
Comprometidos
Contigo
Técnicas analíticas para la monitorización de fármacos biológicos
MD Bellés Medall
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CONGRESO NACIONAL
SOCIEDAD ESPAÑOLA DE FARMACIA
HOSPITALARIA
VALENCIA, DEL 10 AL 13 DE NOVIEMBRE DE 2015
sefh

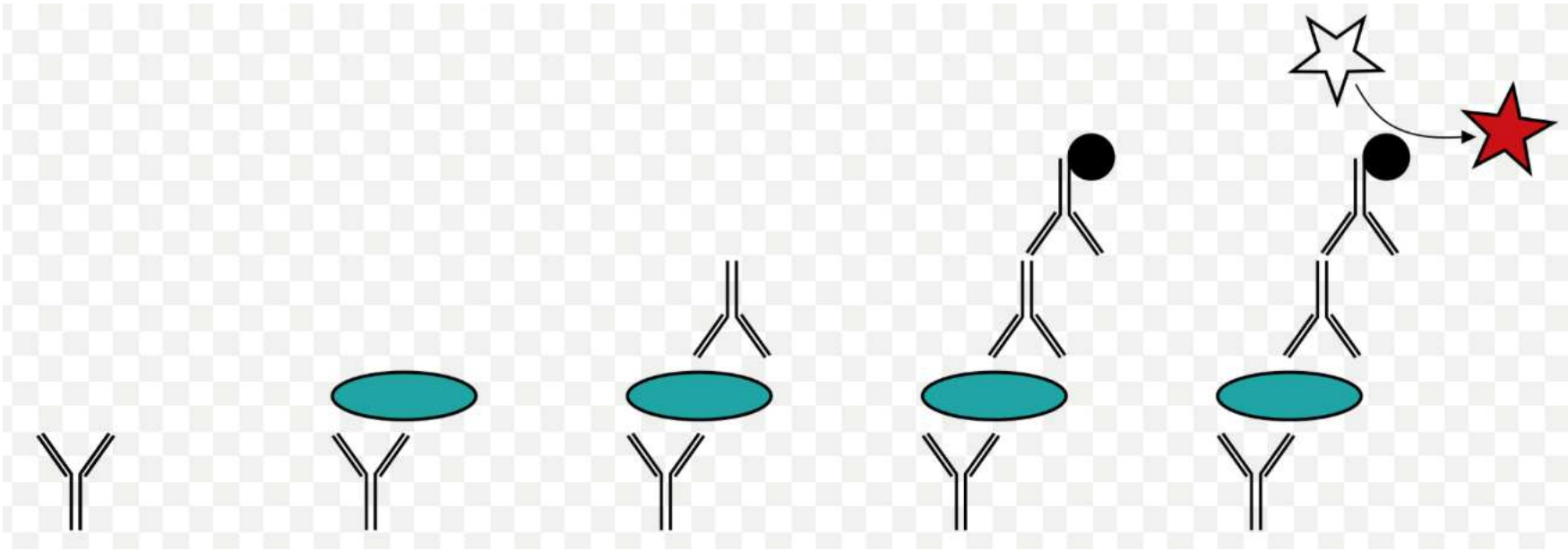
- ✓ Fundamento del E.L.I.S.A
- ✓ Equipos comerciales: diferencias
- ✓ Inmunogenicidad:

  - ELISA puente y sus limitaciones
  - Disociación ácida

- ✓ Otras técnicas
- ✓ Interpretación resultados

E.L.I.S.A.

(Enzyme Linked Immuno Sorbent Assay)



