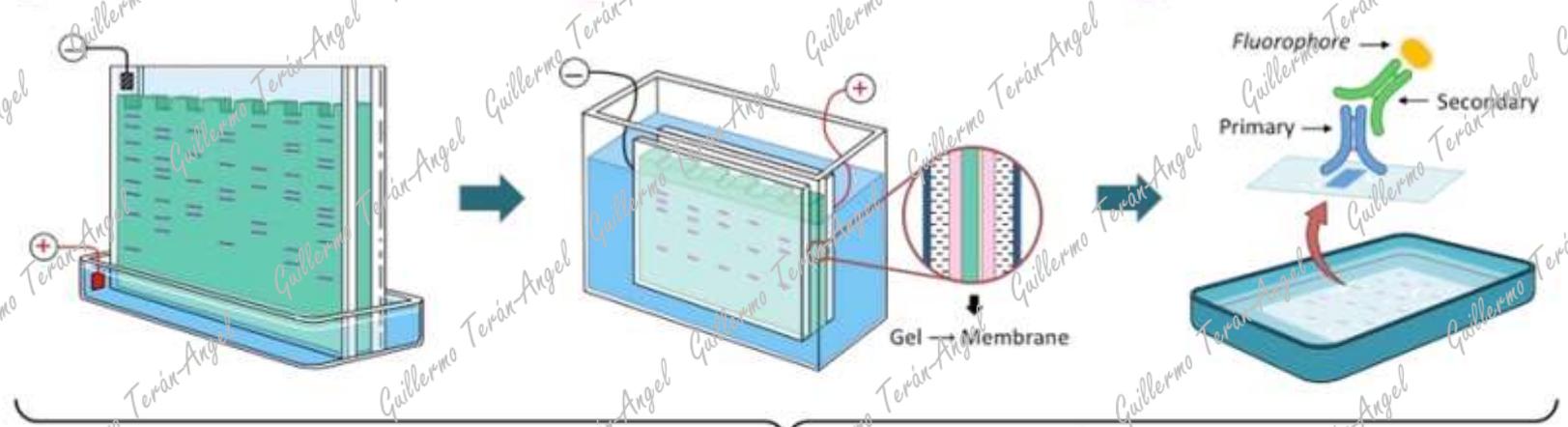
ELECTROFORESIS DE PROTEÍNAS



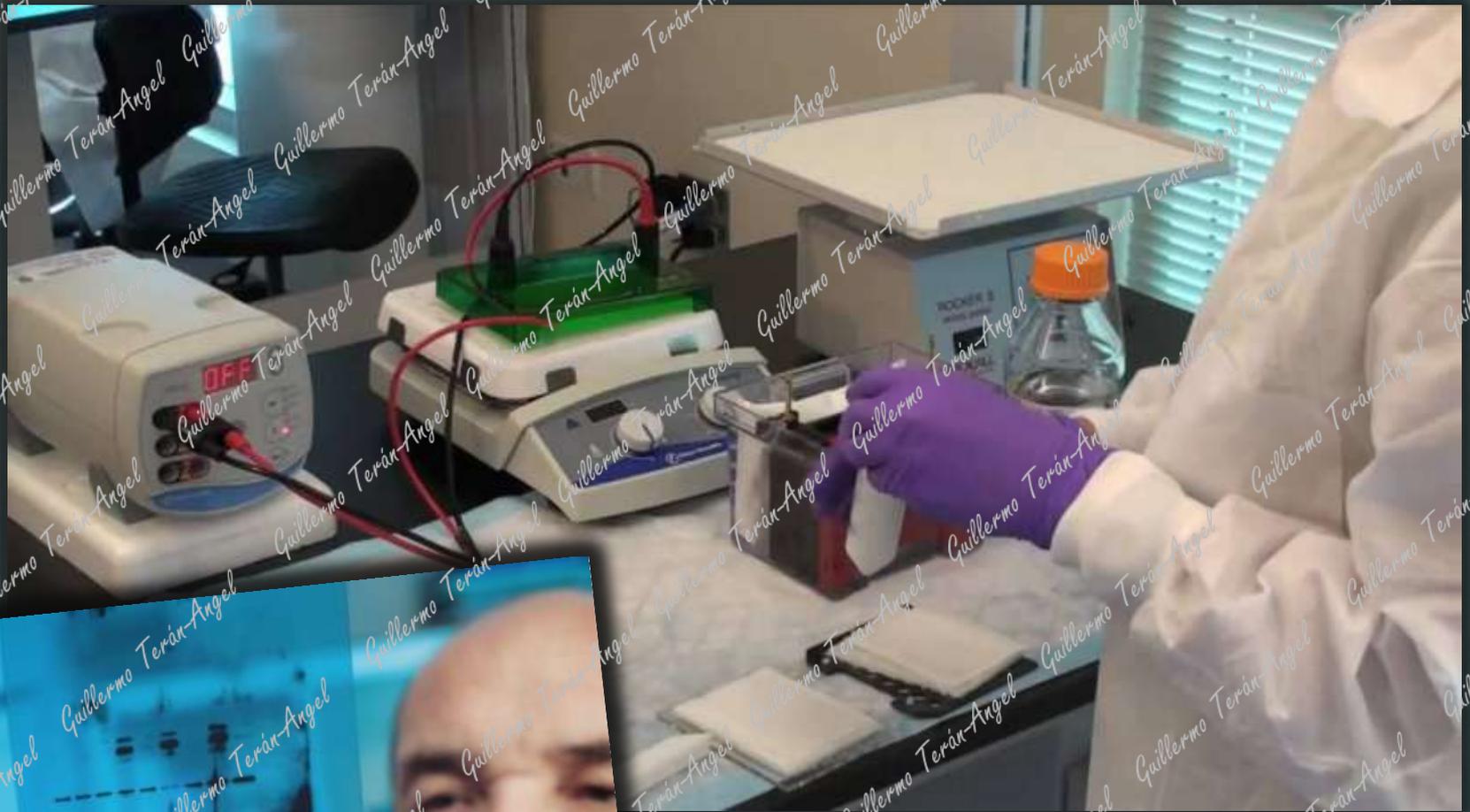
ELECTROFORESIS EN 2D



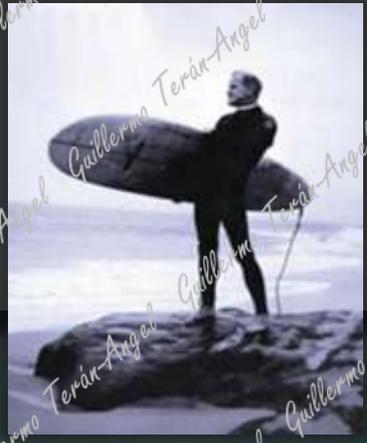
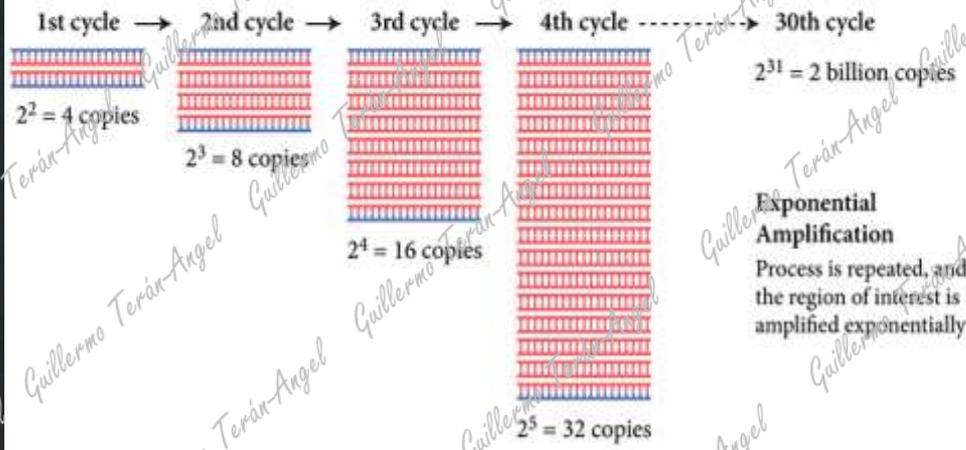
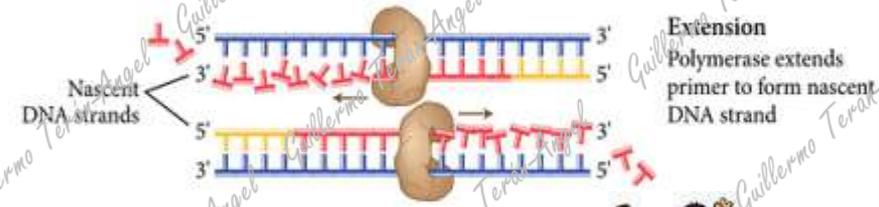
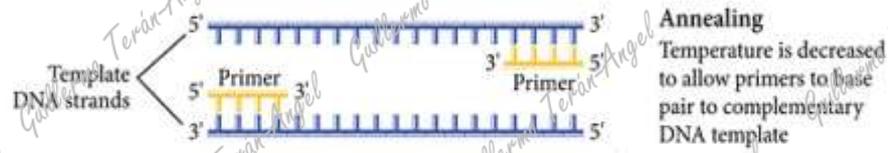
WESTERN BLOT



WESTERN BLOT



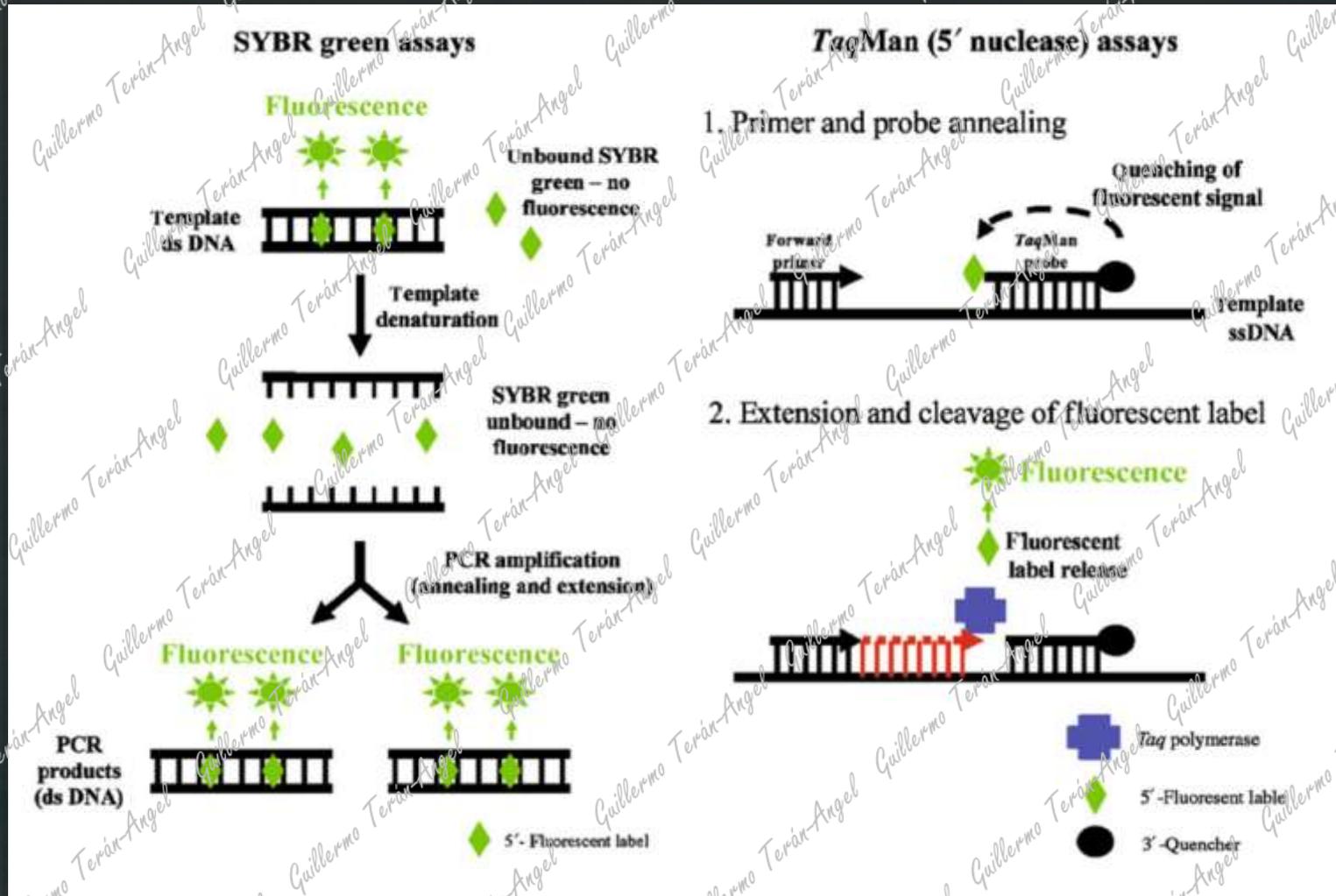
PCR (NO PROTEÍNA C REACTIVA...)



