



MÉTODOS PARA  
EVALUAR LA  
RESPUESTA  
INMUNE

DRA. LUISA BARBOZA

# RESPUESTA INMUNE

Poblaciones celulares

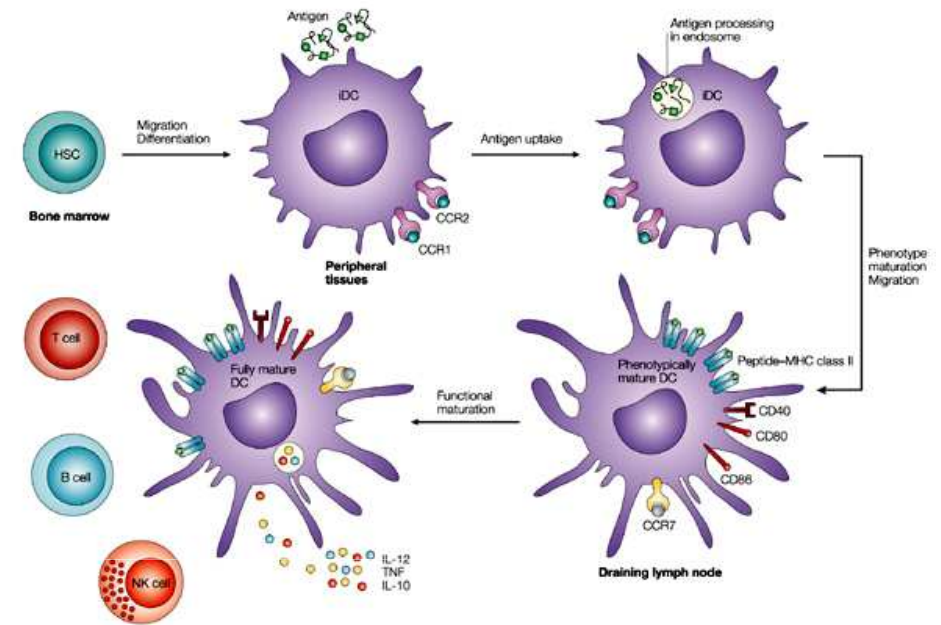
- Células T
- Células B
- Células fagocíticas
- Células citotóxicas

Productos de la respuesta

- Citoquinas
- Inmunoglobulinas/anticuerpos
- Complemento

Diagnóstico

- Patologías inmunes
- Procesos infecciosos

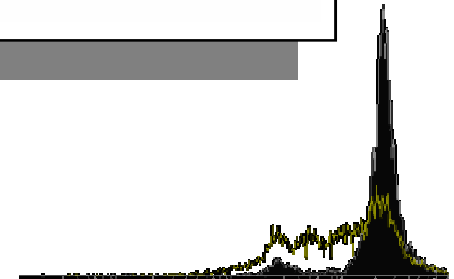
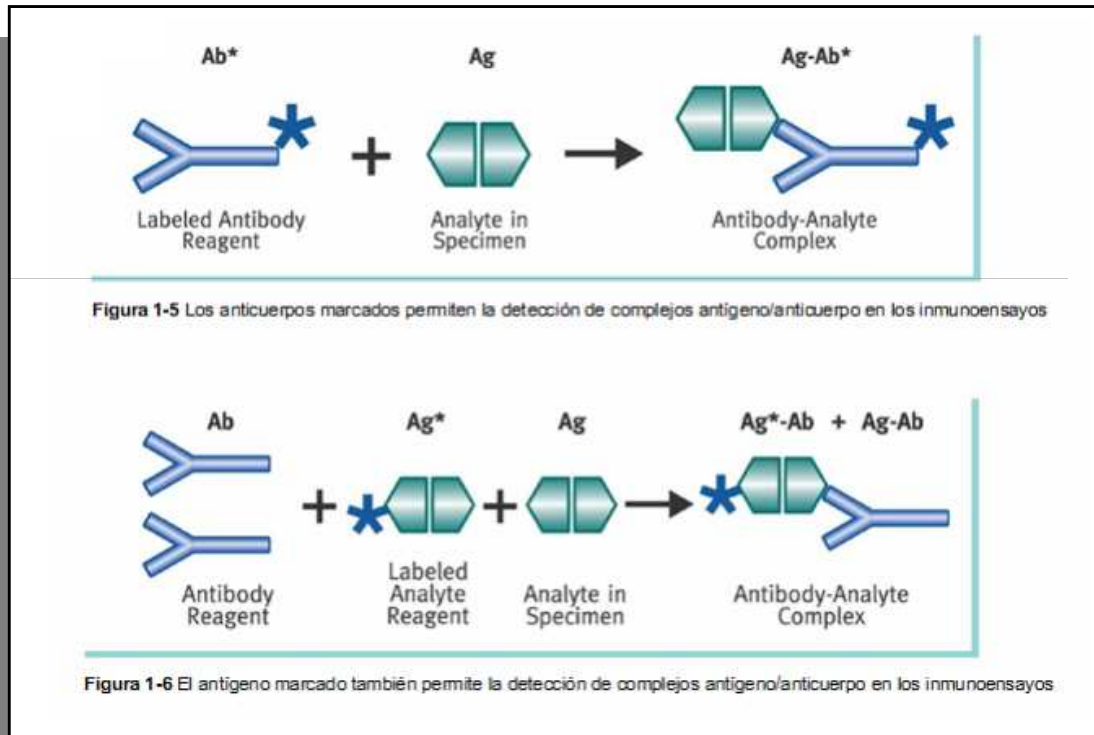


Nature Reviews | Immunology



# Técnicas basadas en interacción antígeno-anticuerpo

- Inmunoensayos
- Western blot
- Inmunoprecipitación
- Inmunohistoquímica
- Inmunofluorescencia





# Tipos de inmunoensayo:



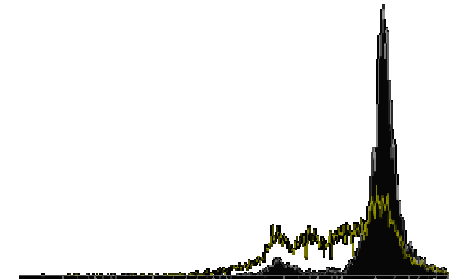
Inmunoensayo:  
Formación de  
inmunocomplejos  
(antígeno/anticuerpo)

Marcados:  
Conjugados a moléculas  
que emiten señales  
detectables

- Radioinmunoensayo (RIA): El marcador es un isótopo radioactivo.
- Análisis inmunoenzimáticos (EIA): El marcador es una enzima.
- Fluoroinmunoanálisis: El marcador es una partícula fluorescente.
- Ensayos inmunoquimioluminiscente: La marca es una sustancia quimioluminiscente.

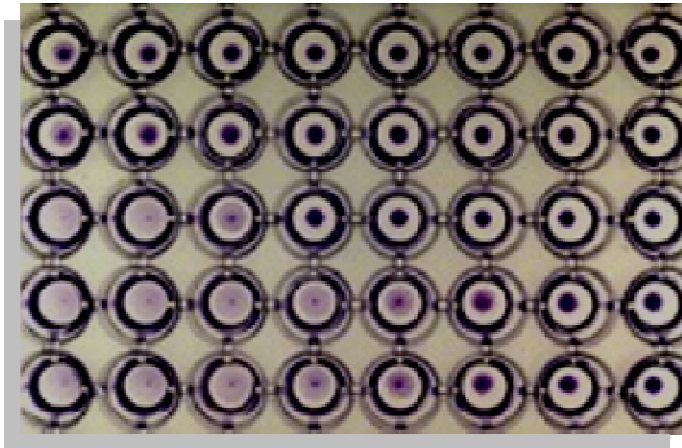
No marcados:  
Son medidos por dispersión  
de luz o por visualización  
directa

- Precipitación
- Aglutinación
- Difusión radial

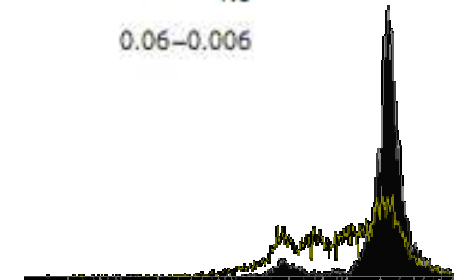


# Inmunoensayos:

## ✓ Comparación



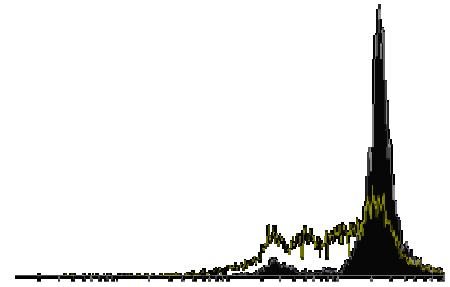
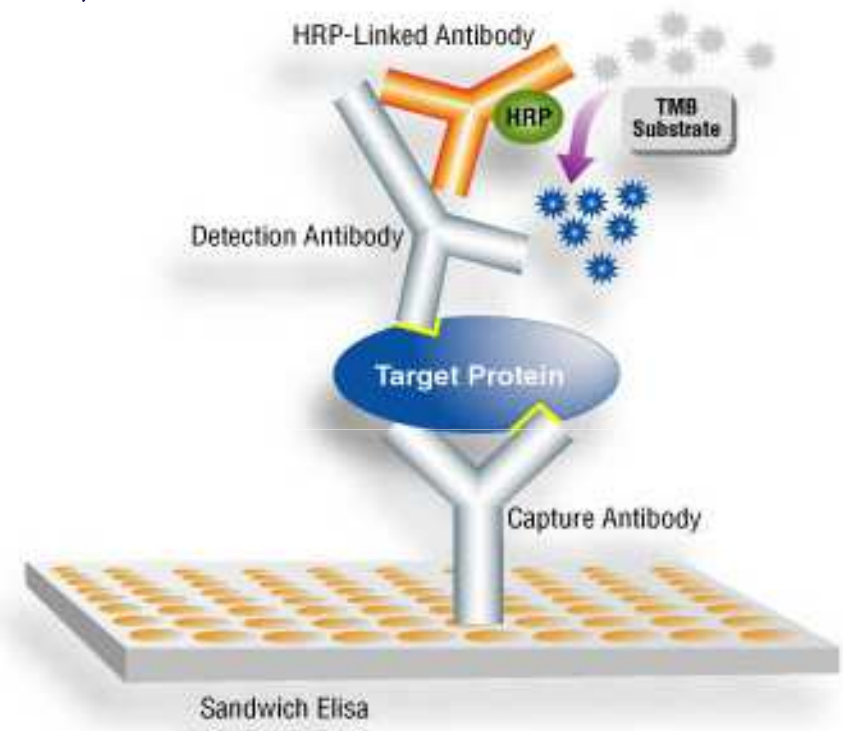
Assay	Sensitivity* ( $\mu\text{g}$ antibody/ml)
Precipitation reaction in fluids	20–200
Precipitation reactions in gels	
Mancini radial immunodiffusion	10–50
Ouchterlony double immunodiffusion	20–200
Immunelectrophoresis	20–200
Rocket electrophoresis	2
Agglutination reactions	
Direct	0.3
Passive agglutination	0.006–0.06
Agglutination inhibition	0.006–0.06
Radioimmunoassay	0.0006–0.006
Enzyme-linked immunosorbent assay (ELISA)	<0.0001–0.01
ELISA using chemiluminescence	<0.0001–0.01 <sup>†</sup>
Immunofluorescence	1.0
Flow cytometry	0.06–0.006



## ELISA (Enzyme linked Immunosorbent Assay):

La detección se realiza colorimétricamente por la interacción de un sustrato cromogénico y una enzima que ha sido acoplada a un anticuerpo detector. La prueba de ELISA se basa en la formación de inmunocomplejos, (reacción antígeno-anticuerpo, uno de los cuales debe ser de reactividad conocida), para detectar la presencia de un analito de interés. La detección se realiza colorimétricamente por la interacción de un sustrato cromogénico y una enzima que ha sido acoplada a un anticuerpo detector.

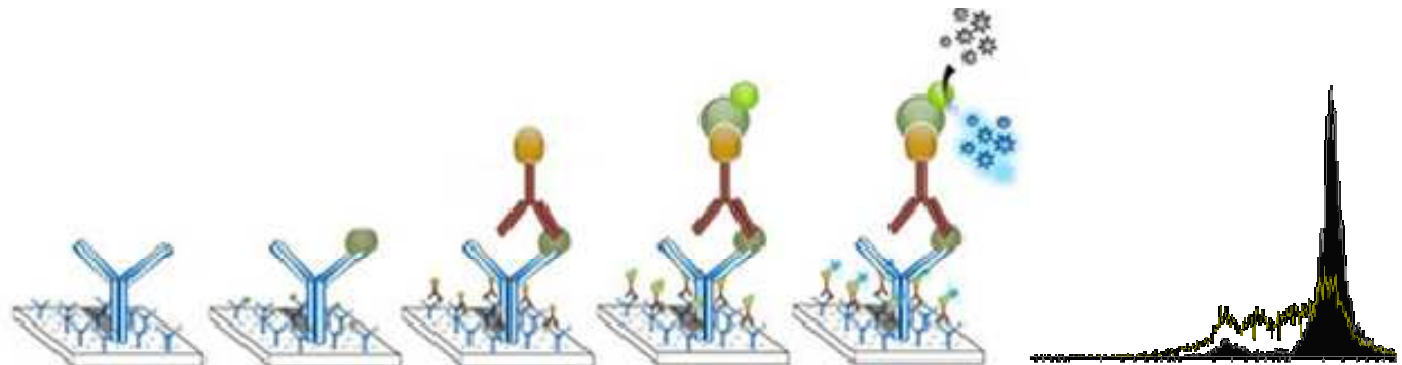
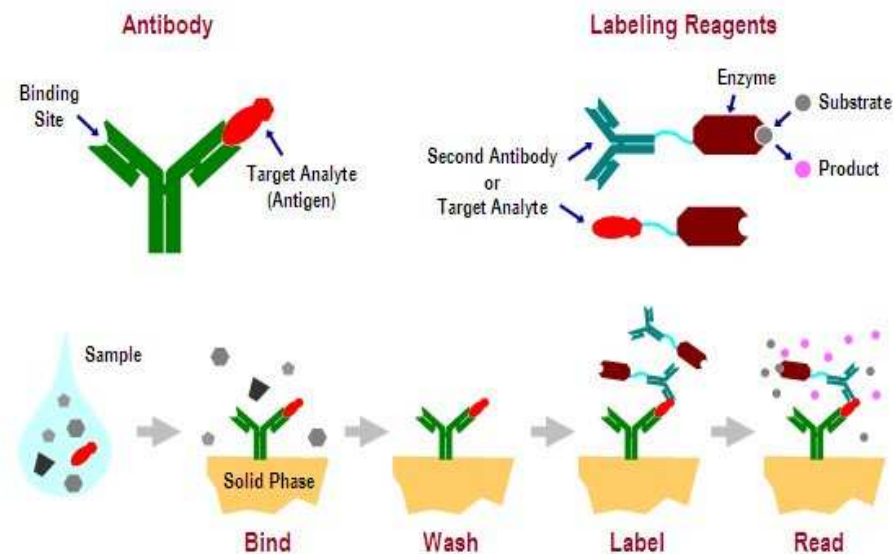
En el ELISA, uno de los reactivos se conjuga con una enzima formando un complejo con actividad inmunológica y enzimática.