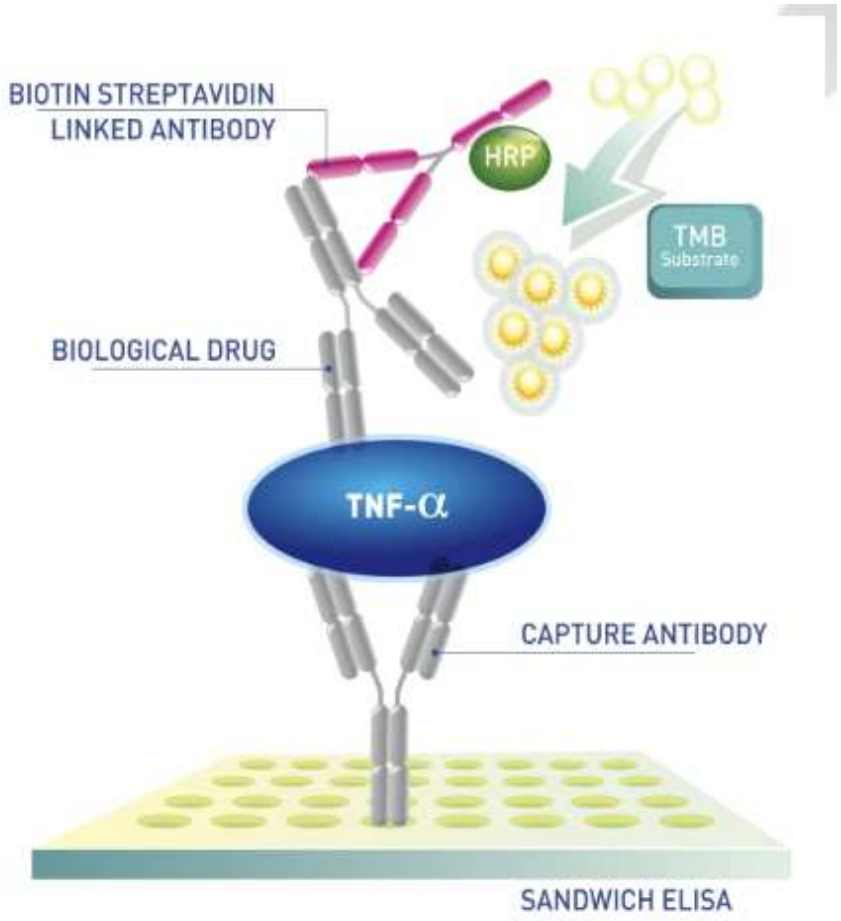
Componentes ELISA

- Fase sólida
- Muestra
- Conjugado: Enzima
- Sustrato
- Solución parada

Equipos	Fabricante	Distribuidor
PROmonitor	PROTEOMIKA	GRIFOLS
LISA-TRACKER	Theradiag	Análisis y Genética
SHIKARI®	MATRIKS BIOTEK innovation for health & wellness	Palex
IDKmonitor®	Immun Diagnostik	leti laboratorios

DAS

(Double Antibody Sandwich)

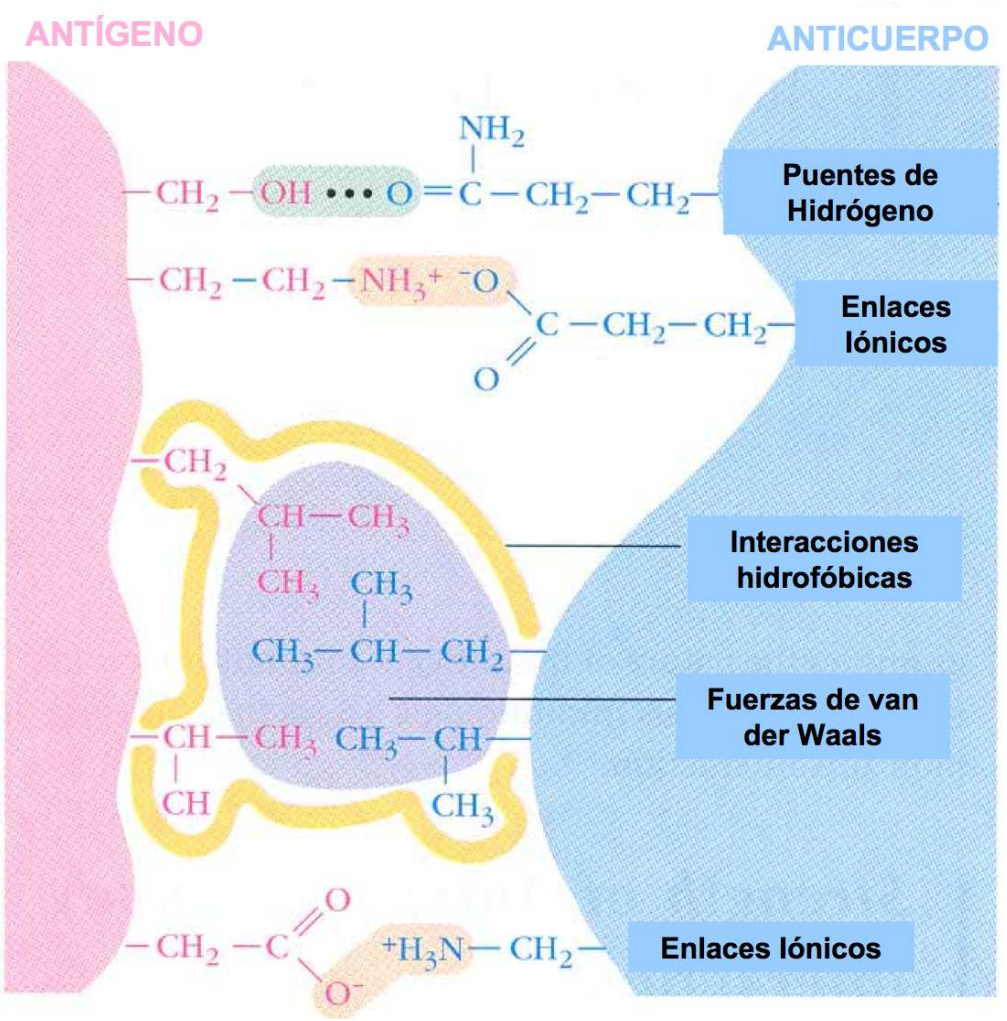


PROmonitor LISA-TRACKER

Conjugado	Ac-HRP	Ac biotinilado Estreptavidina-HRP
Sustrato	TMB	TMB
Solución parada	ClH	H_2SO_4
CP	+	+
CN	+	-
Calibración	6 ptos (0,024-12 mcg/mL)	5 ptos (0,1-16 mcg/mL)
Nº muestras/placa	40	42
Diluciones	1:10; 1:200	1:101

	PROmonitor	LISA-TRACKER	SHIKARI®	IDKmonitor®
Infliximab-AAI	X	X	X	X
Adalimumab-AAA	X	X	X	X
Etanercept-AAE	X	X	X	
Rituximab-AAR	X	X	X	
Golimumab-AAG	X	X		X
Certolizumab-AAC	En desarrollo	X		
Tocilizumab-AAT	En desarrollo	X		
Ustekinumab-AAU	En desarrollo	X		
Bevacizumab-AAB	-	X	X	
Trastuzumab-AAT	-	X	X	