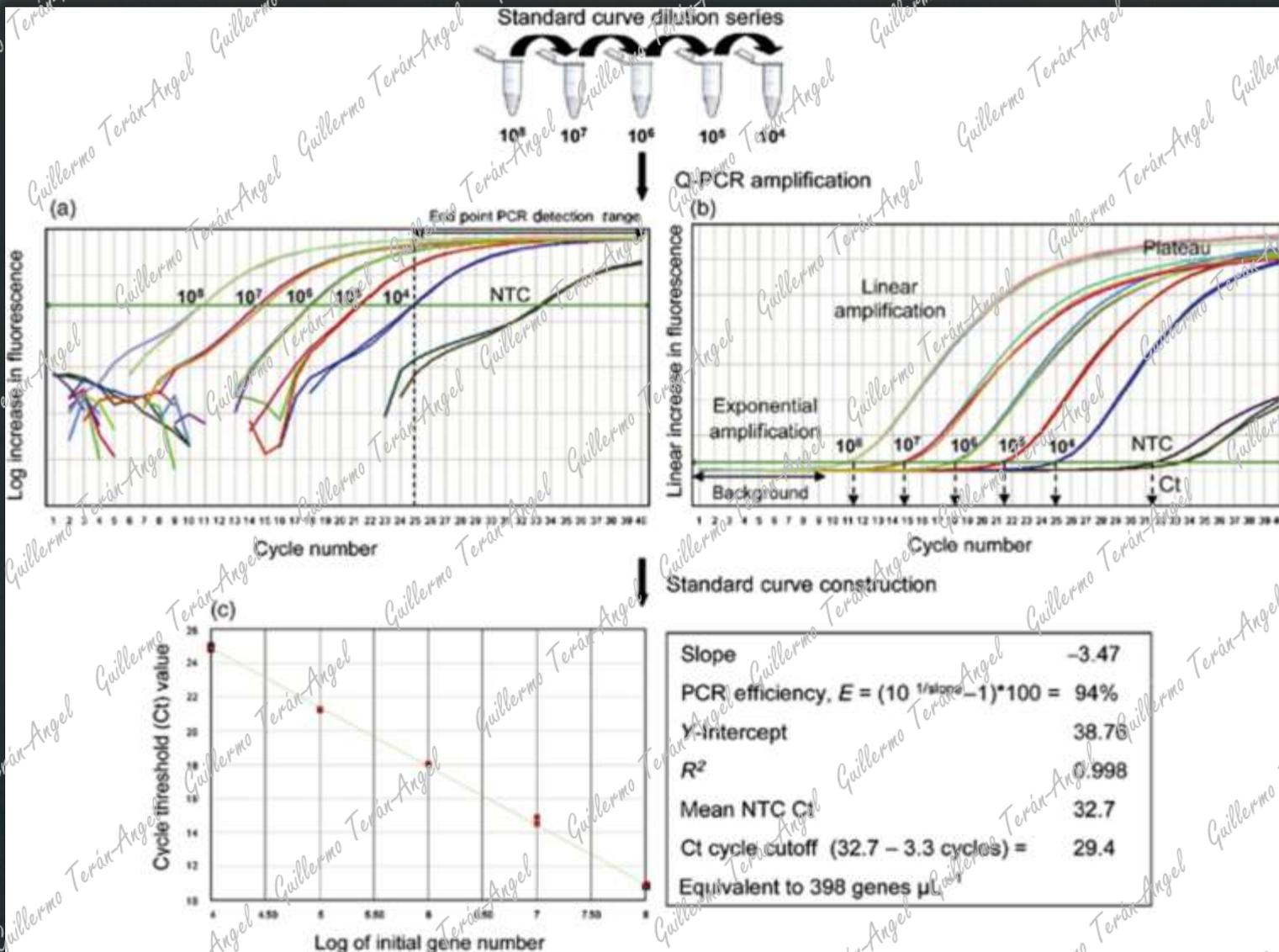
REACCIÓN EN CADENA DE LA POLIMERASA



PCR EN TIEMPO REAL



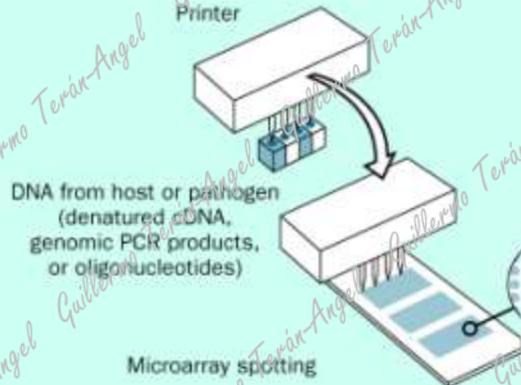
ICUANTIFICABLE!



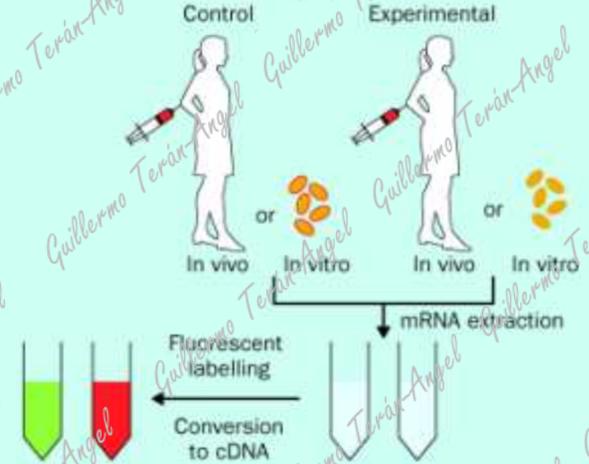
CHIPS DE ADN



Target preparation



Probe preparation



Competitive hybridisation

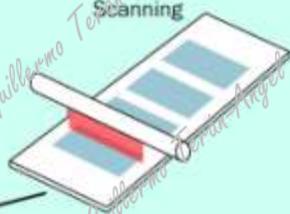
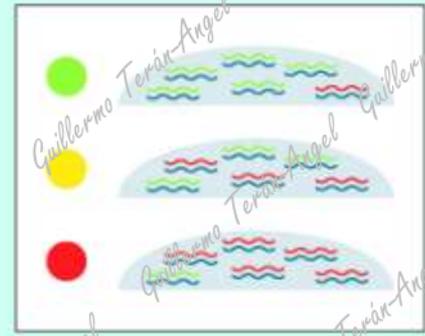
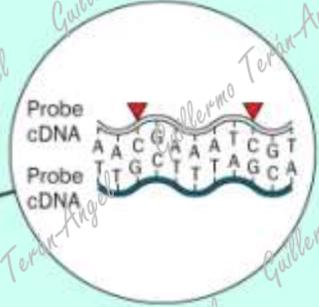
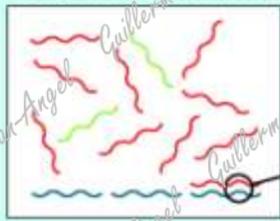
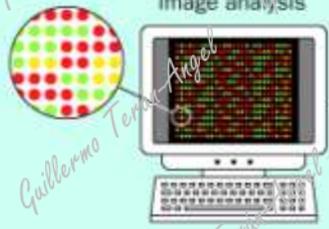


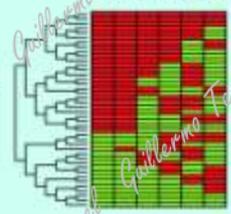
Image analysis



Data normalisation



Data analysis



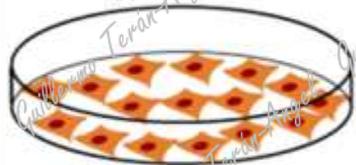
Data interpretation



CHIPS DE ADN

Oligonucleotide microarray

Cells of person 1/condition 1



RNA isolation

mRNA

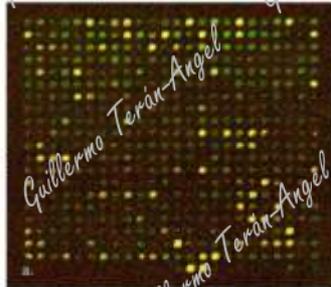
Reverse transcriptase labeling

cDNA

"Green Fluorescent" Targets

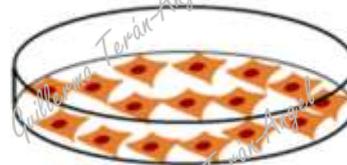
Hybridize to microarray

Microarray with short cDNA spanning the entire genome

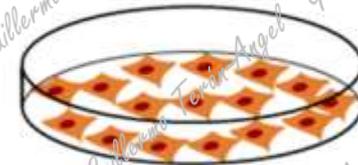


cDNA microarray

Cancer cells



Normal cells



RNA isolation

mRNA

Reverse transcriptase labeling

cDNA

"Red Fluorescent" Targets

mRNA

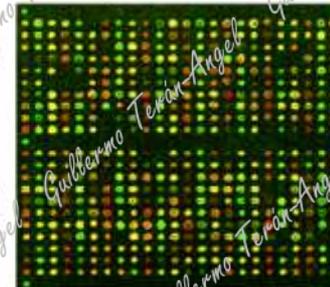
cDNA

"Green Fluorescent" Targets

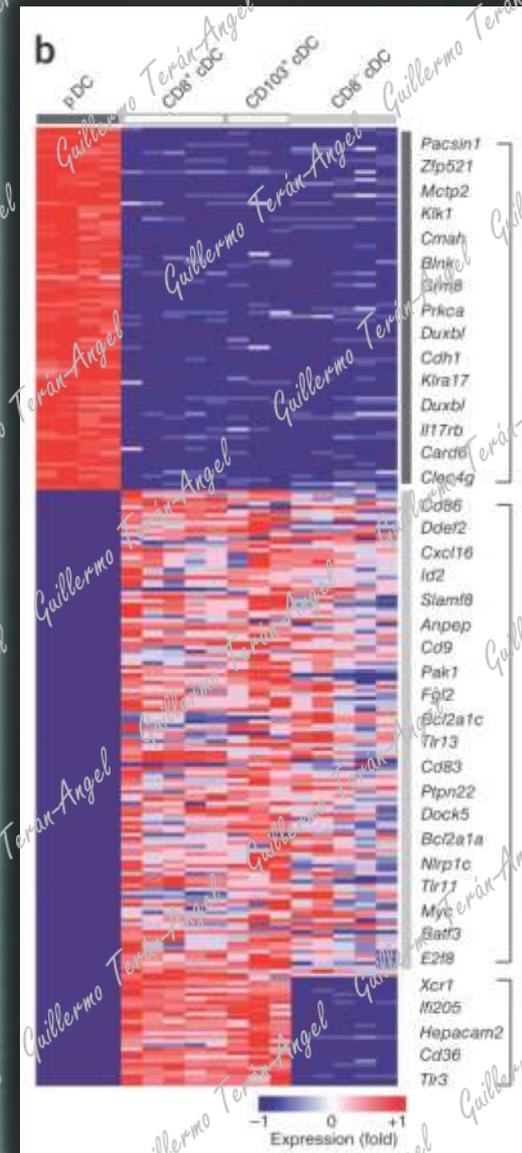
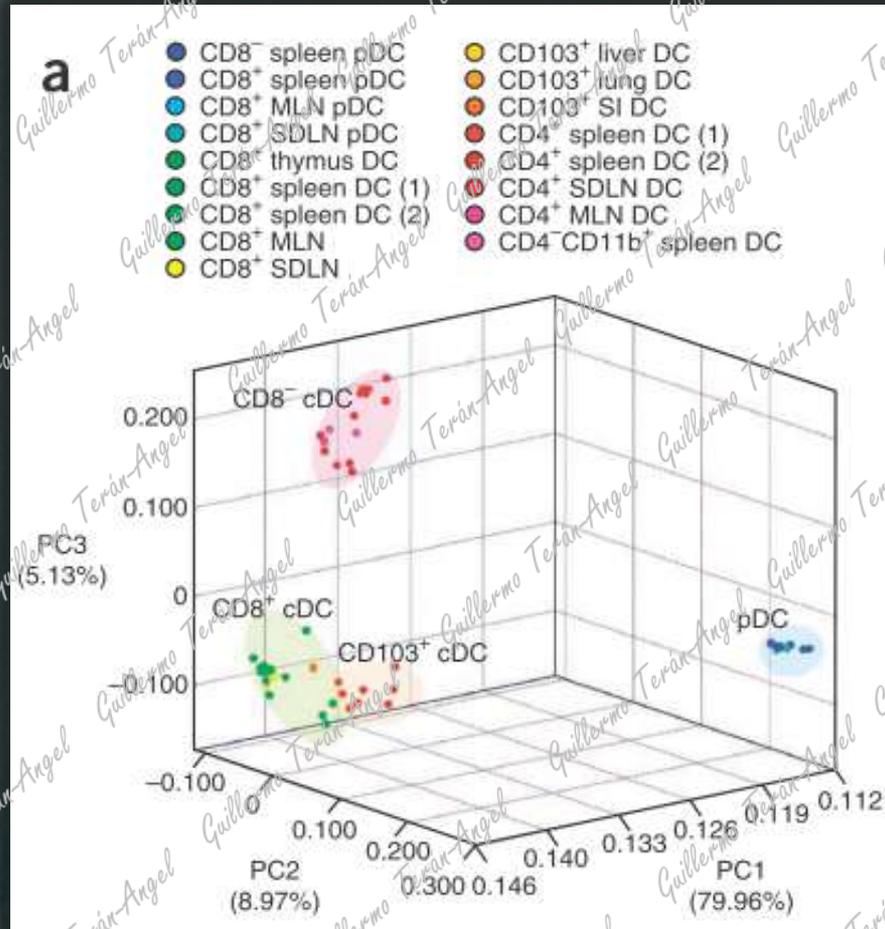
Combine targets

