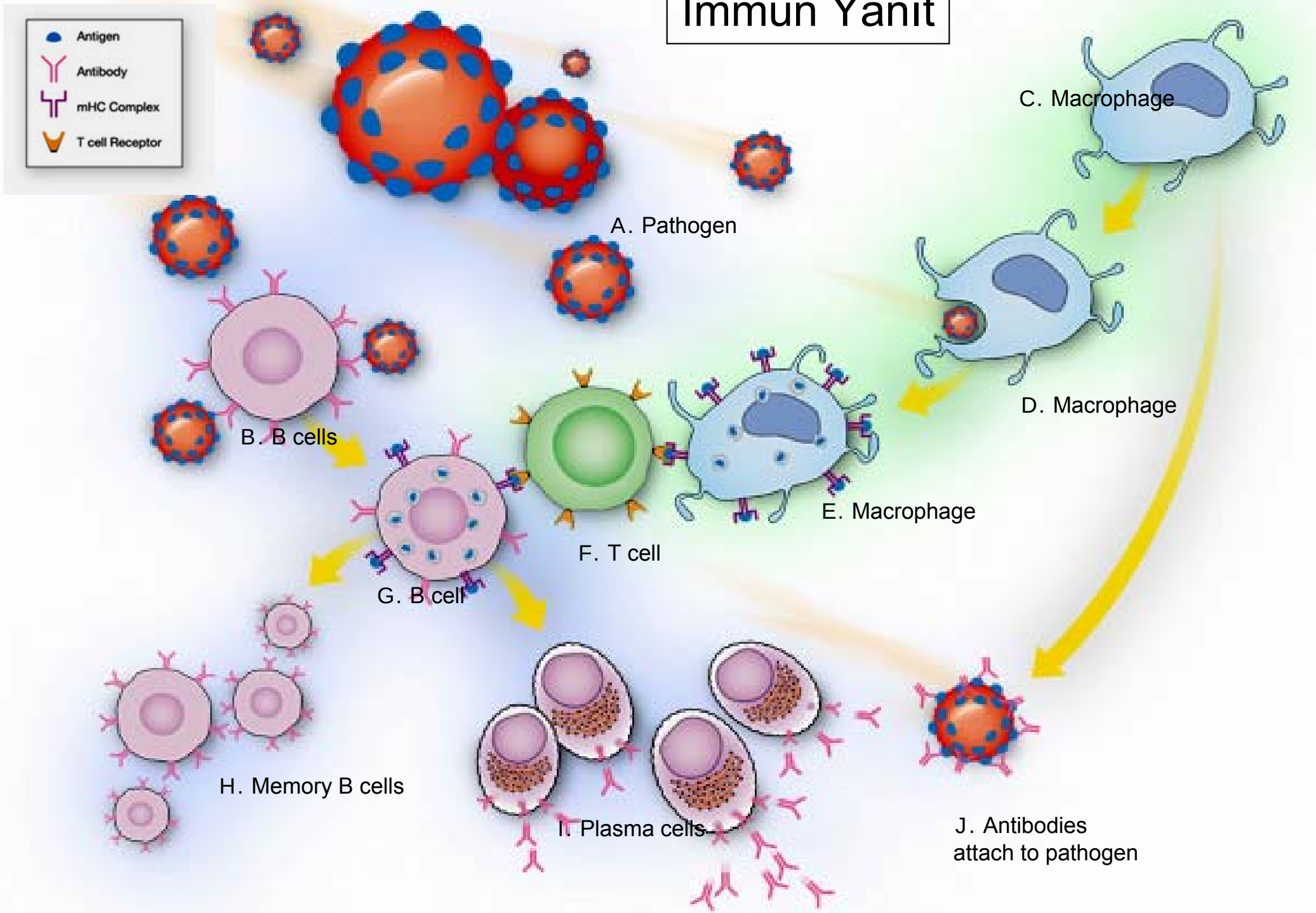
Klinik Mikrobiyoloji'de Enzimli İmmün Deney

"Enzyme Immuno Assay"

Dr. Dilek Çolak

İmmün Yanıt


Antigen
Antibody
mHC Complex
T cell Receptor
A. Pathogen
B. B cells
C. Macrophage
D. Macrophage
E. Macrophage
F. T cell
G. B cell
H. Memory B cells
I. Plasma cells
J. Antibodies attach to pathogen

Radioimmuno assay: RIA

-Dr. Berson & Yalow 1959
(Serumda insülin düzeyi ölçümünde radyoizotop kullanılması 1977'de Nobel ödülü)

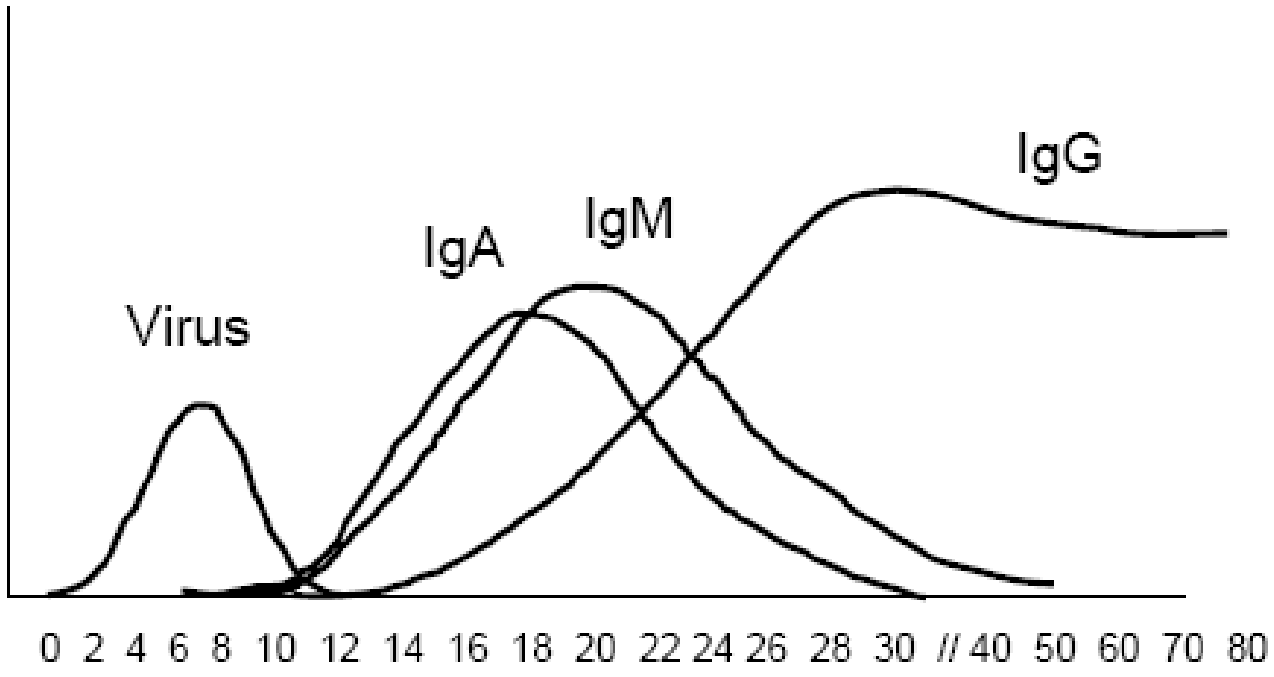
İnfeksiyon hastalıklarının tanısında Ag+Ab reaksiyonlarının kullanıldığı testler

- Aglütinasyon testleri
- Presipitasyon testleri
- Hemaglütinasyon testleri
- Kompleman birleşmesi testleri
- RIA testleri
- İmmünfloresan testleri
- EIA testleri
- Immunoblot testler

Diagnostic Virology

Appropriate samples

Collect the specimens early in the infection process



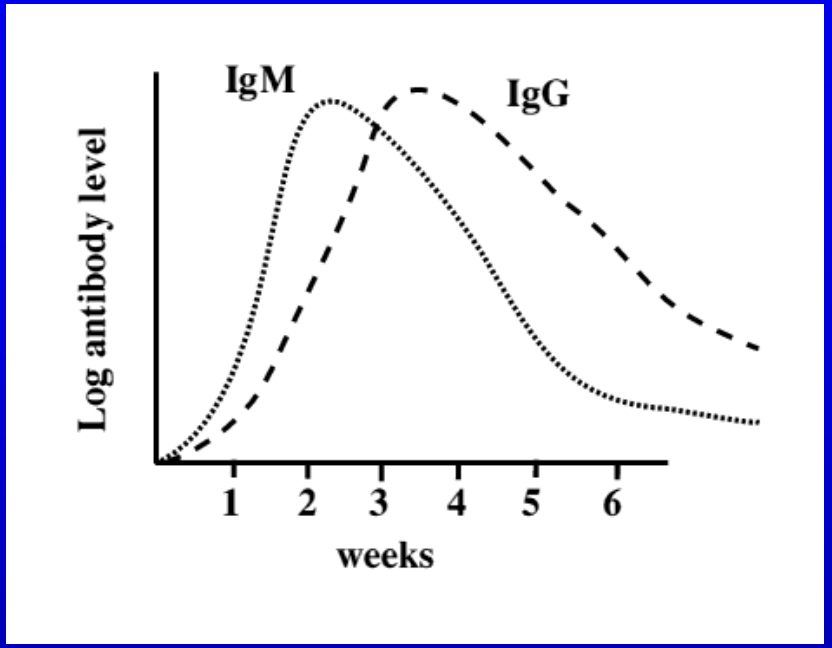
Virus (and its products) is present early in the infection

IgA & IgM are early antibodies
May disappear after 14 to 50 days

IgG remains for life. Is an indicator of past infection

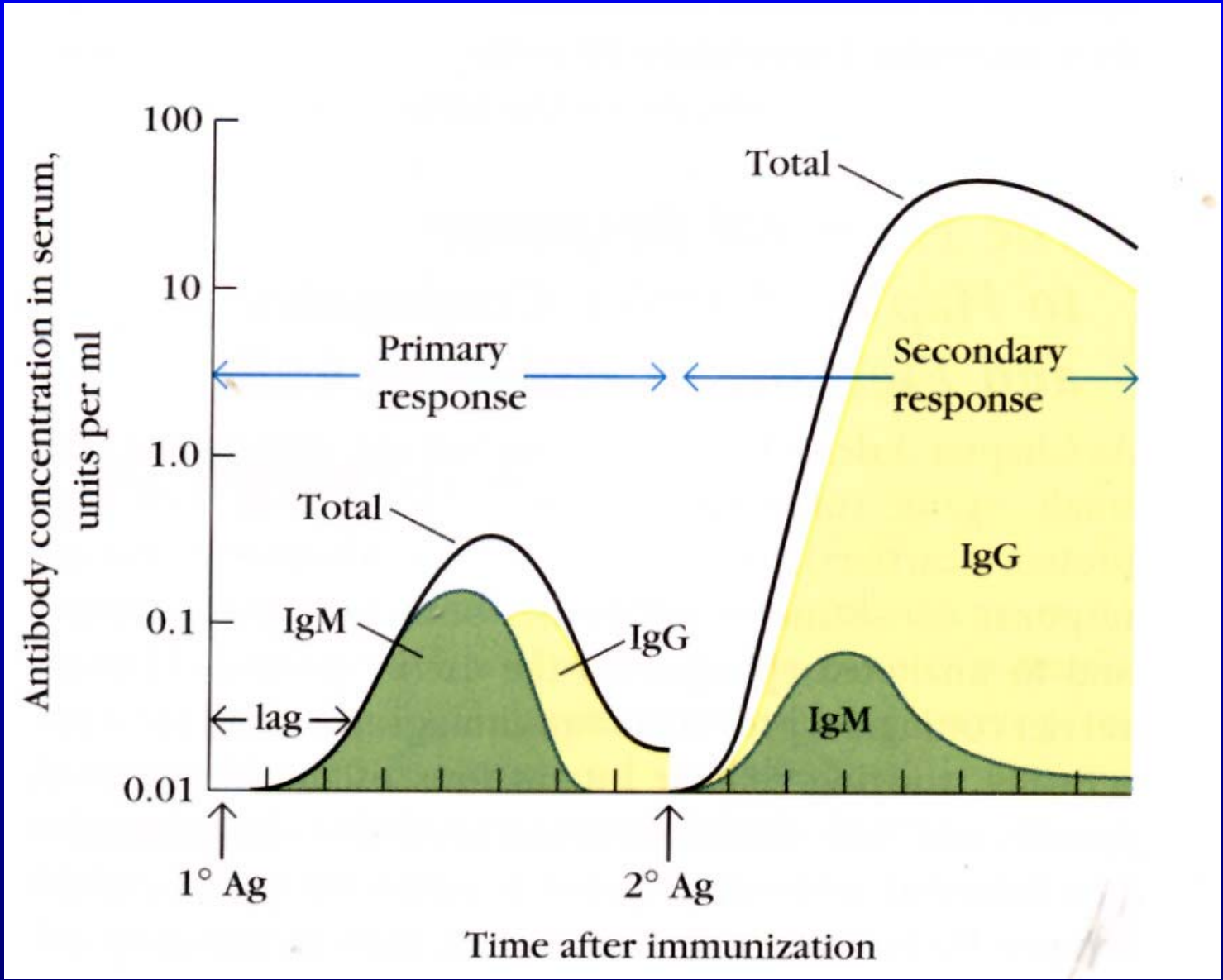
İnfeksiyonlara karşı Ab oluşumu

Anti-viral antikorlar enfeksiyondan haftalar sonra saptanabilirler Virus spesifik IgM, IgG'den daha önce pozitifleşir. IgM yanıtı yaklaşık 2-3 ayda azalmasına rağmen bazı viral enfeksiyonlarda düşük düzeyde bir yıl veya daha fazla devam edebilir. IgG antikorları ise daha uzun süreli, genellikle hayat boyu kalıcıdır. Akut enfeksiyon için serolojik bulgular şunları içerir:

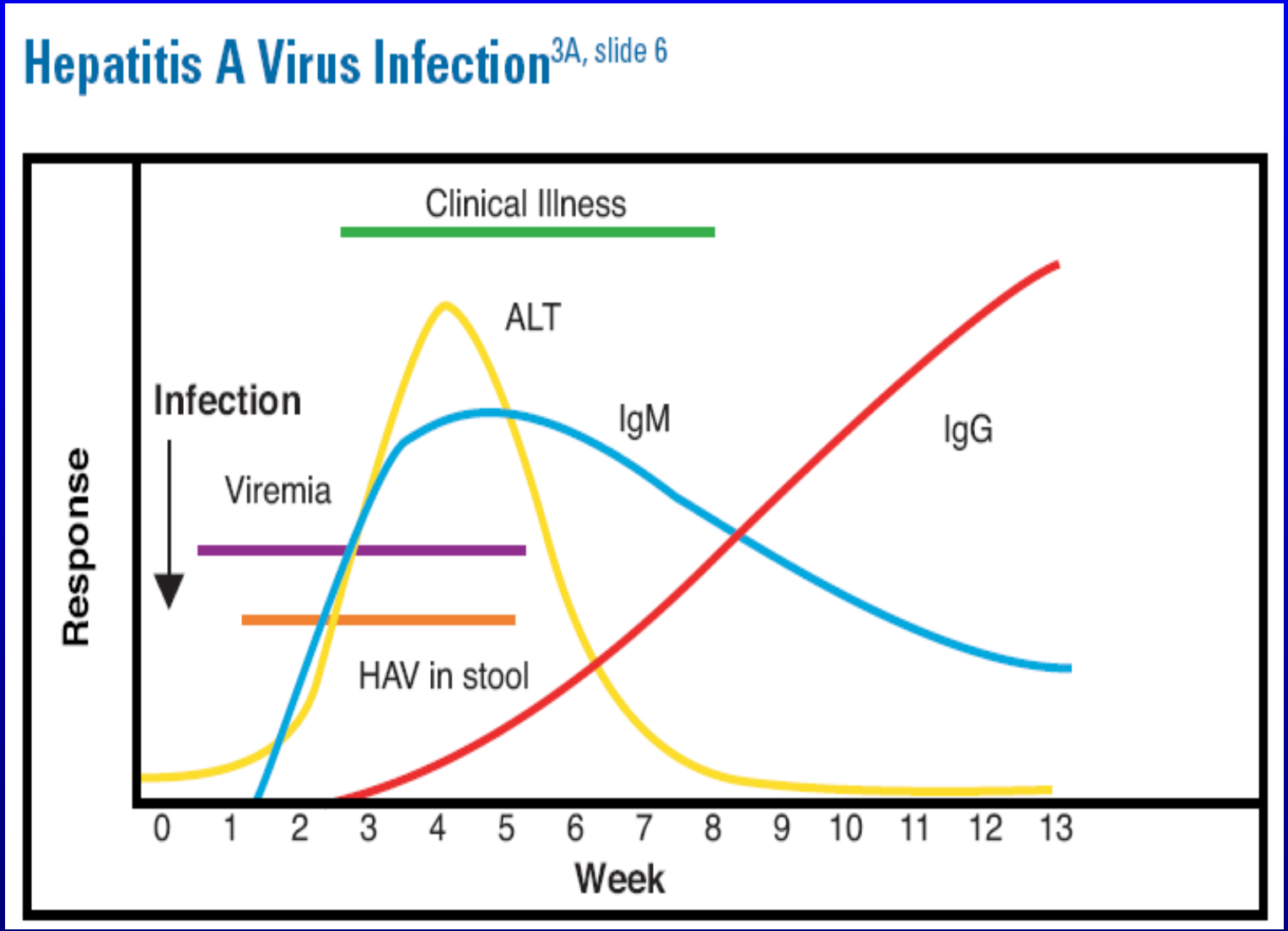


- Serokonversiyon: Hastalığın başlangıcında olmayan anti-viral antikorların birkaç hafta sonra ortaya çıkması (önce IgM ve sonra IgG),
- Akut dönem ile konvelesan dönem arasında anti-viral antikor titrelerinin artması (x4kat, 2 tüp dilüsyonu)
- Akut dönemde anti-viral IgM antikorlarının varlığı (özellikle hastalığın erken dönemlerinde alınan tek bir örnek ile hızlı tanıda kullanılabilir).