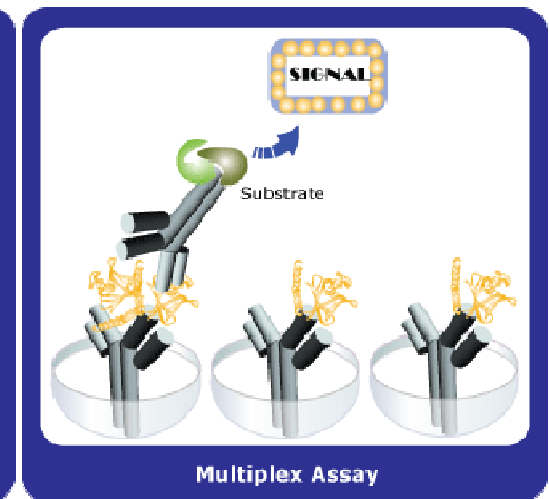
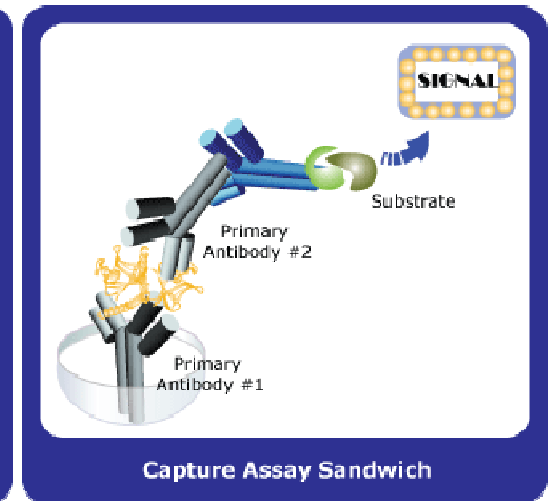
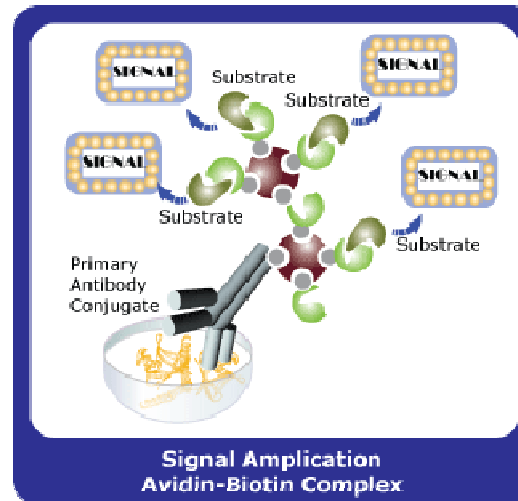
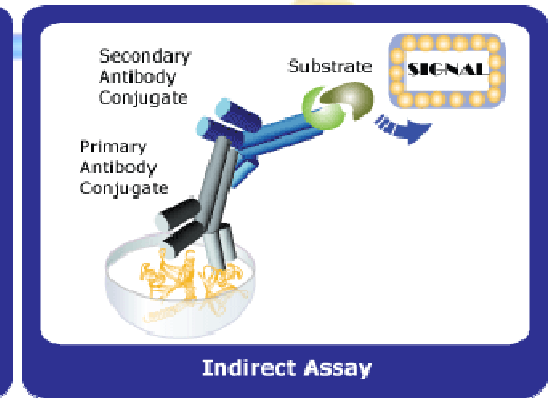
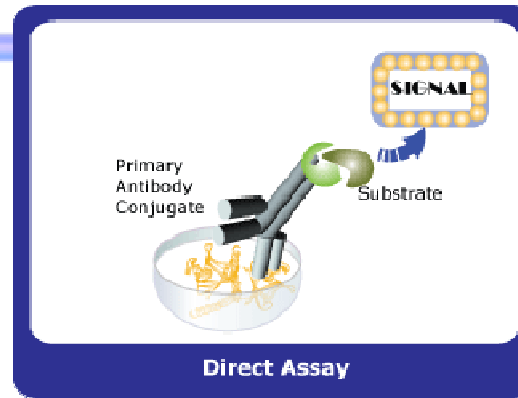
# ELISA. Pasos

- ✓ Conjugación del anticuerpo o del antígeno con la enzima.
- ✓ Unión del antígeno (o del anticuerpo) al soporte.
- ✓ Formación de los inmunocomplejos.
- ✓ Revelado de la reacción enzimática.

## ELISA



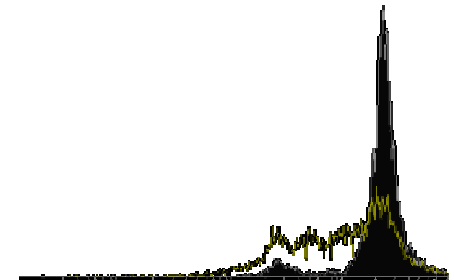
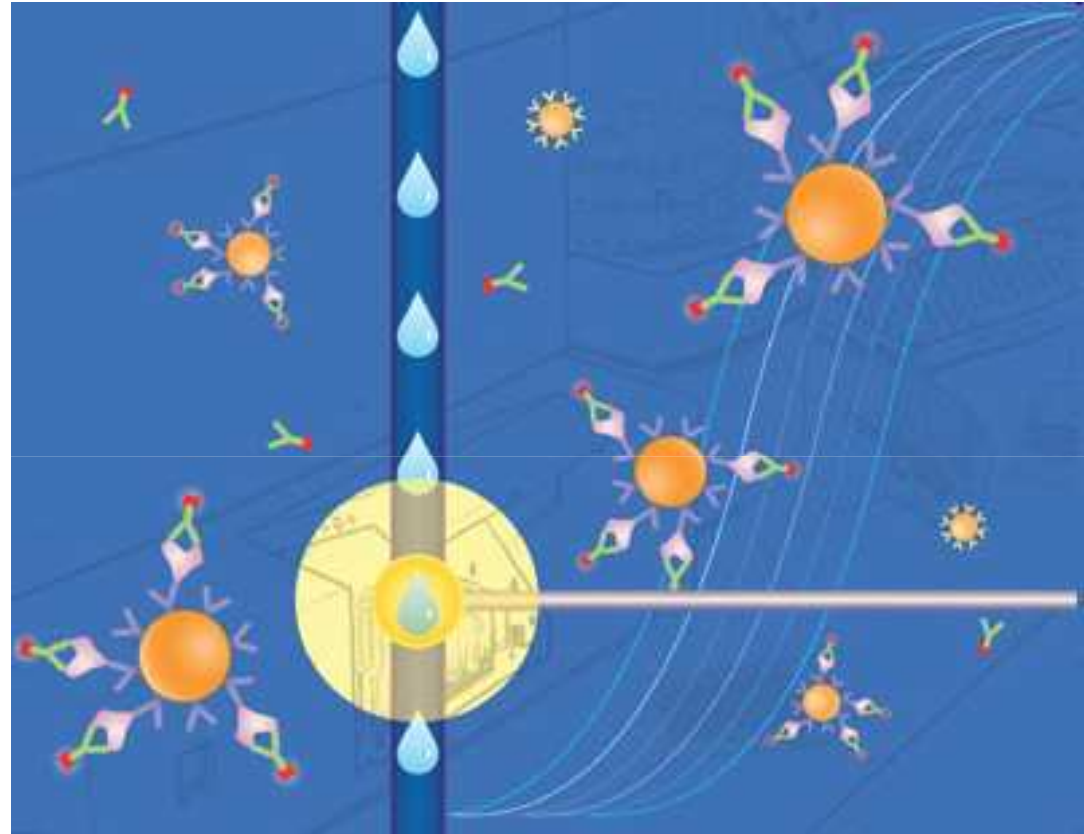
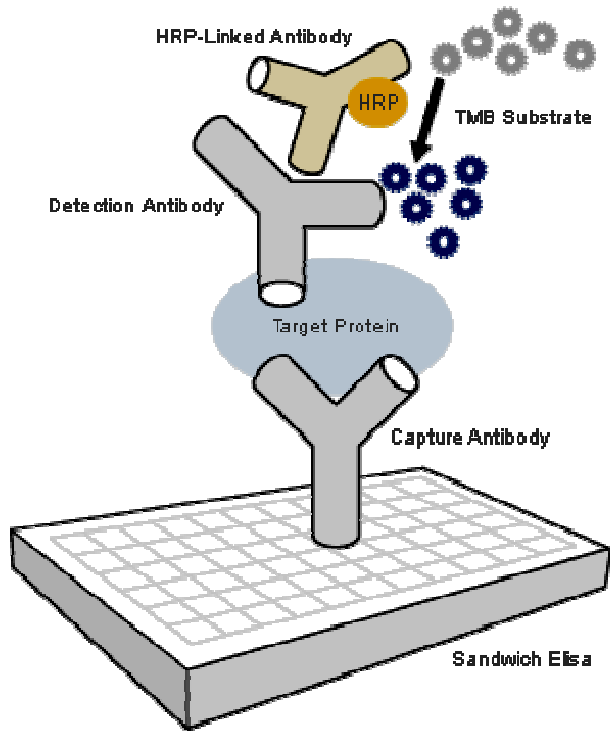
# Tipos de ELISA:





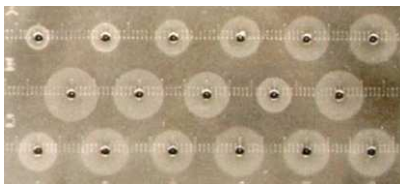
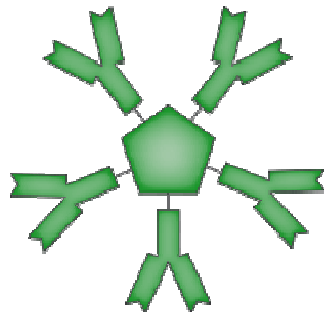
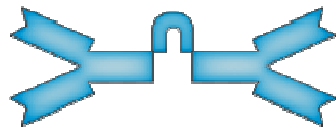
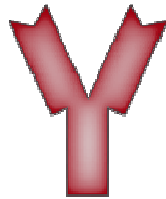
# Evaluación de células B

Anticuerpos / ELISA & CBA



# Utilidad

## Clases y subclases de Ig



**TABLE 4-2** Properties and biological activities\* of classes and subclasses of human serum immunoglobulins

Property/Activity	IgG1	IgG2	IgG3	IgG4	IgA1	IgA2	IgM <sup>‡</sup>	IgE	IgD
Molecular weight <sup>†</sup>	150,000	150,000	150,000	150,000	150,000–600,000	150,000–600,000	900,000	190,000	150,000
Heavy-chain component	γ1	γ2	γ3	γ4	α1	α2	μ	ε	δ
Normal serum level (mg/ml)	9	3	1	0.5	3.0	0.5	1.5	0.0003	0.03
In vivo serum half life (days)	23	23	8	23	6	6	5	2.5	3
Activates classical complement pathway	+	+/-	++	-	-	-	+++	-	-
Crosses placenta	+	+/-	+	+	-	-	-	-	-
Present on membrane of mature B cells	-	-	-	-	-	-	+	-	+
Binds to Fc receptors of phagocytes	++	+/-	++	+	-	-	?	-	-
Mucosal transport	-	-	-	-	++	++	+	-	-
Induces mast-cell degranulation	-	-	-	-	-	-	-	+	-

\*Activity levels indicated as follows: ++ = high; + = moderate; +/- = minimal; - = none; ? = questionable.

<sup>†</sup>IgG, IgE, and IgD always exist as monomers; IgA can exist as a monomer, dimer, trimer, or tetramer. Membrane-bound IgM is a monomer, but secreted IgM in serum is a pentamer.

<sup>‡</sup>IgM is the first isotype produced by the neonate and during a primary immune response.

# Utilidad

Detección de Ag y Ac

- Bacterias
- Parásitos
- Hongos
- Virus

Detección de complejos autoinmunes

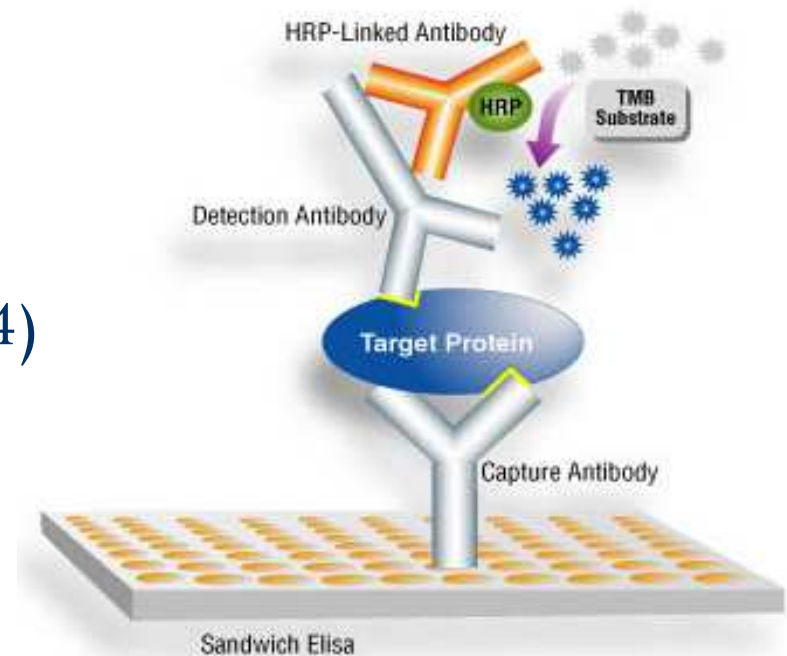
- Anti- DNA (Antígenos nucleares extractables: Sm - Ro - La - RNP)
- Anti-Histonas (H1, H2A, H2B, H3, H4)

LES



UNIVERSIDAD  
DE LOS ANDES

**idic**  
INSTITUTO DE INMUNOLOGIA CLINICA



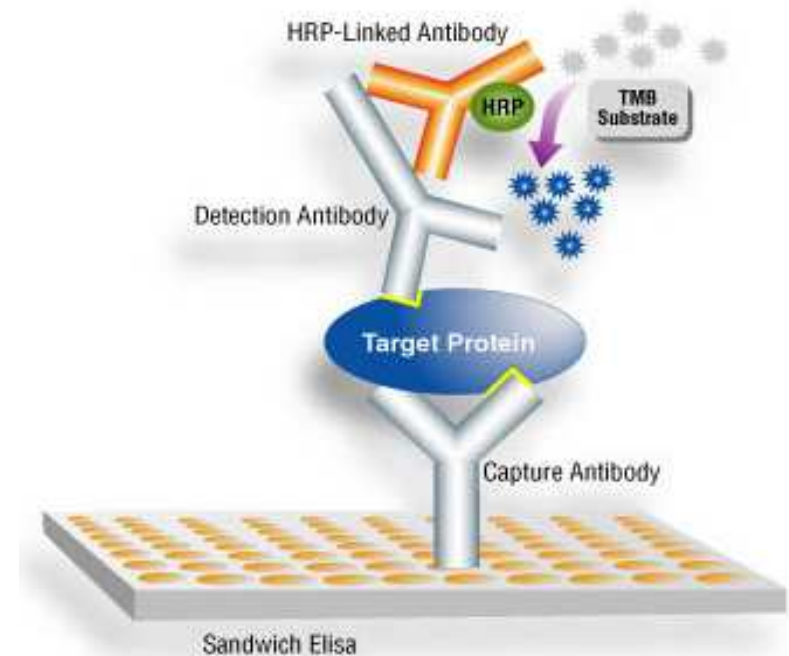
# Utilidad

Detección de Acs contra antígenos de tejido

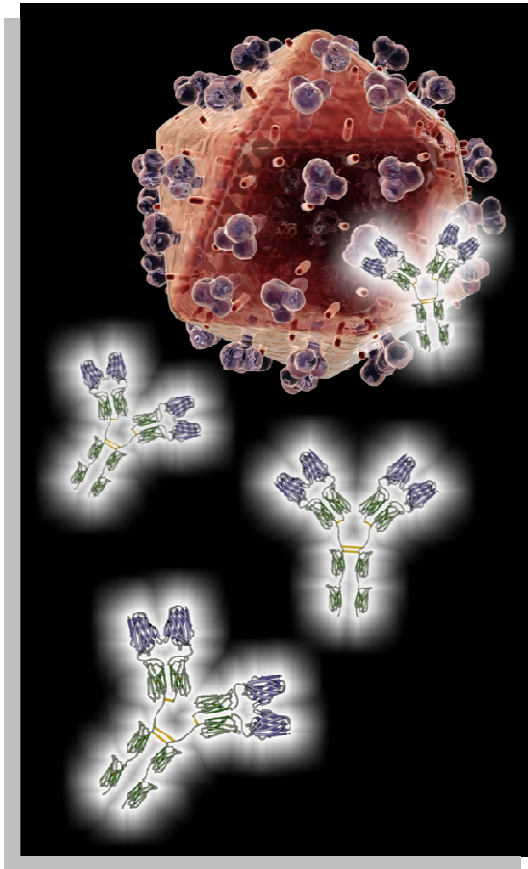
- Anti-tiroglobulina
- Anti-microsomal
- Anticuerpos Antifosfolípidos (Cardiolipina IgG-M)

Detección de Ags asociados a tumores:

- Ag prostático
- Ag ovario (Ca125)
- ACE, alfafetoproteína



## ELISA<sub>s</sub> y Generaciones:



### 1<sup>ra</sup> generación

- Ag: lisado purificado de VIH
- Pocas sensibilidad y especificidad

### 2<sup>da</sup> generación

- Ag: proteínas recombinantes de VIH.  
Detección de VIH-1 y VIH-2
- Poca sensibilidad, mejora la especificidad

### 3<sup>ra</sup> generación

- Ag: proteínas recombinantes de VIH.  
Detección del grupo O del VIH. IgM e IgG
- Mejora la sensibilidad

