DES de Biologie Médicale

Enseignement d'Immunologie

ET01 & ET02

Introduction aux techniques immunologiques (Immunochimie)

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Introduction aux techniques immunologiques (Immunochimie)

1. Principe de la réaction Ag-Ac - Rappel
2. Réactions de précipitation
3. Réactions d'agglutination
4. RIA; ELISA
5. Immunoprécipitation; Western blot
6. Immunofluorescence
7. Techniques de tri cellulaire
8. Conclusion

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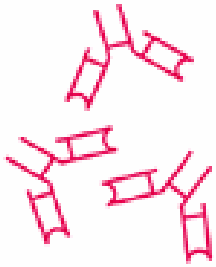
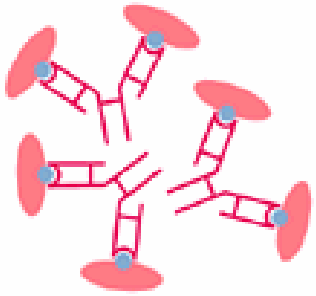
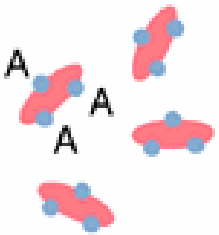
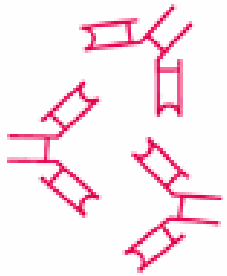
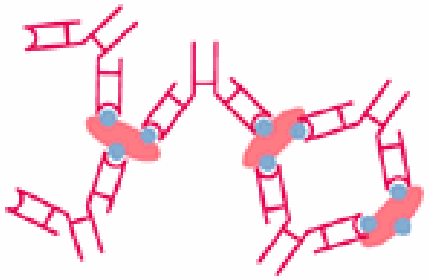
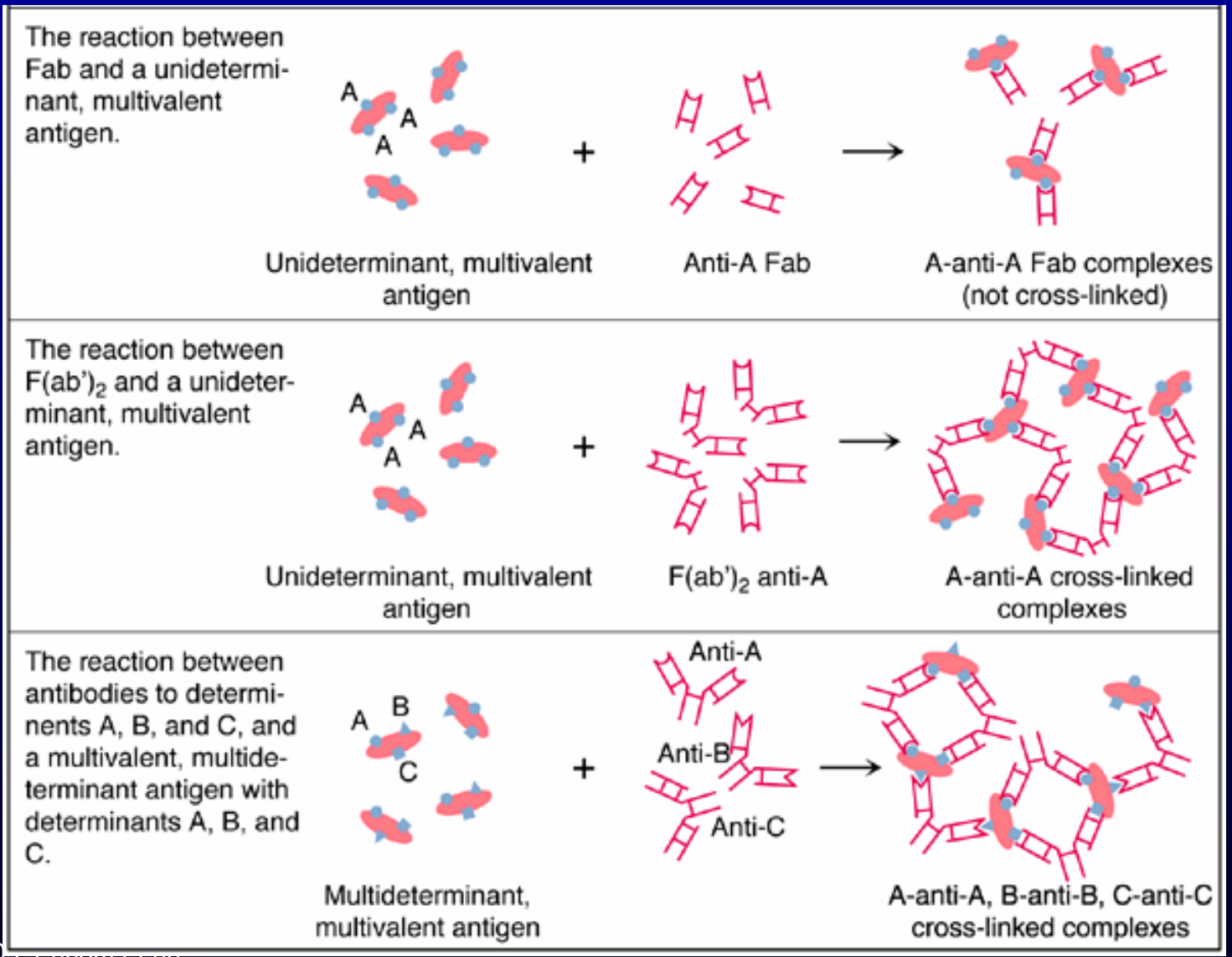
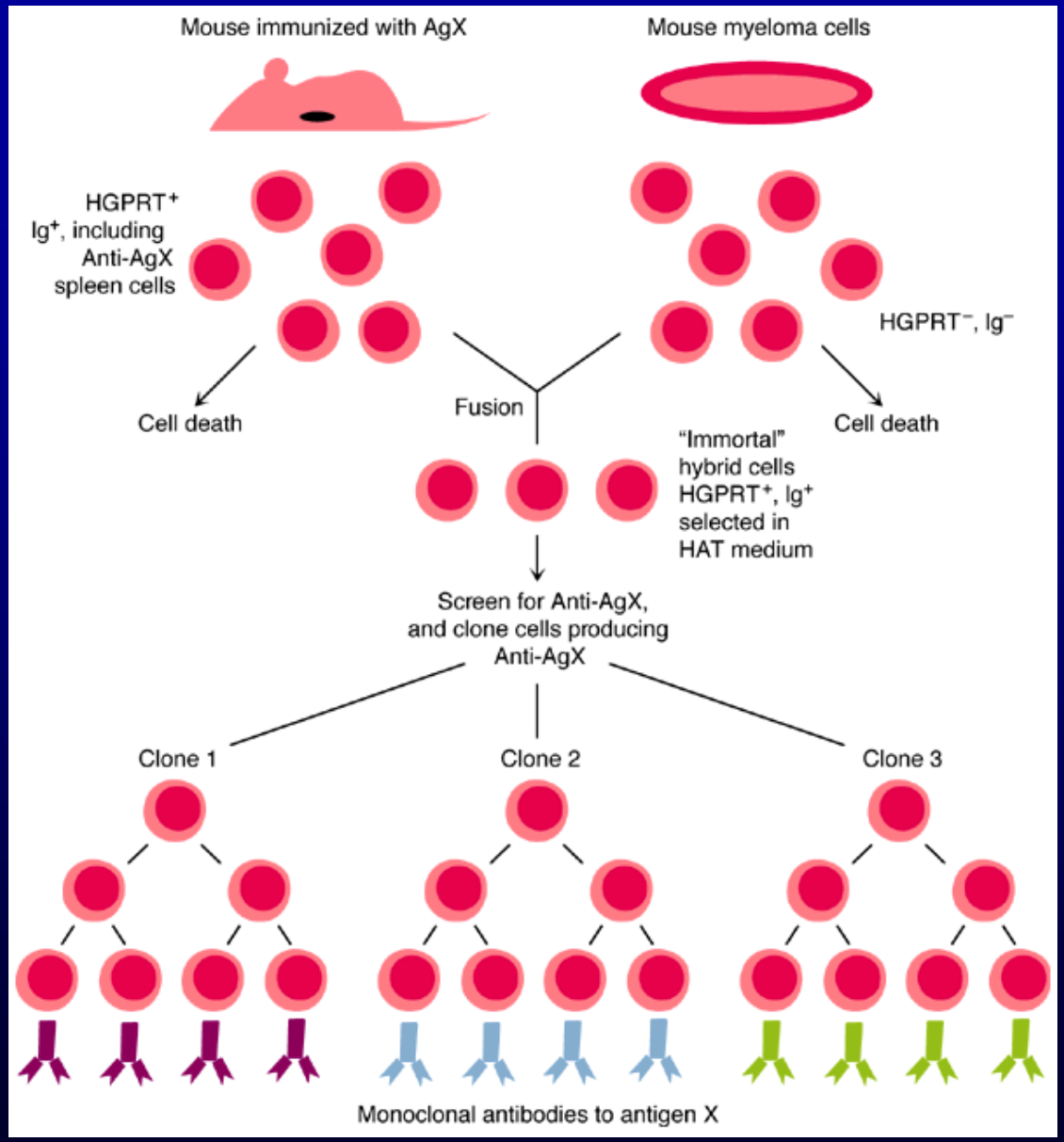
Réaction antigène/anticorps