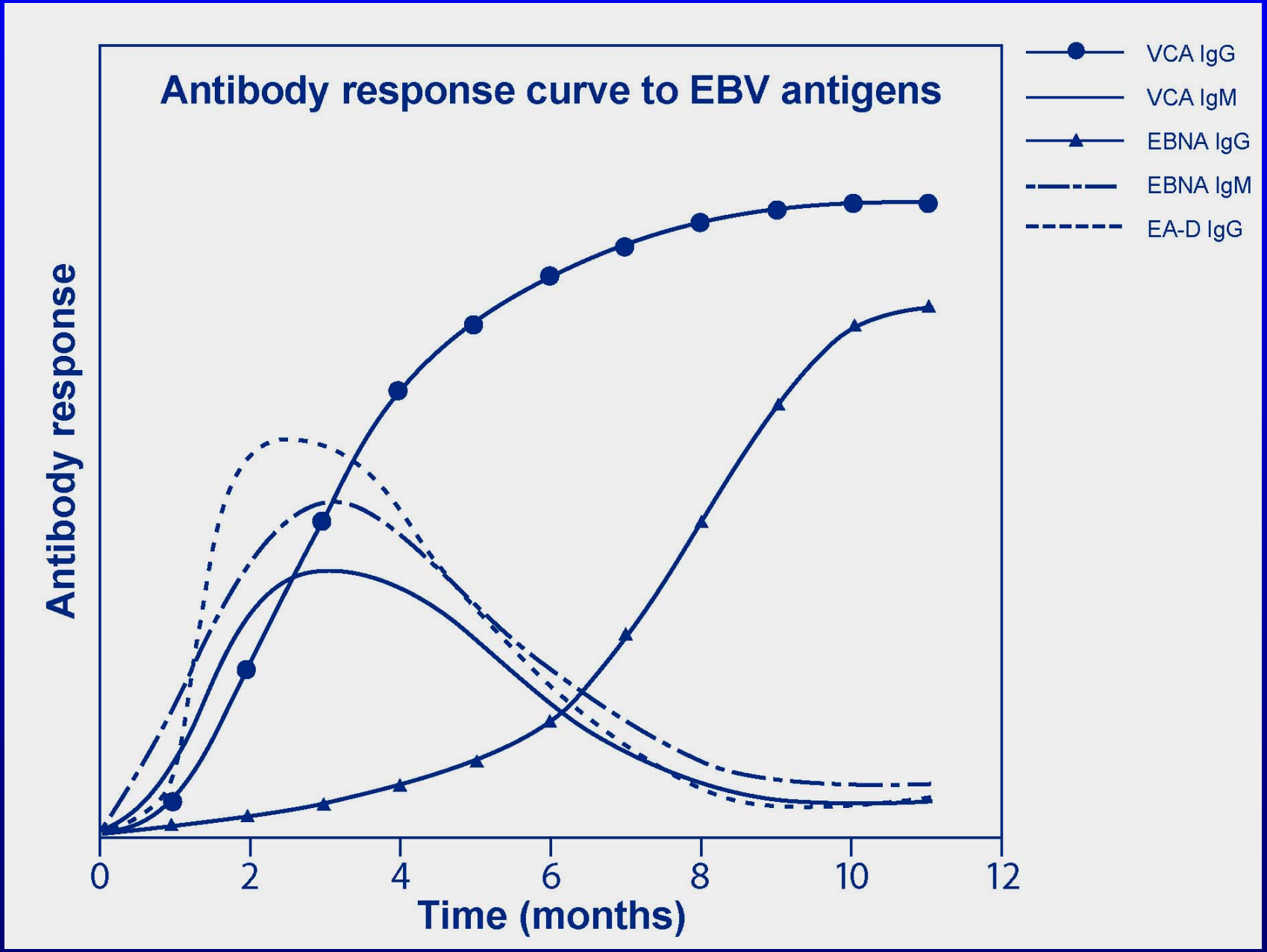
İnfeksiyonlara karşı Ab oluşumu



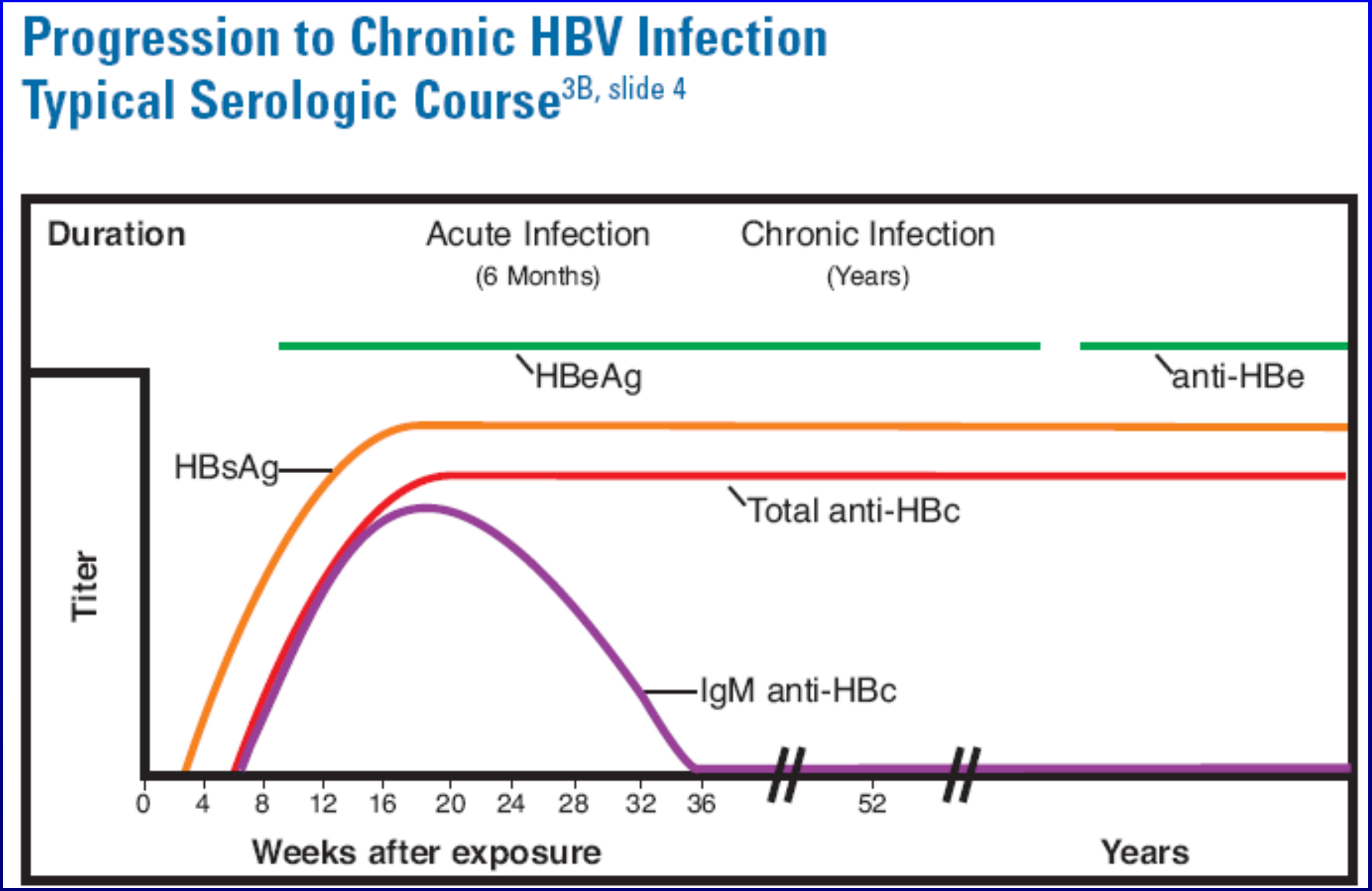
İnfeksiyonlara karşı Ab oluşumu: HAV



İnfeksiyonlara karşı Ab oluşumu: EBV



İnfeksiyonların Serolojik Göstergeleri: HBV



EIA

- Ag veya Ab saptamaya yöneliktir (Serum, plazma, BOS, idrar, tükrük)
- İmmünolojik bir yöntemdir
- Kalitatif (pozitif/negatif) veya kantitatif (sayısal) sonuç alınabilir
- Duyarlılığı yüksektir (ng ölçülebilir)
- Özgüllüğü kantitatif testlerde yüksek, tarama testlerinde daha düşüktür
- Otomasyona uygundur
- Çeşitliliği fazladır
- Aynı anda tek bir mikroorganizmaya ait Ag veya Ab saptanır, multipleks olamaz
- Test sonunda renklenme (absorbans, optik dansite –OD-) spektrofotometrede ölçülür. Belirlenen kriterlere göre değerlendirilir.

EIA

- Ag
- Ab
- Ab-enzim (konjugat)
- Substrat

- Ag veya Ab solid bir yüzeye bağlanır
  Plastik kuyu, tüp, membran, lateks/magnetik partikül
- Ag veya Ab enzimle işaretlenir (konjuge edilir)
  Horseradish peroksidaz, alkalen fosfataz,
  beta-galaktozidaz vb
-Substrat olarak; TMB (tetrametilbenzidin),
  pNPP (paranitrofenilfosfat), kemiluminesan md
  floresan md.ler kullanılır

Ab yapısı

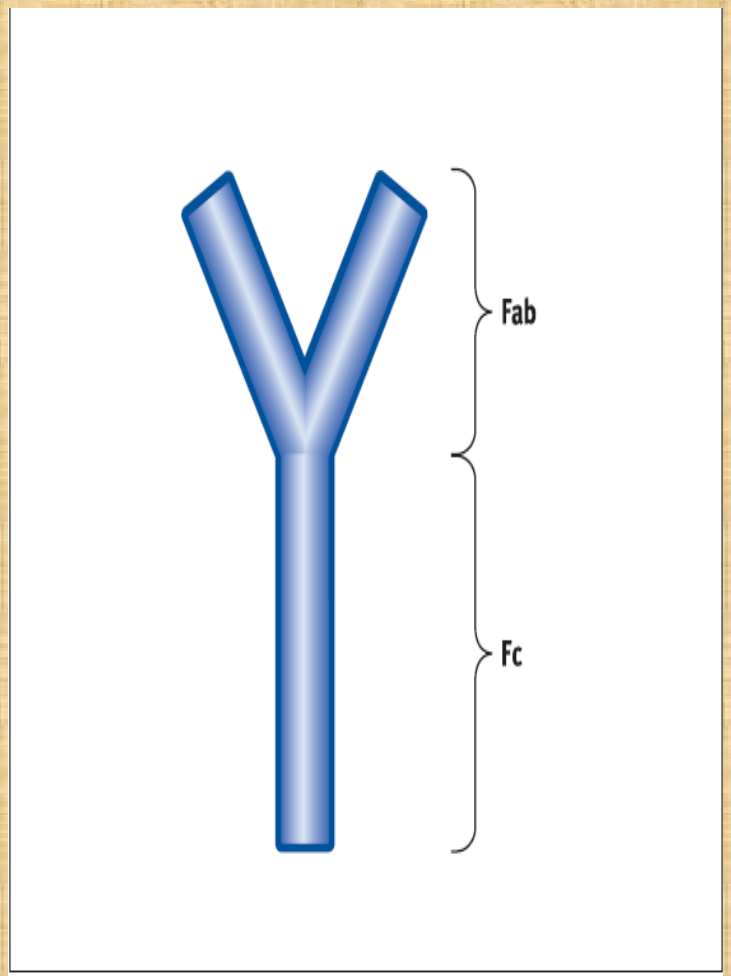
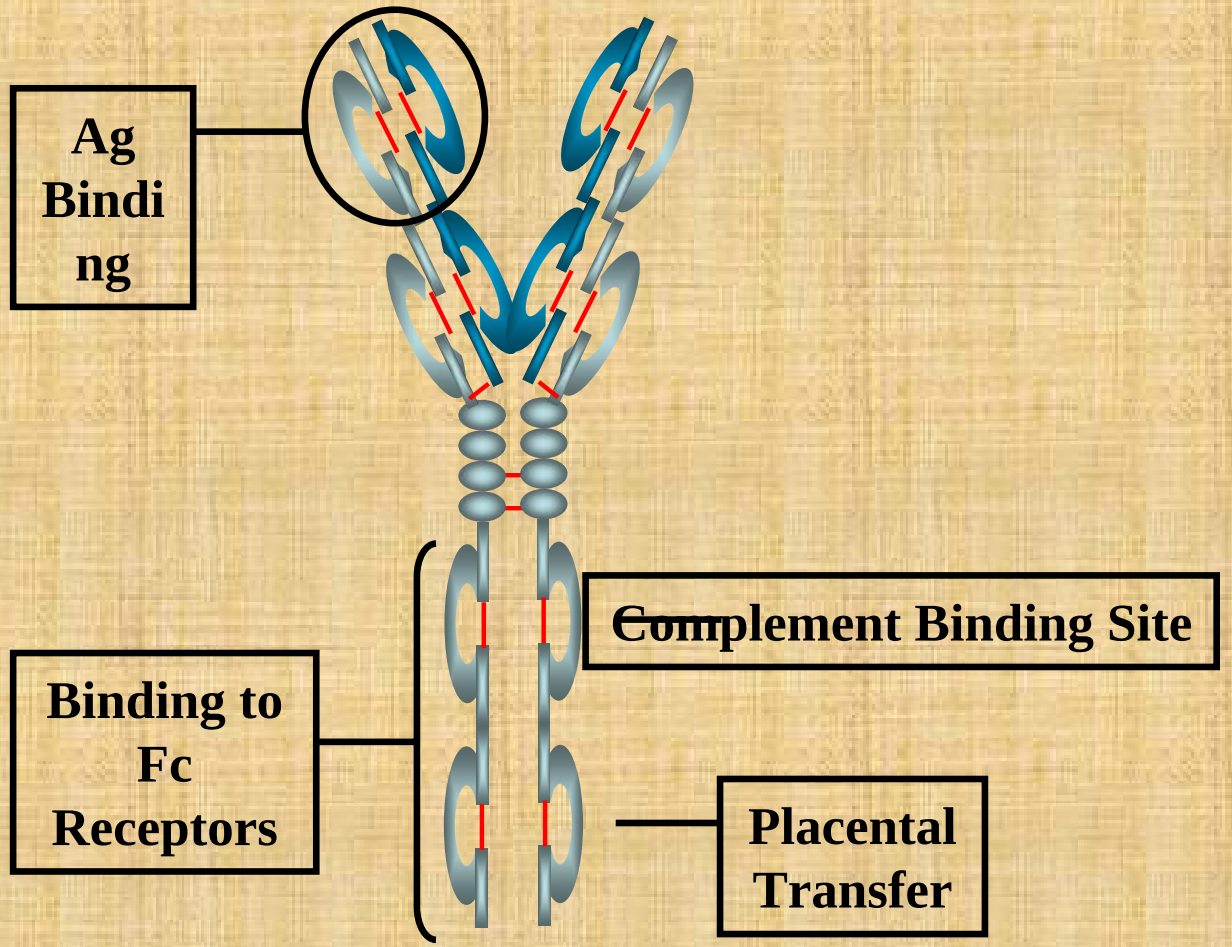