### 4<sup>ta</sup> generación

- Capacidad para detectar al Ag p24 y anticuerpos

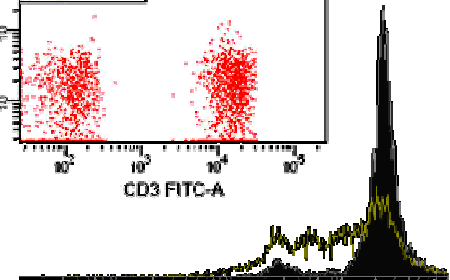
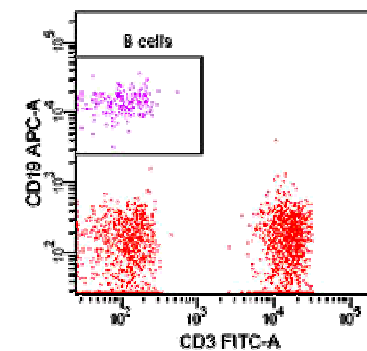
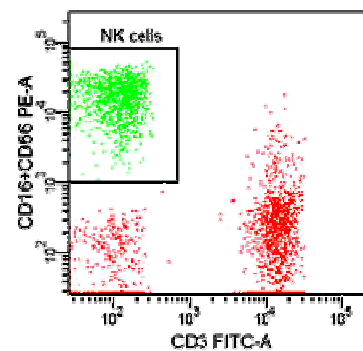
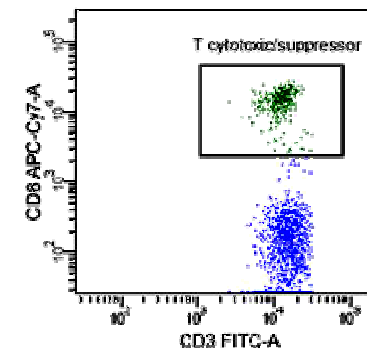
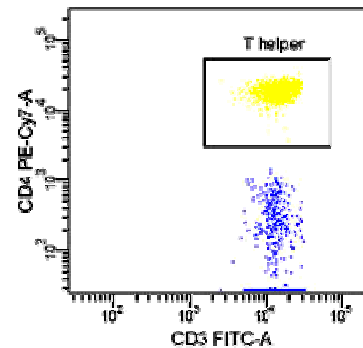
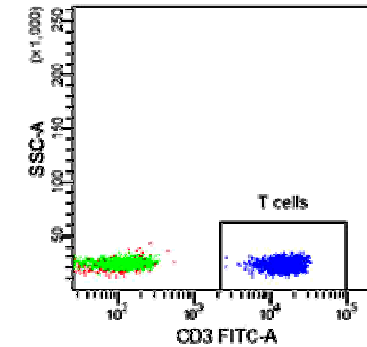
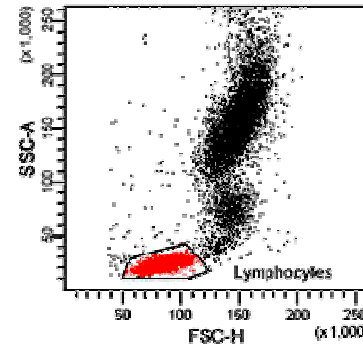
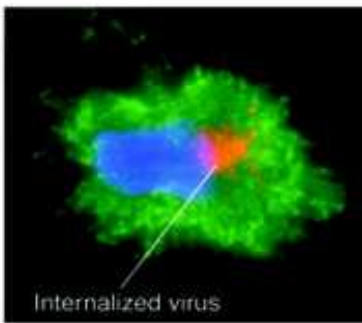


UNIVERSIDAD  
DE LOS ANDES

**idic**  
INSTITUTO DE INMUNOLOGIA CLINICA

# Técnicas basadas en fluorescencia

- Citometría de flujo
- Inmunofluorescencia-  
Microscopia de fluorescencia



UNIVERSIDAD  
DE LOS ANDES

**idic**  
INSTITUTO DE INMUNOLOGIA CLINICA

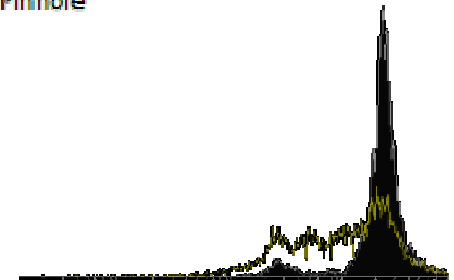
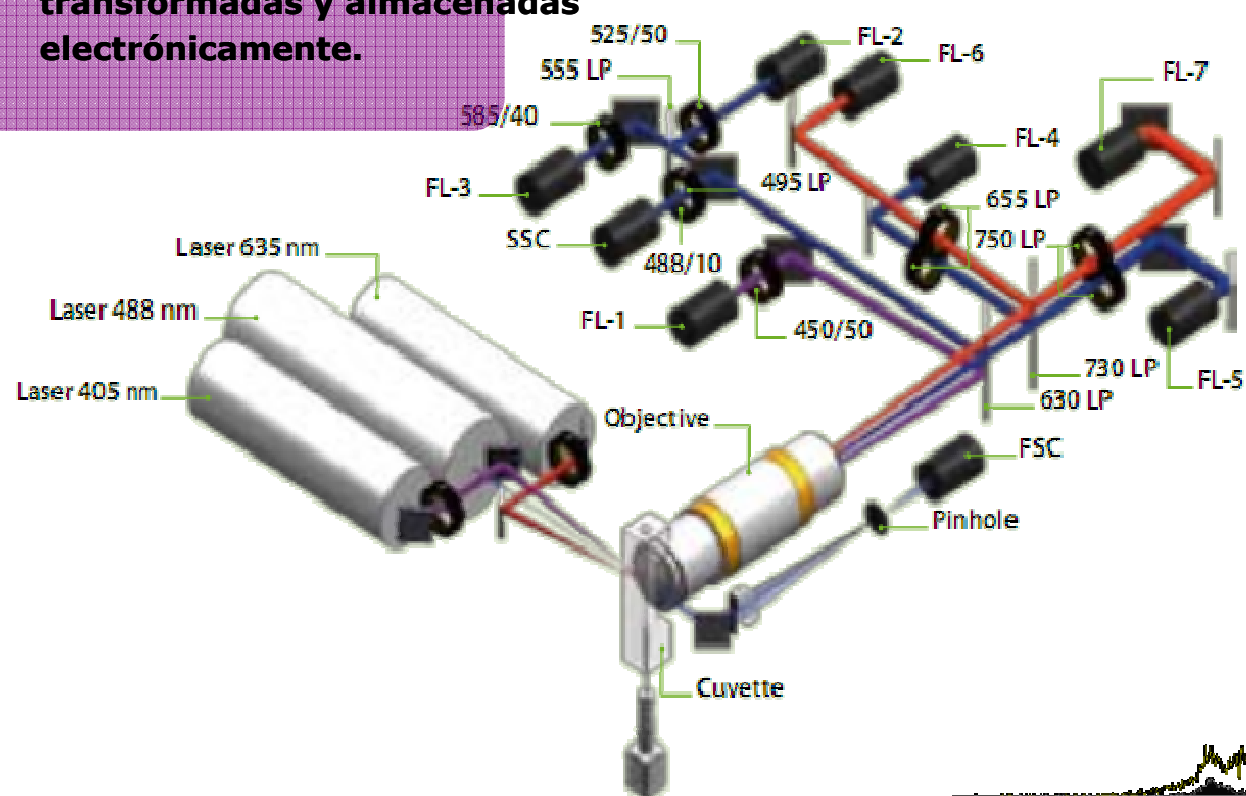
# Fluorescence Activated Cell Sorting (FACS)

Hidrodinámica

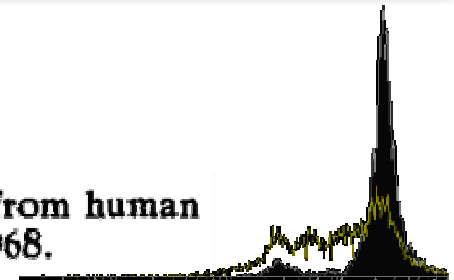
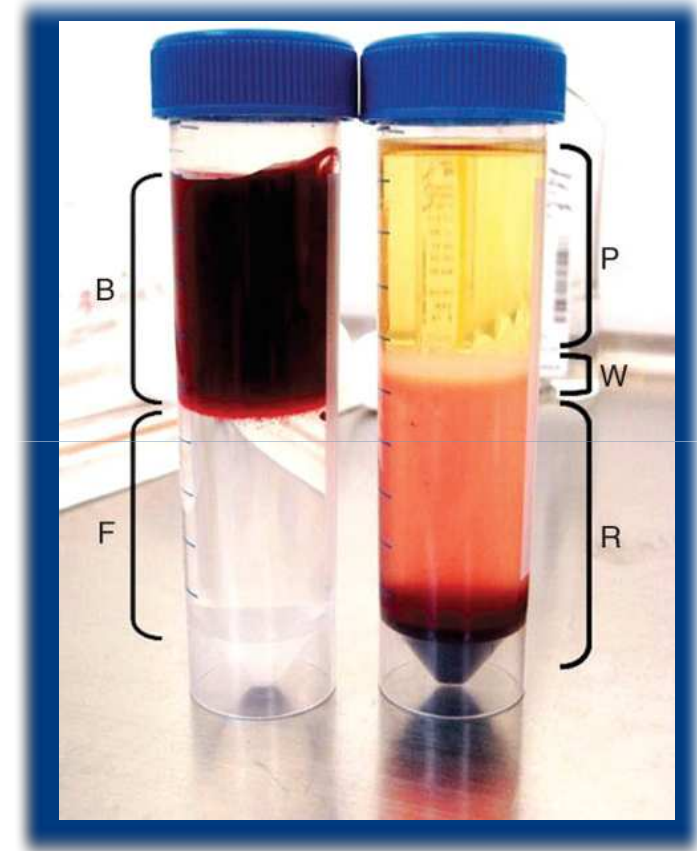
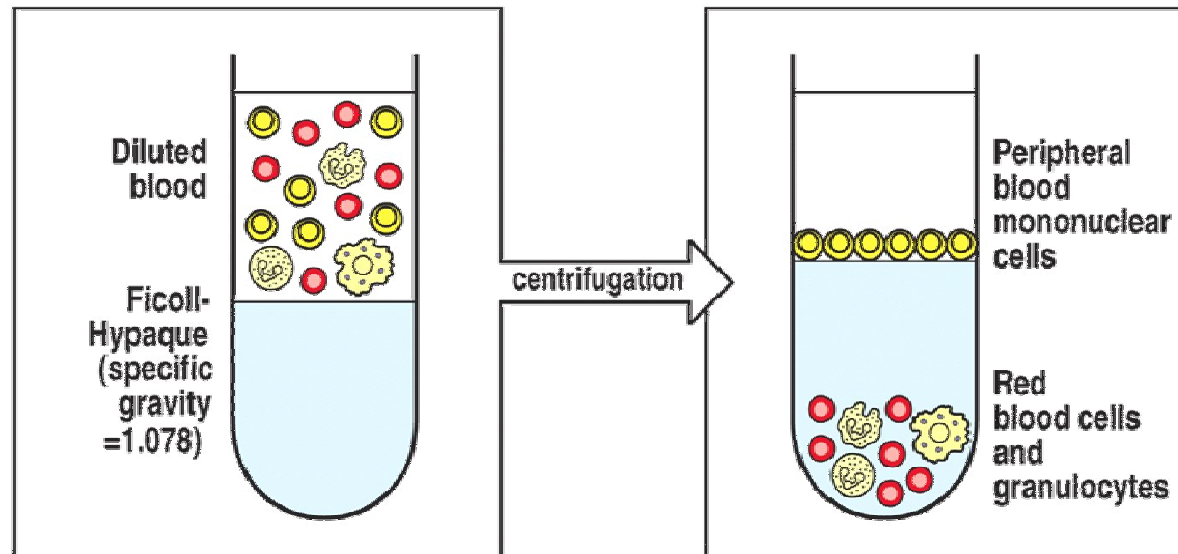
Óptica

Electrónica

Técnica que permite analizar partículas en suspensión que se hacen fluir a través de un rayo de luz, las partículas interactúan dispersando este haz de luz y emitiendo fluorescencia, estas señales ópticas son detectadas, transformadas y almacenadas electrónicamente.

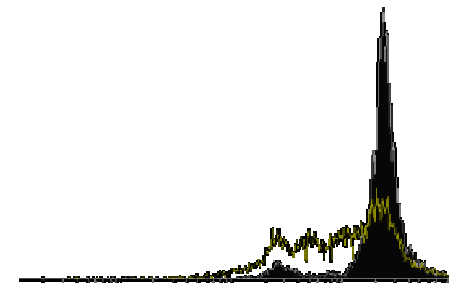
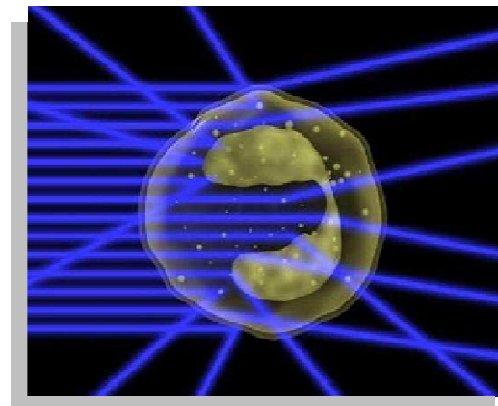
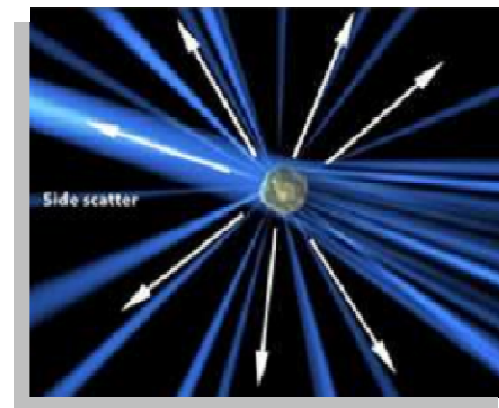
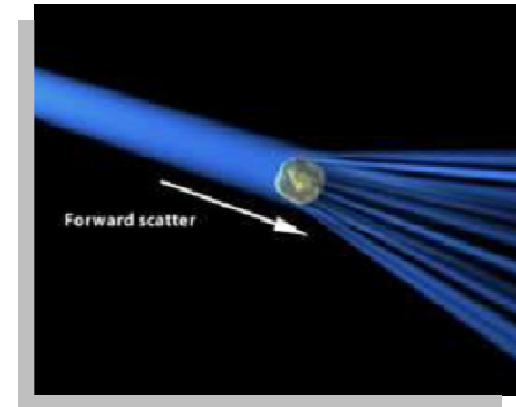
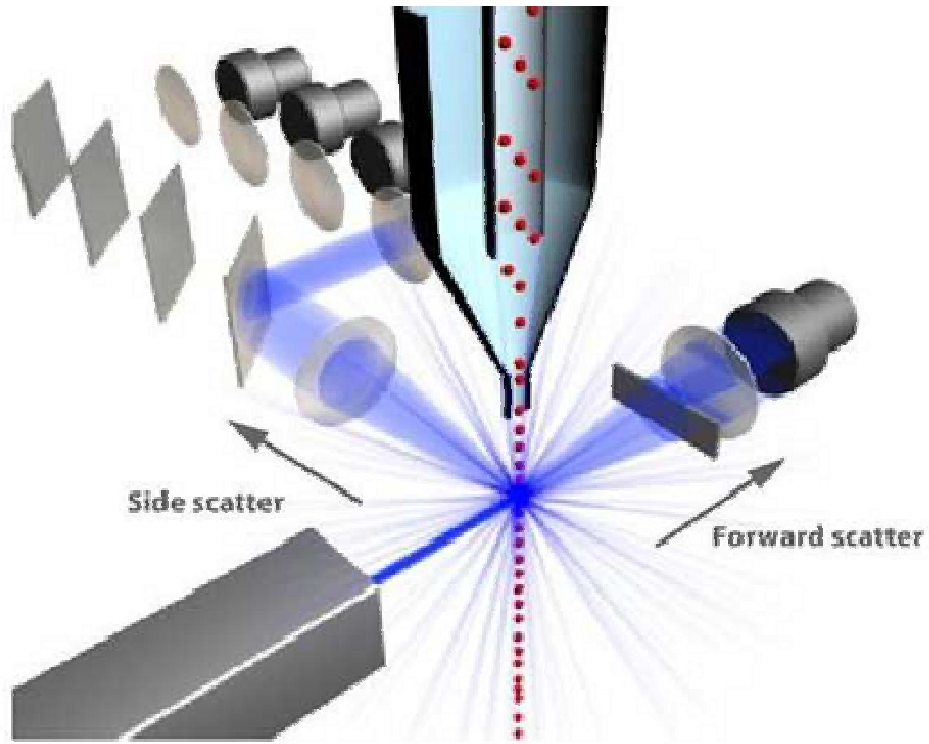