	Antigen/Hapten	Antibody/Fragment	
The reaction between antibody and a hapten.	 Univalent, unideterminant antigen (hapten)	+  Anti-A	→  A-anti-A complexes (not cross-linked)
The reaction between antibody and a unide-terminant, multivalent antigen.	 Unideterminant, multivalent antigen	+  Anti-A	→  A-anti-A cross-linked complexes

Réaction antigène/anticorps



Production d'anticorps monoclonaux



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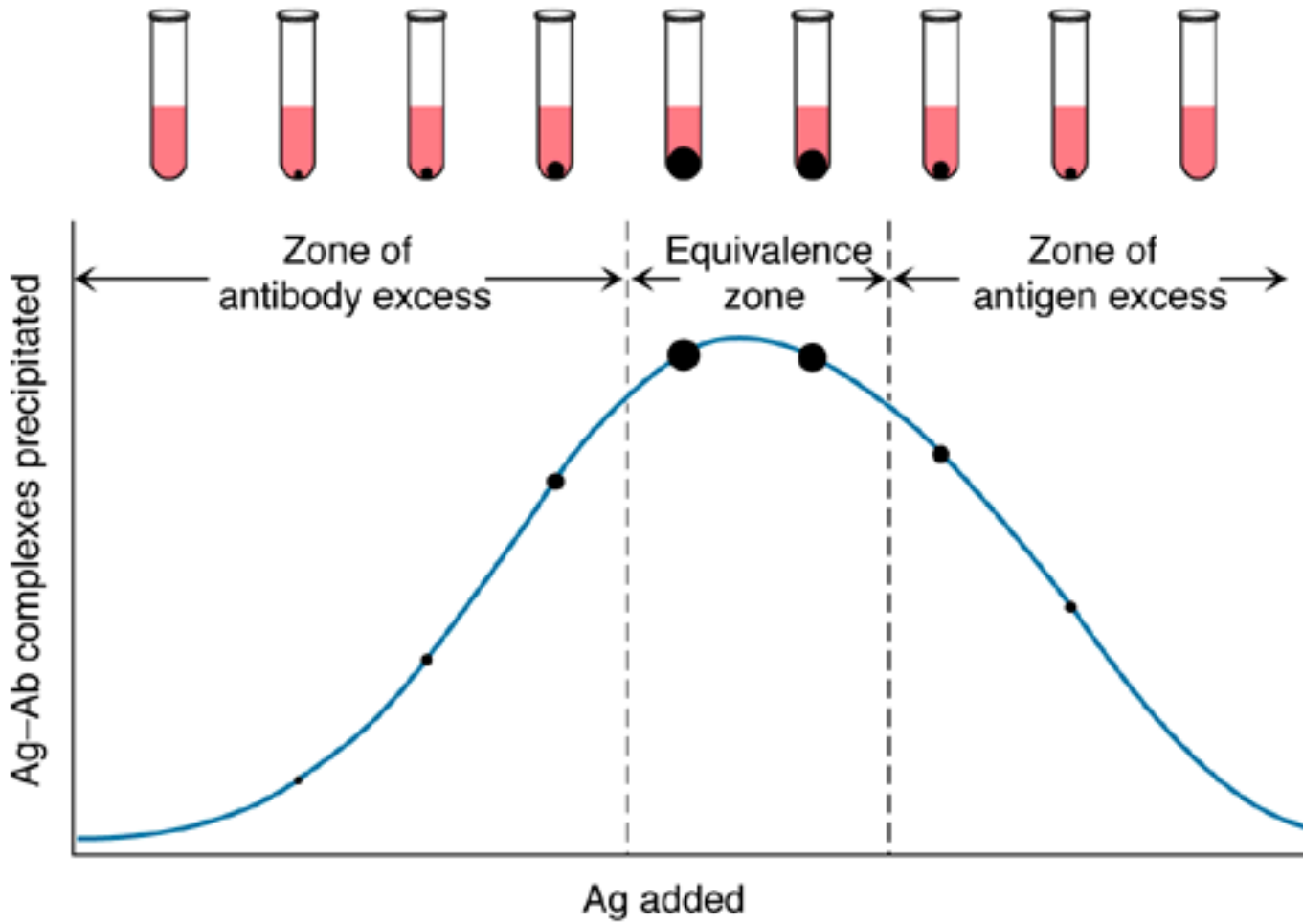
Précipitation en milieu liquide



Précipitation interfaciale

Technique
du “ring test”
(test qualitatif)