FIGURE 1-1 Antibody Structure and Functional Sites



Poliklonal ve Monoklonal Ab'lar

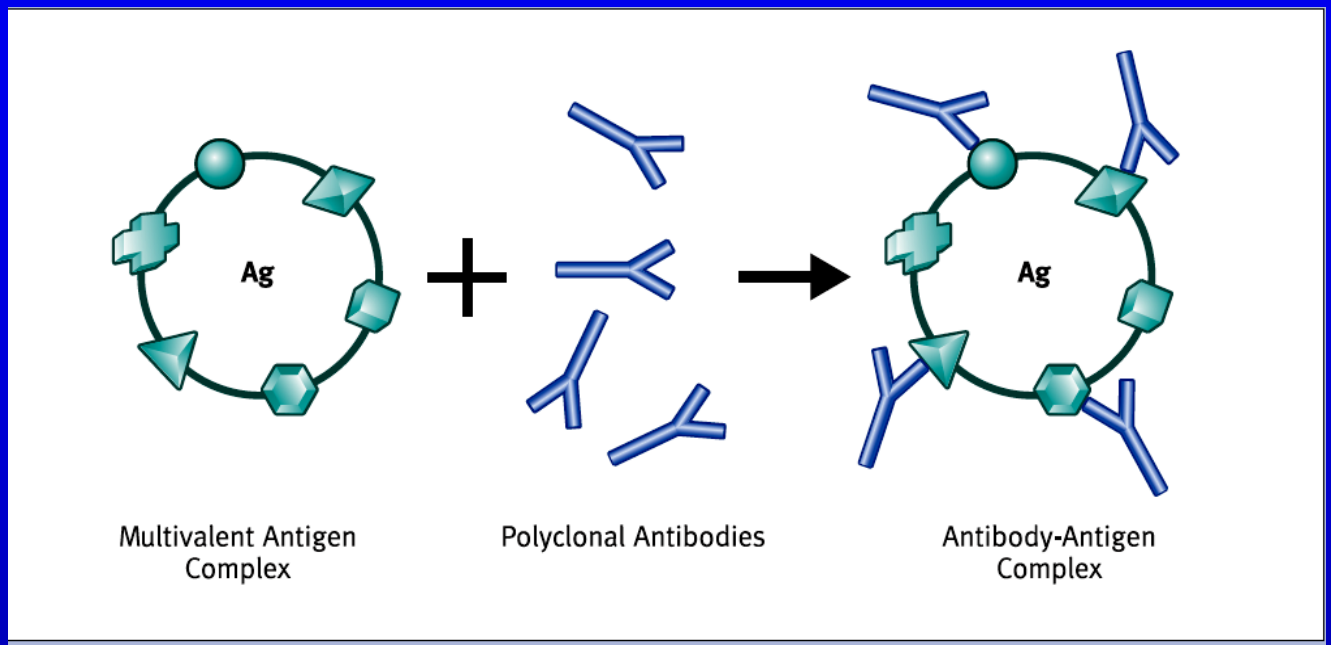


FIGURE 1-2 Multiple antigen specificities of polyclonal antibodies

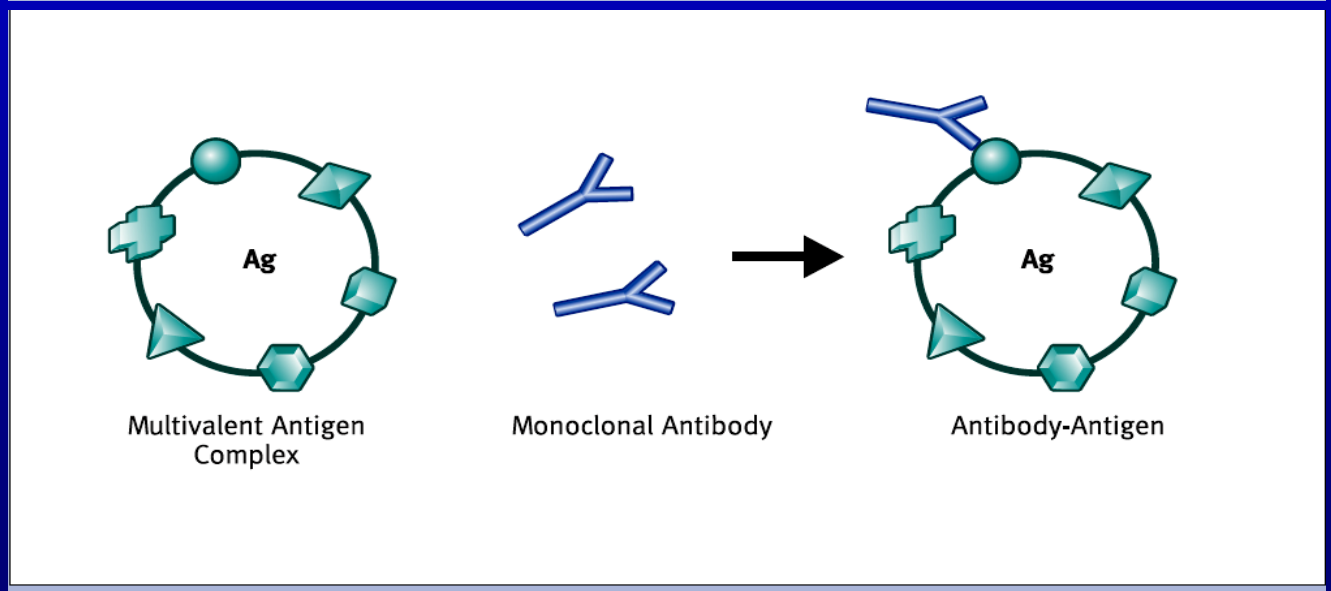
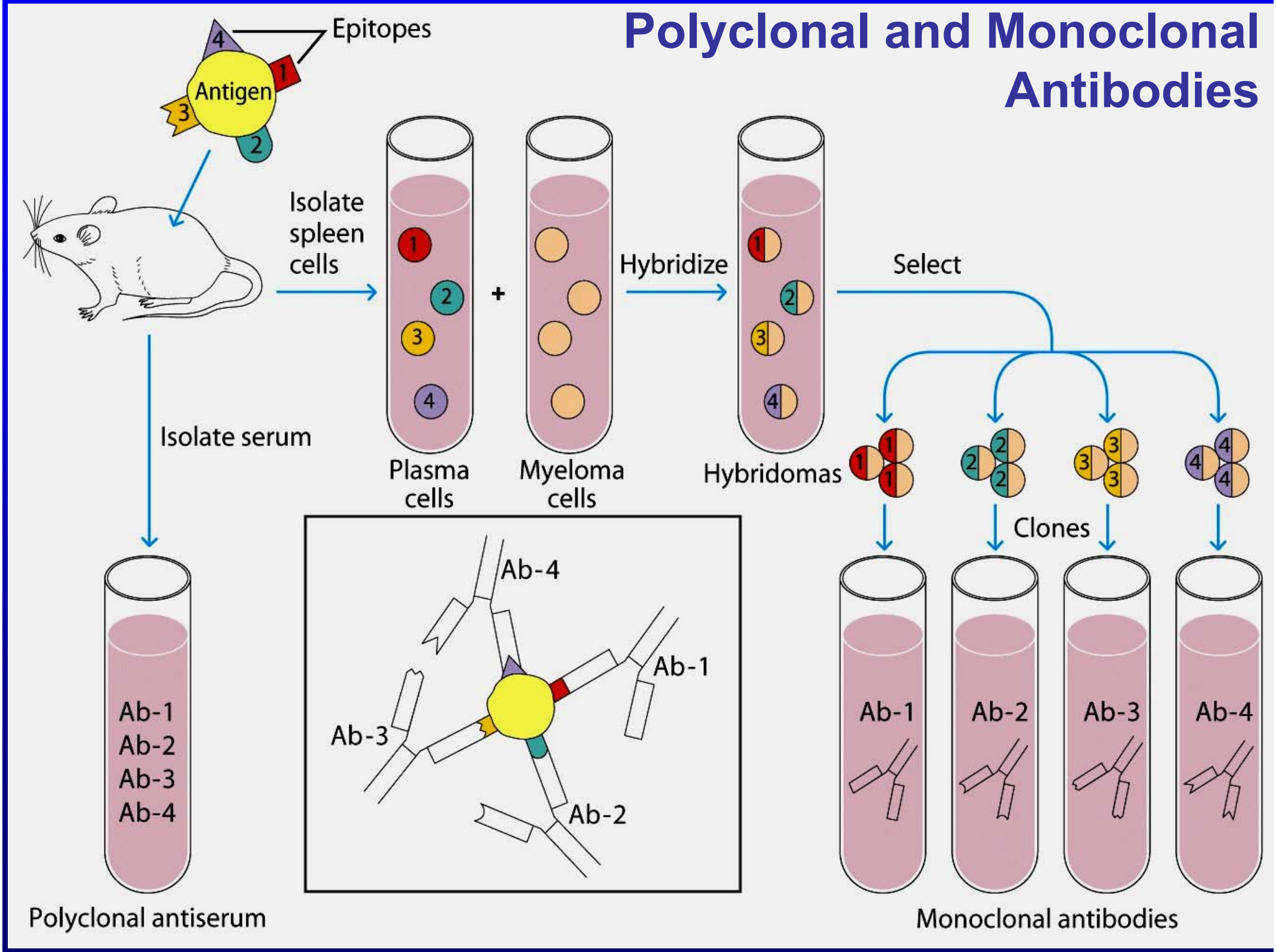
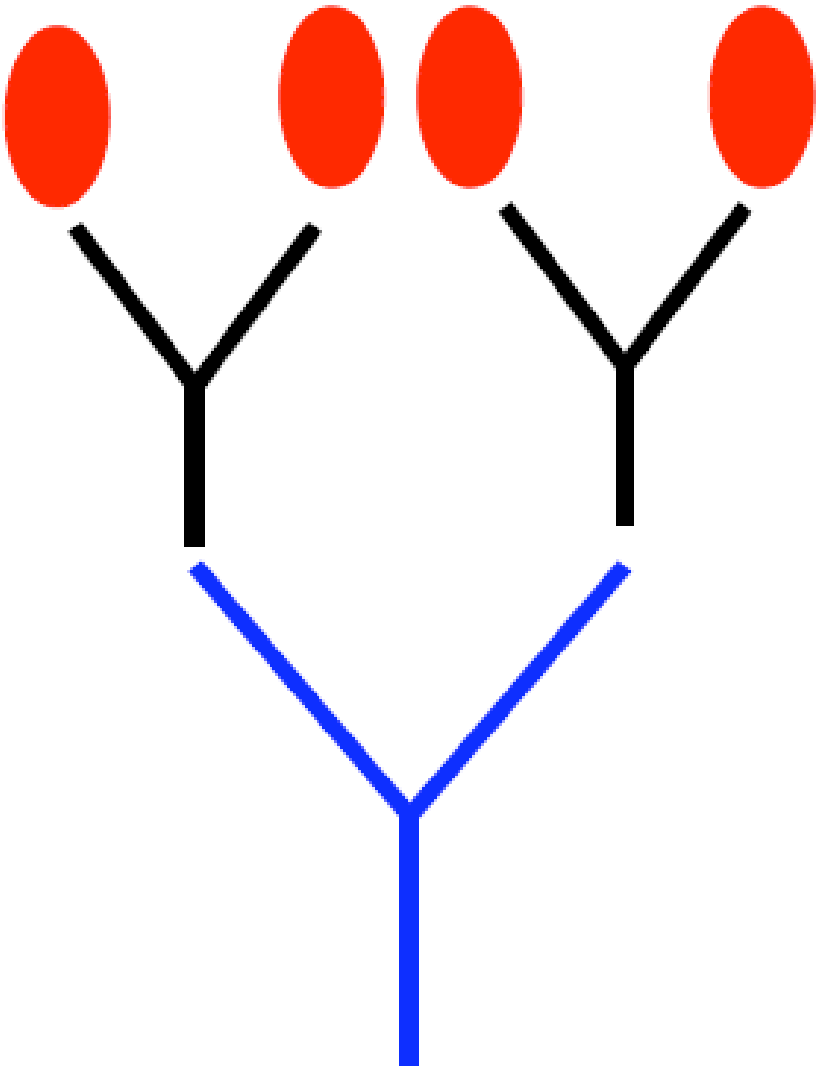


FIGURE 1-3 Uniform specificities of monoclonal antibodies

Polyclonal and Monoclonal Antibodies



Technical Terminology



Antigen

"Insulin"

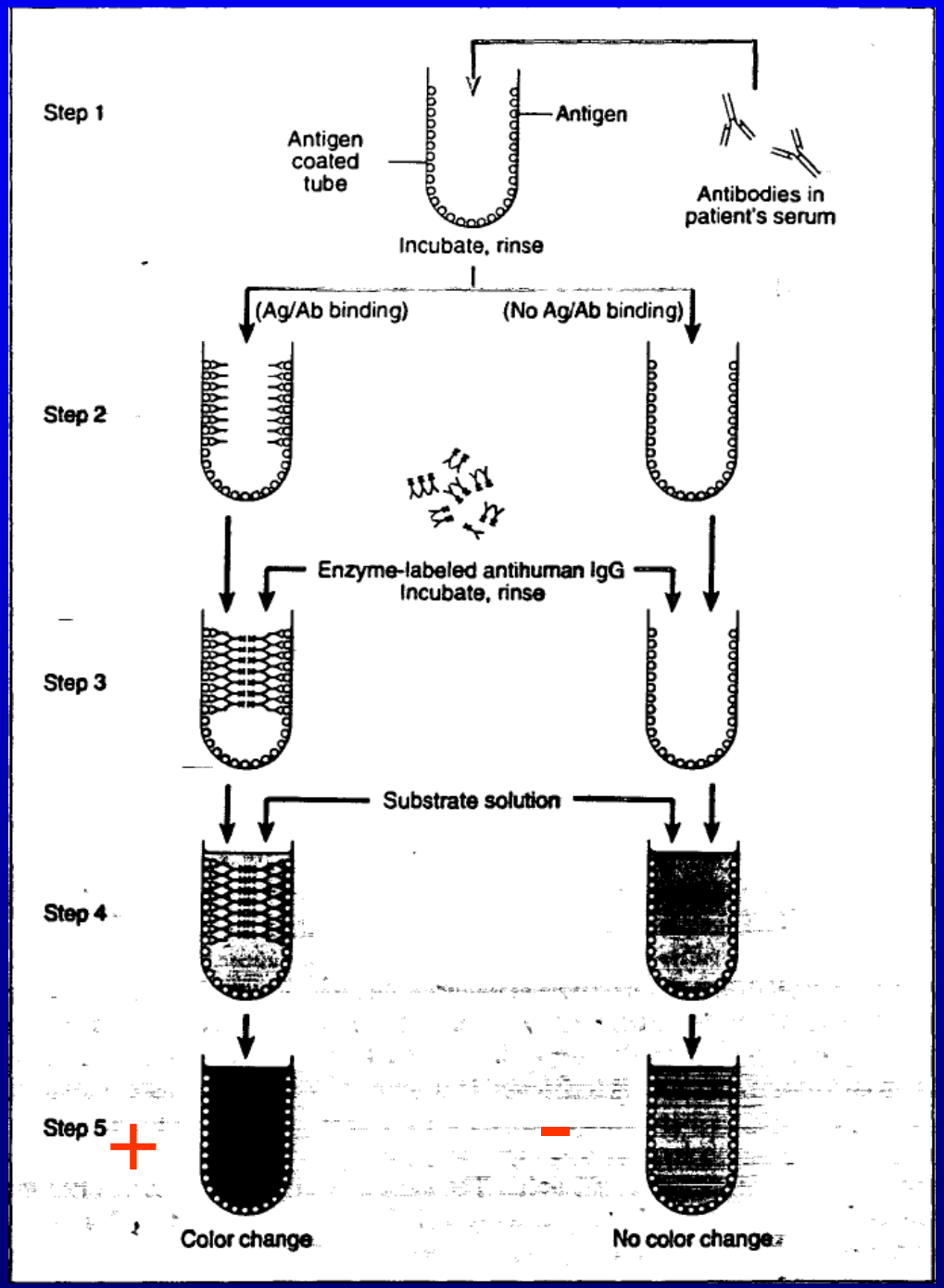
1° Antibody

"Mouse anti-insulin IgG"