# Separación Celular

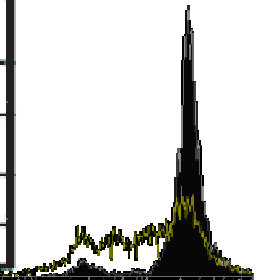
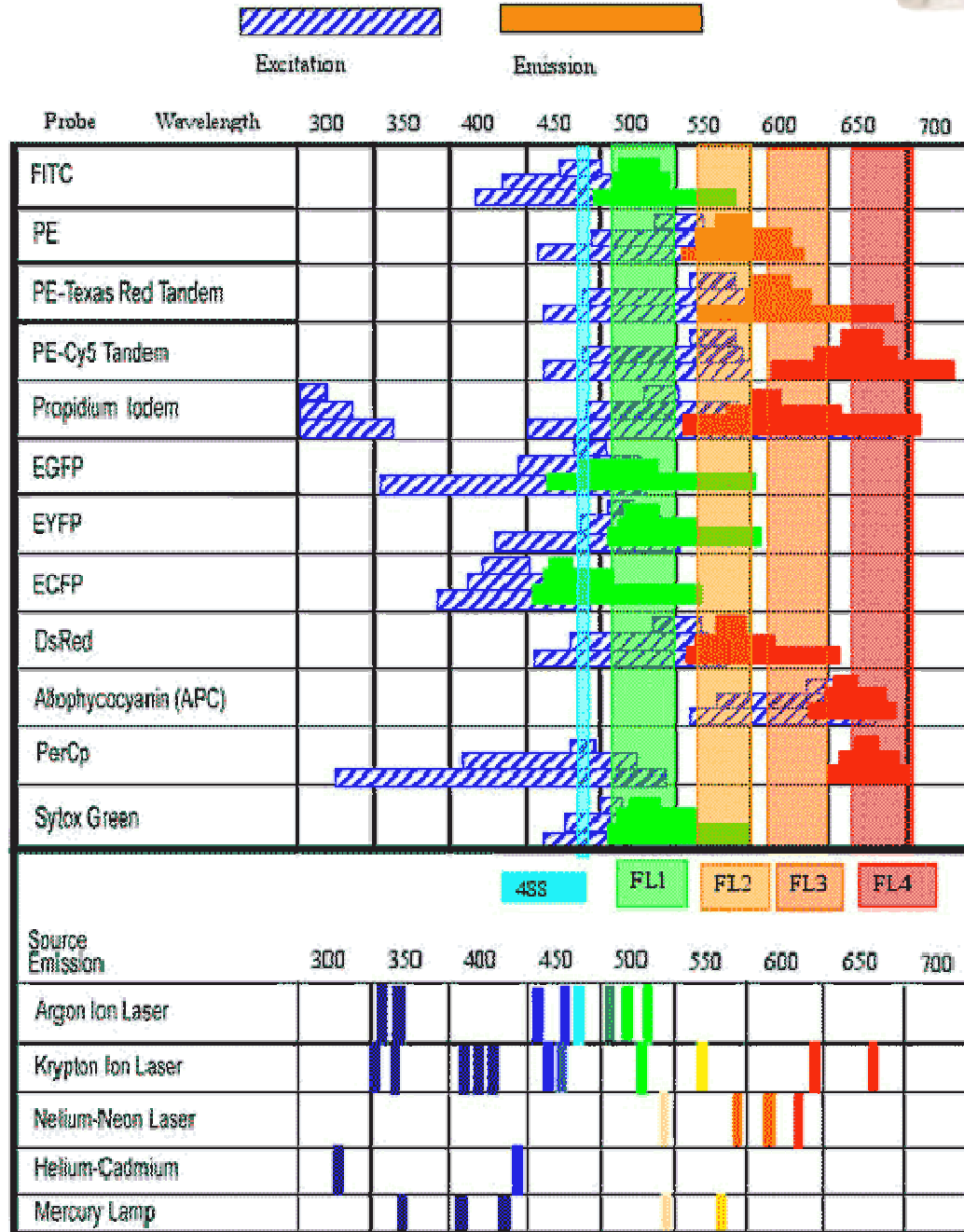
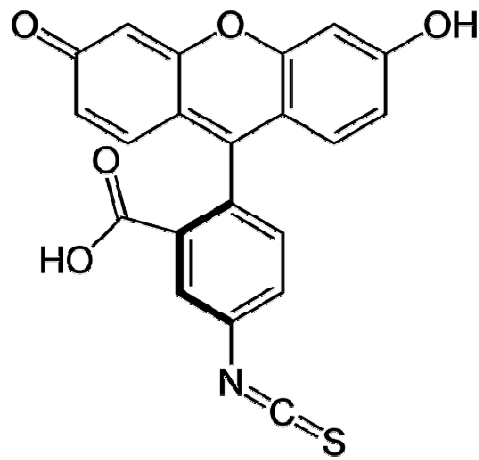




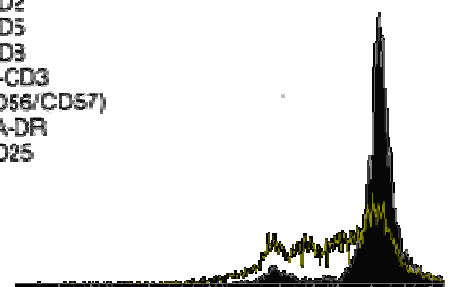
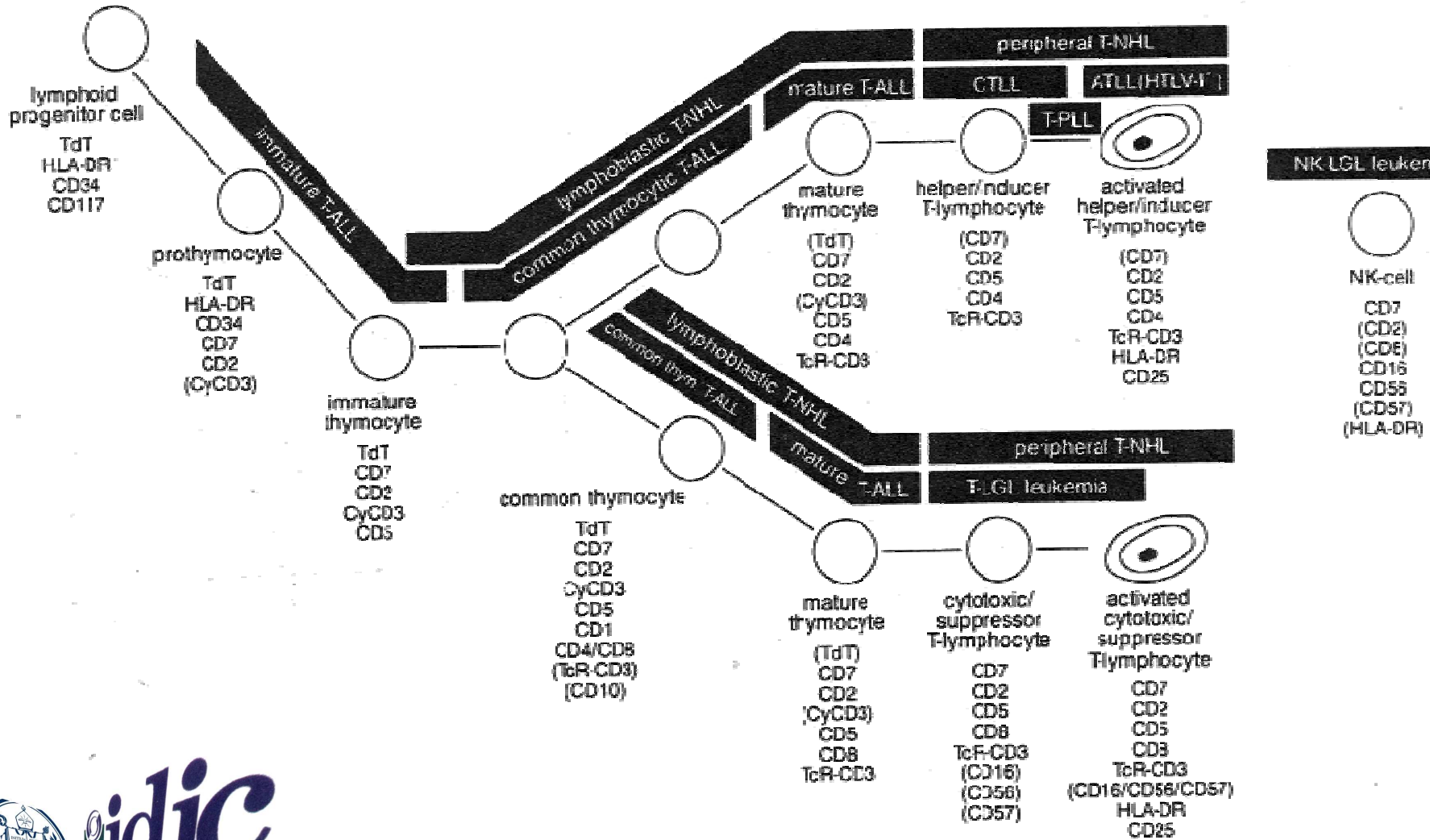
# Óptica



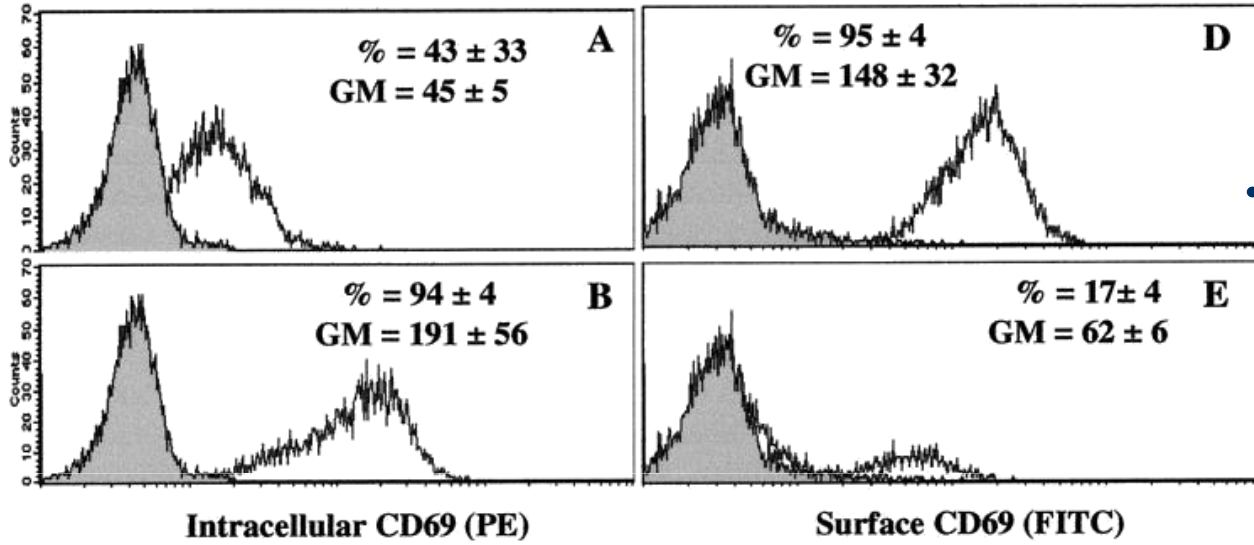
# Fluorocromos



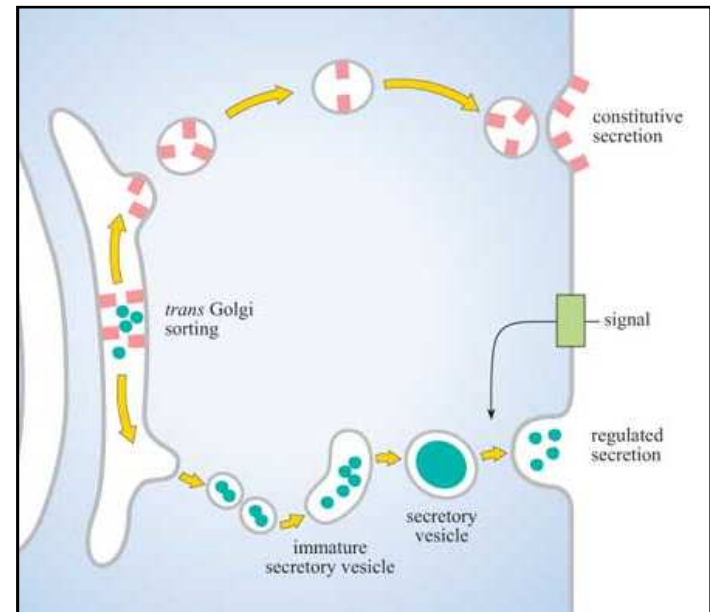
# Utilidad: Evaluación de la función hematopoyética y de leucemias/linfomas



# Producción de citocinas/ marcadores intracelulares

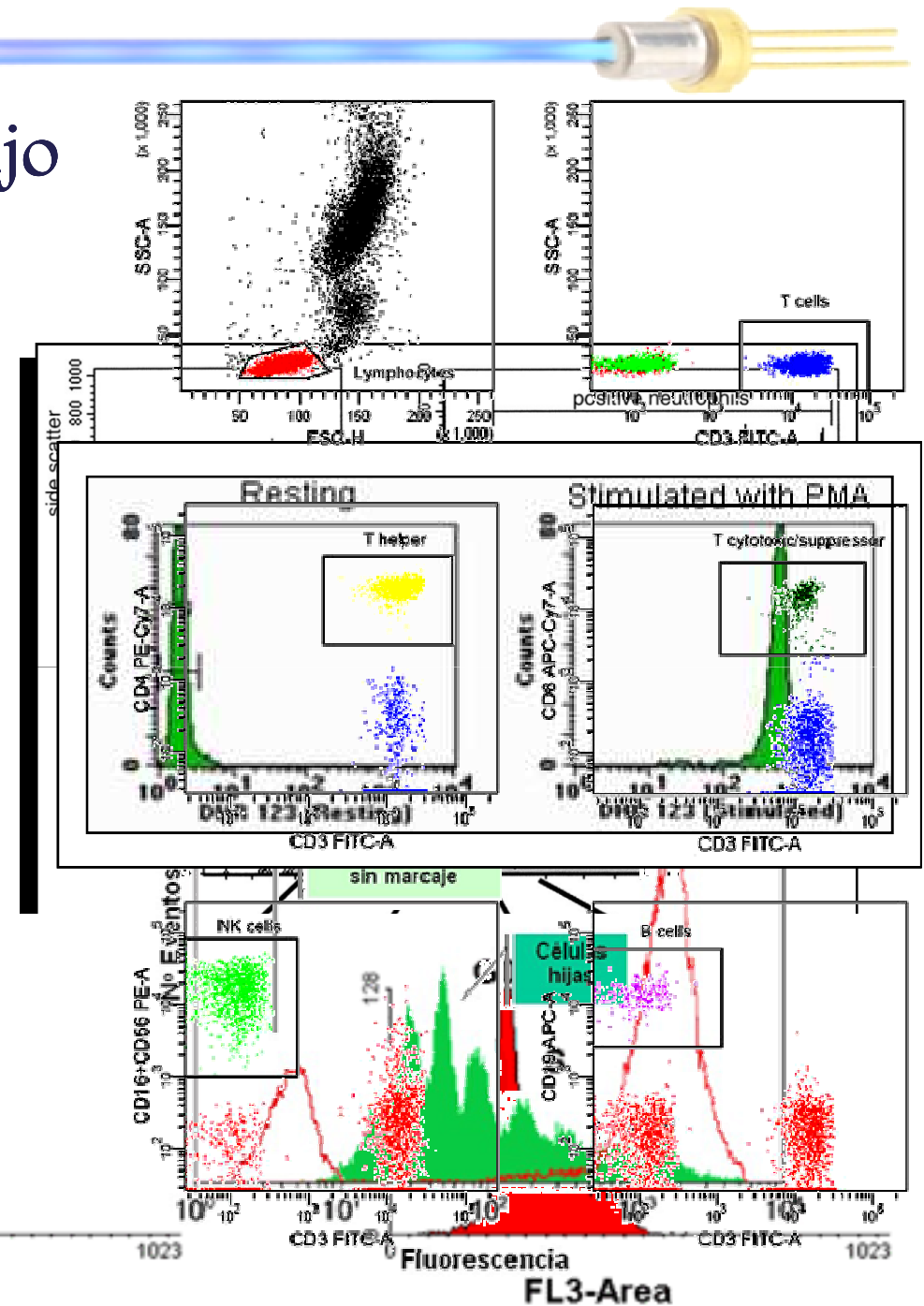


• Ionomicina / Brefeldina A

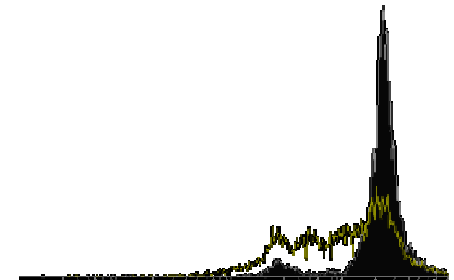
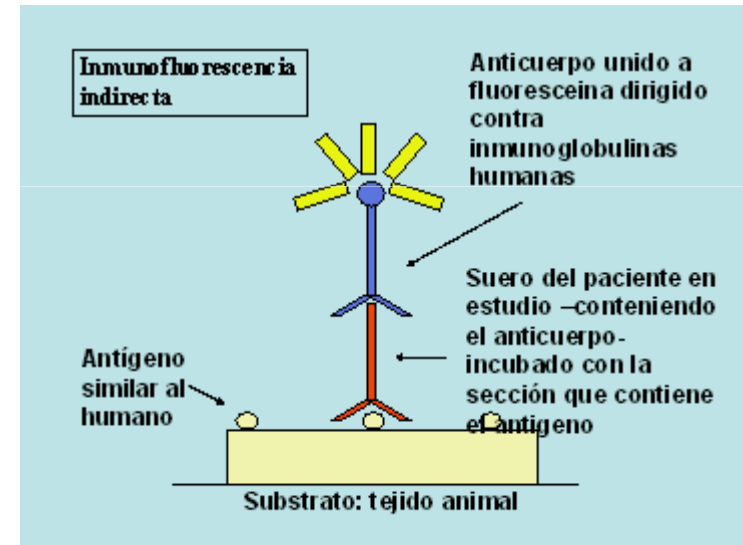
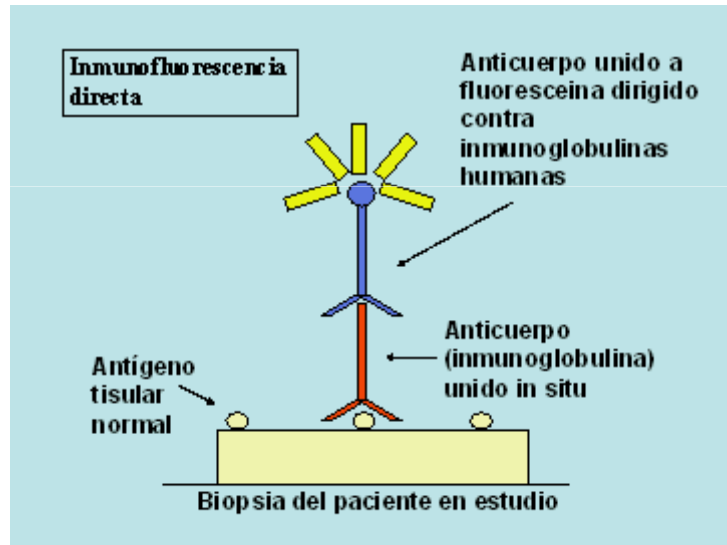