Hybridize to microarray

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



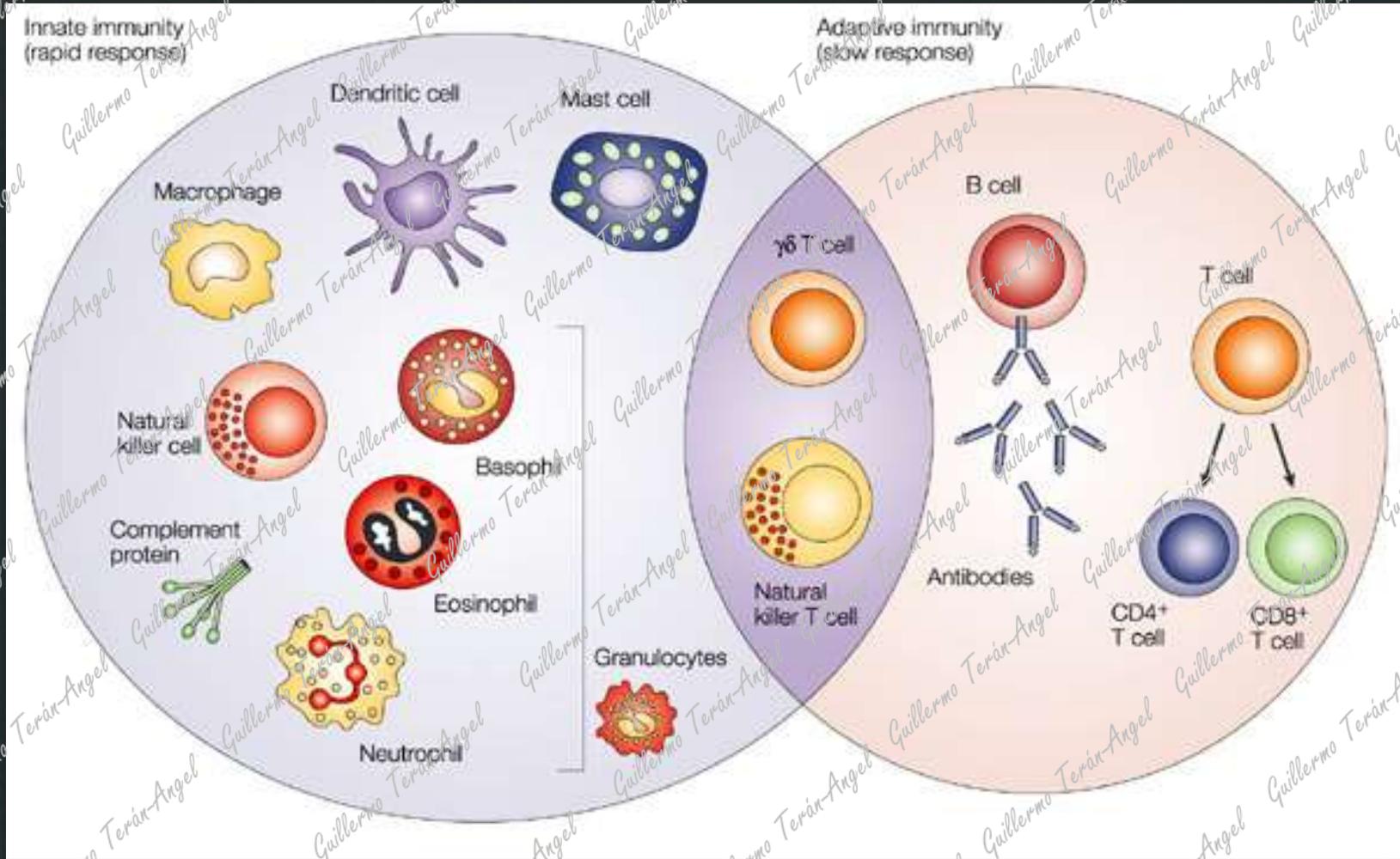
ANÁLISIS DE COMPONENTES PRINCIPALES



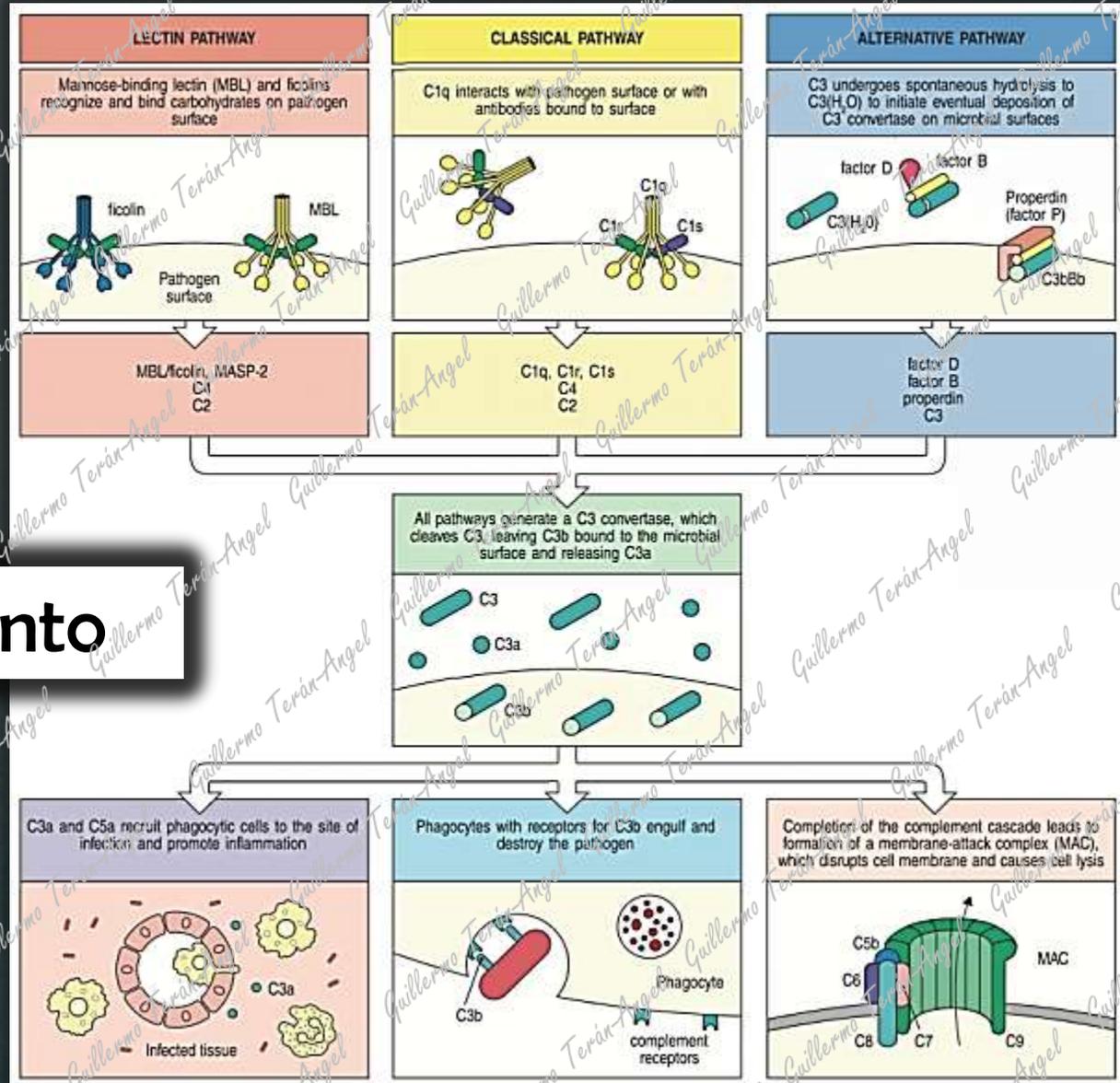


**AHORA SI: LA CLASE
MÉTODOS PARA EVALUAR LA RESPUESTA
INMUNE HUMORAL Y CELULAR**

EL OBJETO DE ESTUDIO

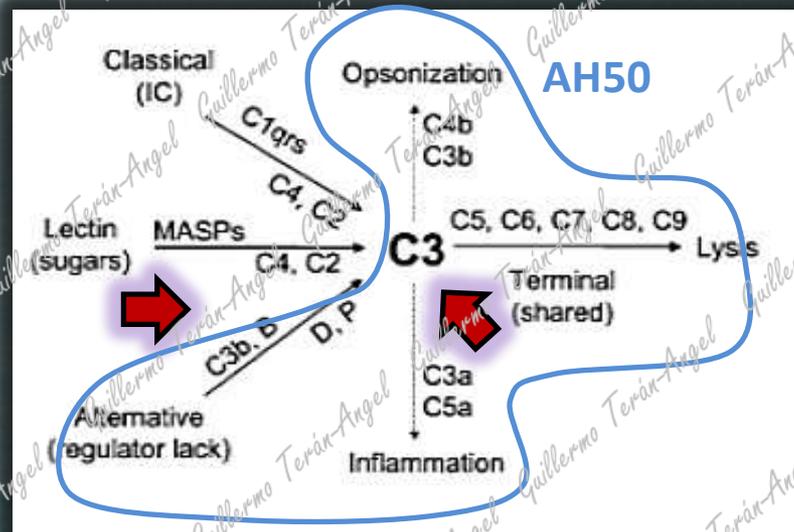
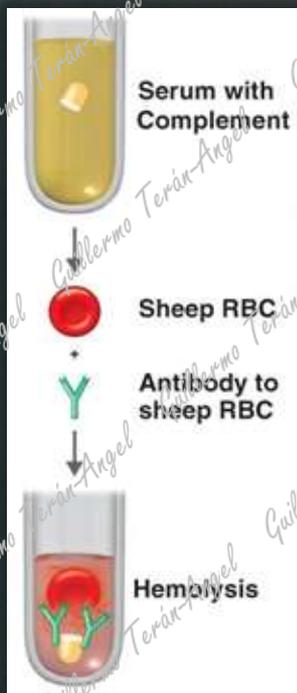
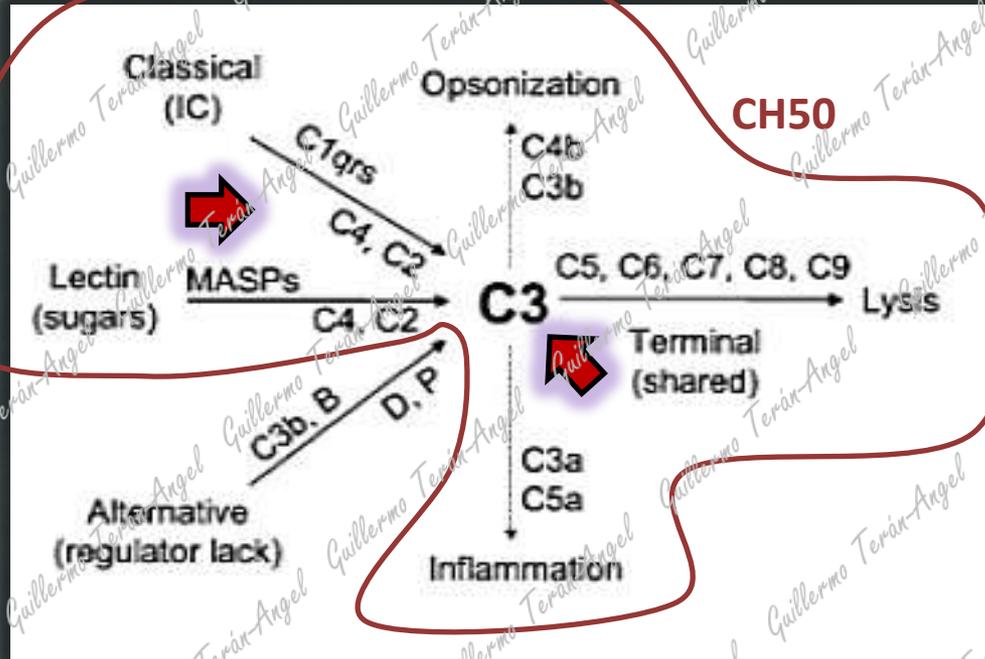