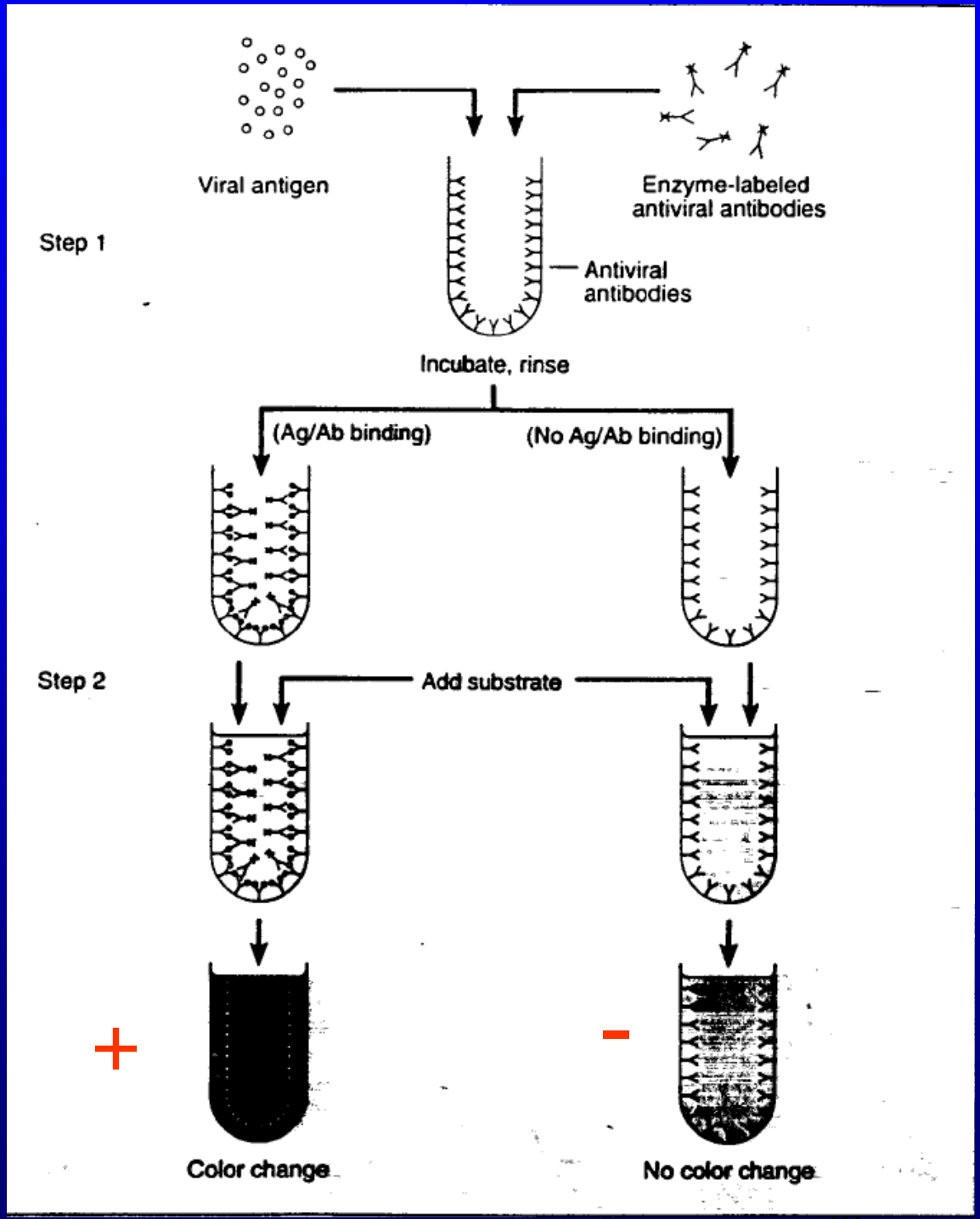
2° Antibody

"Goat anti-mouse IgG"

Ab Saptanmasında Solid Faz EIA



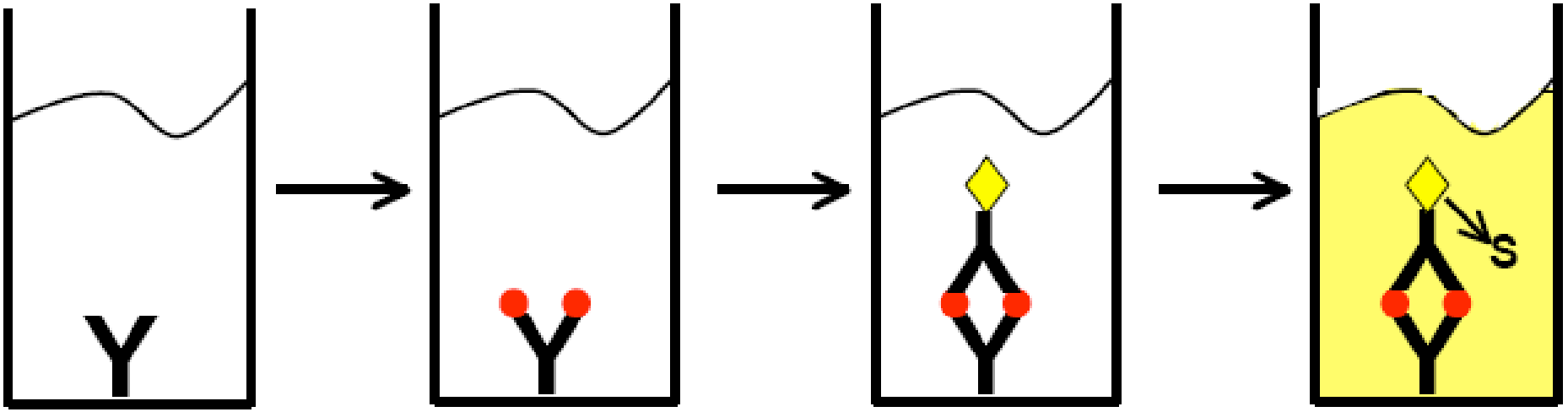
Ag Saptanmasında Solid Faz EIA



Enzyme-Linked ImmunoSorbant Assay (ELISA)

Qualitative or quantitative detection of antigen or antibody from a complex mixture

Sandwich Method



Coat well with 1° antibody

Add antigen (purified or mixture)

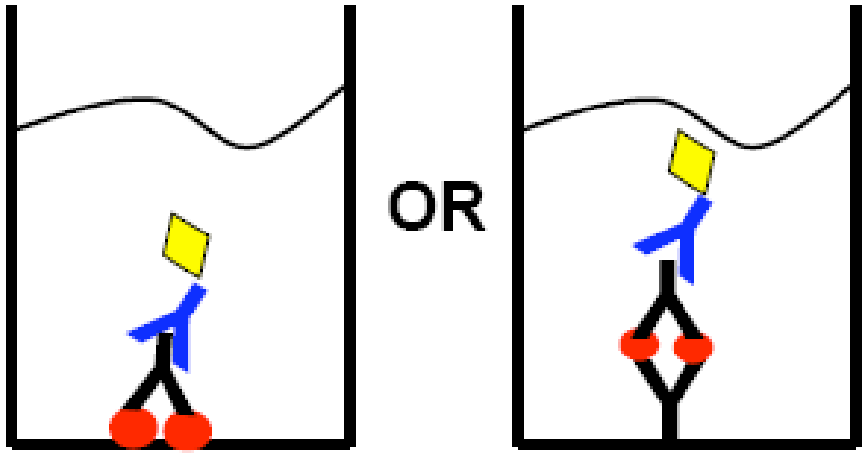
Add enzyme-linked 1° Ab

Add colored substrate

Native Antigen

Variations on ELISA

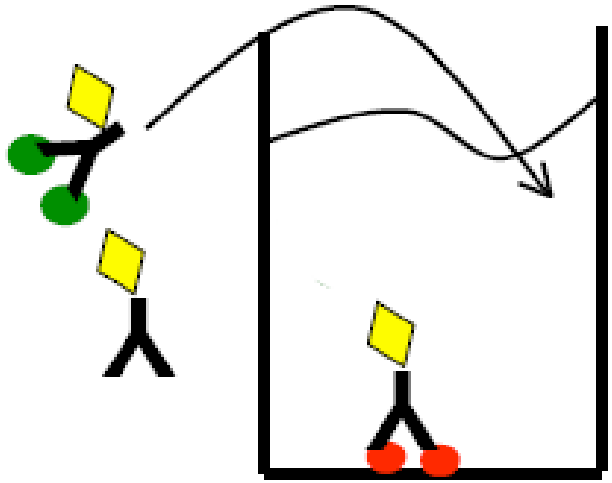
Indirect Method



Detect antibody

Detect antigen

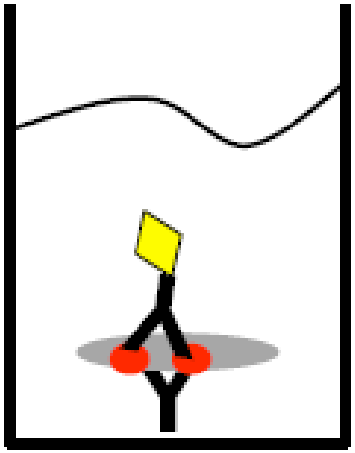
Competitive Method



Pre-react antigen1 with labeled antibody before adding to antigen2-labeled well

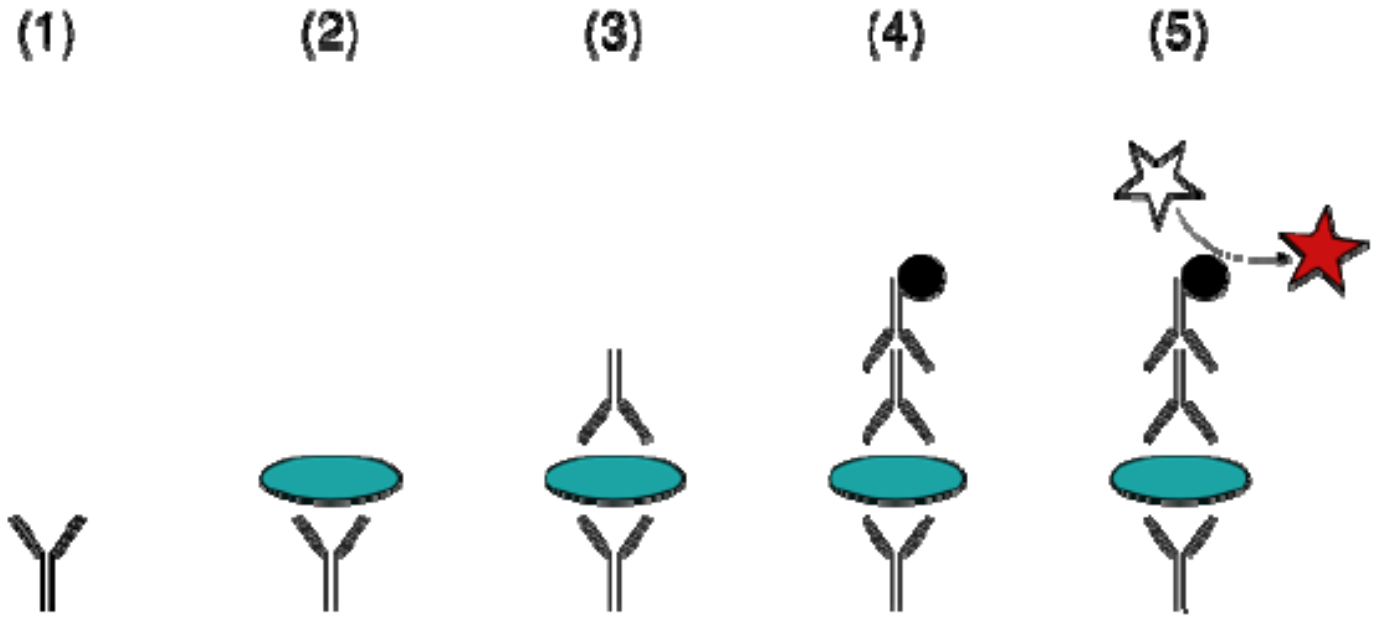
less color = better binding to antigen1

ELISPOT Method



Detect cells expressing or secreting antigen

ELISA (Enzyme-Linked ImmunoSorbent Assay)



A sandwich *ELISA*. (1) Plate is coated with a capture antibody; (2) sample is added, and any antigen present binds to capture antibody; (3) detecting antibody is added, and binds to antigen; (4) enzyme-linked secondary antibody is added, and binds to detecting antibody; (5) substrate is added, and is converted by enzyme to detectable form.

Nonkompetitif EIA

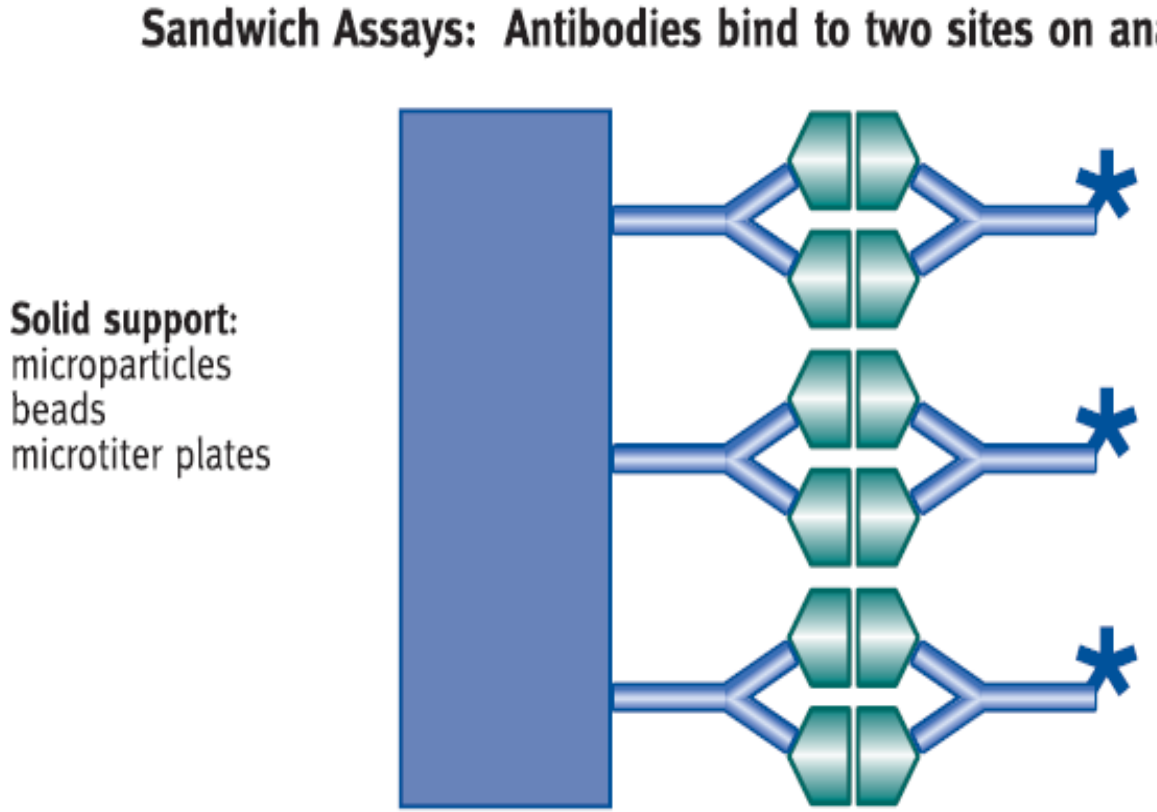
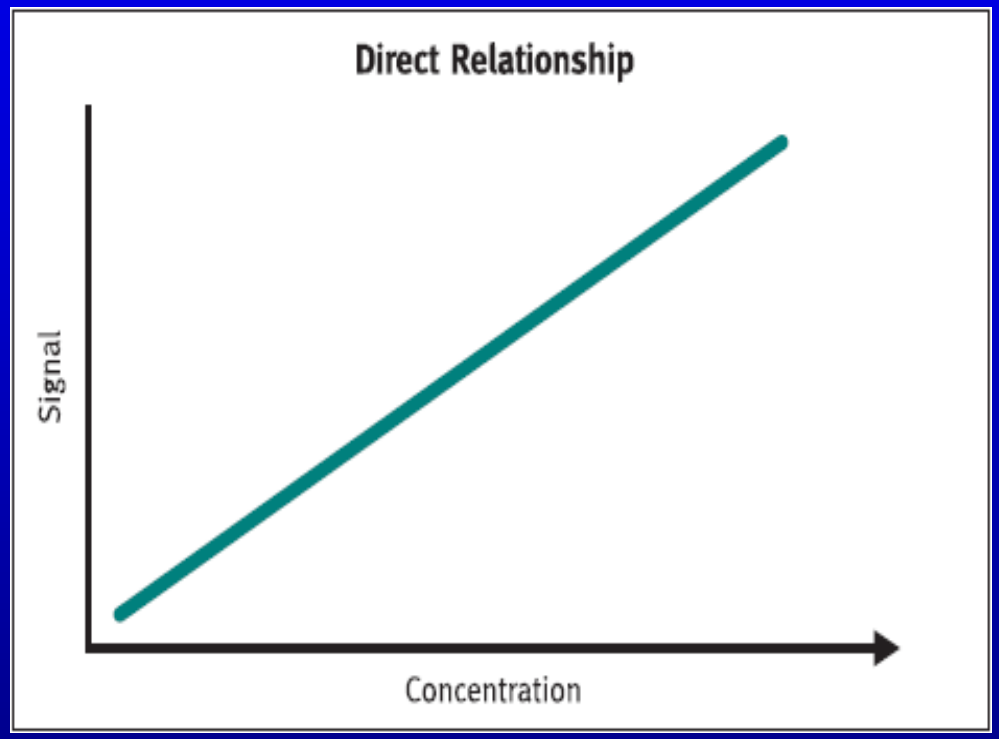