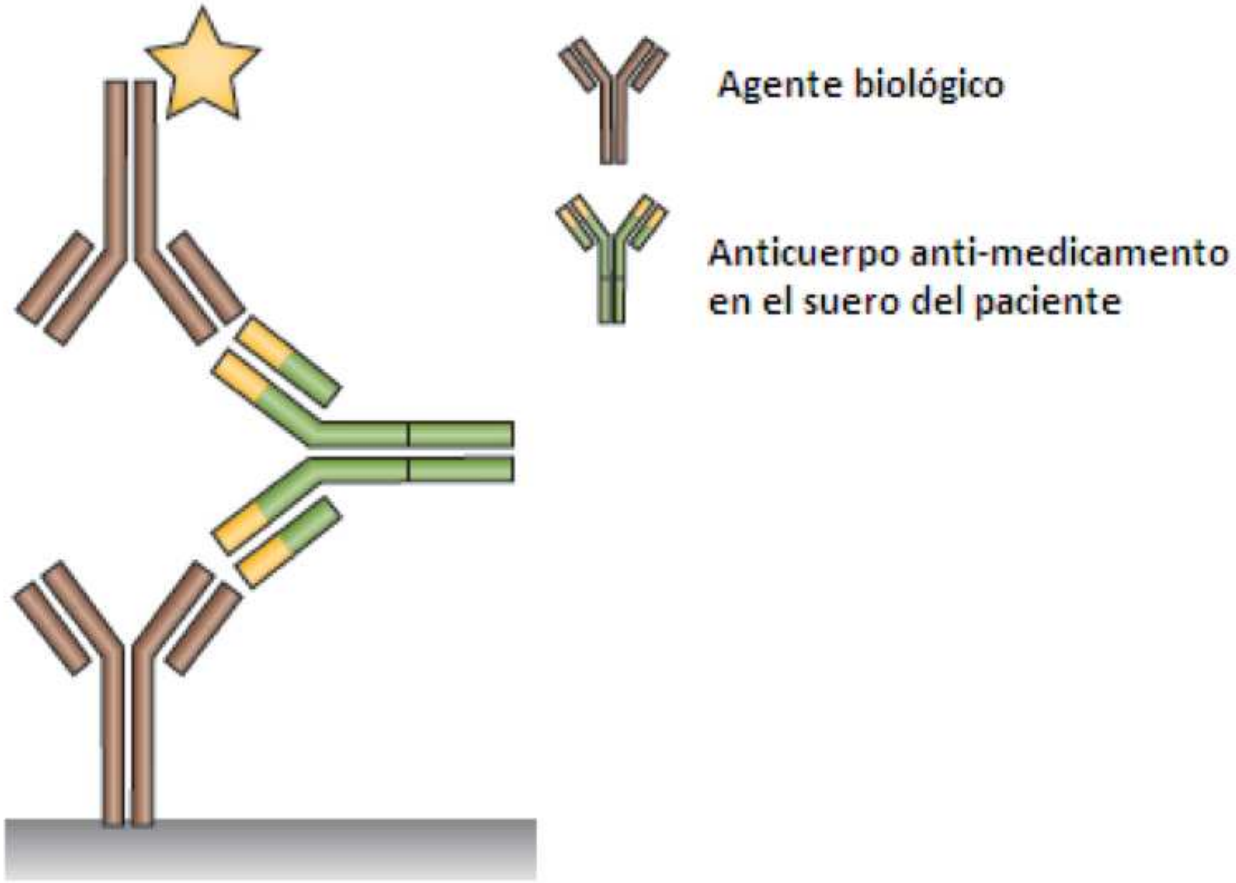
- ✓ Unión No-covalente
- ✓ Tª, pH, humedad, luz
- ✓ Aspectos prácticos:
  - o Almacenamiento micropocillos
  - o Alicuotar muestras
  - o Homogeneizar
  - o Atemperar reactivos



- Automatizado
- Sencillo y rápido (3,5-4h)
- Nº muestras/sesión = 40-42
- Cuantificación del fármaco libre
- Poco espacio (80x150cm, 1,9m^3)

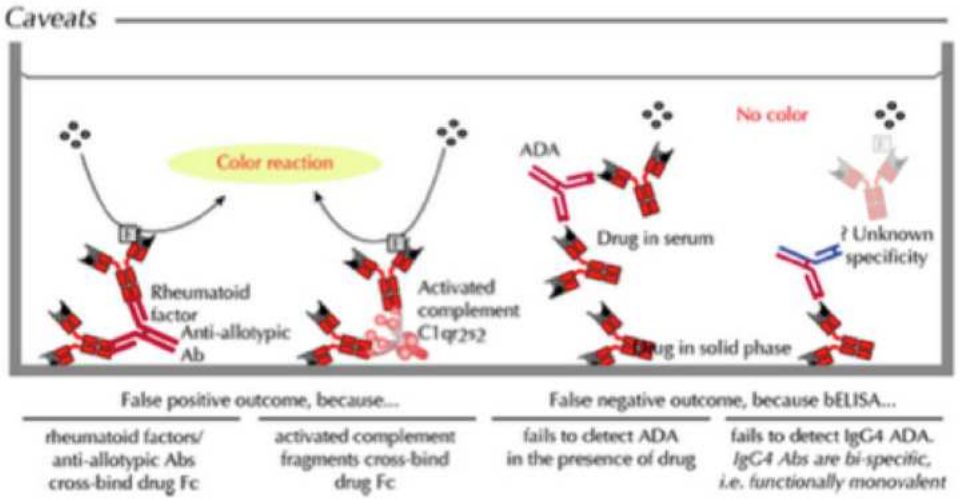
- Amplificación de la señal
- ↓ especificidad
- Pérdida de reactividad (humedad, Tª, luz)
- Curva calibración/sesión
- Ausencia patrón estándar