RESPUESTA HUMORAL

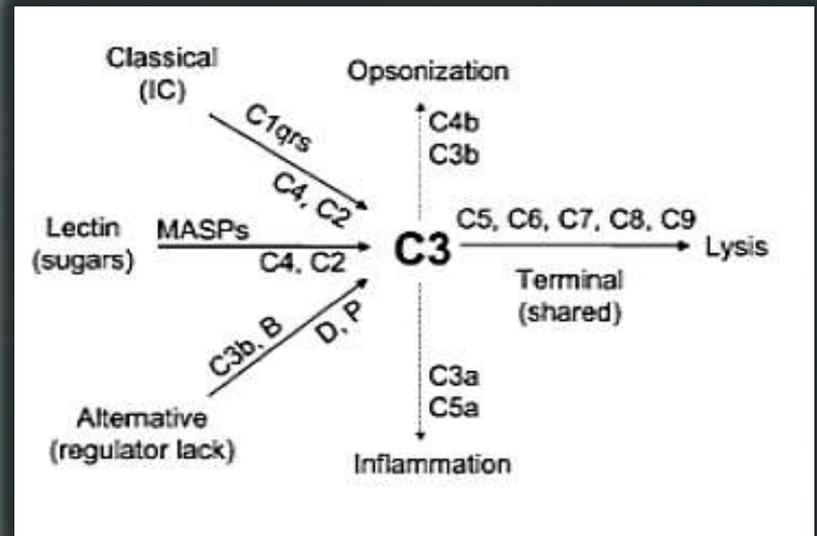
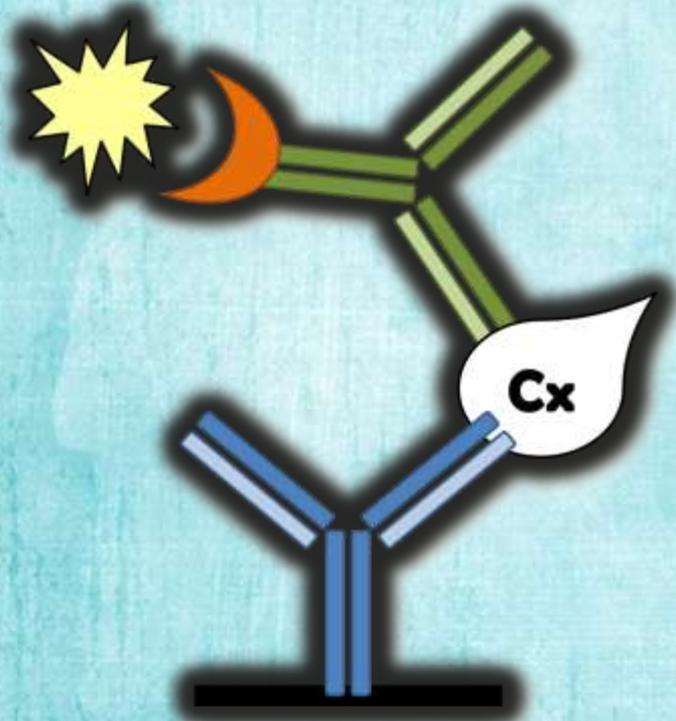


Complemento

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)

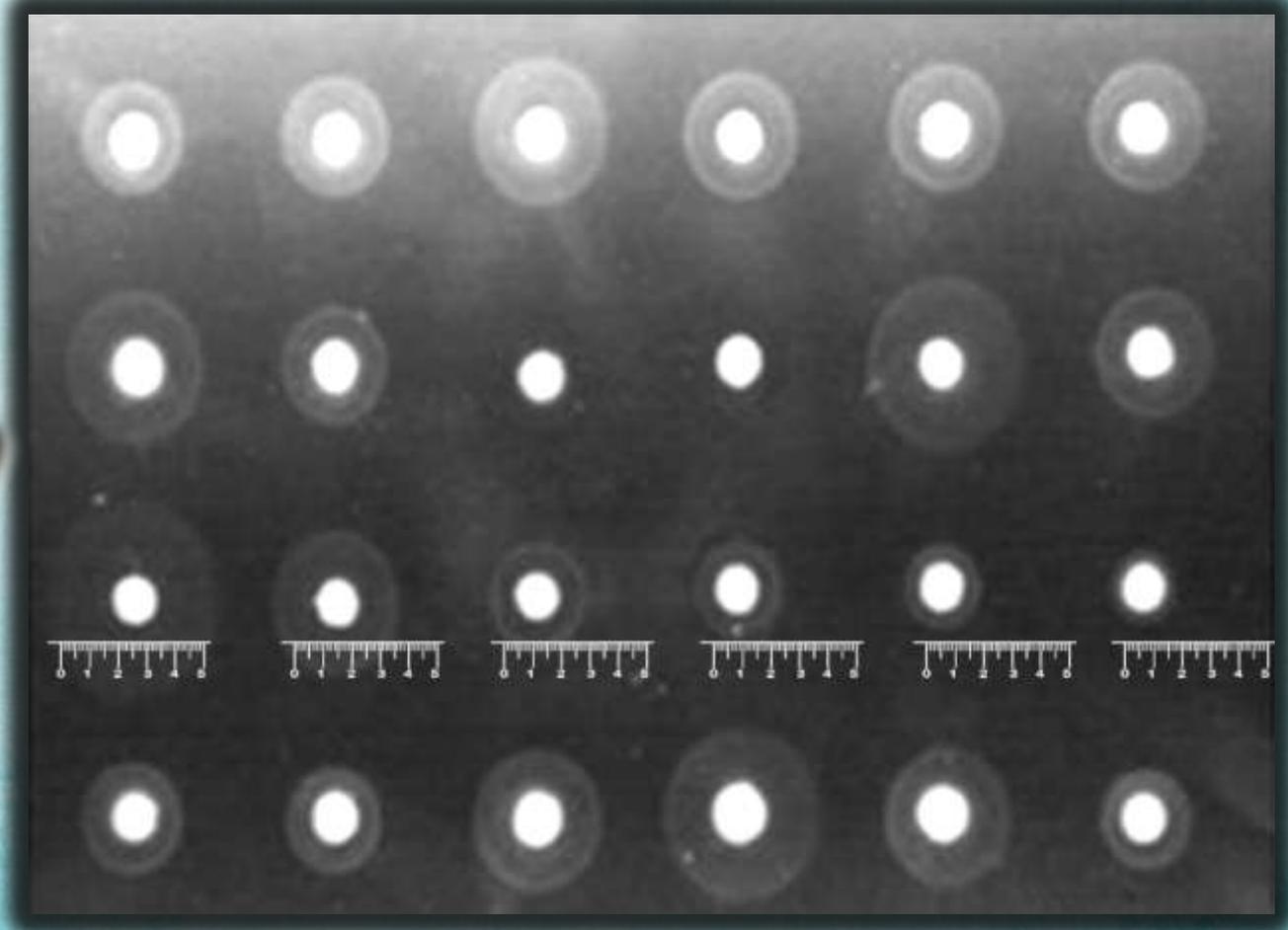
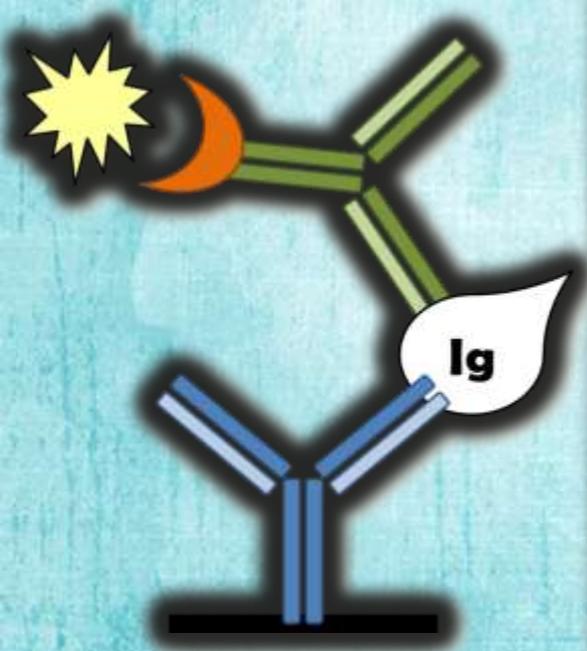
CH50=0 & AH50=0 ► Común

INMUNOGLOBULINAS

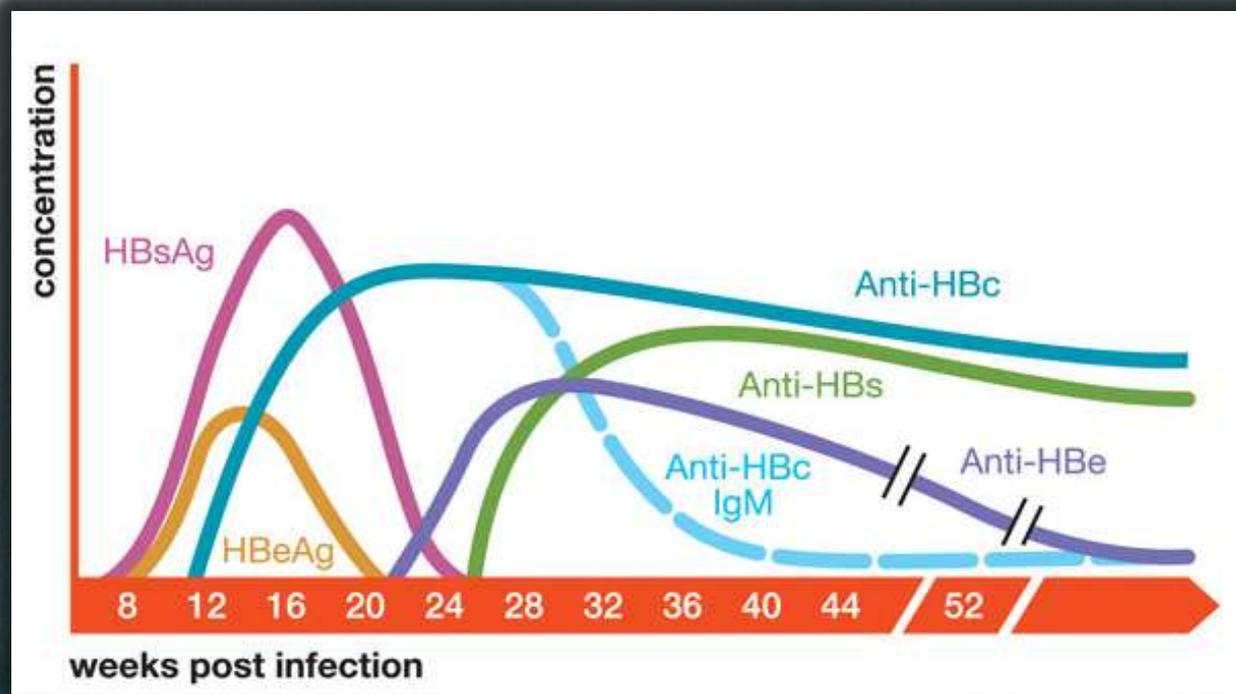
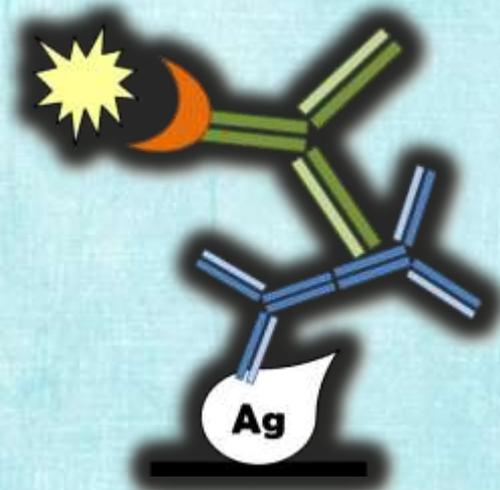
Property	IgG	IgA	IgM	IgD	IgE
Usual molecular form	Monomer	Monomer, dimer	Pentamer, hexamer	Monomer	Monomer
Other chains	None	J chain, SC	J chain	None	None
Subclasses	G1, G2, G3, G4	A1, A2	None	None	None
Heavy chain allotypes	Gm (=30)	No A1, A2m (2)	None	None	None
Molecular mass, kDa	150	160, 400	950, 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.