FIGURE 1-10 Noncompetitive sandwich method of immunoassay

Nonkompetitif EIA



Renklenme ↑

Kompetitif EIA

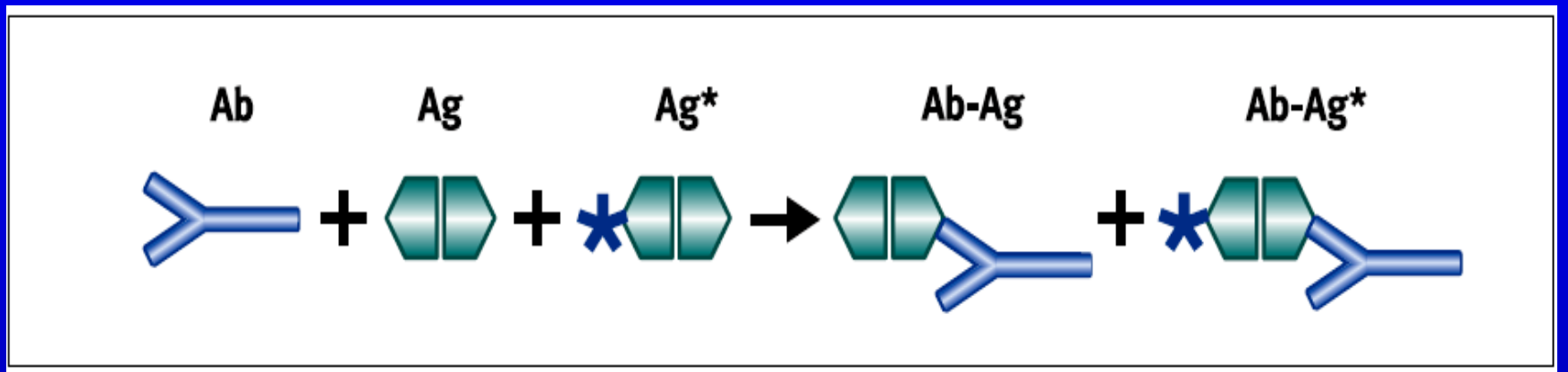


FIGURE 1-8 One step competitive immunoassay

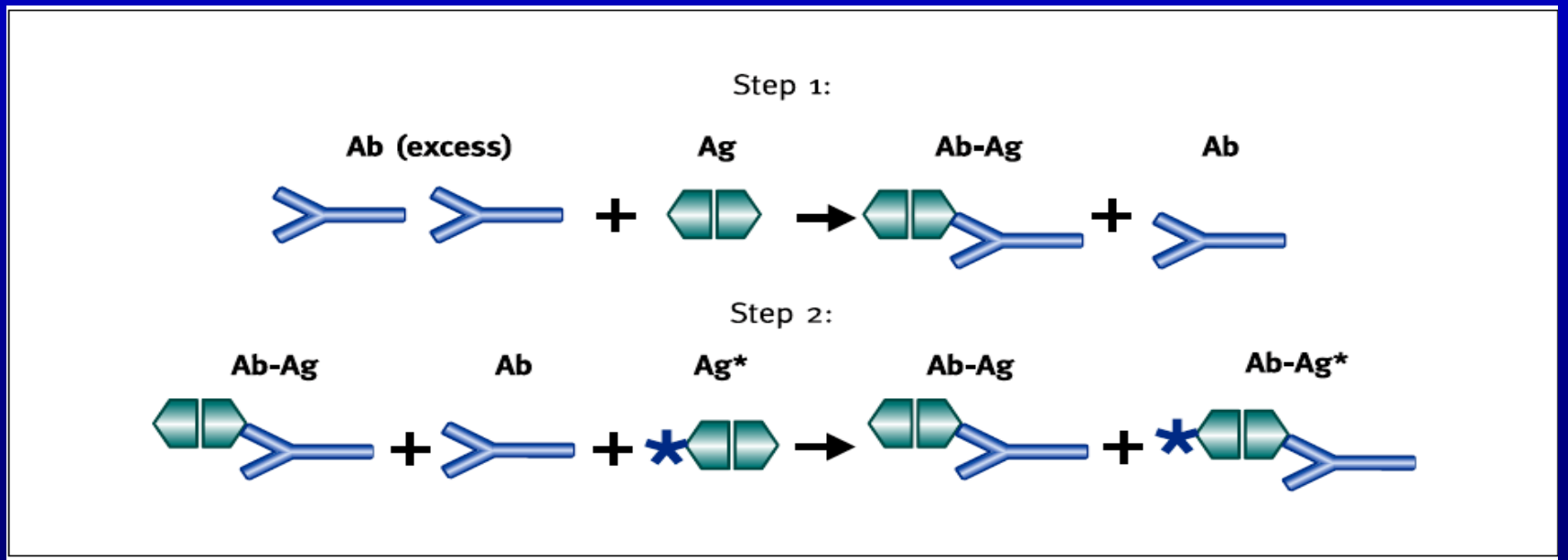
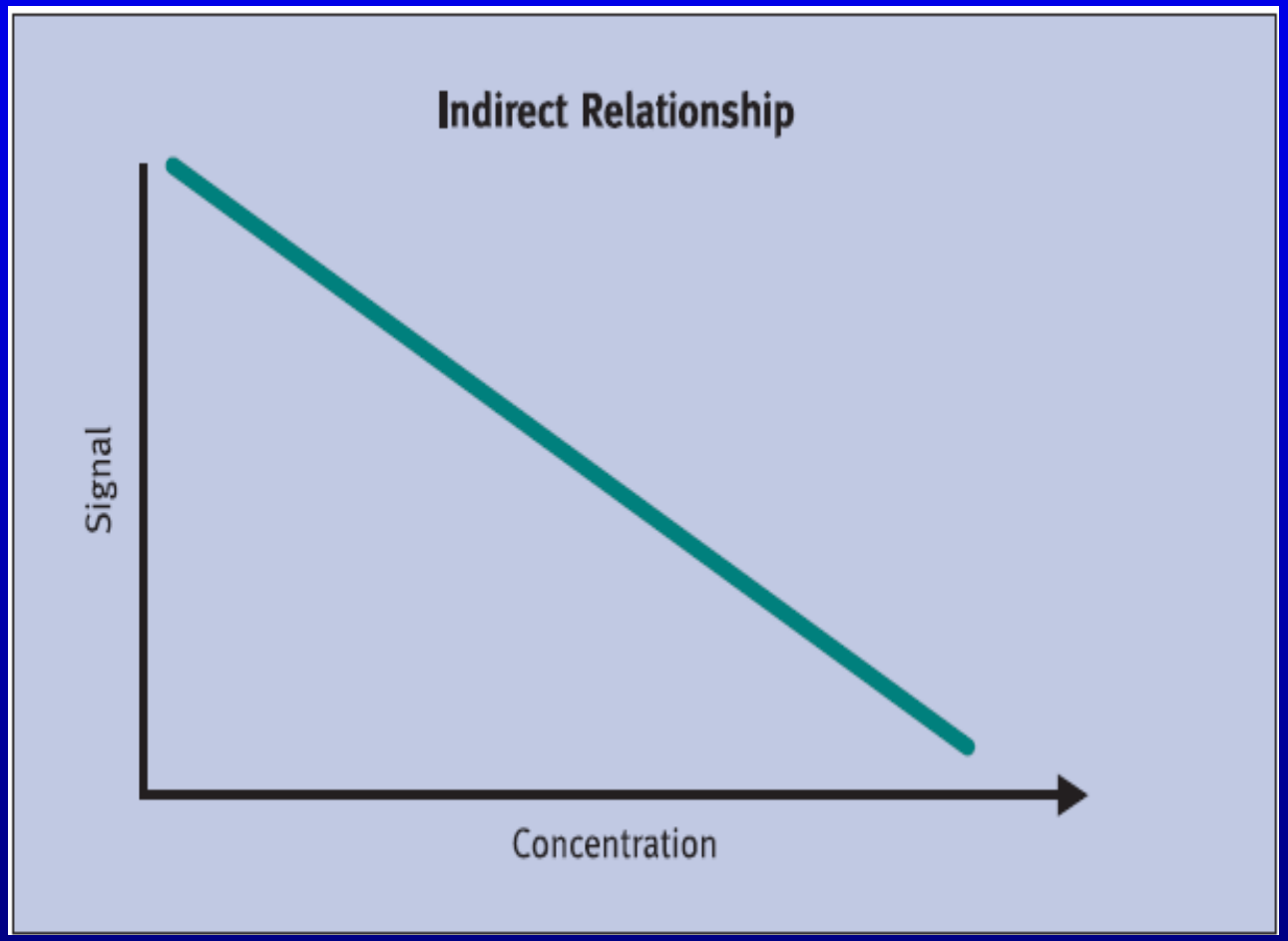