# Usos de la citometría de flujo

- ✓ Evaluación de subpoblaciones celulares
- ✓ Análisis de contenido de ADN (grado de ploidía)
- ✓ Proliferación celular
- ✓ Producción de citocinas/marcadores celulares
- ✓ Fagocitosis
- ✓ Estrés oxidativo



# Inmunofluorescencia



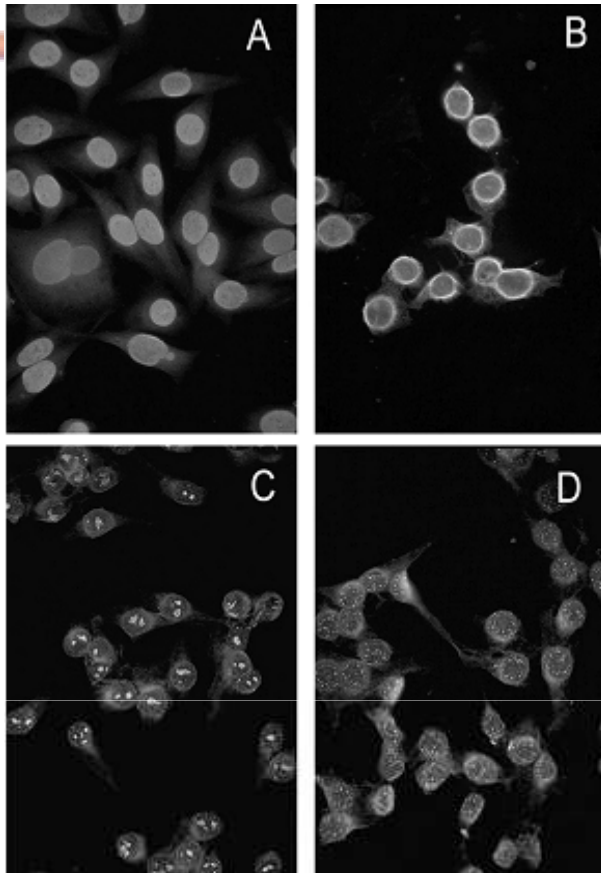
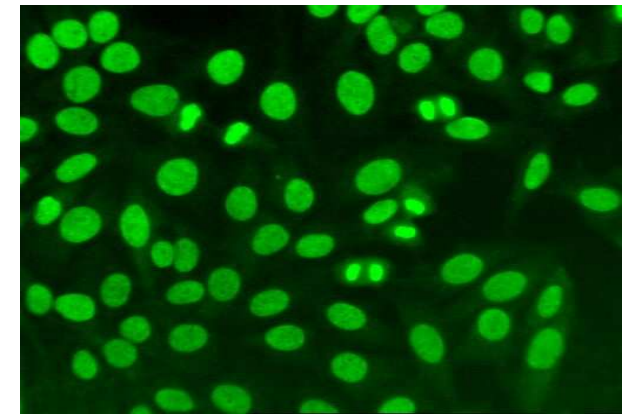


Figura 12.3: Patrones de inmunofluorescencia en la determinación de anticuerpos antinucleares. (A) patrón difuso; (B) patrón periférico; (C) patrón nucleolar; (D) patrón moteado.

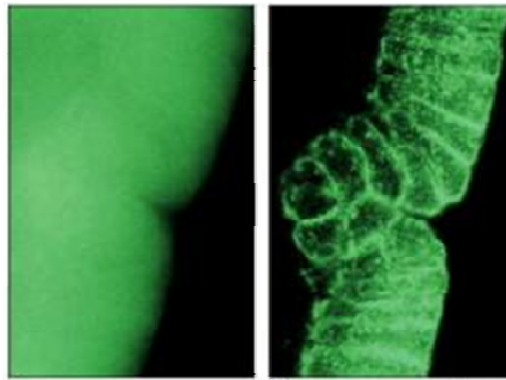
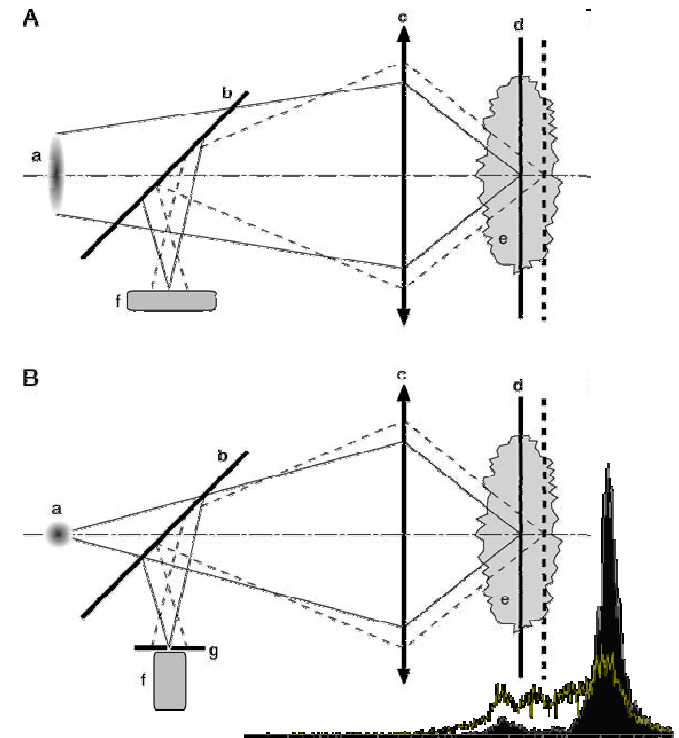
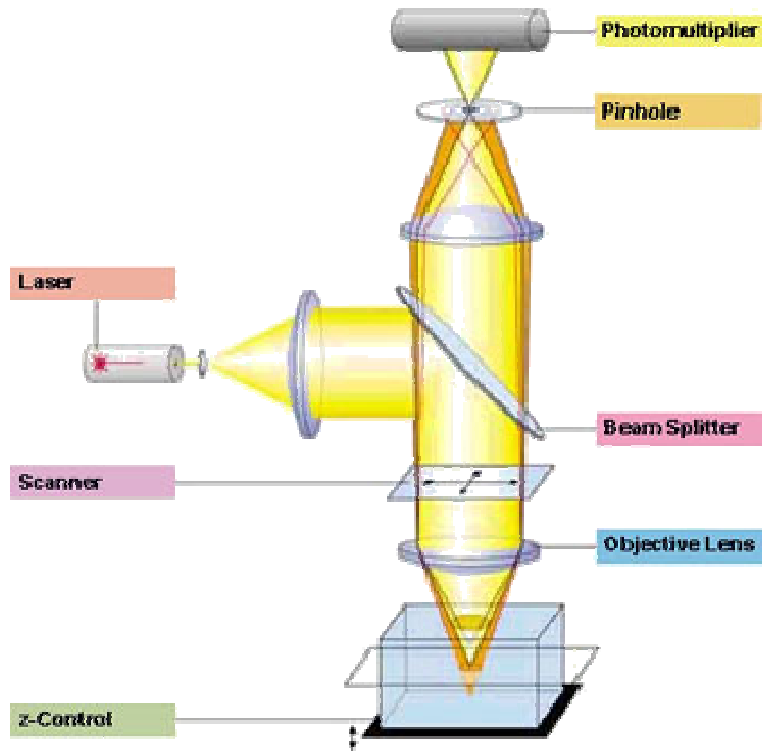


## Inmunofluorescencia

- ✓ Anticuerpos Antinucleares (IFI Hep-2)
- ✓ Anticuerpos Anti DNA
- ✓ Anticuerpos Antimitocondria
- ✓ Anticuerpos Antimúsculo Liso
- ✓ Anticuerpos contra Polimorfonuclear Neutrófilo (ANCA)
- ✓ Anti-FTA



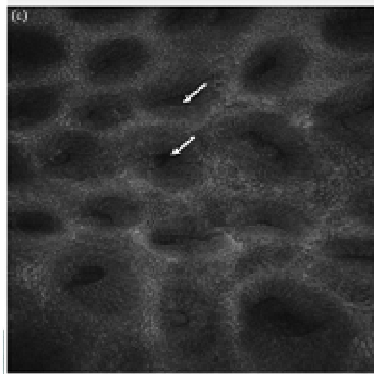
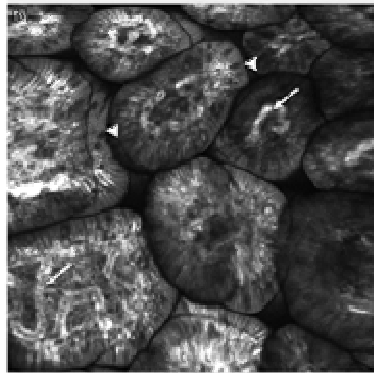
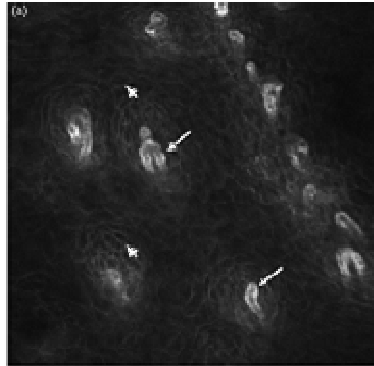
# Microscopía confocal





# Microscopía confocal

Aplicaciones!!!



INSTITUTO DE INMUNOLOGIA CLINICA

## Confocal endomicroscopy

Kerry Dunbar and Marcia Canto

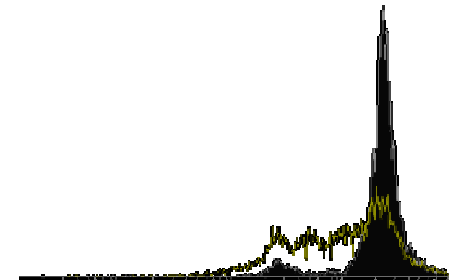
Current Opinion in Gastroenterology 2008, 24:631-637

Table 2 Comparison of reported performance characteristics in endomicroscopy studies

	Sensitivity (%)	Specificity (%)	Accuracy (%)
CLE pattern classification for colorectal pathology [5]	97.4	99.4	99.2
GI neoplasia-miniprobe CLE [4*]	93.1	92.1	92.4
Chromoendoscopy-guided CLE in UC [7**]	94.7	98.3	97.8
Chromoendoscopy-guided CLE in UC [8*]	94	92	-
CLE for DALM and ALM [9**]	100	96.6	97
Chromoendoscopy-guided CLE for polyps [10]	97.4	99.3	99.1
CLE for distinguishing adenoma vs. hyperplastic polyps [11]	83	100	89
Confocal Barrett's esophagus classification [12]	92.3	98.4	97.4
CLE-guided EMR [13]	94	50	-
Esophageal squamous cell carcinoma [14*]	100	87	95
Gastric pit pattern			
Neoplasia	90	99.4	97.1
Atrophy	83.6	99.6	97.5
Gastritis [15**]	81.9	99.3	95.8
Confocal celiac criteria [16]	70	95	80
Fluorescent peptide for colon dysplasia-confocal miniprobe [17**]	81	82	

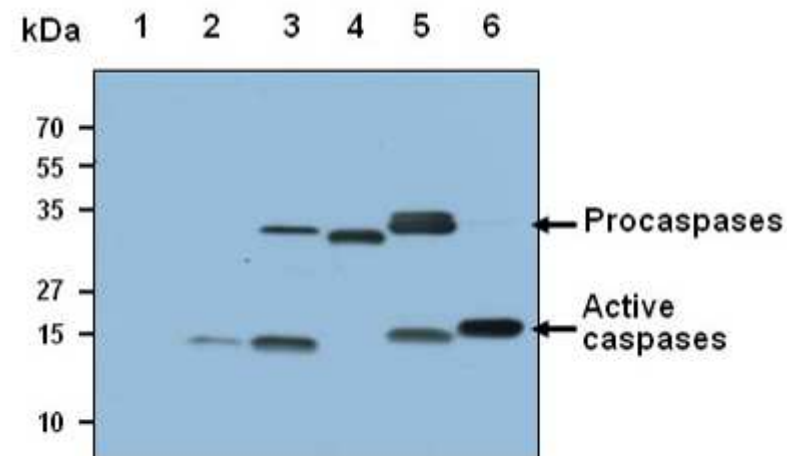
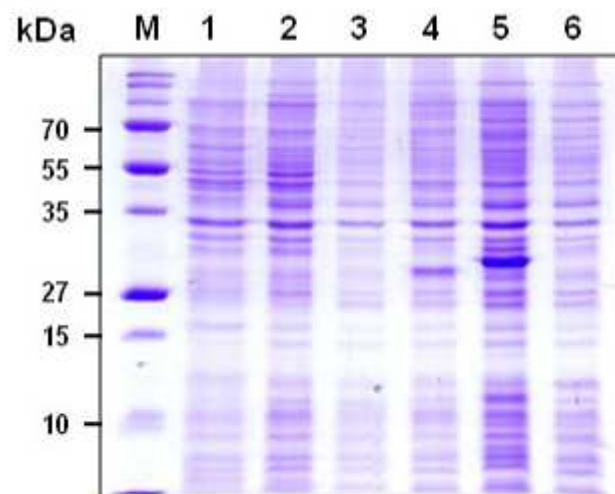
ALM, adenoma-like masses; CLE, confocal laser endomicroscopy; DALM, dysplasia-associated lesion masses; EMR, endoscopic mucosal resection; GI, gastrointestinal; UC, ulcerative colitis.