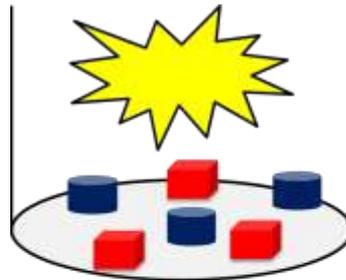
CUANTIFICACIÓN DE IG



IG ESPECÍFICAS

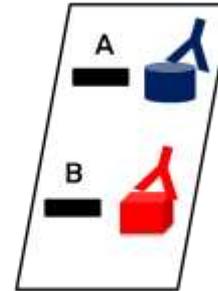


DIAGNÓSTICO

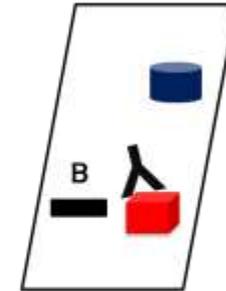


**Ensayos en
tiritas**

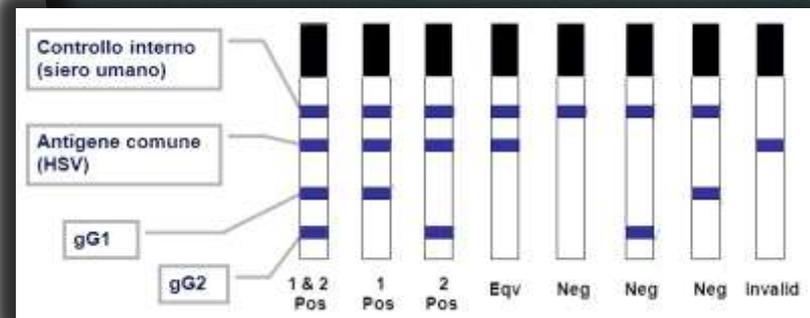
**Ensayos en
suspensión**



**POSITIVO
crónico o agudo**



**REACCIÓN
CRUZADA**



RESPUESTA CELULAR



Separando células

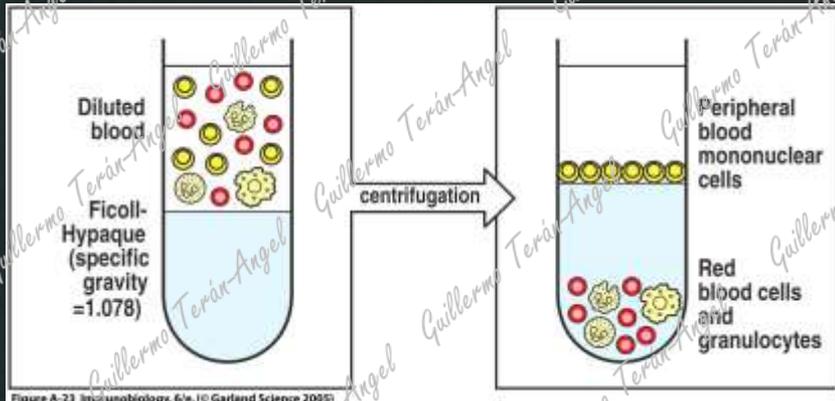
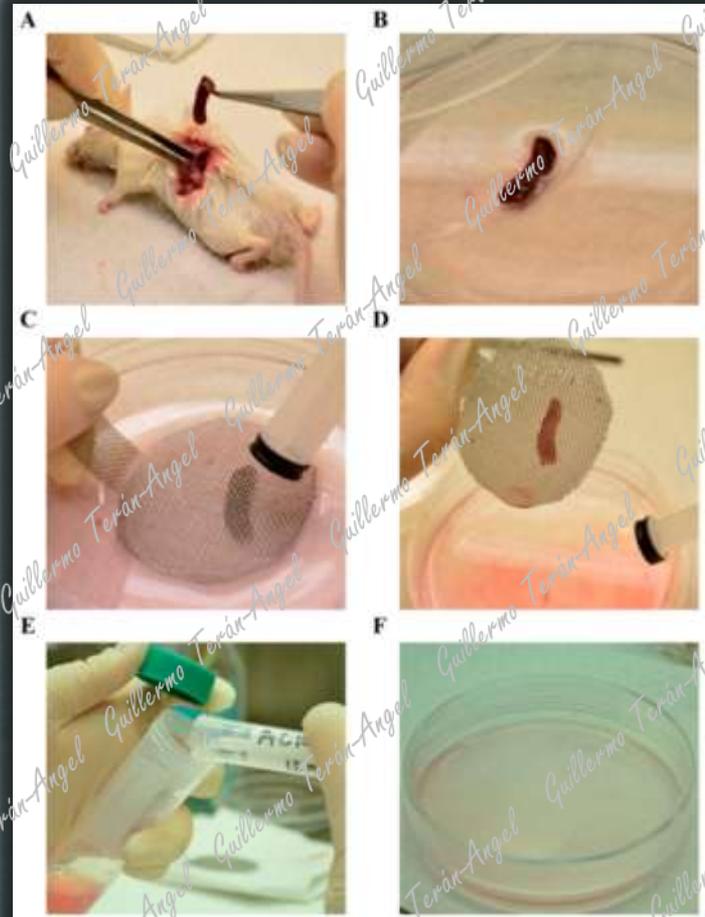
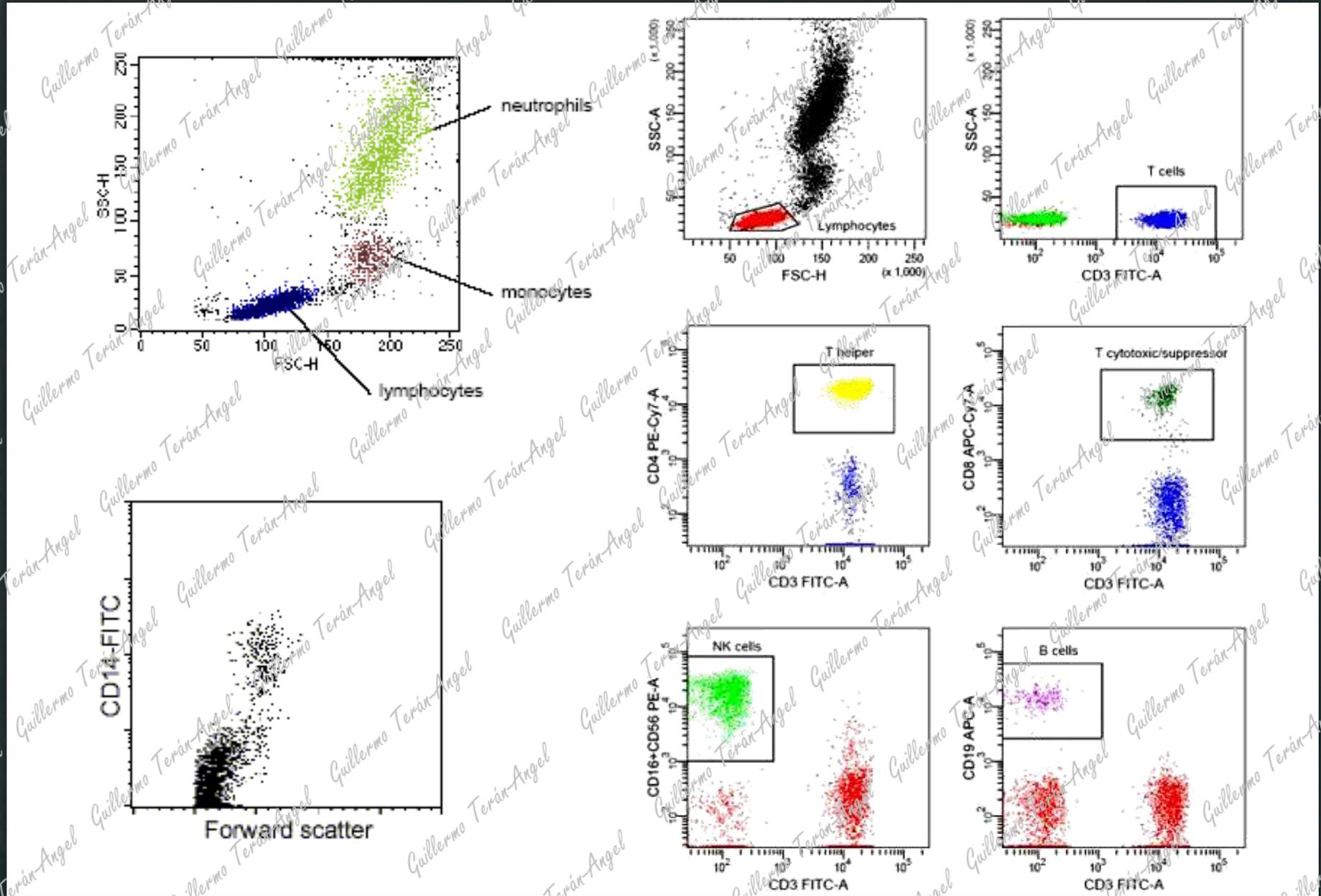
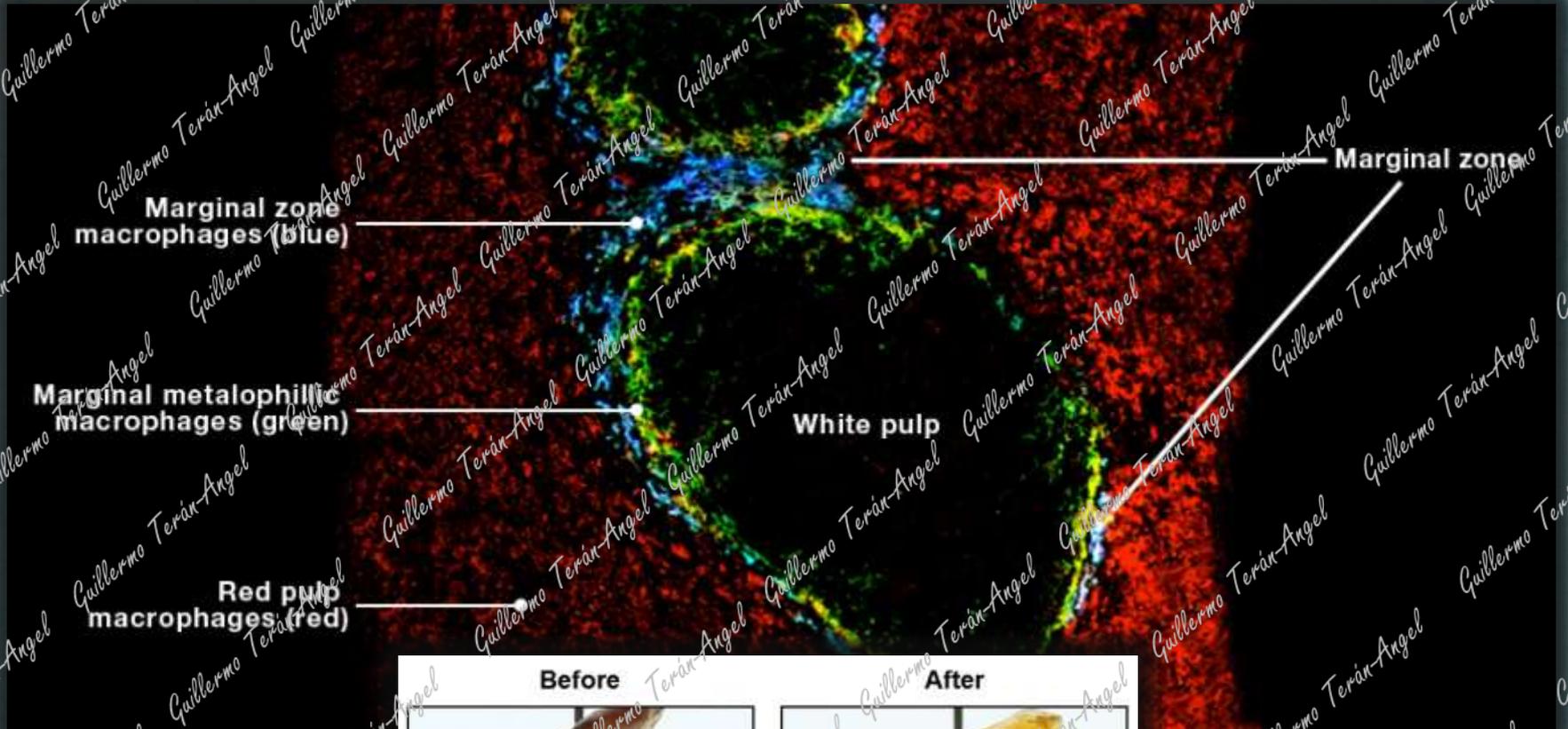