FIGURE 1-9 Two step competitive immunoassay

Kompetitif EIA

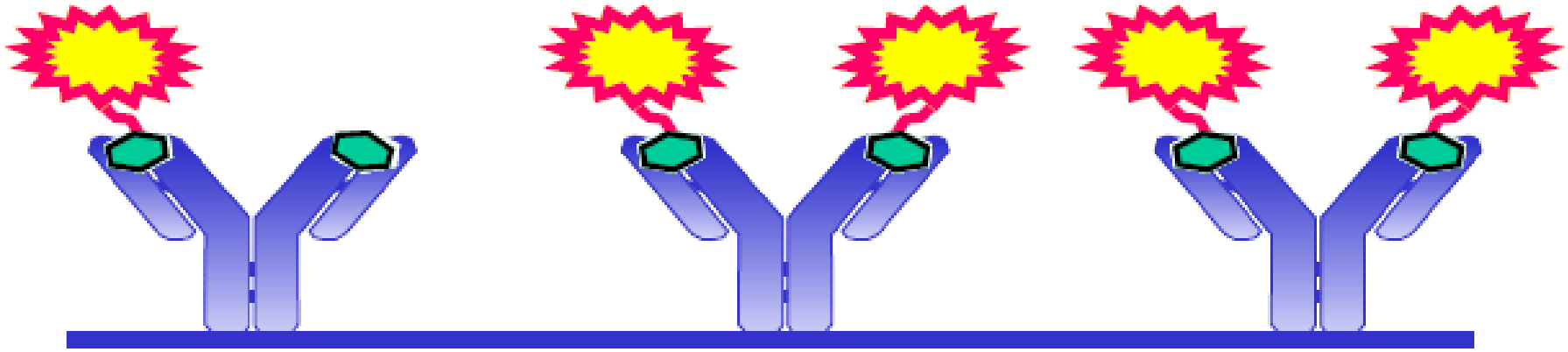


Renklenme ↓

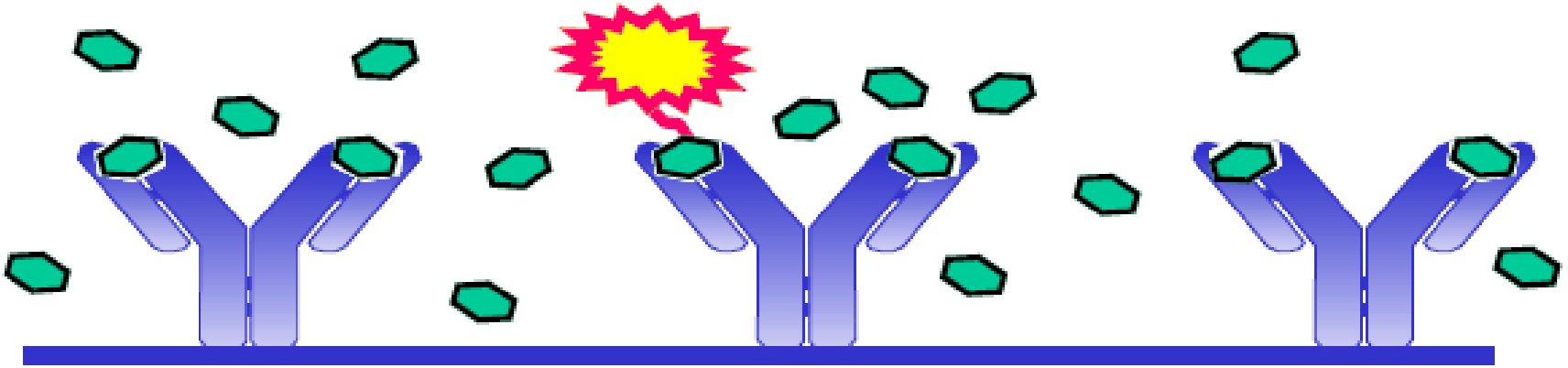
Competitive Immunoassay

AEIC AEIC AEIC AEIC AEIC AEIC AEIC AEIC AEIC

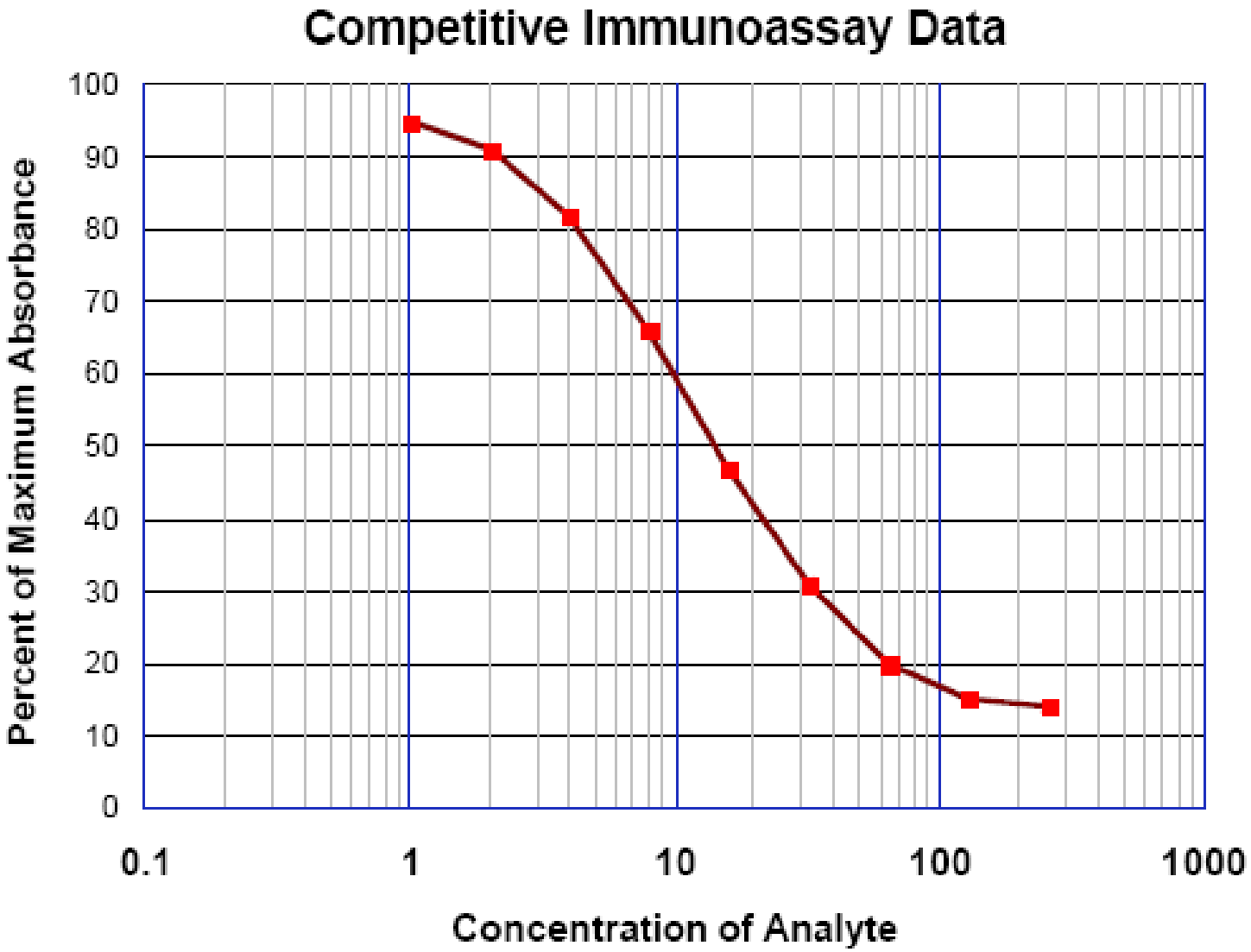
I. No analyte - high detection signal



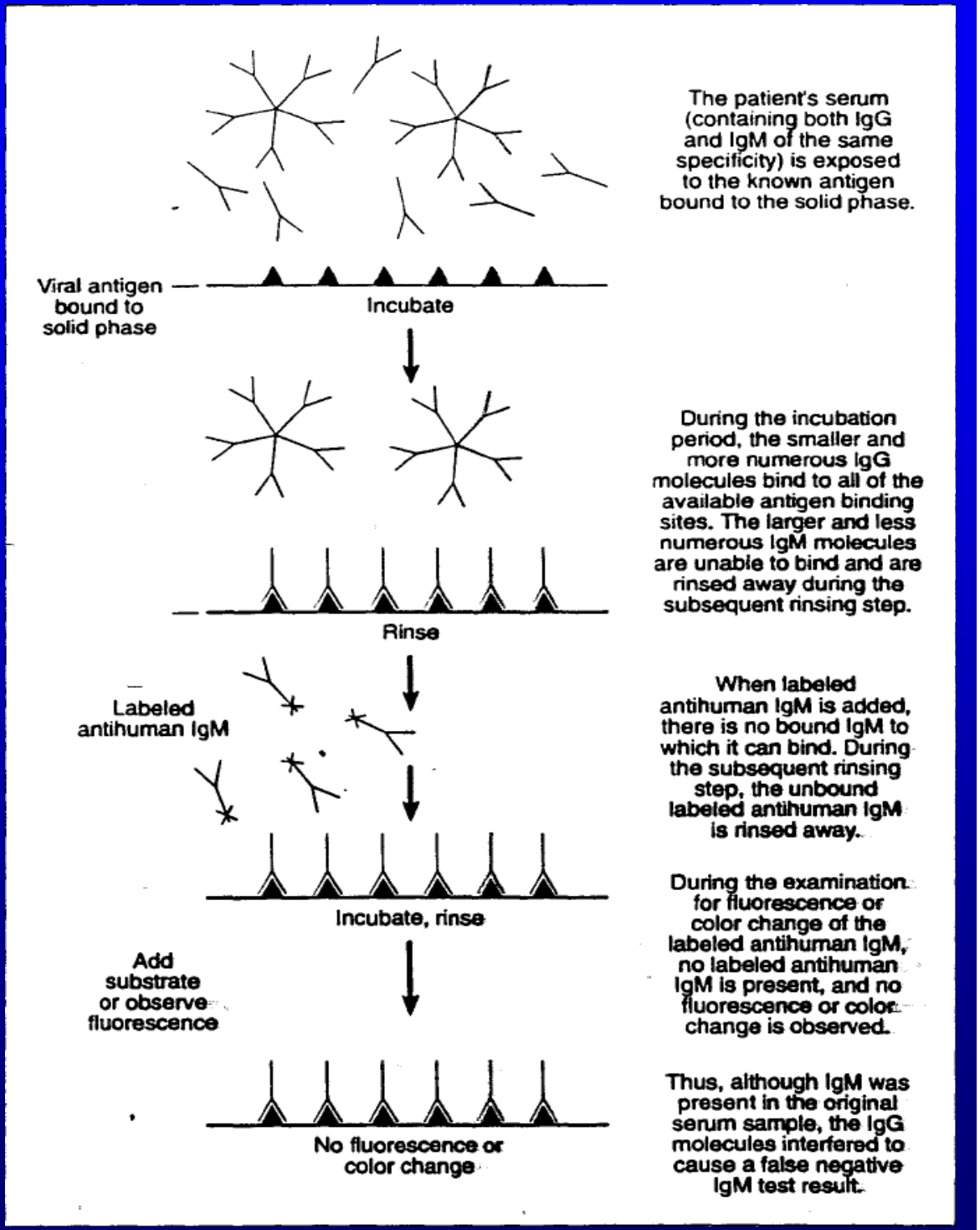
II. Analyte present - detection signal reduced



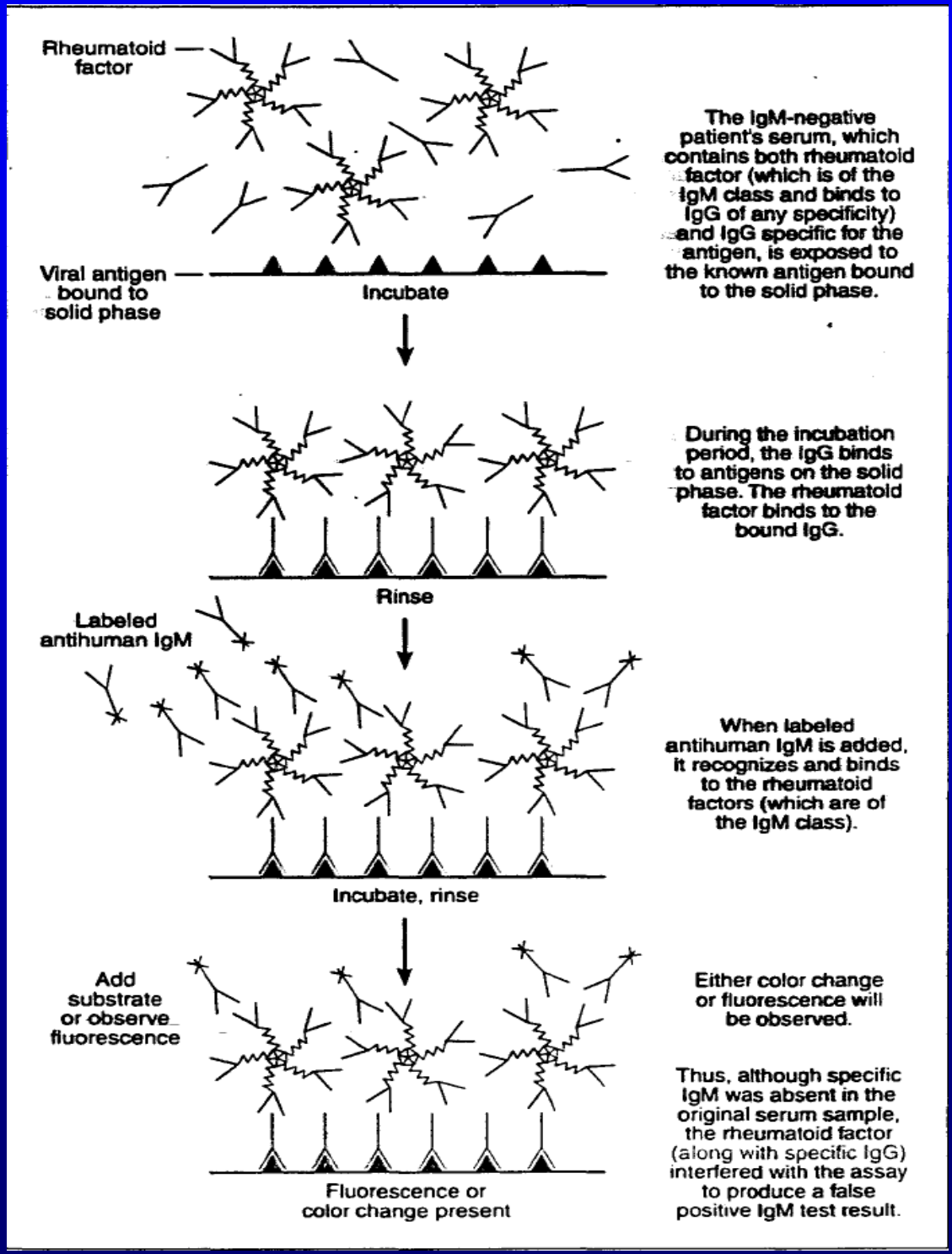
Competitive Immunoassay Data Format



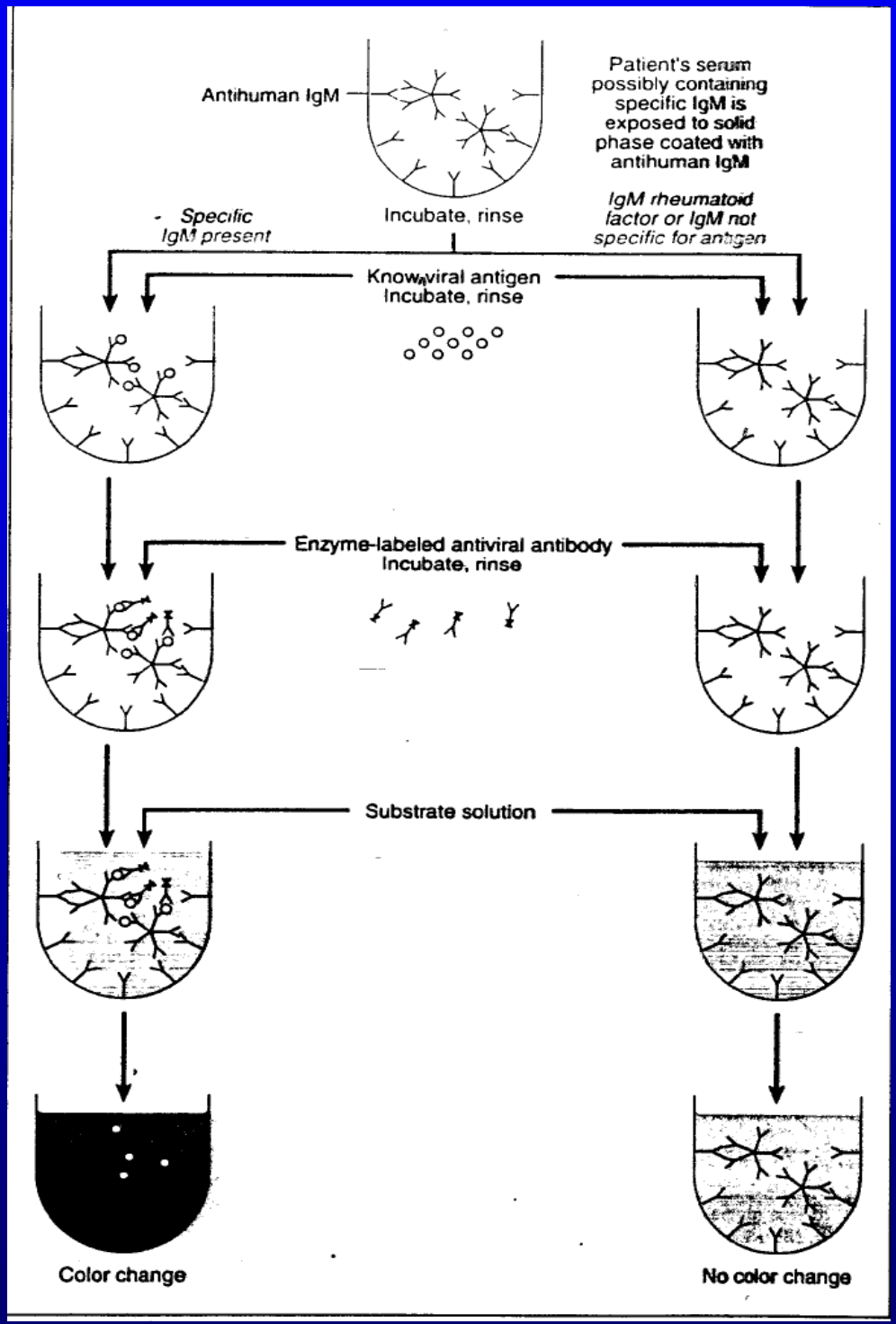
IgM EIA testinde yalancı negatif sonuç

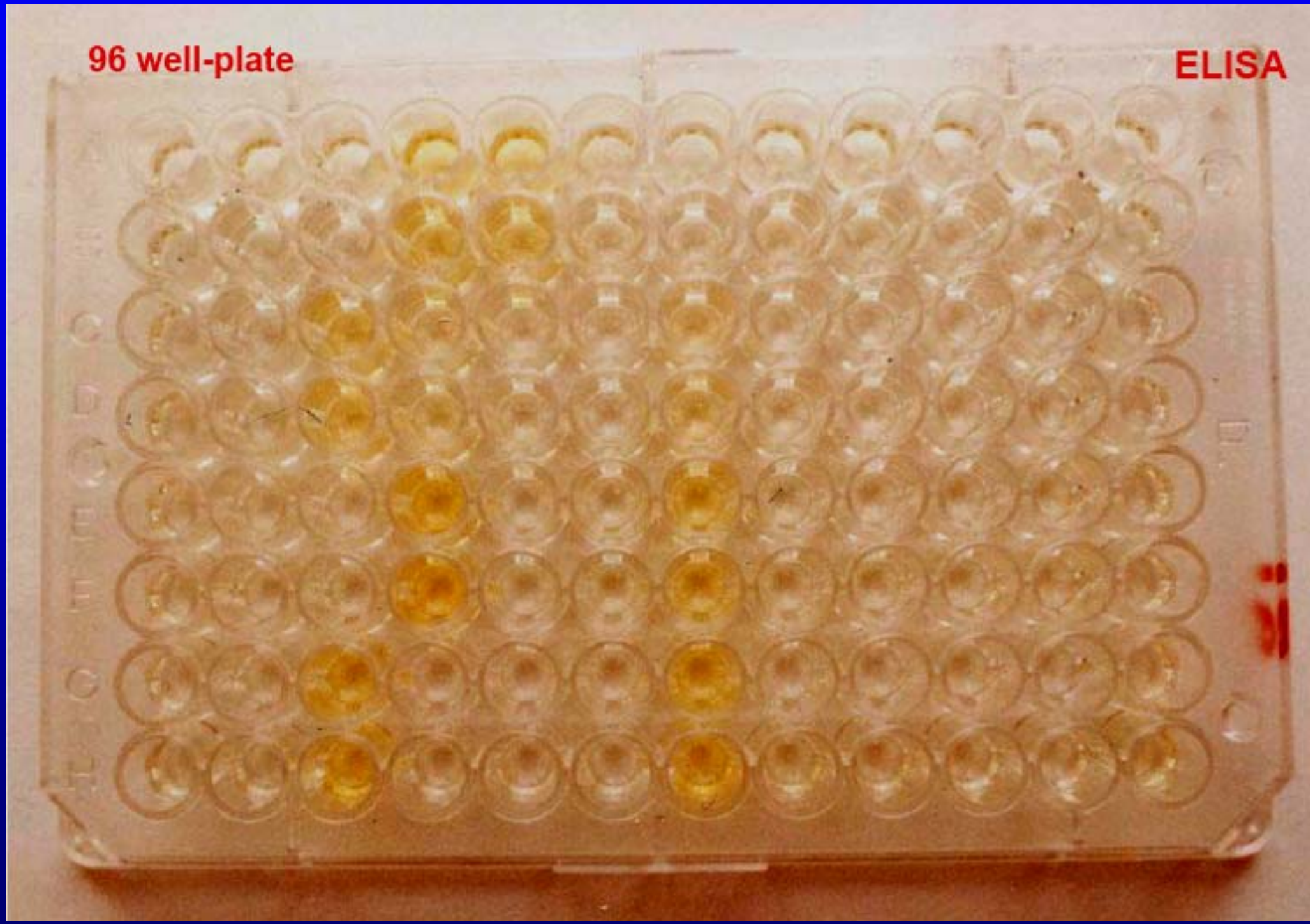


IgM EIA testinde yalancı pozitif sonuç (RF etkisi)