Inmunogenicidad: ELISA puente



ELISA puente

- Ac (-): datos “no–concluyentes”
- Baja especificidad:
  - No detecta IgG_4
  - Falsos positivos: FR, complemento activado



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ORIGINAL ARTICLE

Practical application of acid dissociation in monitoring patients treated with adalimumab

Francisca Llinares-Tello · José Rosas-Gómez de Salazar · José Miguel Senabre-Gallego · Gregorio Santos-Soler · Carlos Santos-Ramírez · Esteban Salas-Heredia · Xavier Barber-Vallés · Juan Molina-García · AIRE-MB Group

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Disociación ácida

20 µl suero + 100 ml ácido acético 300 mM (pH=3)

↓ **15 min , Tª amb**

+ 31 µl Tris base sol (neutralizar)

↓ **5 min, agitar**

+ 49 µl sol buffer (dil 1:10)

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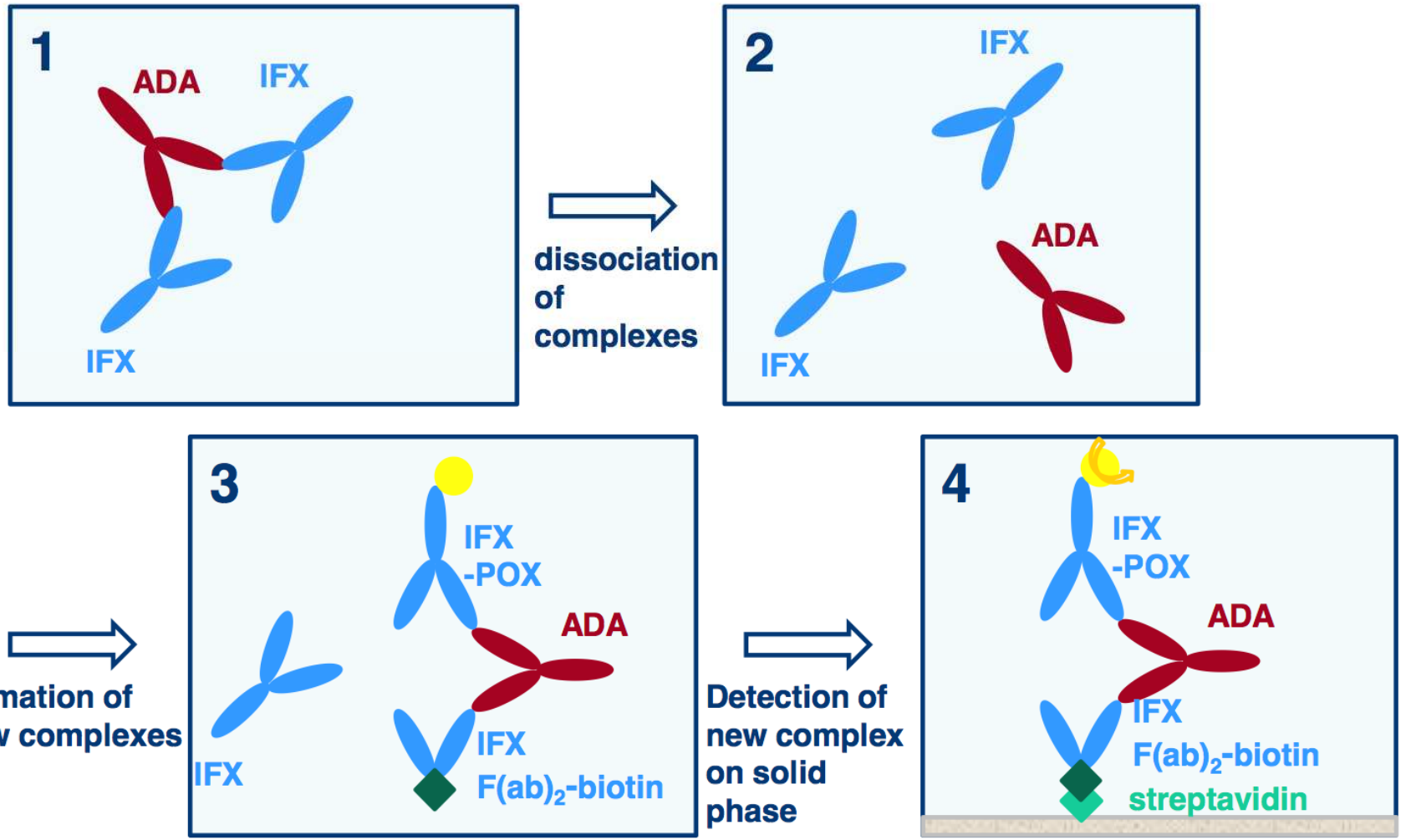
Disociación ácida

- Estrategia para detectar precozmente inmunogenicidad
- Técnica cualitativa
- No se detecta **IgG_4**
- % recuperación AAF ↓:
  - Desnaturalización de los AAF tras acidificación
  - AAF baja afinidad pérdida por lavados
  - Parcial formación de inmunocomplejos tras neutralización.