Figure A-21 Immunobiology, 6/e. © Garland Science 2005

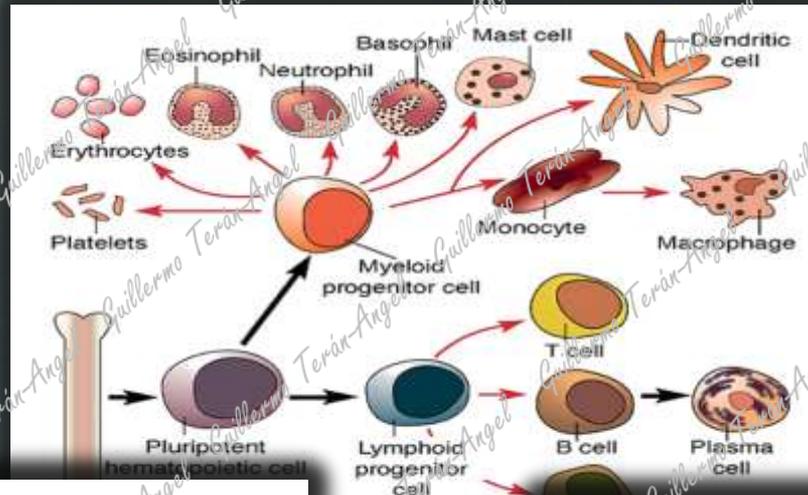
LAS CÉLULAS BLANCAS



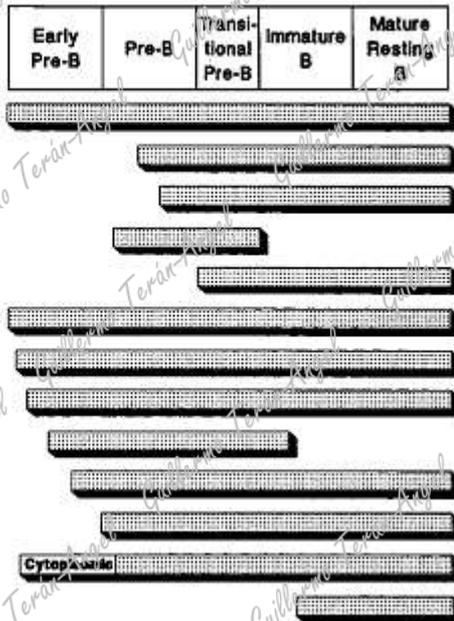
IN SITU



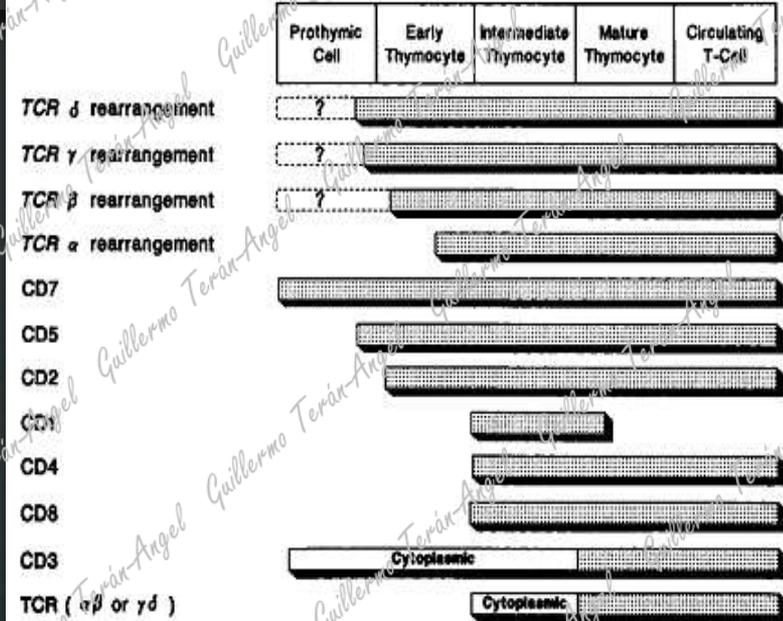
EVALUANDO EL ORIGEN



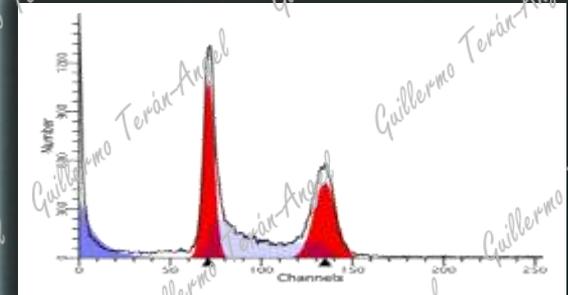
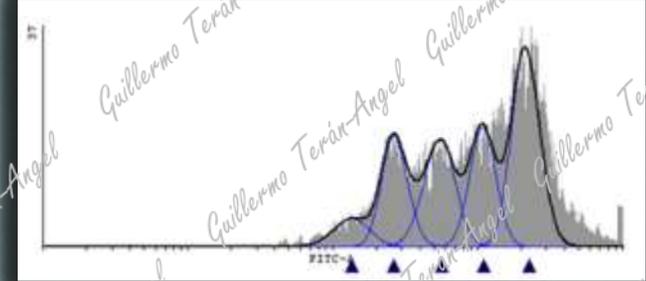
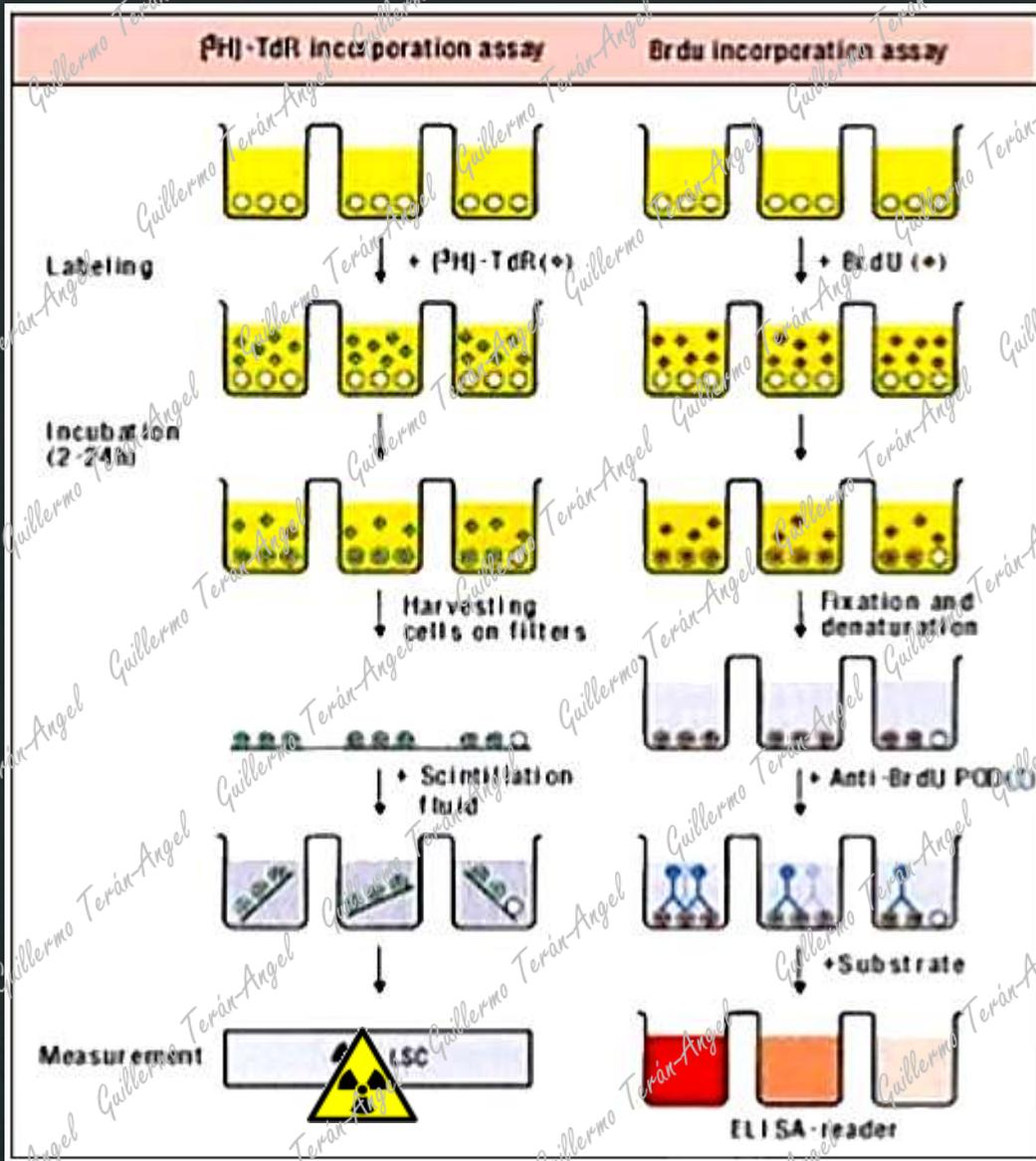
B-Cell Ontogeny



T-Cell Ontogeny

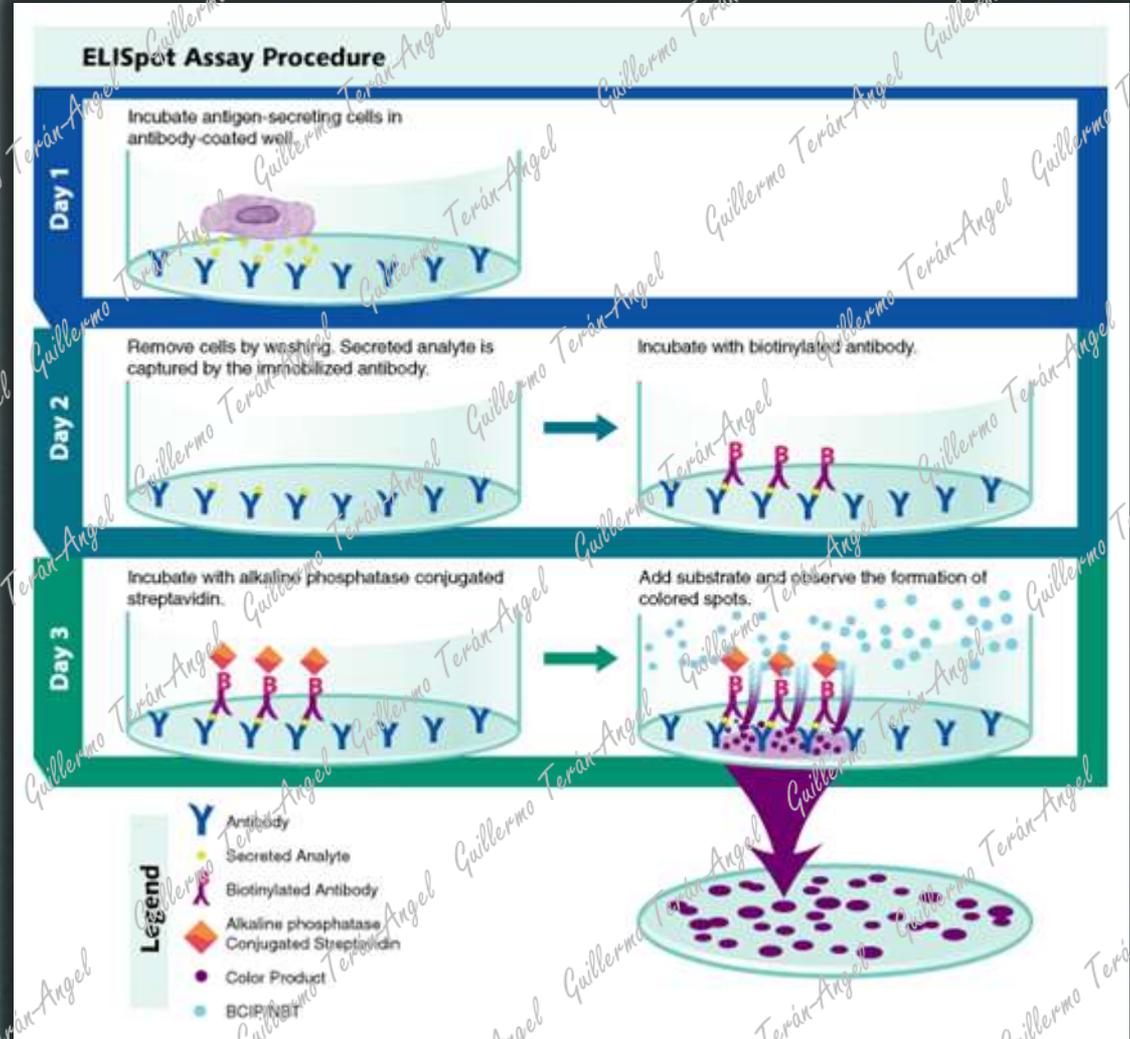
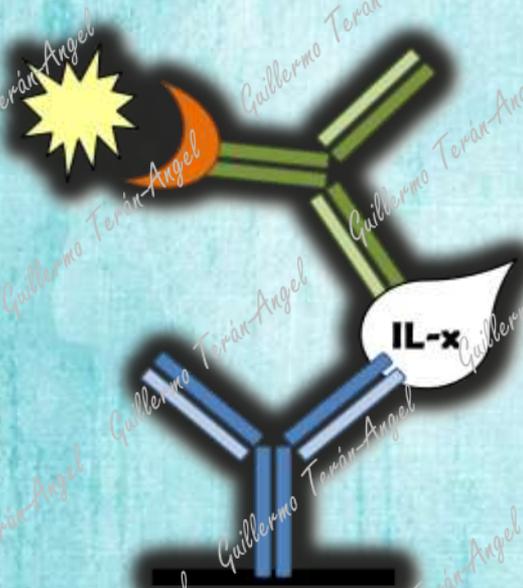