IgM Capture EIA




96 well-plate
ELISA

Electrochemiluminescence EIA

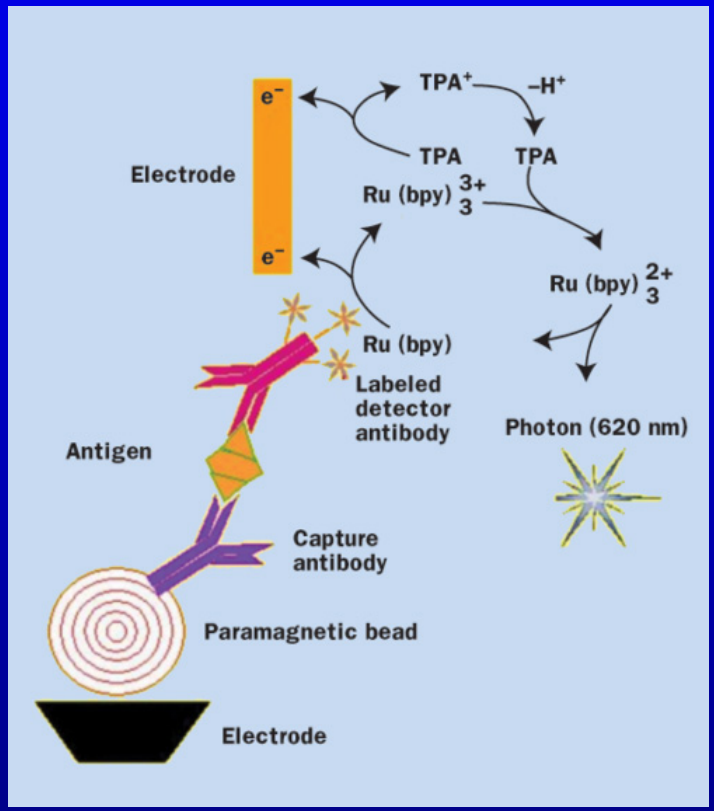
- HBsAg içeren örnek+ biotin-monoklonal antiHBs + ruthenium complex- monoklonal antiHBs “sandviç”
- Streptavidin kaplı mikropartiküller

  Biotin-streptavidin kompleksi oluşur
  Elektrod yüzeyine magnetik bağlanma

  Yıkama bağlanmayan materyal uzaklaştırılır

  Elektroda gelen elektrik ile ışıma

- Saptama sınırı çok düşük: 200 fmol/L



Kompetitif floresan polarizasyon EIA

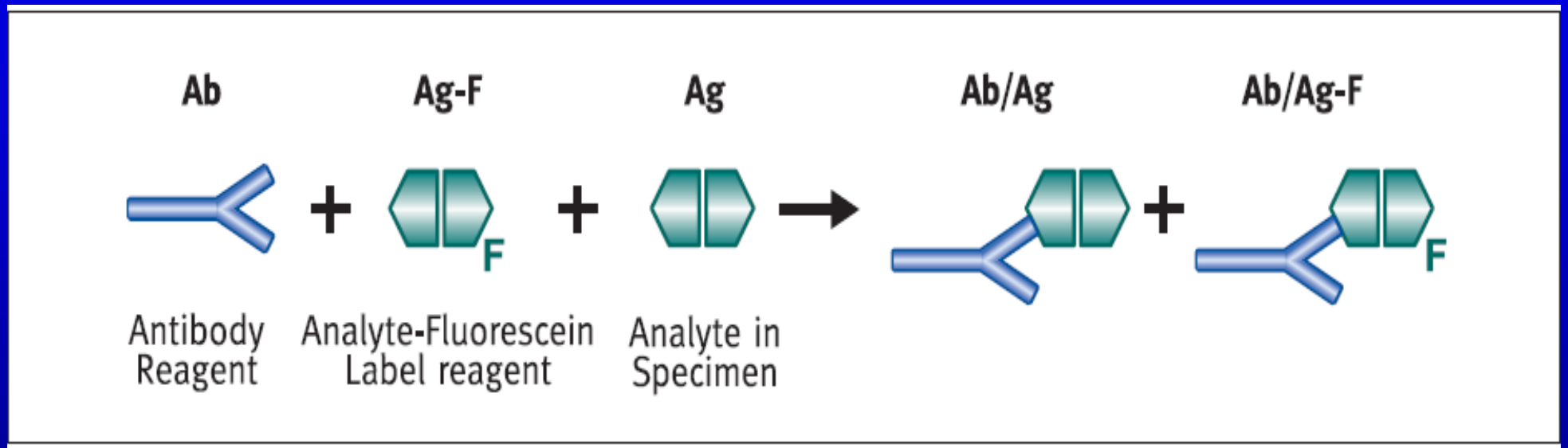


FIGURE 2-2 Competitive fluorescence polarization immunoassay (FPIA)

MEIA

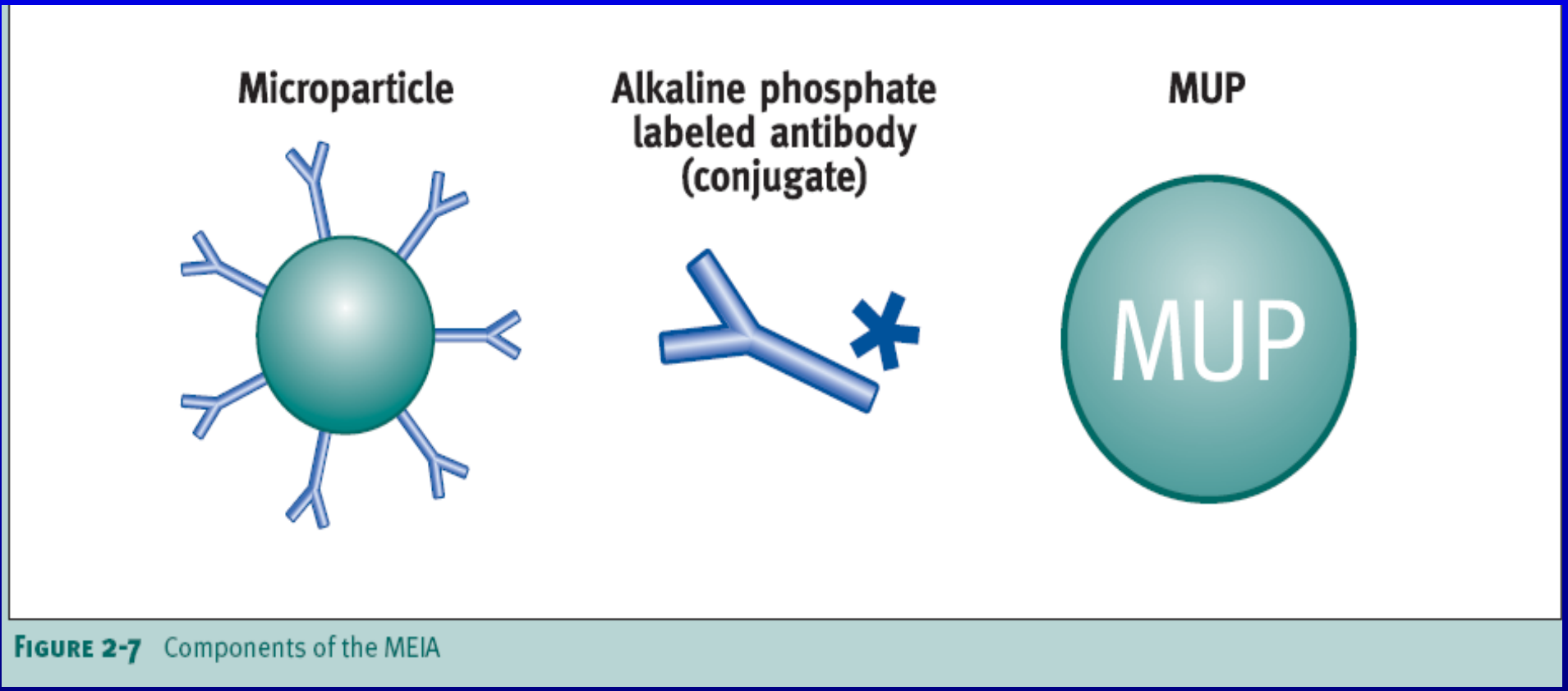


FIGURE 2-7 Components of the MEIA

MEIA

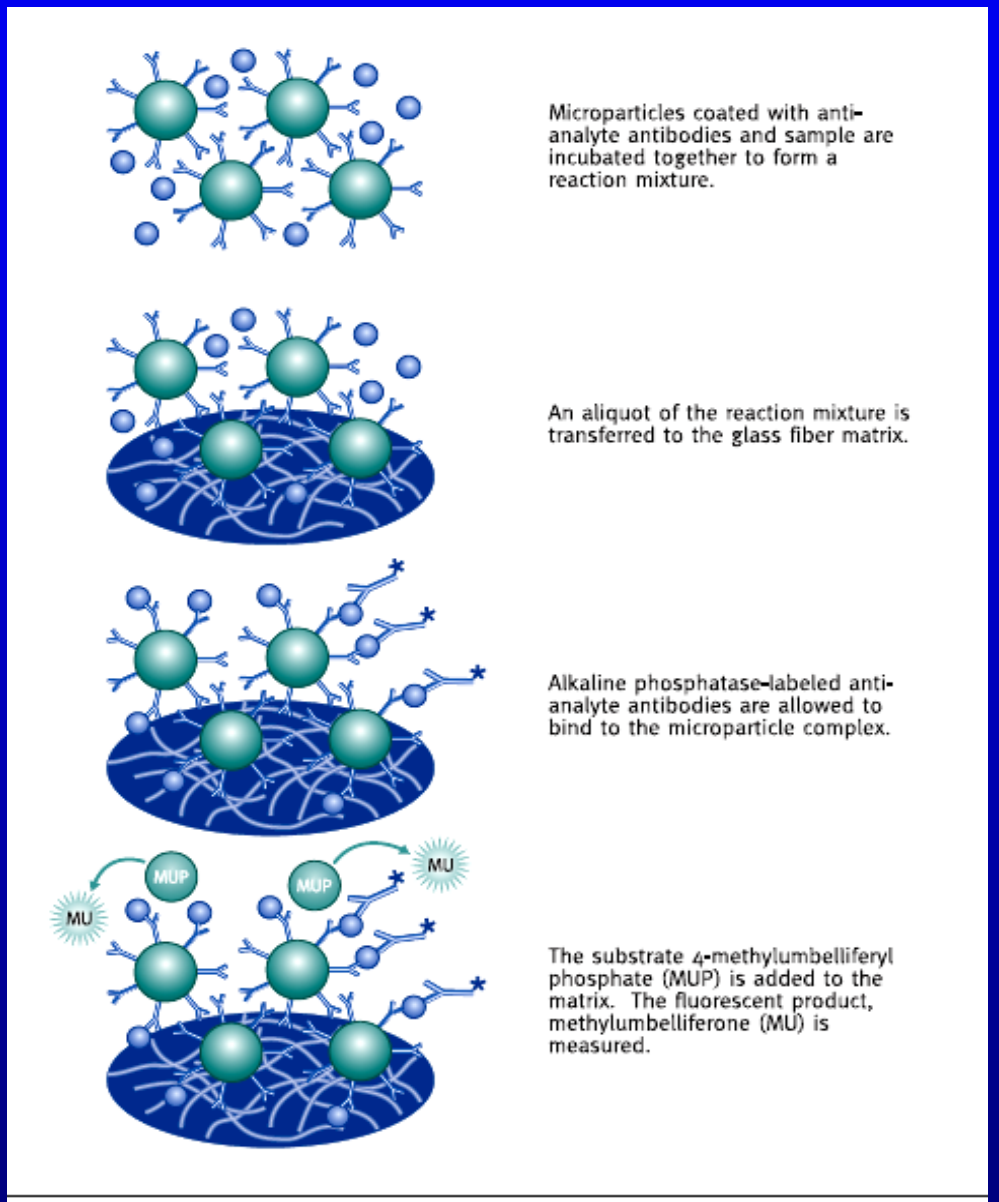
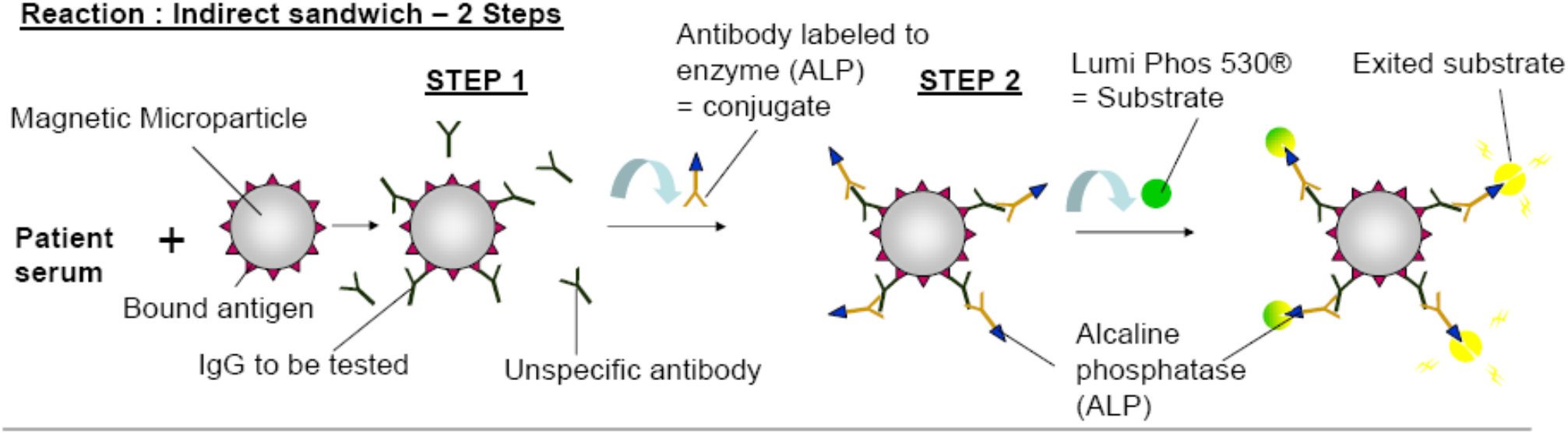
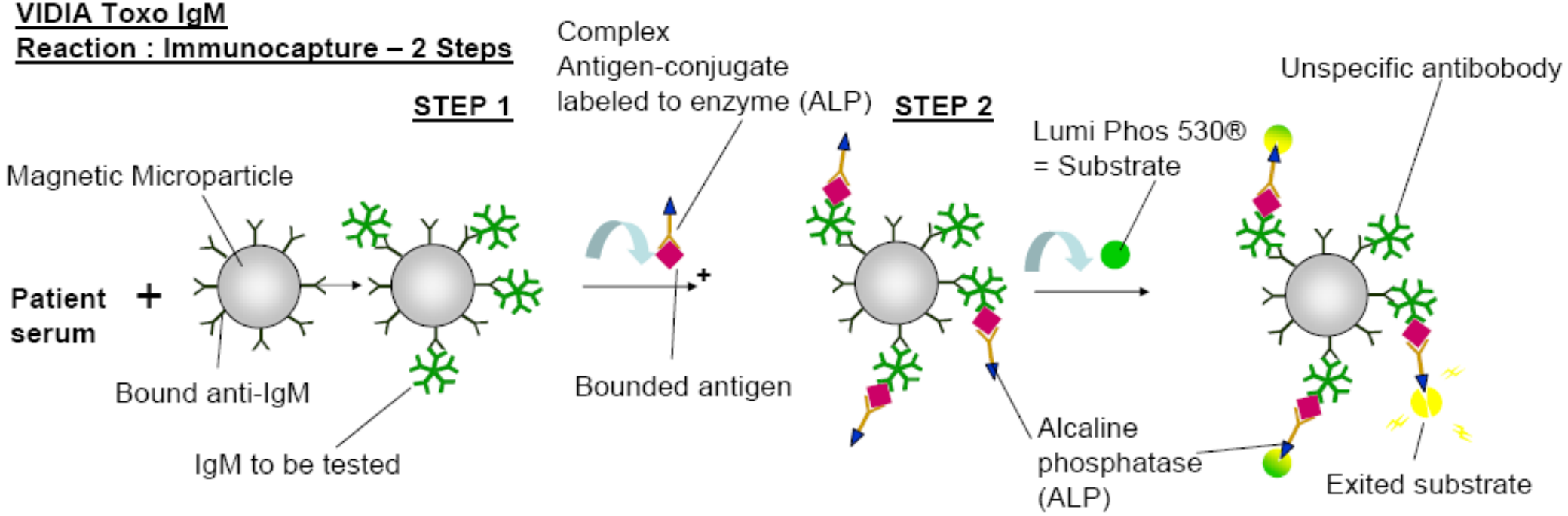