- ✓ FITC sódica
- ✓ Violeta de Cresilo
- ✓ Acriflavina HCL





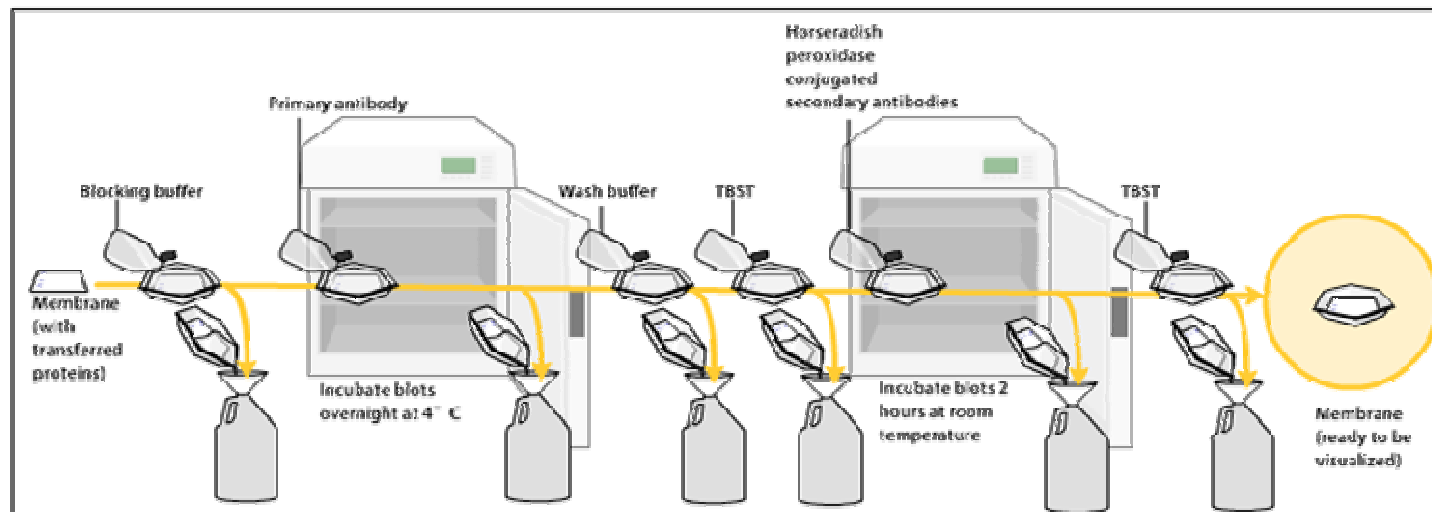
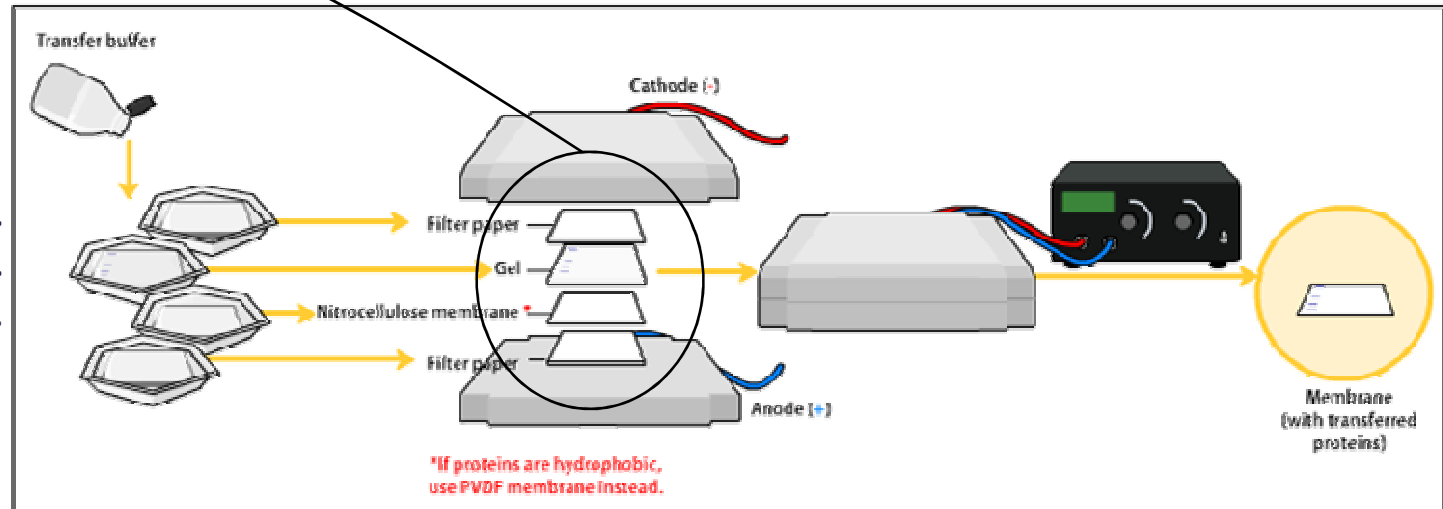
# WESTERN BLOTTING



# WESTERN BLOTTING

## Pasos básicos

- Difusión.
- Capilaridad (southern like).
- Electroforética.

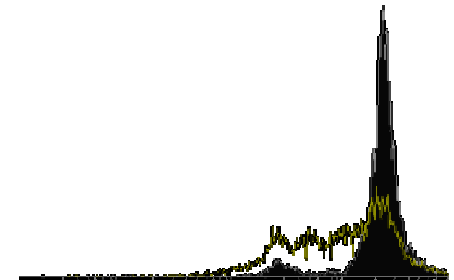
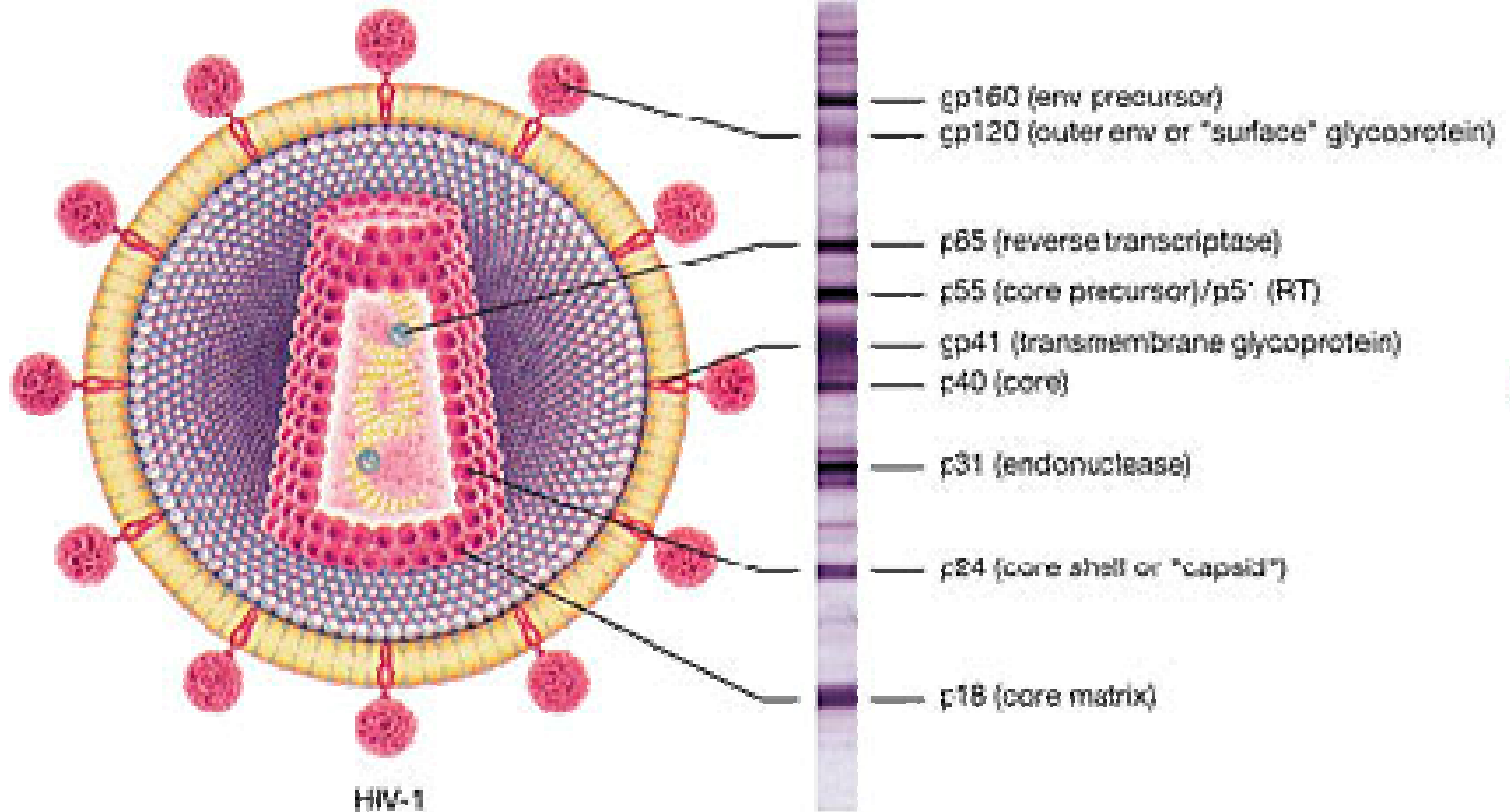


UNIVERSIDAD  
DE LOS ANDES

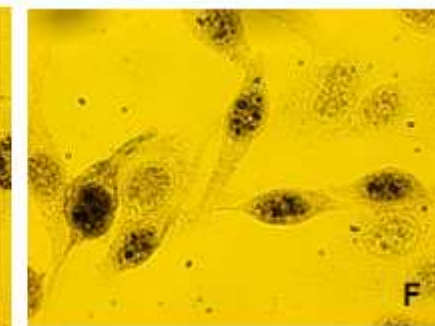
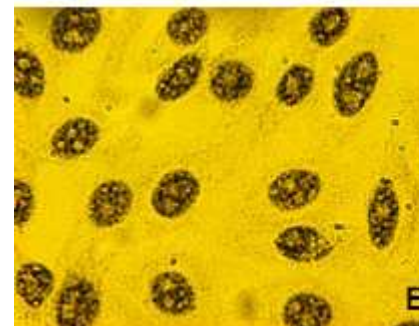
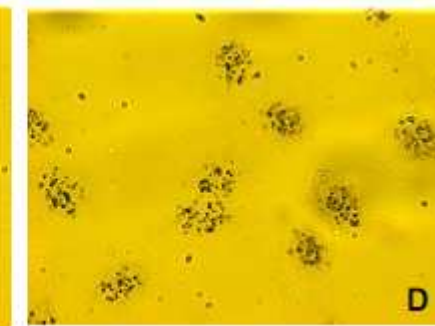
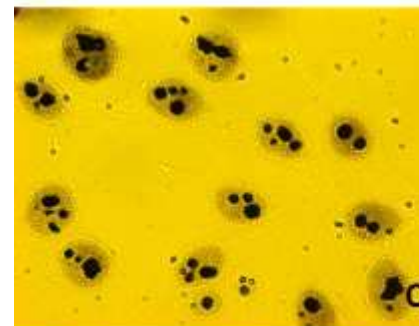
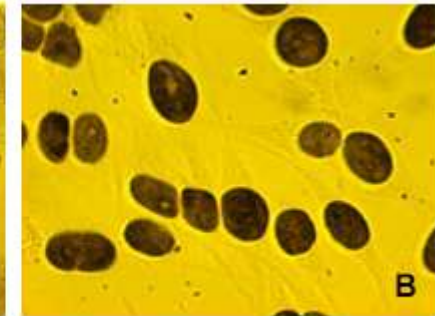
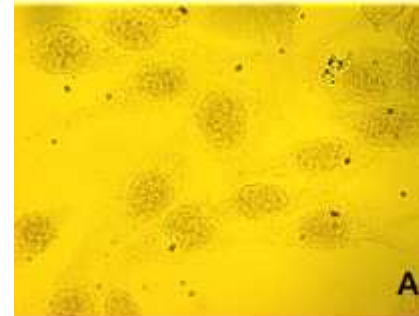
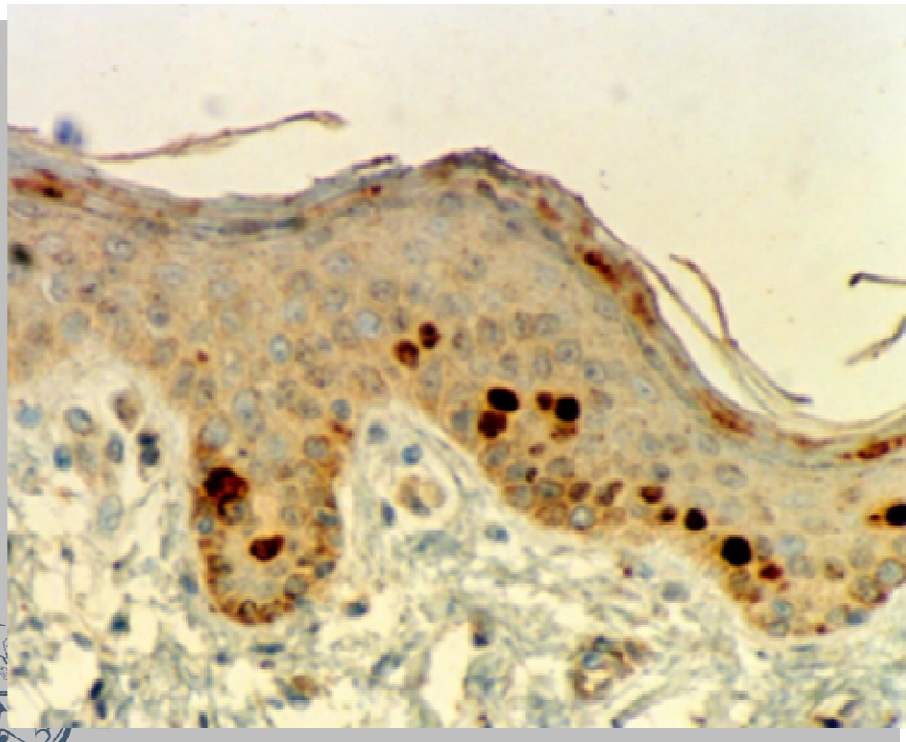
**idic**  
INSTITUTO DE INMUNOLOGIA CLINICA

# WESTERN BLOTTING

Everybody knows western



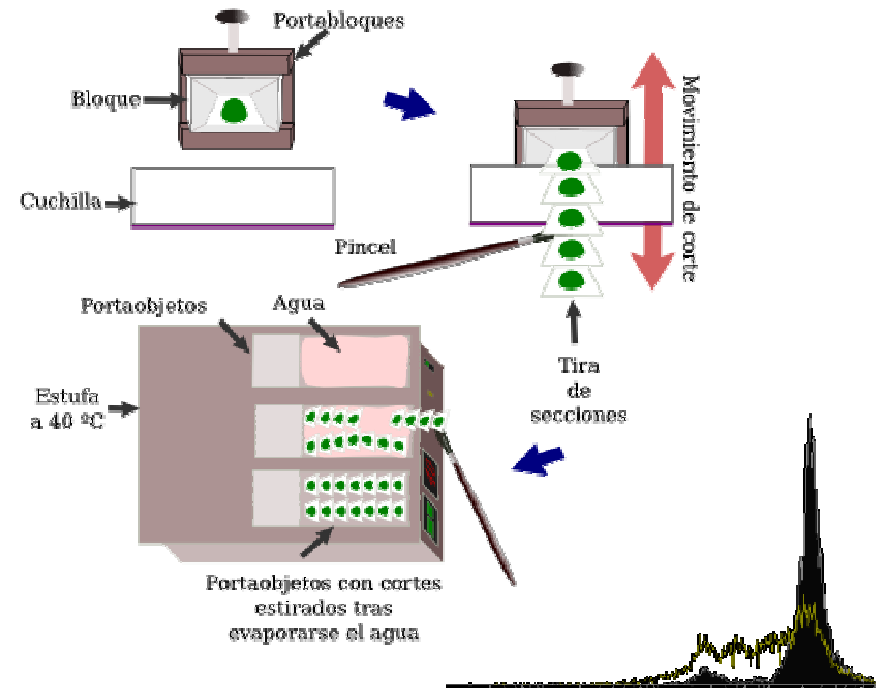
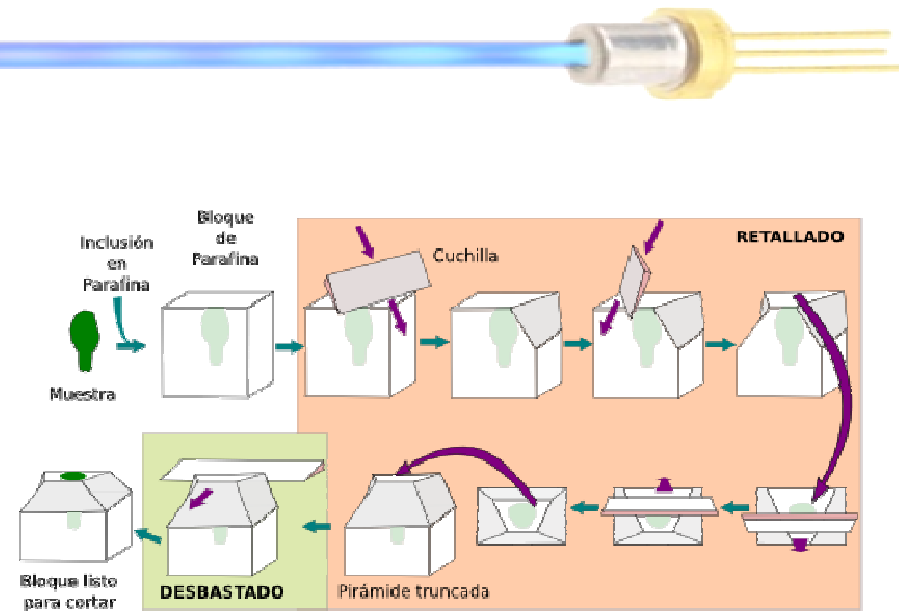
# Inmunohistoquímica