EVALUANDO LA PROLIFERACIÓN

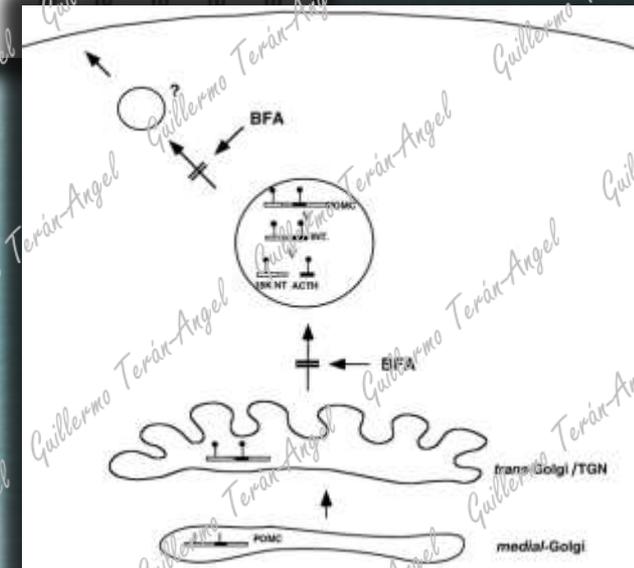
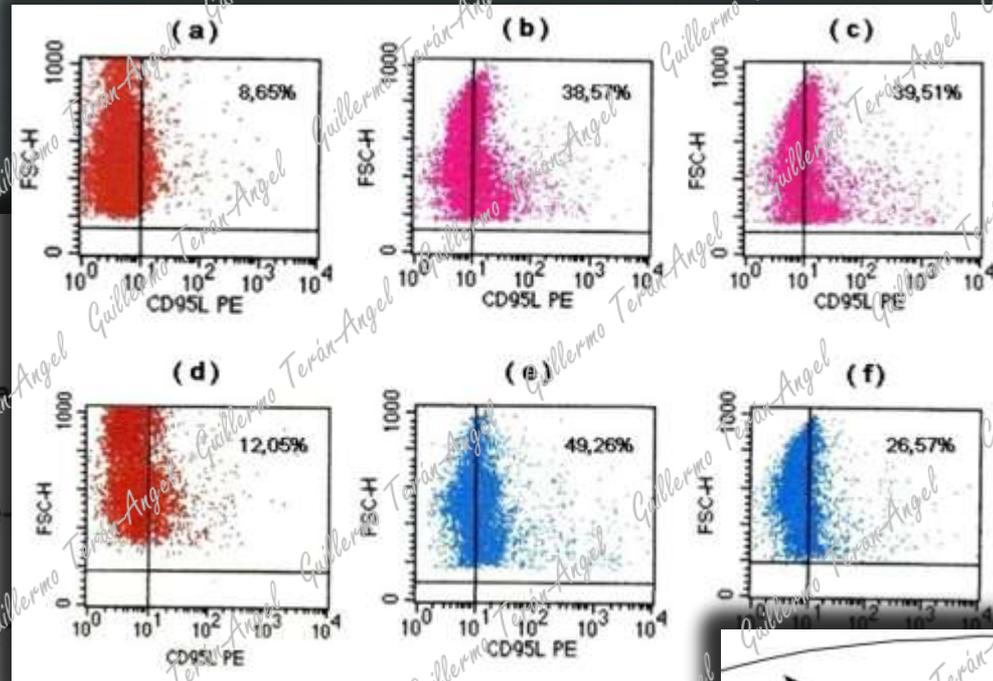
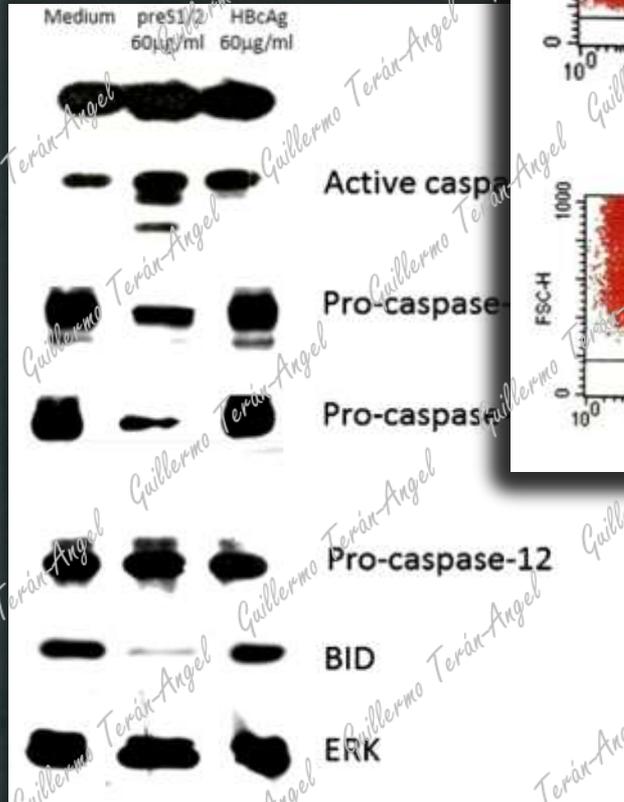


TIA
BrdU
CFSE
PI

PRODUCCIÓN DE CITOCINAS

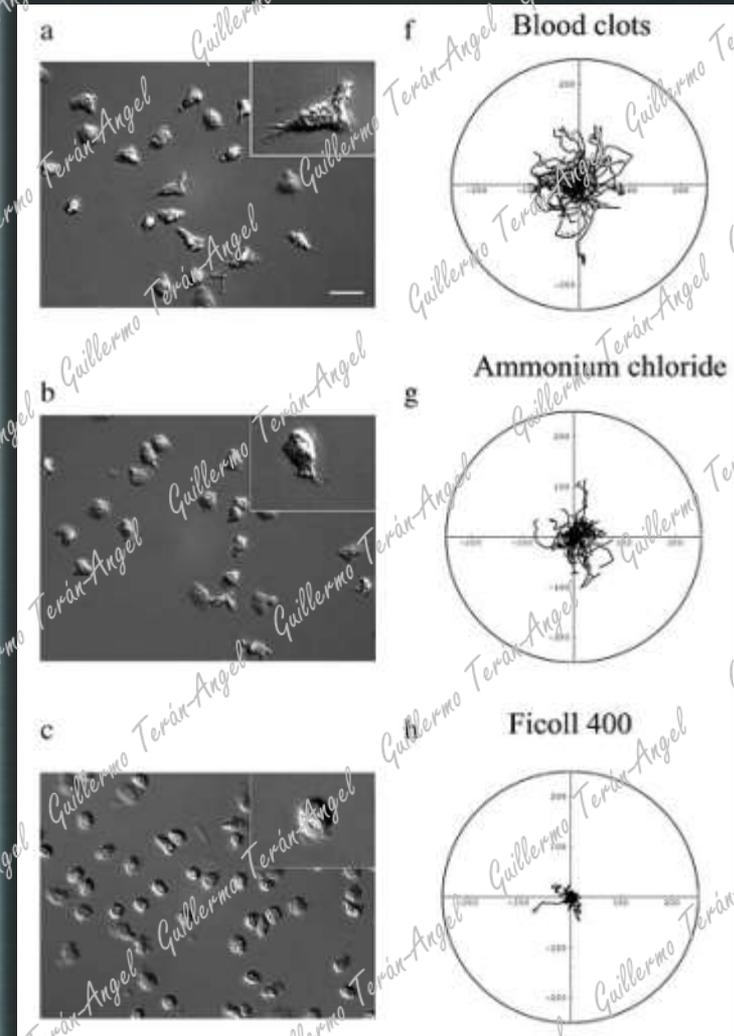
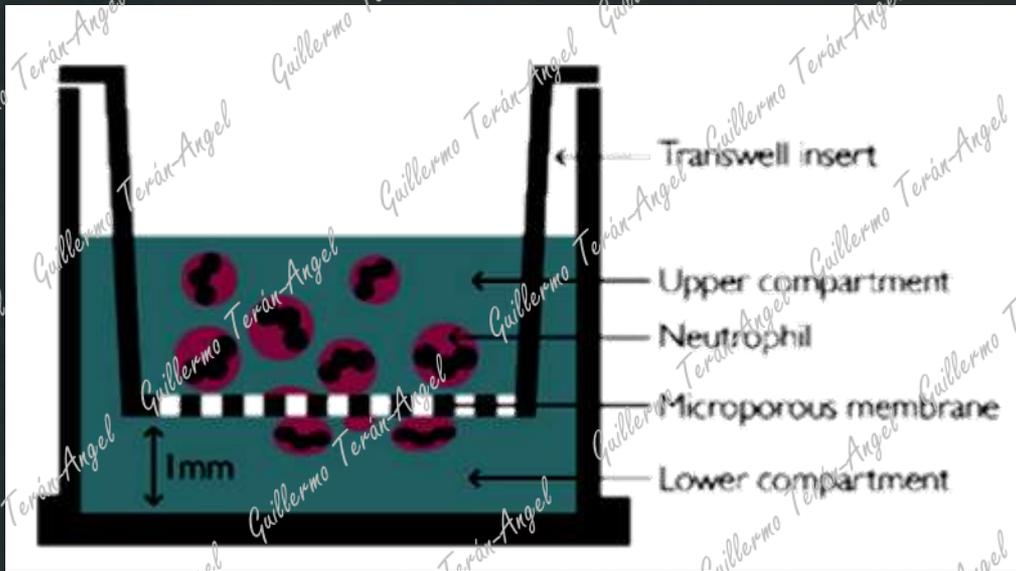


MOLÉCULAS INTRA Y EXTRA

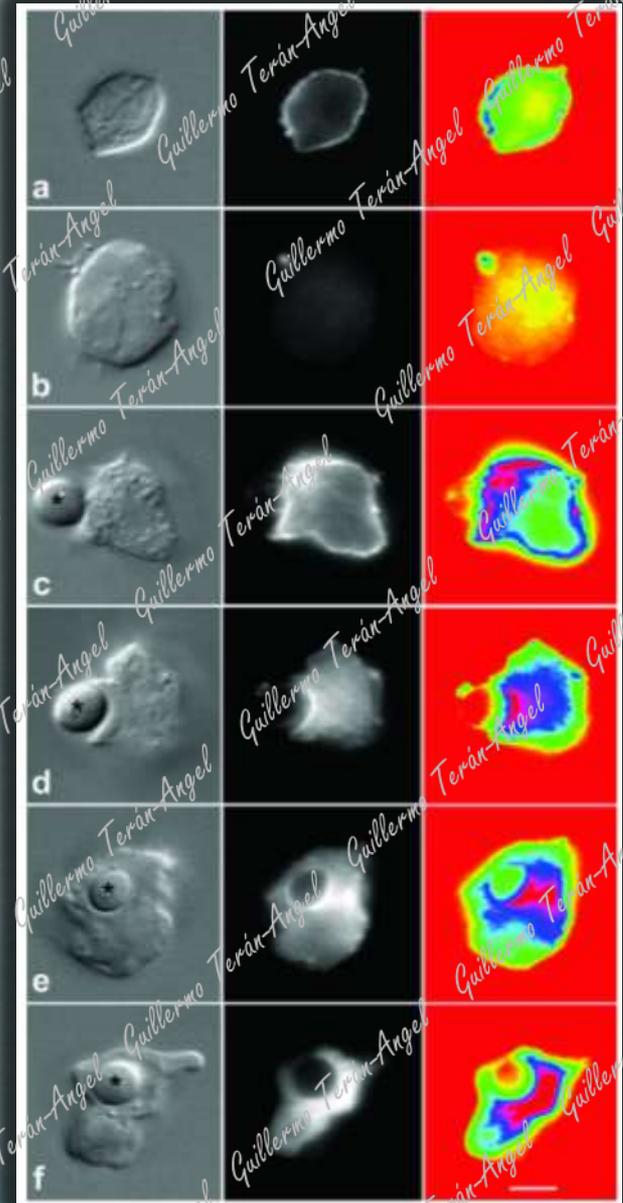
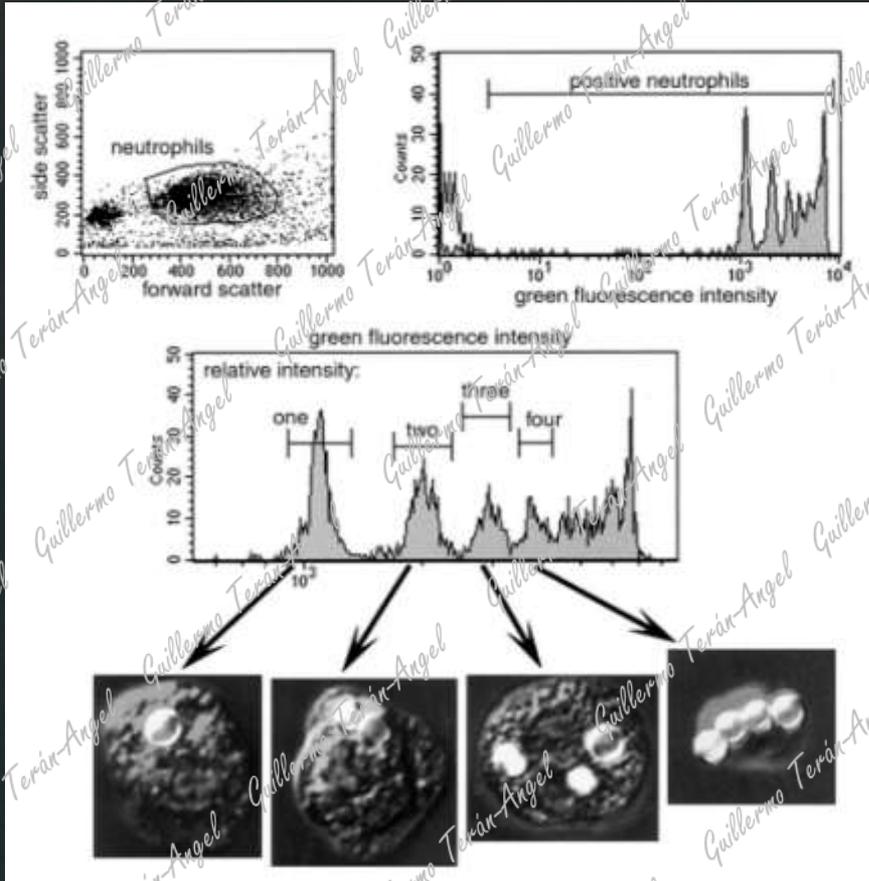