Principle of new ADA assay

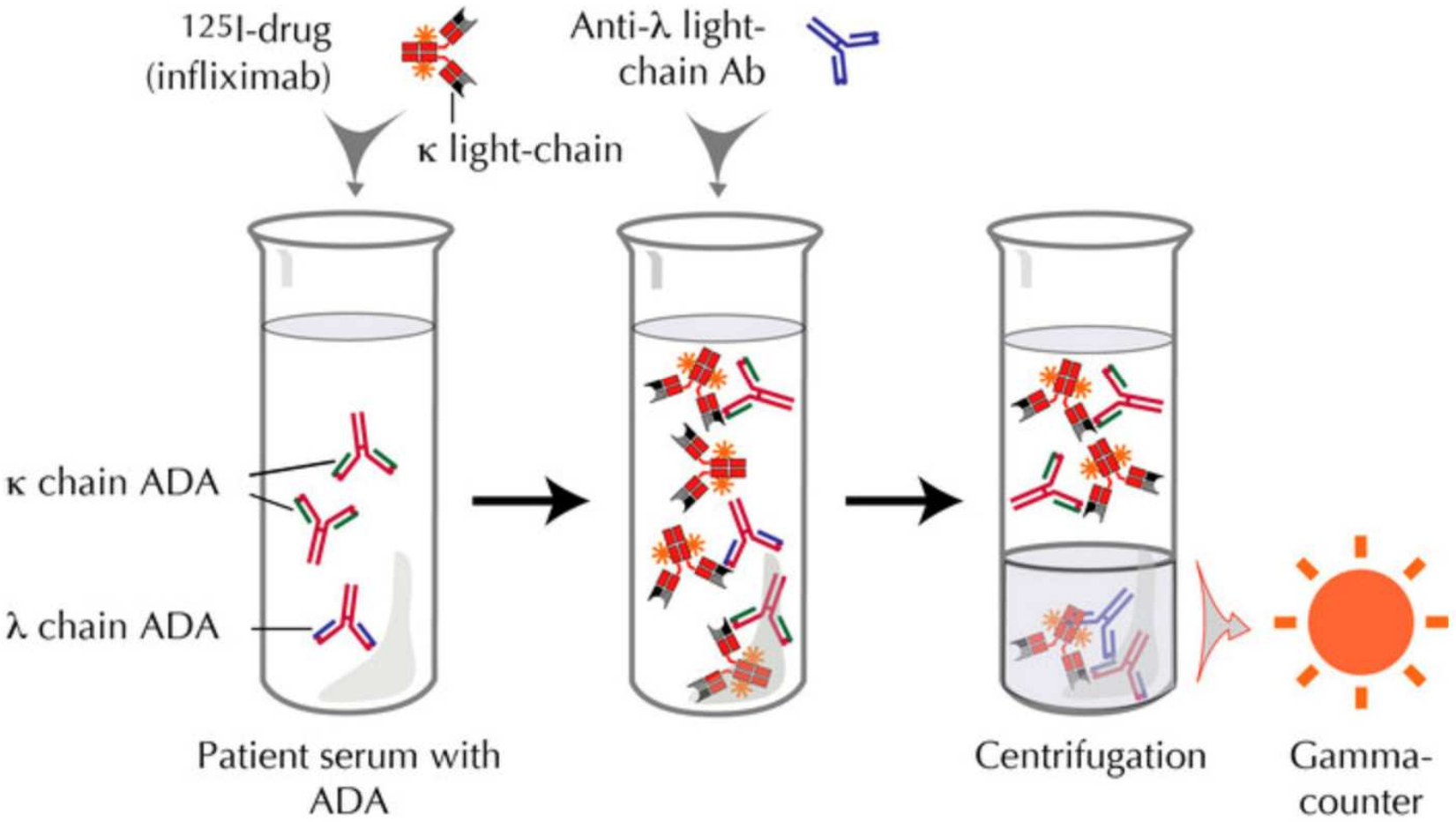
*IDK*monitor®



Otras técnicas

- RIA
- HMSA
- Gen reportero

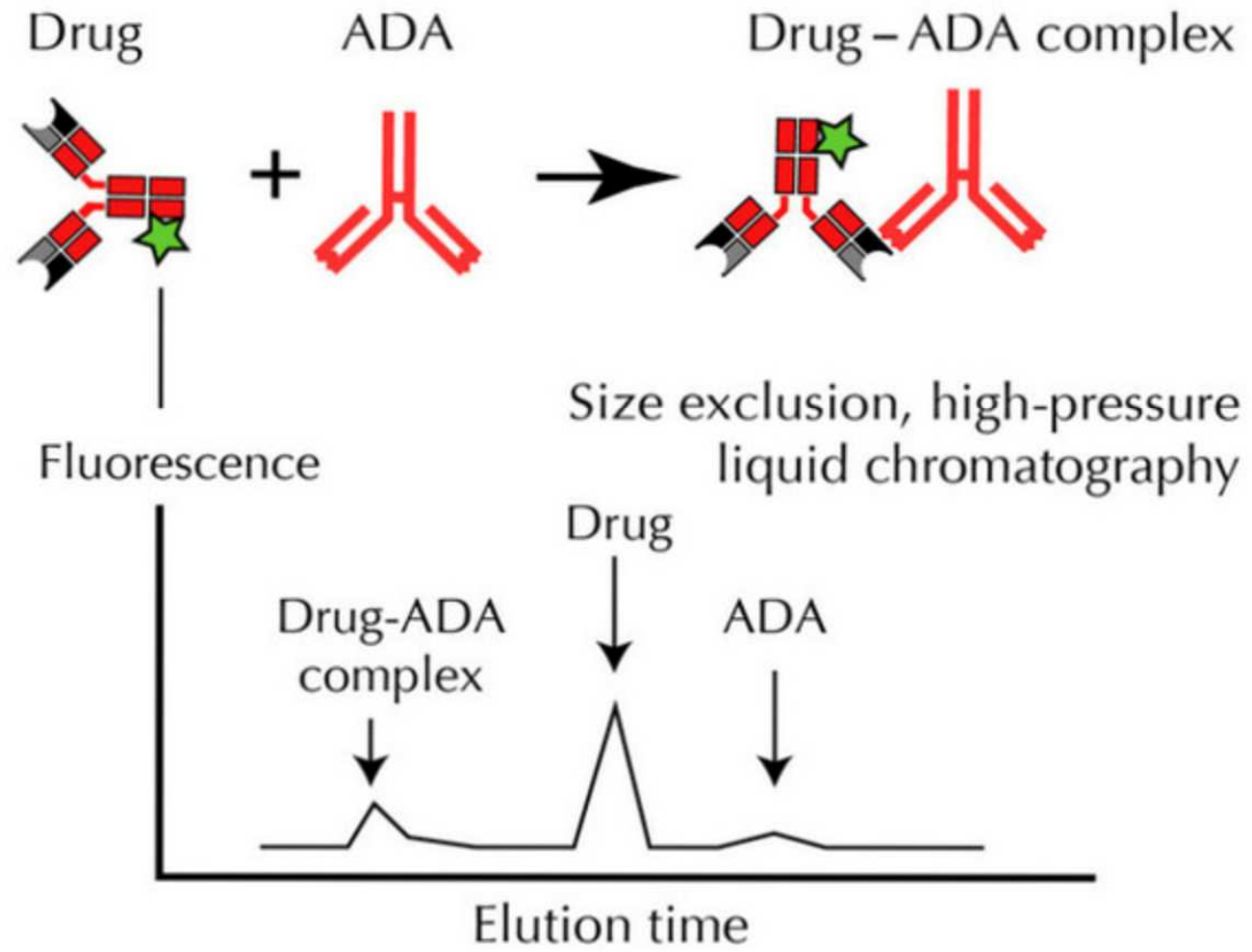
Radioimmunoassay (RIA) for ADA



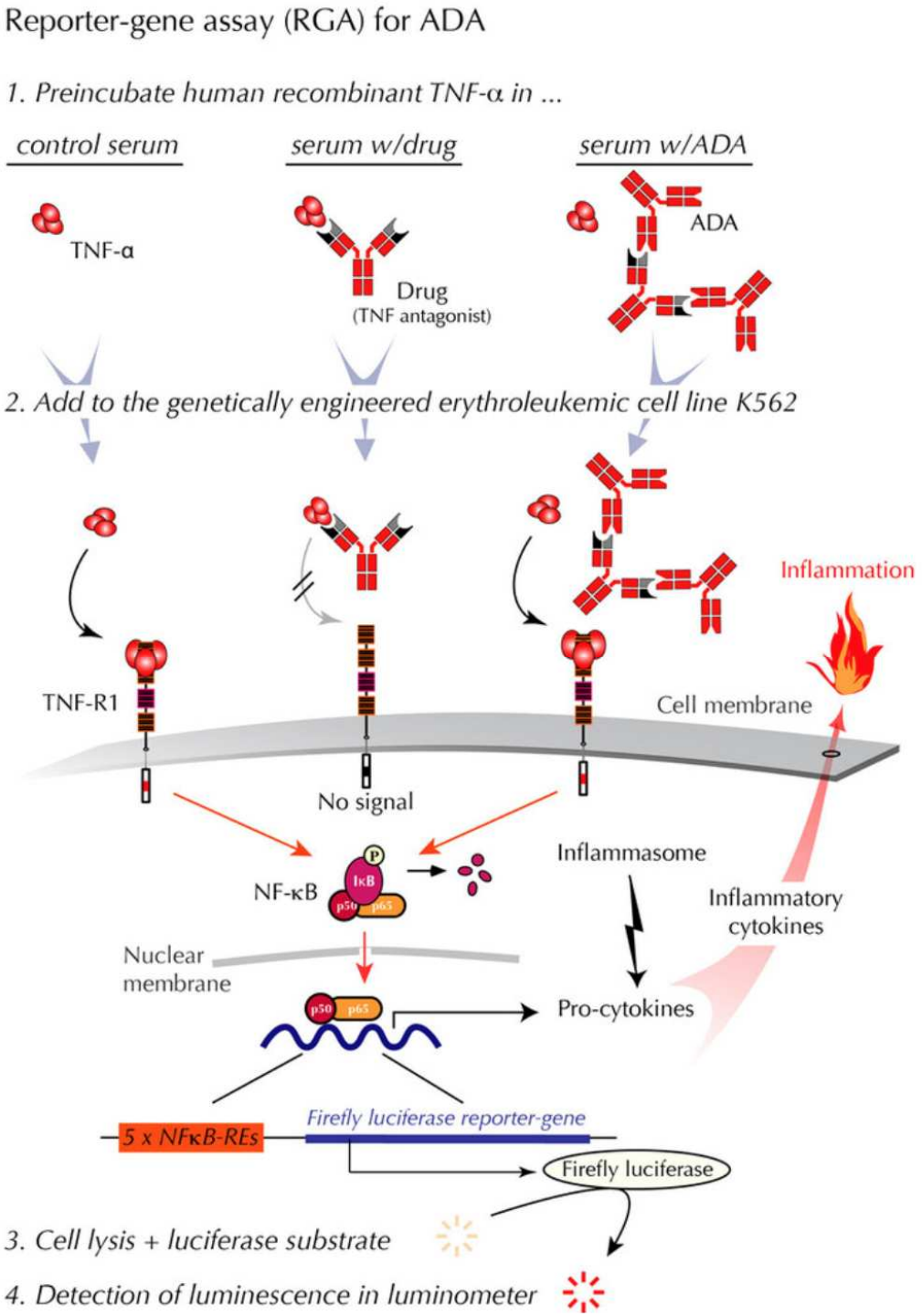
- ↑Especificidad
- ↑Sensibilidad

- Complejidad
- Caro
- ↑tiempo incubación

Homogeneous mobility-shift assay (HMSA) for ADA

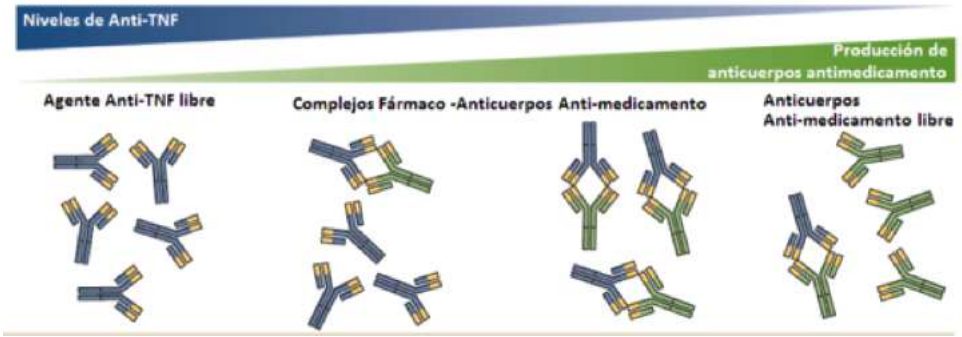


- ↑↑Especificidad
- ↑↑Sensibilidad
- Investigación
- Fraccionamiento immunocomplejos



- ⊙ Actividad biológica
- ⊙ Universal
- ⊙ Rápido

- ⊙ Cultivo celular
- ⊙ Precio (??)



AAF

ELISA captura	++	+	-	-
ELISA puente	-	-	-	+
RIA	-	-	-	++
RGA	-	-	+	+
HMSA	-	+/-	+	++

Clinical Implications of Measuring Drug and Anti-Drug Antibodies by Different Assays When Optimizing Infliximab Treatment Failure in Crohn's Disease: *Post Hoc* Analysis of a Randomized Controlled Trial

Casper Steenholdt, MD, PhD[1], Klaus Bendtzen, MD, DMSc[2], Jørn Brynskov, MD, DMSc[1], Ole Ø Thomsen, MD, DMSc[1] and Mark A. Ainsworth, MD, PhD, DMSc[1]