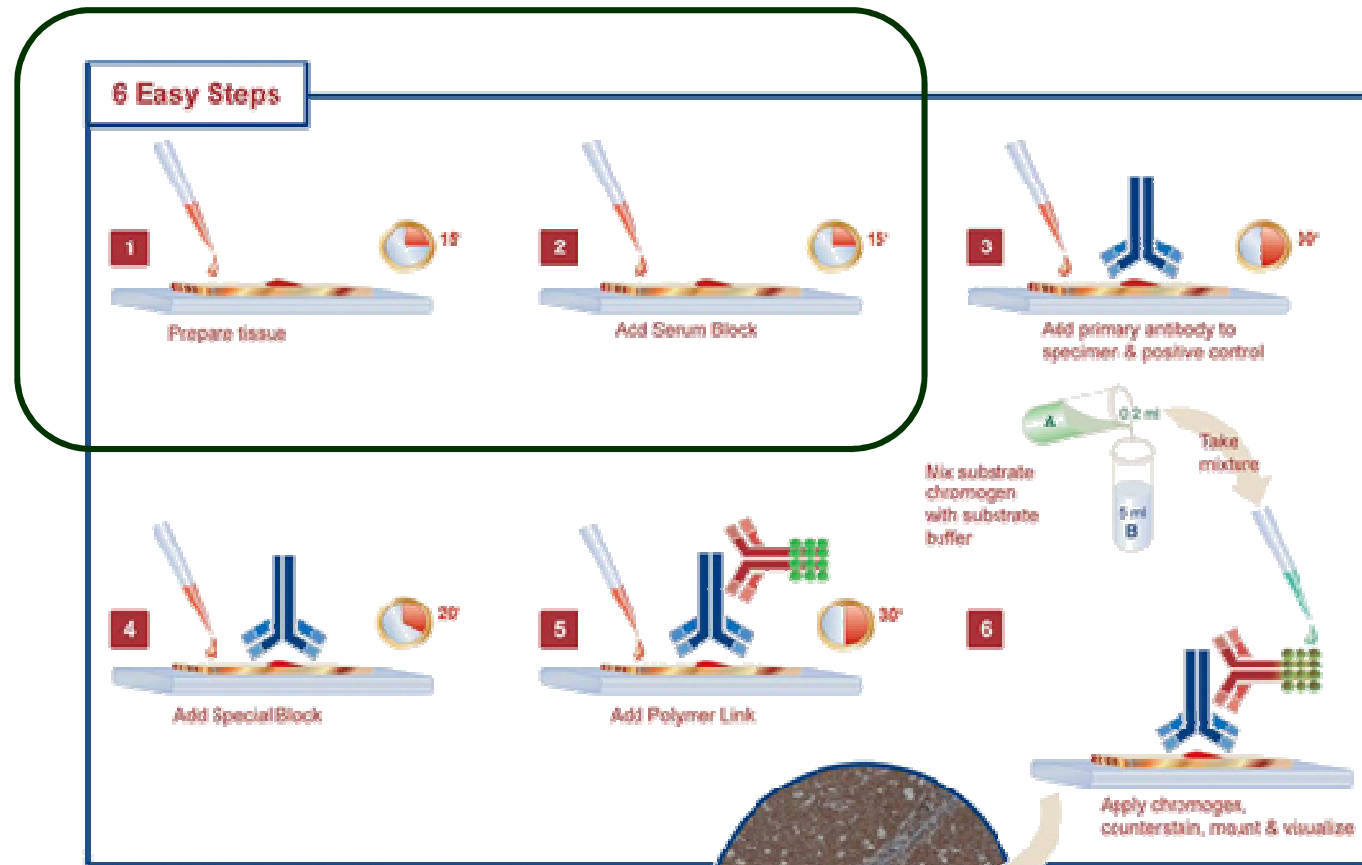
# Inmunohistoquímica

## Montaje de la muestra (Preservación del tejido)

- ✓ Fijado e inclusión en parafina
- ✓ Cortado ultrafino
- ✓ Montaje en laminas

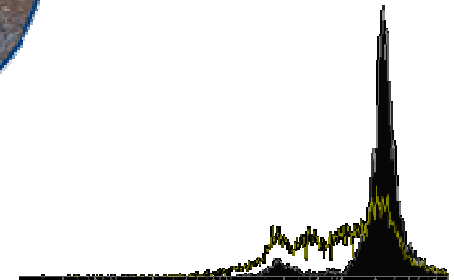


# Inmunohistoquímica



## Pretratamiento del tejido

- ✓ Tratamientos proteolíticos o por calentamiento
- ✓ Inhibición de actividades endógenas indebidas
- ✓ Bloqueo

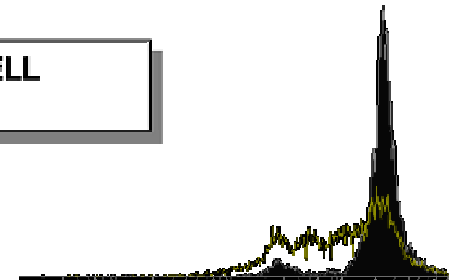
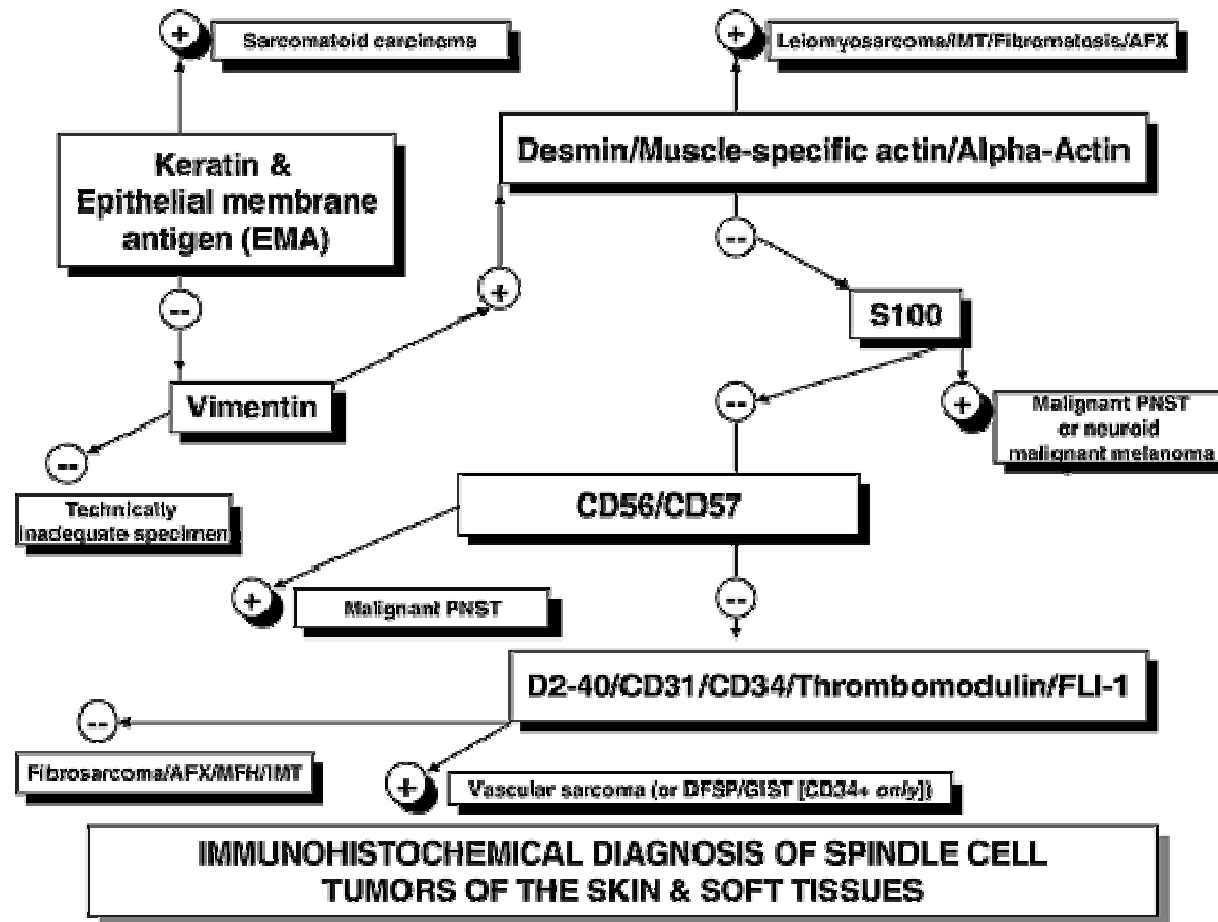


# Inmunohistoquímica

Aplicaciones!!!

Immunohistochemical approaches to the diagnosis of undifferentiated malignant tumors

Annals of Diagnostic Pathology 12 (2008) 72–84



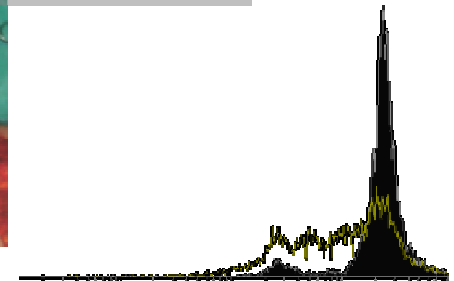
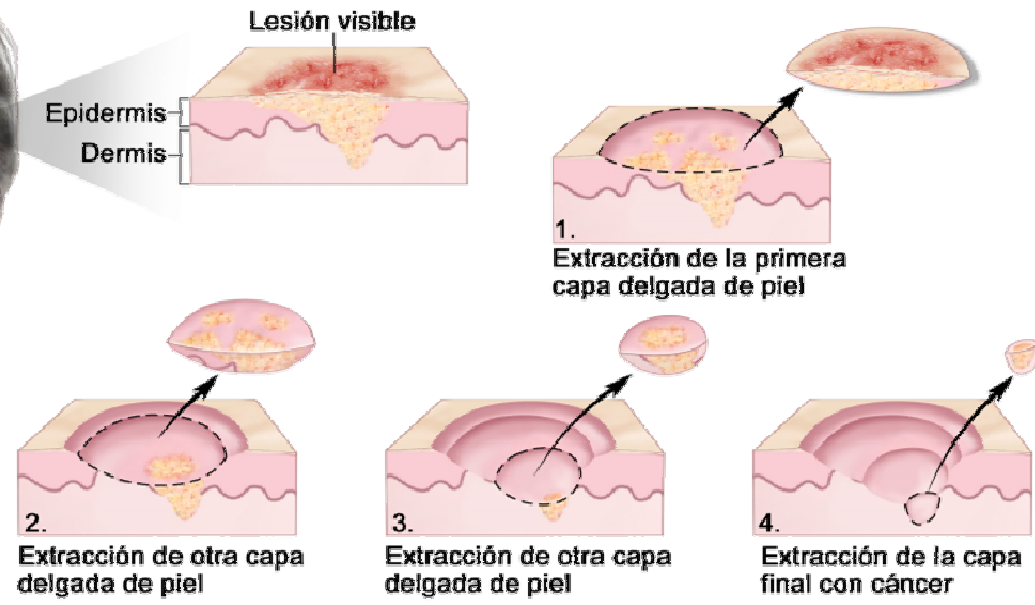
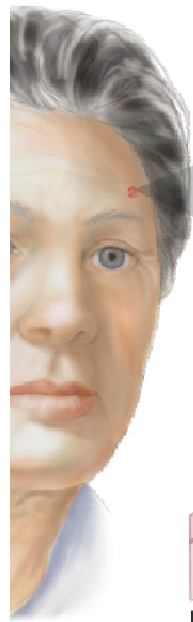




# Inmunohistoquímica

Aplicaciones!!!

## Current Progress of Immunostains in Mohs Micrographic Surgery: A Review



UNIVERSIDAD DE LOS ANDES

idic  
INSTITUTO DE INMUNOLOGÍA CLÍNICA

# Inmunohistoquímica

Aplicaciones!!!

*Dermatol Surg* 2008;34:1621–1636

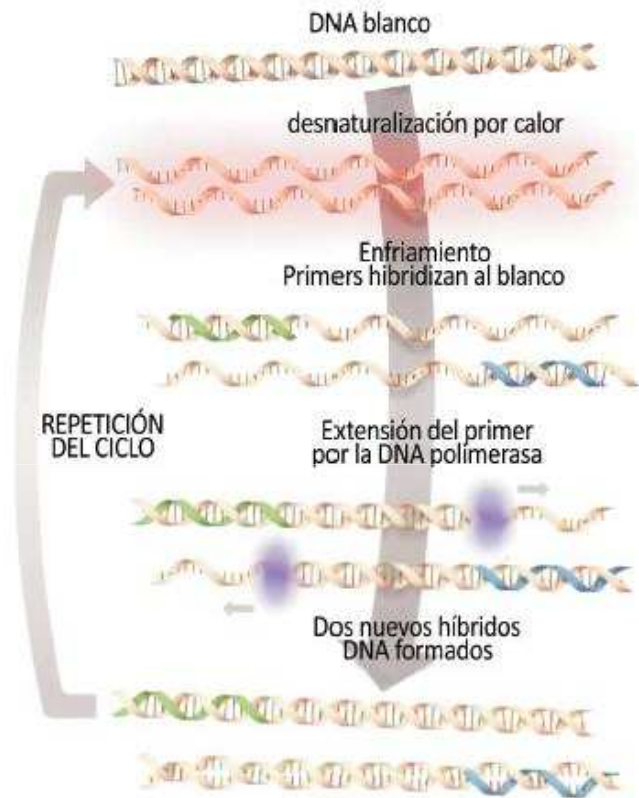
**TABLE 1. Tumor Types Treated with Mohs Micrographic Surgery and Immunohistochemistry**

Tumor	Immunostain
Melanoma, lentigo maligna, lentigo maligna melanoma	Mel-5, human melanoma black-45, Melan-A/melanoma antigen recognized by T-cells, S100
Desmoplastic melanoma, spindle cell melanoma	S100
Basal cell carcinoma	(+) stains = cytokeratins (AE1/AE3), Ki67, Ber-EP4, proliferating cell nuclear antigen (-) stains = desmogleins, CD34
Squamous cell carcinoma	(+) stains = cytokeratins (AE1/AE3) (-) stains = desmogleins
Microcystic adnexal carcinoma	(+) stains = CK1, AE1/AE3, CK19, EMA, CEA (-) stain = CK20
Dermatofibrosarcoma protuberans	(+) stain = CD34 (-) stains = factor XIIIa, tenascin (negative at DEJ only), HMGA1, HMGA2, CD163
Mucinous carcinoma <sup>118</sup>	Low molecular weight cytokeratin (Cam 5.2)
Extramammary Paget's disease	CK7
Atypical fibroxanthoma	(+) stain = CD10 (-) stain = S100, CD34
Malignant nodular hidradenoma <sup>117,118</sup>	(+) stains = estrogen receptor, cytokeratin, EMA, CEA (-) stain = progesterone receptor
Sebaceous carcinoma	(+) stains = AE1/AE3, Cam 5.2, p53, Ki67, EMA, BRST-1 (-) stains = p21, bcl-2
Merkel cell carcinoma	(+) stains = CK20, synaptophysin (-) stains = thyroid transcription factor 1
Atypical cellular neurothekeoma <sup>119</sup>	(+) stains = nonspecific esterase, vimentin (-) stain = S100
Syringomatous carcinoma <sup>120</sup>	(+) stains = high- and low-molecular-weight cytokeratins, CEA (-) stain = patchy S100
Trichilemmal carcinoma <sup>121</sup>	(+) stains = CK17, c-erb-B2 (-) stain = CK15
Embryonal rhabdomyosarcoma <sup>122</sup>	Vimentin, S100, MyoD1
Granular cell tumor <sup>123-125</sup>	S100
Infantile digital fibroma <sup>126</sup>	Actin

EMA = epithelial membrane antigen; CEA = carcinoembryonic antigen; HMG = high mobility group; DEJ = dermo-epidermal junction.