FIGURE 2-8 Process of the MEIA method

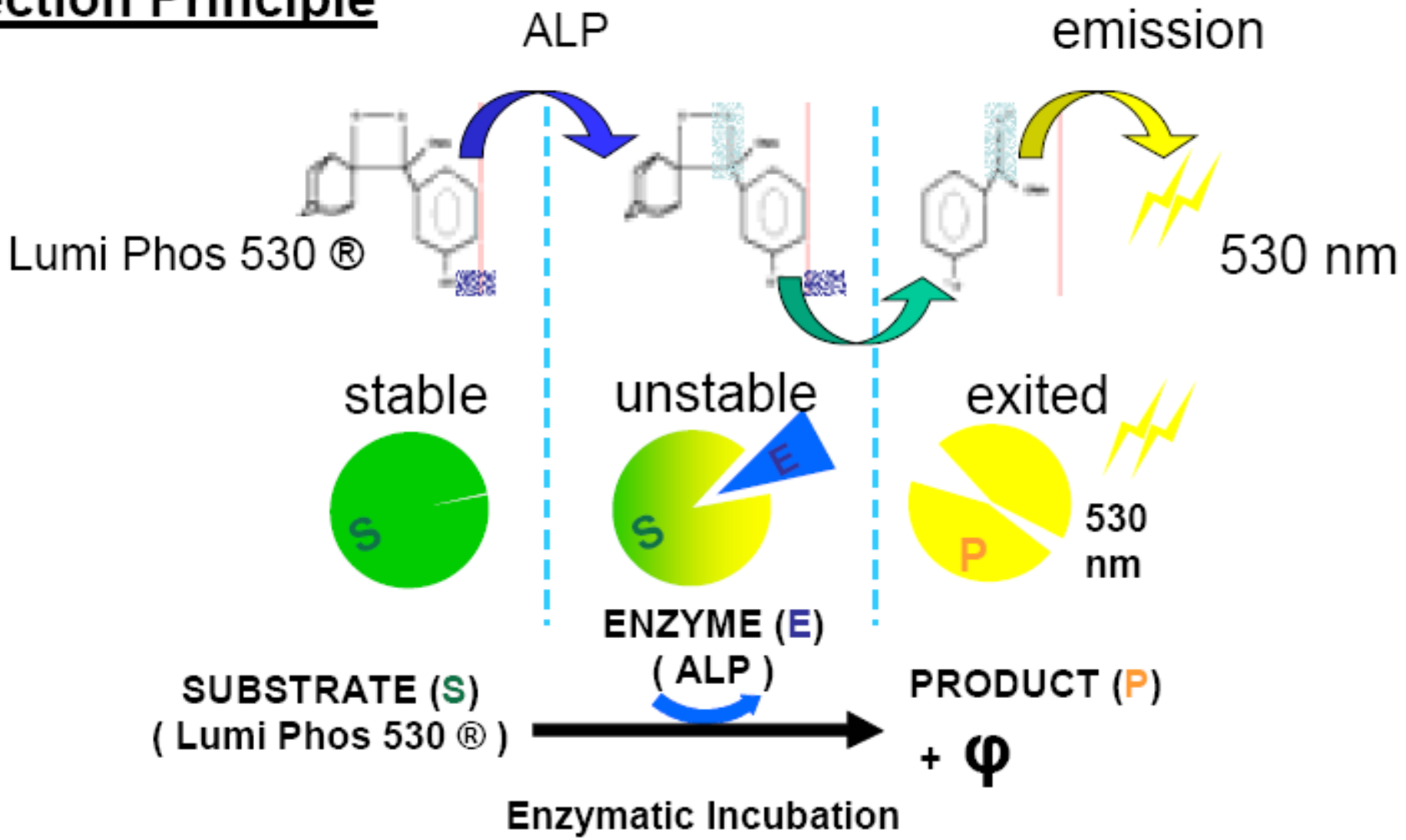
VIDIA Toxo IgG
Reaction : Indirect sandwich – 2 Steps



VIDIA Toxo IgM
Reaction : Immunocapture – 2 Steps



Detection Principle

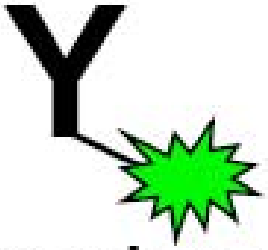


The signal of the reaction mixture is generated via chemi-luminescence

Applications for Immunoassays:

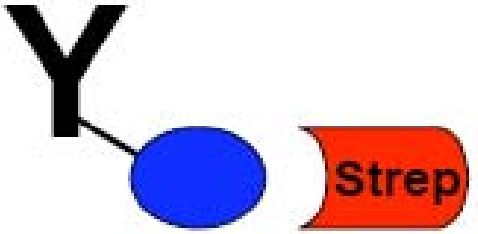
- Tumor Markers, e.g. AFP, CEA, hCG, PSA …
- Cardiac Markers, e.g. CK-MB, CRP, Digoxin, Myoglobin …
- Cell based Assays, e.g. cell cytotoxicity …
- Allergy, e.g. histamines, egg, milk, allmonds …
- Growth Deficiency, e.g. hGH
- Enzyme activity
- Hormone and Steroid Screening, e.g. T4, fT3, TSH …
- Drug Abuse Screening, e.g. amphetamines, cocaine, LSD …
- Immunological Screening
- Infectious Diseases, e.g. Chlamydia, CMV, Hepatitis, Rubella …
- Veterinary, e.g. bacterial infection, fertility, drugs, BSE …
- Food and Beverages, e.g. pathogens, toxins…
- Water Analysis, e.g. bacterial contamination, toxins, heavy metalls …
- Agriculture, e.g. endotoxins, pesticides …
- Environment, e.g. industrial chemicals, pesticides, surfactants …

Antibody Modifications

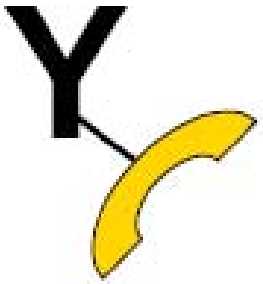


Fluorophores

fluorescein, rhodamine, phycoerythrin

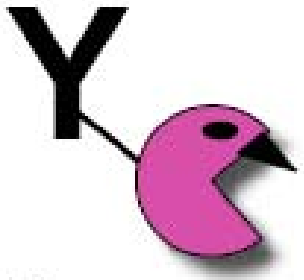


Biotin



Beads/Solid support

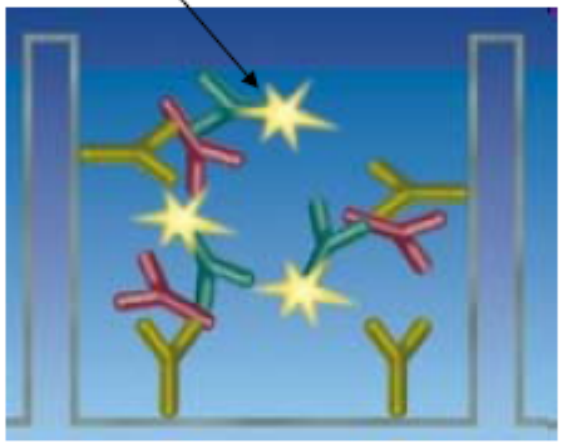
agarose, magnets, ProteinA

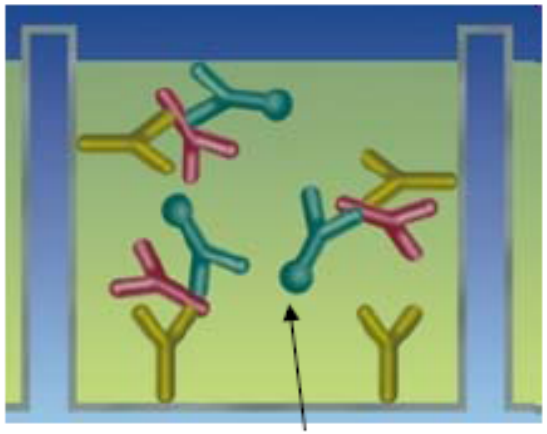


Enzymes

AP, HRP

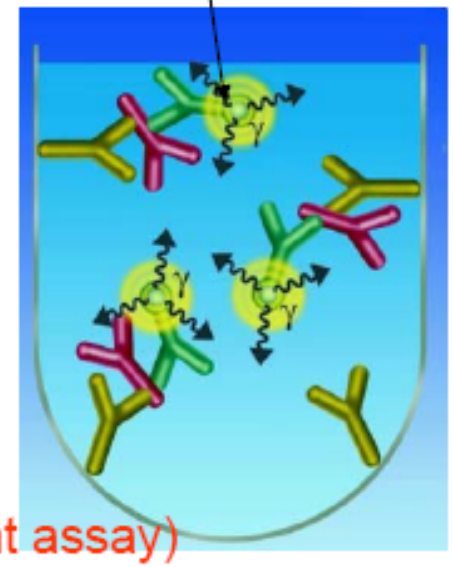
LIA (Luminescence immunoassay)



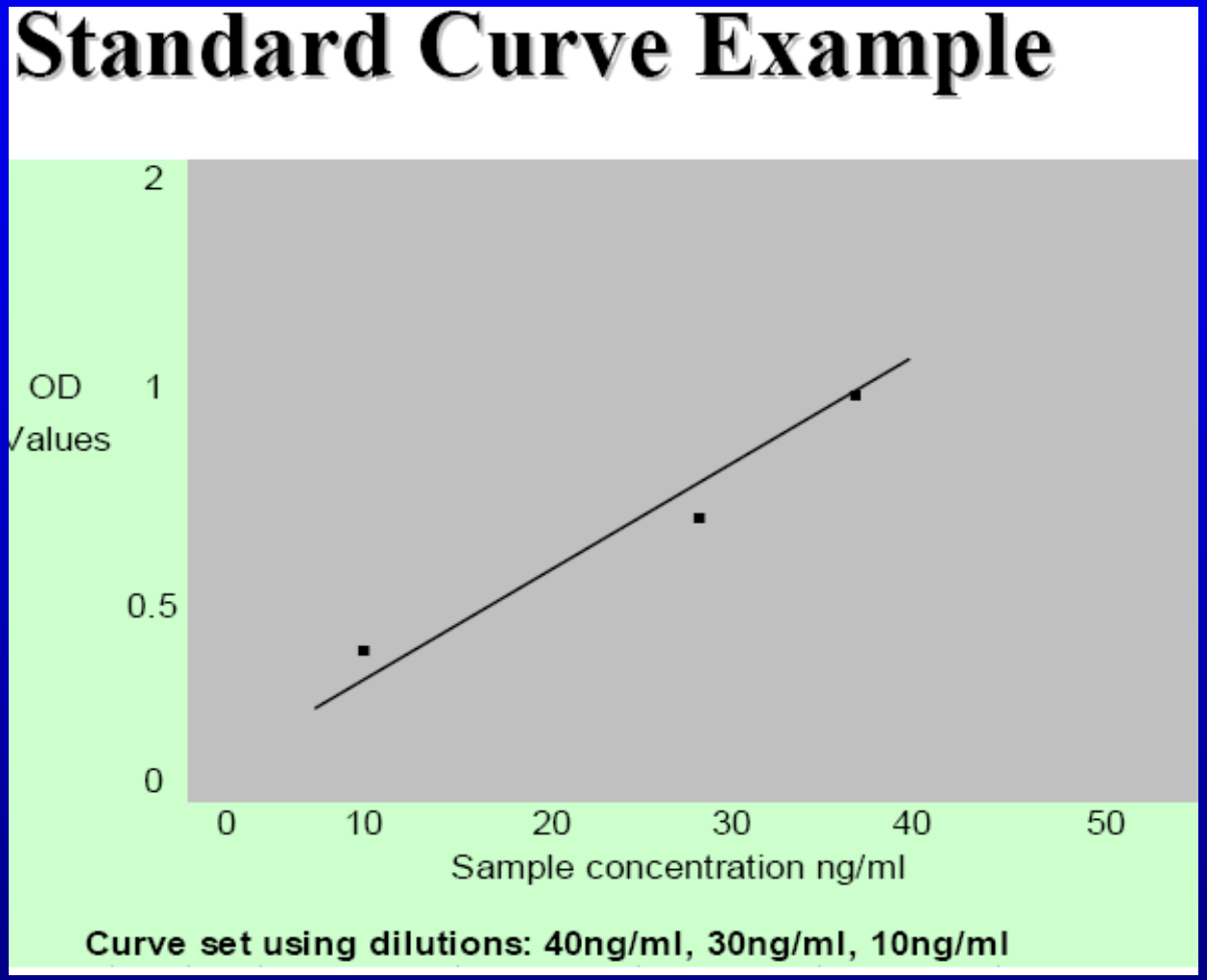


ELISA (Enzyme-linked immuno-sorbent assay)

Radio-Immunoassay



Kantitatif EIA



Cut off: Hesaplanan eşik değer

Gray zone: Eşik değer ±%10

Cut off indeksi (COI): Örnek.OD/Cut off.OD

BOS'ta Ab Araştırılması

- **Beyin Omurilik Sıvısı (BOS) Serolojisi:** Santral sinir sistemi (SSS) enfeksiyonlarının tanısı için serolojik testler BOS örneklerinde çalışılabilir. BOS'ta kuduz virusuna özgül antikorların saptanması aktif enfeksiyon açısından tanı koydurucudur. Alphavirus veya flavivirus ensafalitlerinin tanısında, virus spesifik IgM antikorlarının varlığı tanı koydurucudur. Daha sık rastlanan HSV ensefalitinde ise, BOS'ta virusa özgül antikorların saptanması tanısal değildir. Kandaki antikorlar, SSS enfeksiyonu olmasa da BOS'ta saptanabilmektedir. Çünkü nörolojik hastalıklara bağlı olarak kan beyin bariyerinin bozulması, kandan BOS'a antikor geçişine neden olmaktadır. Bu yüzden sık rastlanan viruslarda, SSS enfeksiyonunun kanıtı için, intratekal antikor sentezinin gösterilmesi gereklidir.

BOS'ta Ab Araştırılması

- Bu amaçla BOS'taki spesifik anti-viral antikor düzeyinin serum spesifik anti-viral antikor düzeyine oranının; BOS'taki total IgG düzeyinin serum total IgG düzeyi oranına bölünmesi gereklidir . Yani;

BOS spesifik antikor : Serum spesifik antikor /
BOS IgG : Serum IgG

- Bu oranın 1.5'den büyük olması spesifik intratekal antikor sentezini gösterir. BOS antikor düzeyleri genellikle serum düzeyinden 1000 kat daha düşük olduğundan BOS'da antikor saptamaya yönelik yöntem çok duyarlı olmalıdır. BOS/serum özgül antikor düzeyi kantitatif olarak elde edilebilmelidir. Bu amaçla genellikle EIA yöntemi kullanılmaktadır. Kantitatif antikor düzeyi elde etmek amacıyla BOS ve serumun seri dilüsyonlarının çalışılması gereklidir.