OBJECTIVES: **Cost-effective guidance of therapeutic strategy in Crohn's disease patients with secondary infliximab (IFX) treatment failure may be achieved by serum IFX and anti-IFX antibody (Ab) measurements by radioimmunoassay (RIA). This study investigated implications of using other techniques for this purpose.**

METHODS: **This is a *post hoc* analysis of randomized clinical trial including 66 Crohn's disease patients with IFX failure in whom IFX and anti-IFX Ab measurements by RIA had been used for therapeutic guidance. Samples were additionally assessed by enzyme-linked immunosorbent assay (ELISA), homogeneous mobility shift assay (HMSA), and functional cell-based reporter gene assay (RGA).**

RESULTS: **IFX detection was comparable between assays (82% RIA, 76% ELISA, 88% HMSA, and 74% RGA), and it correlated significantly (Pearson's r=0.91–0.97, P<0.0001). However, IFX concentrations varied systematically between all pair of assays except RIA-RGA. Anti-IFX Ab detection was variable (27% RIA, 9% ELISA, 33% HMSA, and 11% RGA), but correlated significantly (Pearson's r=0.77–0.96; P<0.0001). Anti-IFX Abs detected by RIA and HMSA were often from sera without drug-neutralizing activity (RGA). Assays agreed on classification of underlying mechanism for treatment failure in most cases (79–94%). The majority (74–88%) failed IFX owing to pharmacodynamic problems, or had noninflammatory pathophysiology for symptoms resembling relapse. Applied threshold for therapeutic vs. subtherapeutic IFX level influenced classifications. The four different assays did not differ in terms of the ability to predict response to interventions defined by the algorithm.**

CONCLUSIONS: **Despite variable analytical properties, common assays result in similar classifications and interventions in patients with IFX treatment failure, and with comparable clinical outcomes. Implications are, however, profound for the minority classified differently.**

SUPPLEMENTARY MATERIAL is linked to the online version of the paper at http://www.nature.com/ajg

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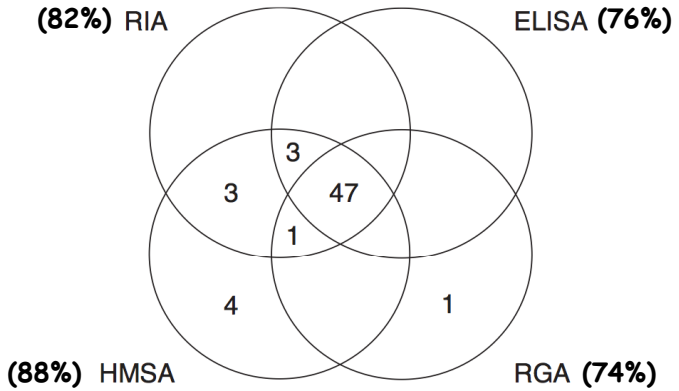


Figure 2. Detection of IFX by RIA, ELISA, HMSA, and RGA in patients with secondary IFX treatment failure (n=66). IFX was undetectable in the same seven patients by all assays (not shown on figure).