# PCR (Polymerase Chain Reaction)



Desarrollada en 1986 por Kary Mullis

Obtener un gran número de copias de un fragmento de ADN particular, partiendo de una cantidad muy pequeña

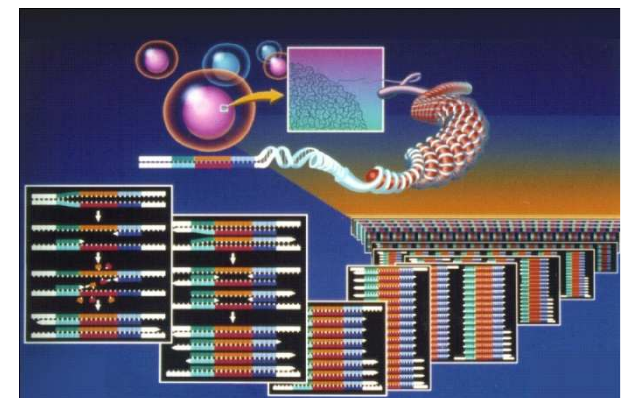
Amplificar un fragmento de ADN o ARN (RT-PCR)

Identificación de:

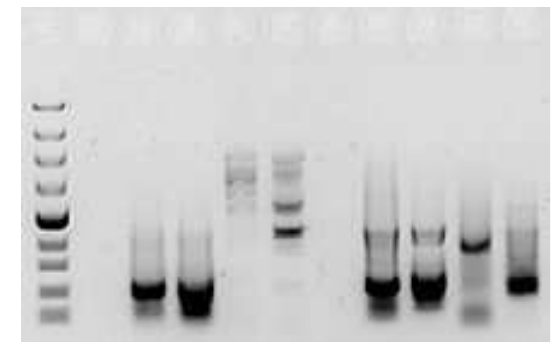
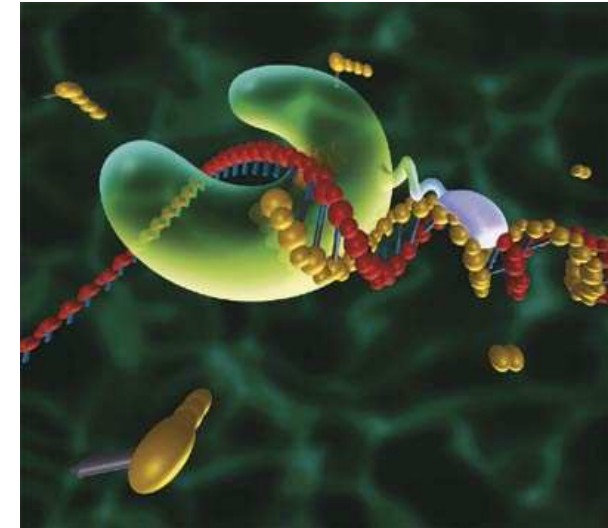
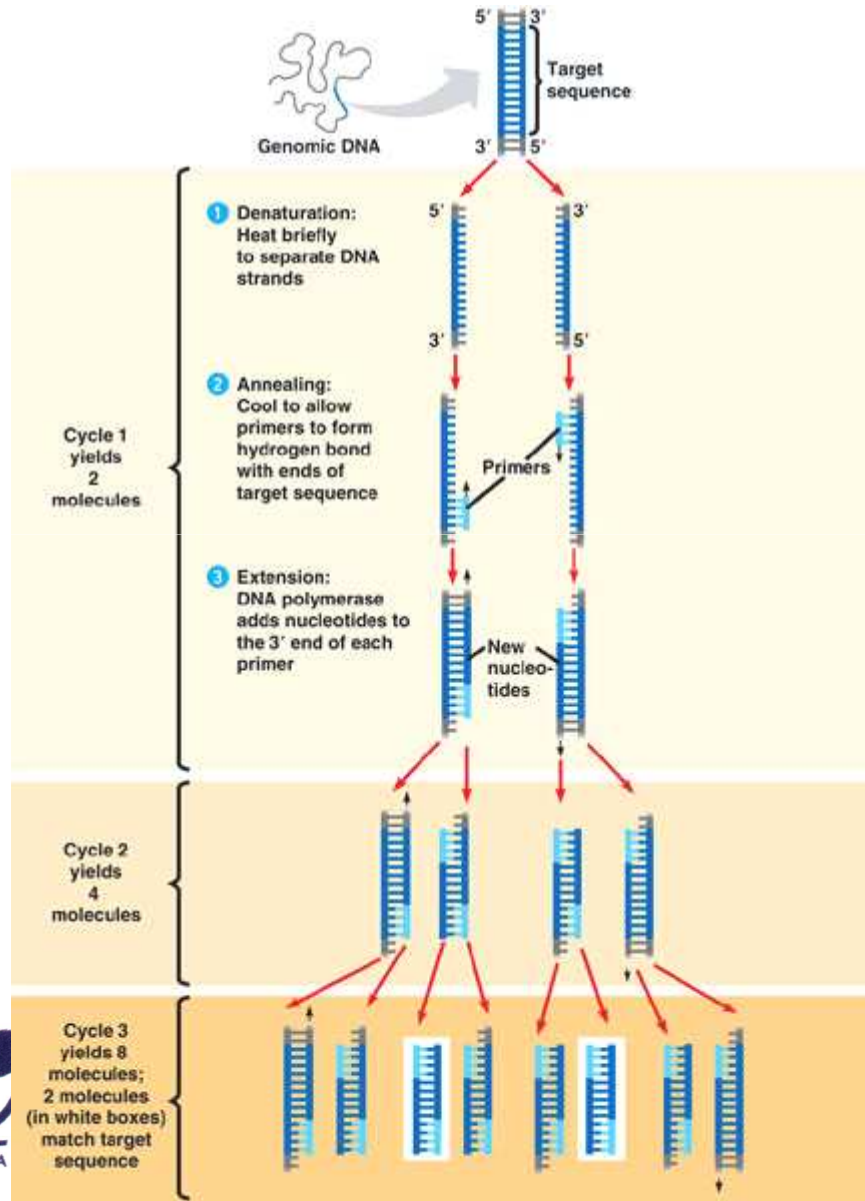
✓ Microorganismos

✓ Personas

Investigación



# Pasos de la PCR



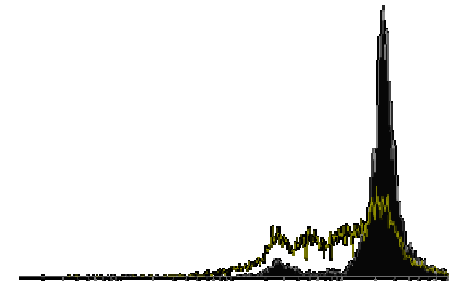


# PCR en tiempo Real

Real Time PCR

Real Time PCR:  
The basics in a few words

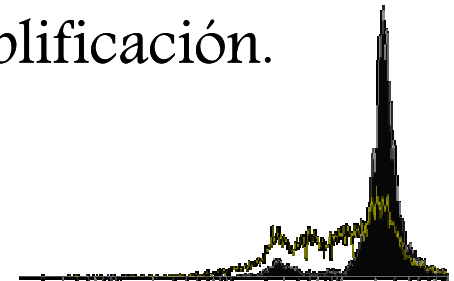
Real Time PCR



# PCR en tiempo Real



- La amplificación y detección se producen de manera simultánea
- La detección por fluorescencia permite medir durante la amplificación la cantidad de ADN sintetizado en cada momento.
- Los termocicladores para llevar a cabo la PCR a tiempo real incorporan un lector de fluorescencia y están diseñados para poder medir, en cualquier momento, la fluorescencia emitida en cada uno de los viales donde se realice la amplificación.



# PCR en tiempo Real

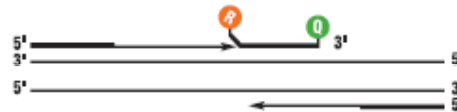
## Marcadores fluorescentes

### TAQMAN® PROBE-BASED ASSAY CHEMISTRY

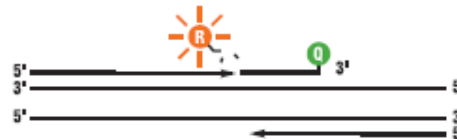
1. **Polymerization:** A fluorescent reporter (R) dye and a quencher (Q) are attached to the 5' and 3' ends of a TaqMan® probe, respectively.



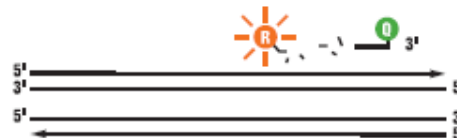
**Strand displacement:** When the probe is intact, the reporter dye emission is quenched.



3. **Cleavage:** During each extension cycle, the DNA polymerase cleaves the reporter dye from the probe.



4. **Polymerization completed:** Once separated from the quencher, the reporter dye emits its characteristic fluorescence.



### SYBR® GREEN I DYE ASSAY CHEMISTRY

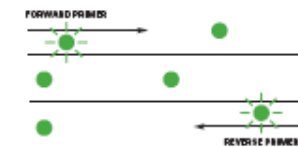
1. **Reaction setup:** The SYBR® Green I Dye fluoresces when bound to double-stranded DNA.



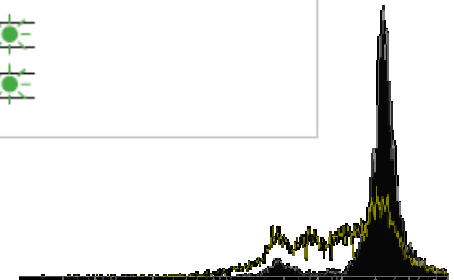
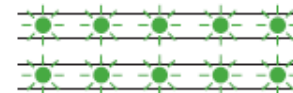
2. **Denaturation:** When the DNA is denatured, the SYBR® Green I Dye is released and the fluorescence is drastically reduced.



3. **Polymerization:** During extension, primers anneal and PCR product is generated.



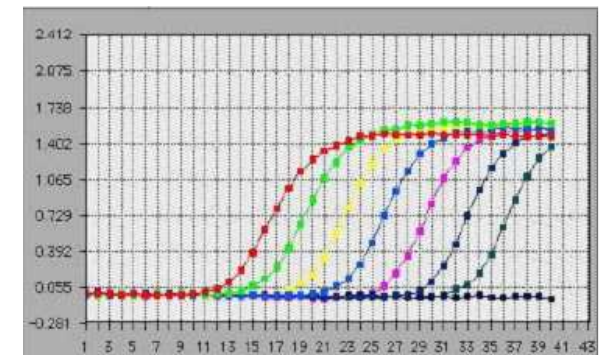
4. **Polymerization completed:** When polymerization is complete, SYBR® Green I Dye binds to the double-stranded product, resulting in a net increase in fluorescence detected by the 7900HT system.





# PCR en tiempo Real. Ventajas

- ❖ Mayor precisión, exactitud y sensibilidad
- ❖ Permite hacer detecciones múltiples
- ❖ No requiere procesamiento post-PCR.
  - ❖ Evita la contaminación
  - ❖ Mayor rapidez en la obtención de los resultados
- ❖ Cuantificación del contenido del material genético (ADN, ARN)

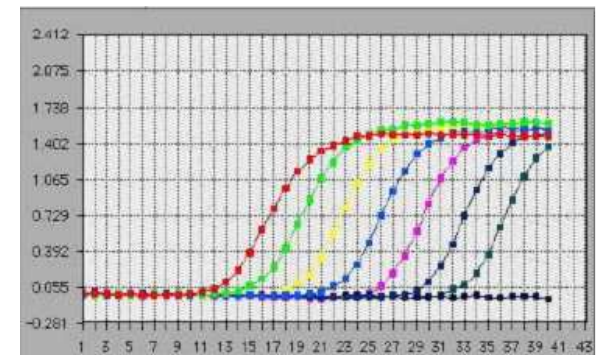




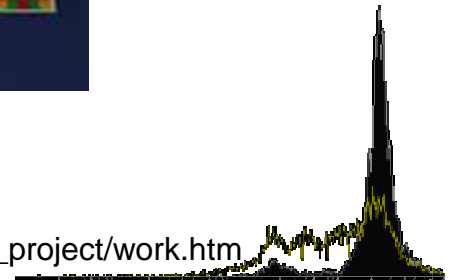
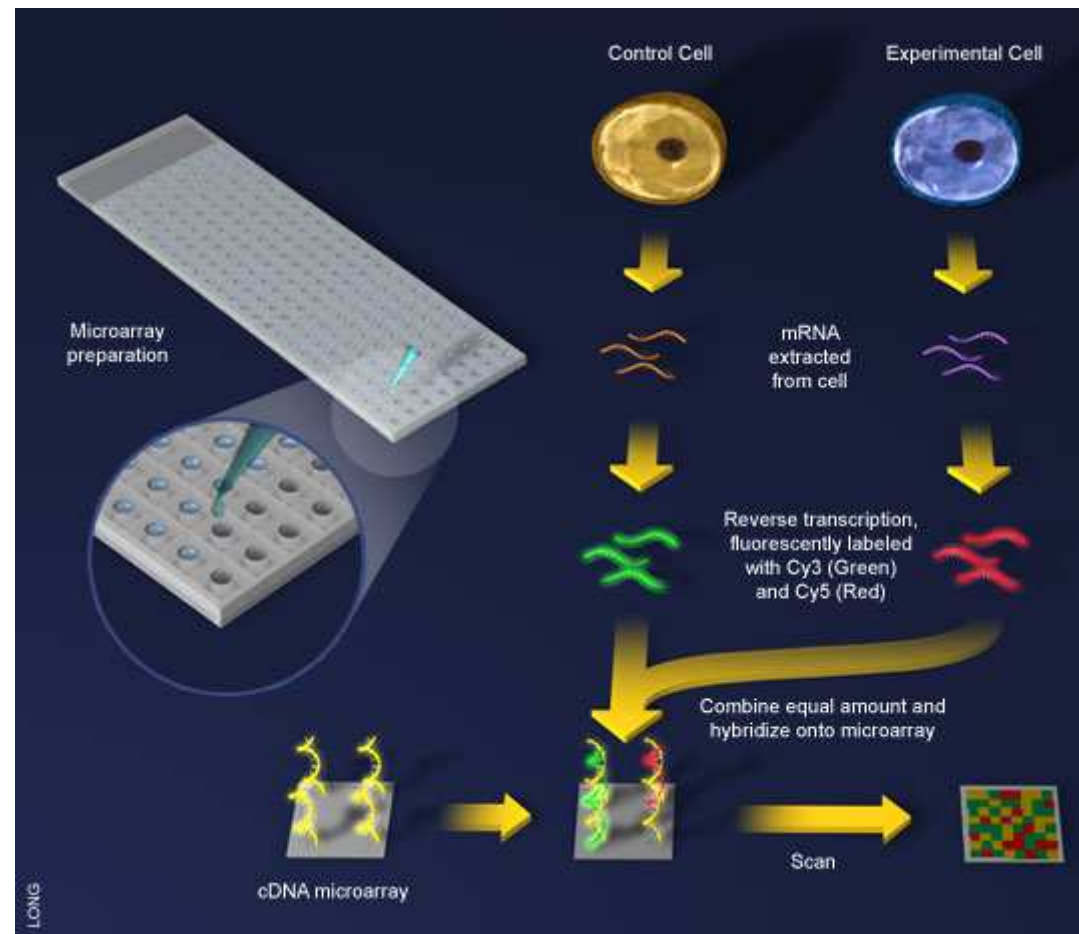


# PCR en tiempo Real. Utilidad

- ❖ Identificación de microorganismos
  - ❖ Cuantificación
  - ❖ Monitoreo de resistencia a tto.
- ❖ Expresión de genes

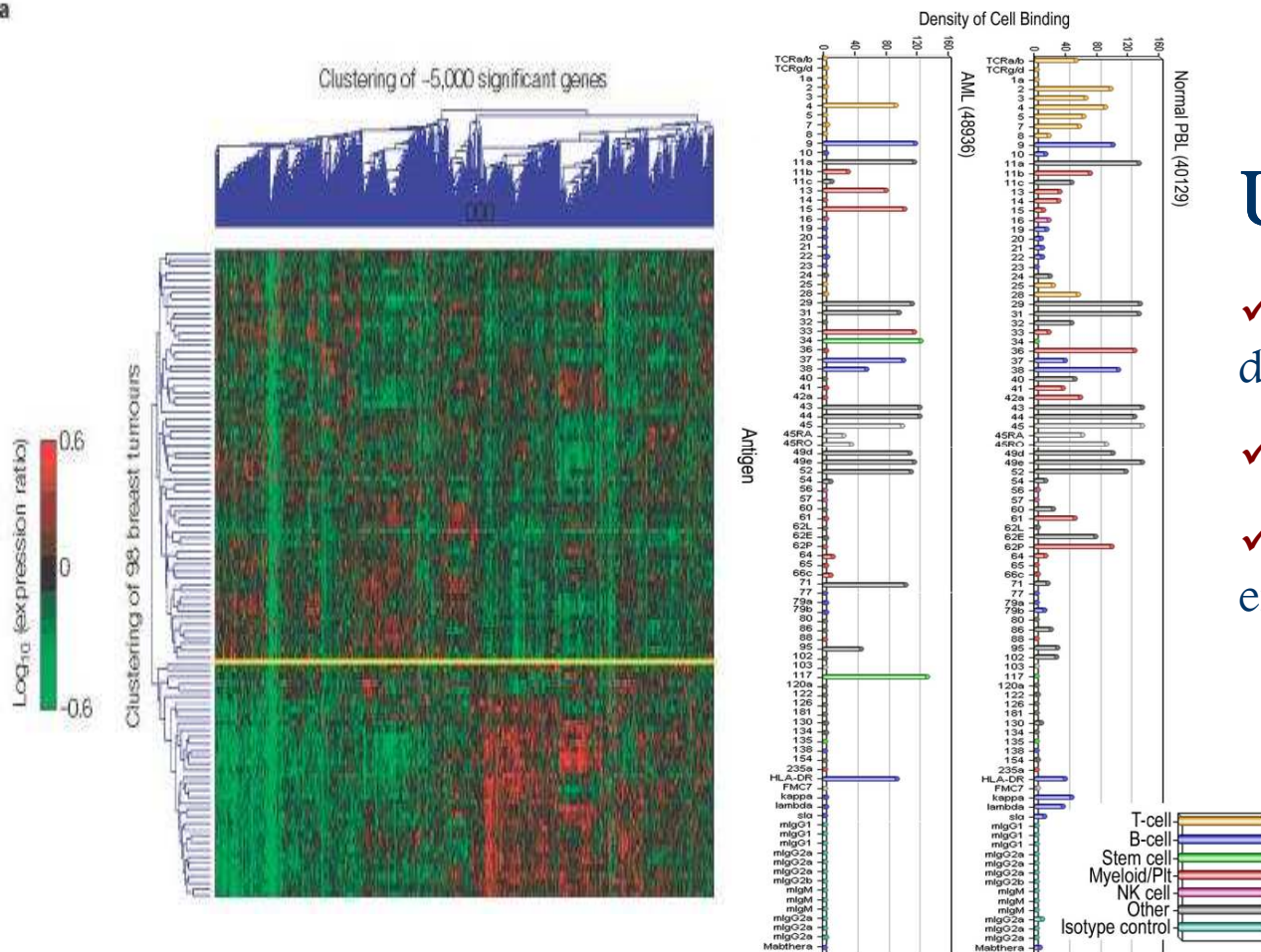


# Microarray





a



## Utilidad:

- ✓ Expresión de genes en diferentes patologías
- ✓ Valor pronóstico- Cáncer
- ✓ Susceptibilidad a enfermedad
- ✓ Infecciones
- ✓ Alergias