Journal of Medical Virology 86:496–504 (2014),
Guillermo Terán Angel ULA 2001

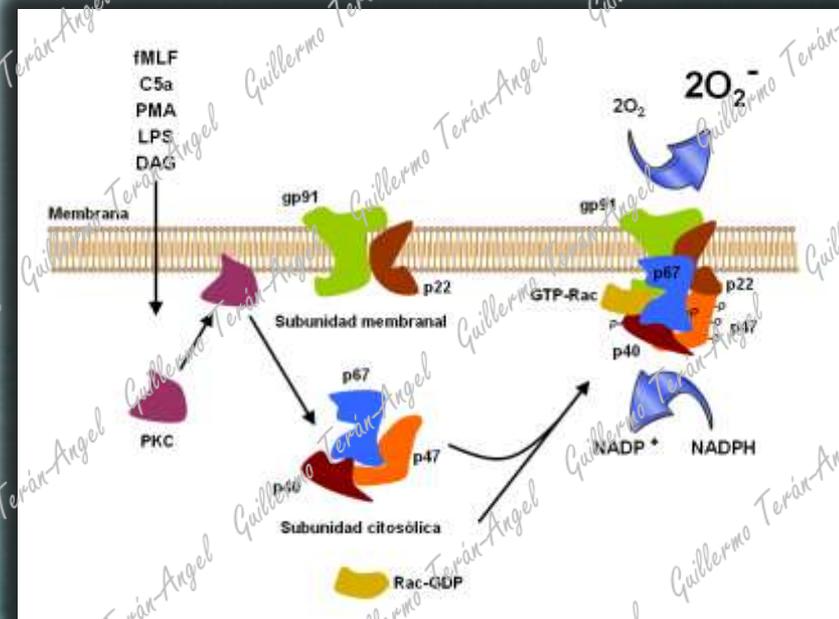
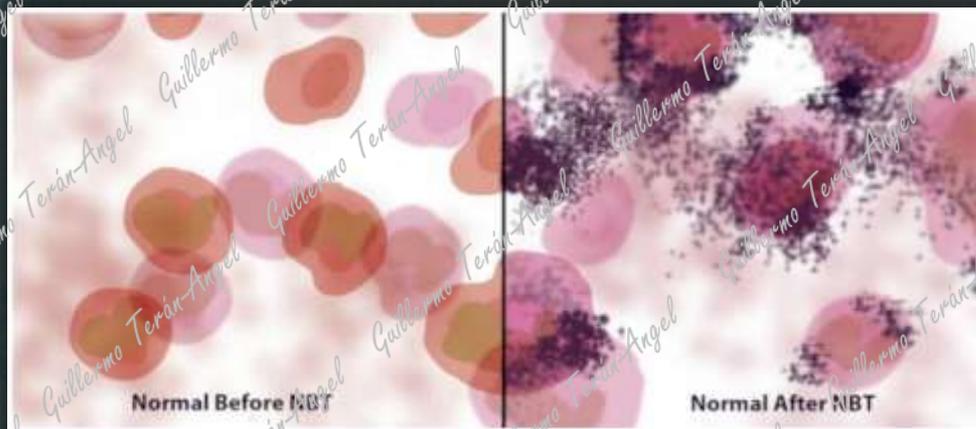
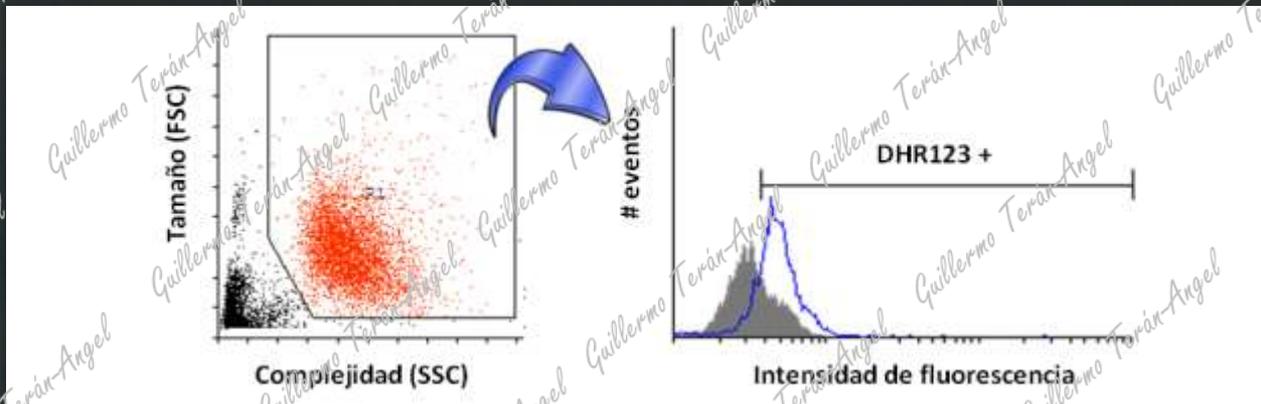
MOVILIDAD Y QUIMIOTAXIS



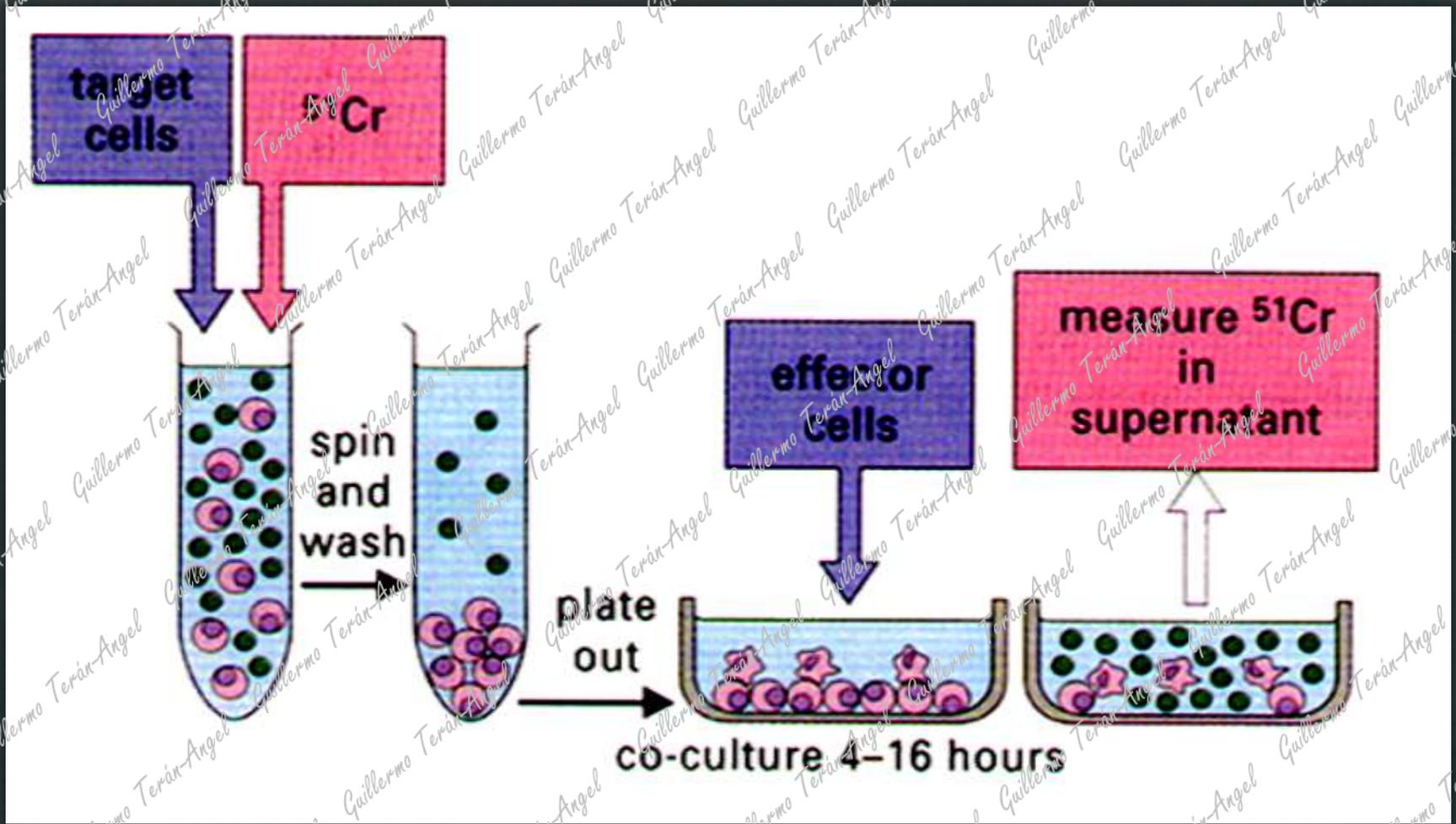
EVALUACIÓN DE CÉLULAS FAGOCÍTICAS



ESTRÉS OXIDATIVO



CELULAS CITOTÓXICAS



¡Pregunten Ahora o Callen Para Siempre!



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

ACTIVIDAD

PROBLEMA:

Un joven de 24 años se expone a una situación de riesgo de infección por VIH:

- a) Ud. como médico ¿qué le recomendaría al paciente?
- b) Por otra parte, el individuo decide ir a la semana a un laboratorio a hacerse una prueba de ELISA para evaluarse su estatus serológico, en el laboratorio le informan que ellos realizarán un ELISA de 4ta generación, dicha prueba resulta positiva. Así mismo, acude a un segundo laboratorio donde le realizan un ELISA de tercera generación, que arroja un resultado negativo. Explique cuál es la diferencia entre ambos tipos de ELISA y el porqué de la disparidad de los resultados
- c) En vista de los resultados obtenidos se le realiza una carga viral de VIH, la cual da un resultado de 500.000 copias/ml. Explique en qué consiste la cuantificación de carga viral por PCR real time, que sistemas de detección fluorescente se pueden emplear para llevar a cabo dicha cuantificación
- d) Luego de todas estas pruebas, explique cuál es el estado serológico de dicho paciente
- e) ¿Qué estudios adicionales le recomendaría Ud. a dicho joven?

Docencia en el IDIC-ULA (no somos sólo inmuno de 3ro)

Pregrado

Inmunología 3er año

Electivas

Investigación 6to año

Investigación 3er año (próximamente)

Servicio comunitario
4to y 5to año

Control ambiental de alergias

Prevención y bioseguridad VIH

Diagnóstico VIH

Postgrado

Maestría en Inmunología

CNCG Inmunodiagnóstico

Inmunología Residentes IAHULA

Doctorado en Inmunología (próximamente)

Cursos

Inmunodiagnóstico

Metodología y estadística