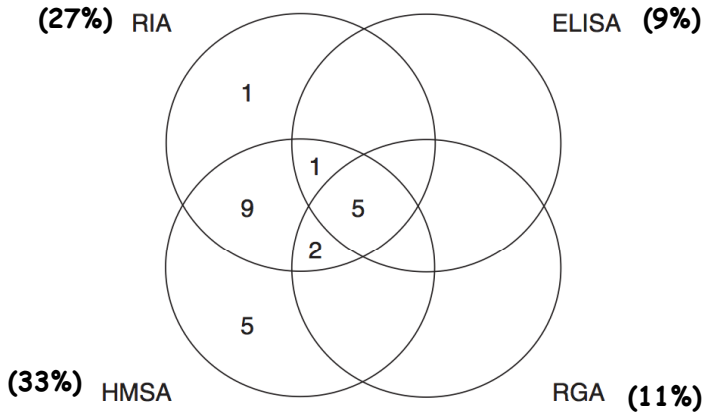
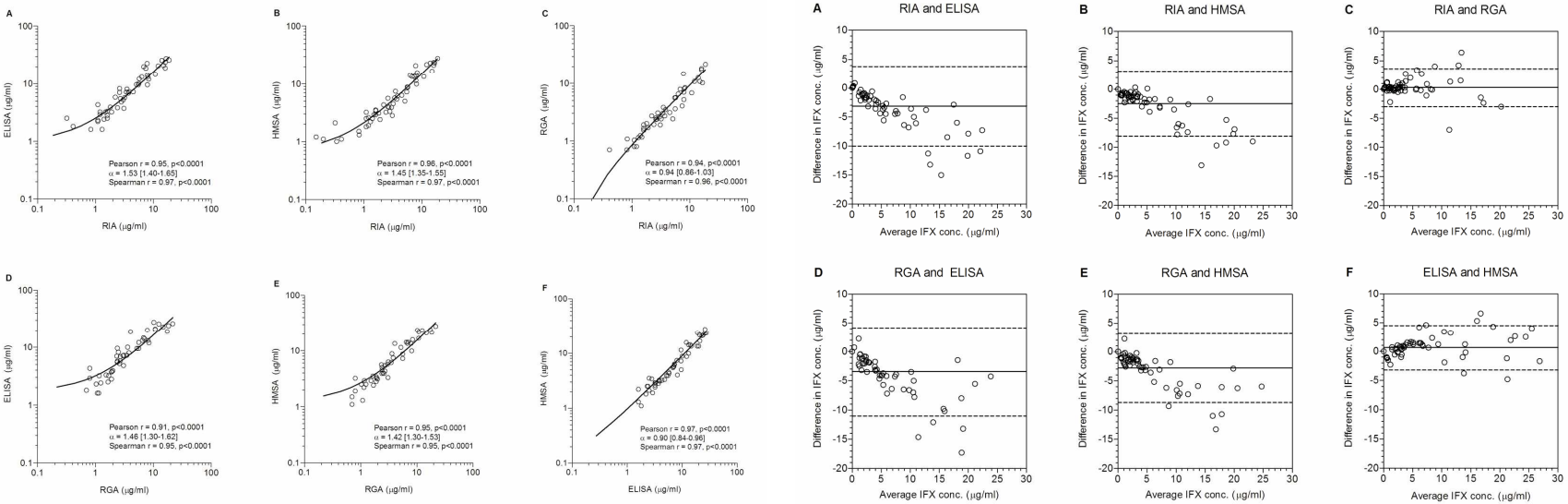


Figure 3. Detection of anti-IFX Abs by RIA, ELISA, HMSA, and RGA in patients with secondary IFX treatment failure (n=66). Anti-IFX Abs were undetectable in the same 43 patients by all assays (not shown on figure).

Supplementary Table 1: Agreement between IFX assays

	AGREEMENT ON RANKING*			AGREEMENT ON CONCENTRATIONS#
	ICC	[95% CI]	P	Mean difference, µg/ml [95% CI]
RIA vs. ELISA	0.76	[0.29–0.90],	<0.0001	-3.12 [-3.98 – -2.25]
RIA vs. HMSA	0.82	[0.41–0.93],	<0.0001	-2.48 [-3.18 – -1.77]
RIA vs. RGA	0.94	[0.90–0.96],	<0.0001	0.32 [-0.09 – 0.74]
RGA vs. ELISA	0.72	[0.23–0.88],	<0.0001	-3.44 [-4.39 – -2.49]
RGA vs. HMSA	0.80	[0.30–0.92],	<0.0001	-2.80 [-3.54 – -2.06]
ELISA vs. HMSA	0.96	[0.94–0.98],	<0.0001	0.64 [0.15 – 1.12]



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- Técnicas: elevada variabilidad con diferencias sistémicas.
- Resultados no comparables.
- Ensayos > sensibilidad para AAF: HMSA y RIA.
- ↑ concordancia en clasificación de los pacientes según fallo secundario.
- Tras seguimiento a largo plazo: resultados comparables.

Interpretación resultados

- No comparar resultados entre técnicas.
- ELISA puente: datos “no concluyentes”, técnica cualitativa. Unidades medida no estandarizadas (ng/mL; AU/mL)
- Trabajar con controles propios
- Titulaciones bajas de Ac :
  - Falso positivo
  - Ac “transitorios”
  - Fracaso secundario por inmunogenicidad


Tobra
Teofilina
Paracetamol
CysA
MTX
€
60 €/determinación
4 sesiones trabajo/placa (6 niveles/sesión)
48 €/determinación
3 sesiones trabajo/placa (10 niveles/sesión)
40 €/determinación
2 sesiones trabajo/placa (18 niveles/sesión)
34 €/determinación
1 sesión trabajo/placa (42 niveles/sesión)

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