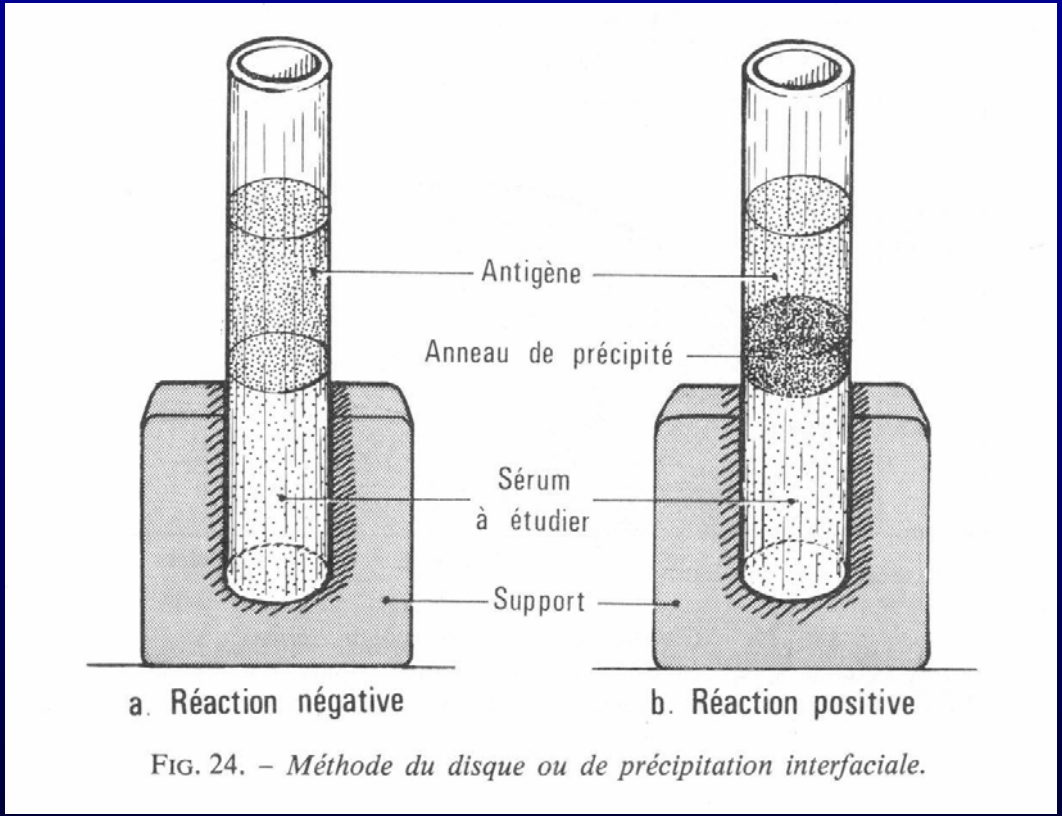


FIG. 24. – *Méthode du disque ou de précipitation interfaciale.*

Diffusion unidimensionnelle

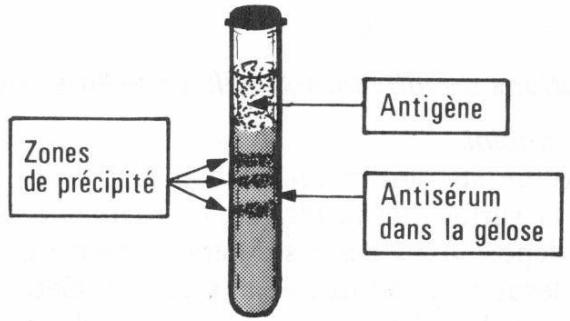


FIG. 29. – *Technique de Oudin : réaction de précipitation avec diffusion simple unidimensionnelle.*

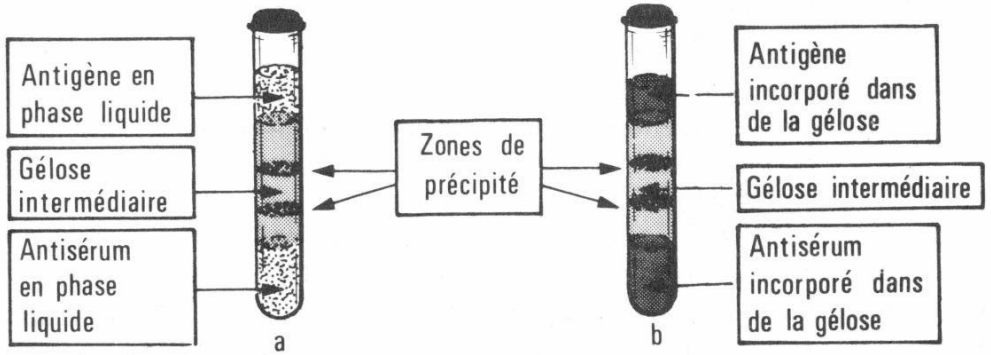


FIG. 30. – *Réaction de précipitation avec diffusion double et unidimensionnelle.* *a)* Technique de Preer. *b)* Technique d'Oakley-Fulthorpe.

Diffusion simple bidimensionnelle

Technique de Mancini

Immunodiffusion radiale

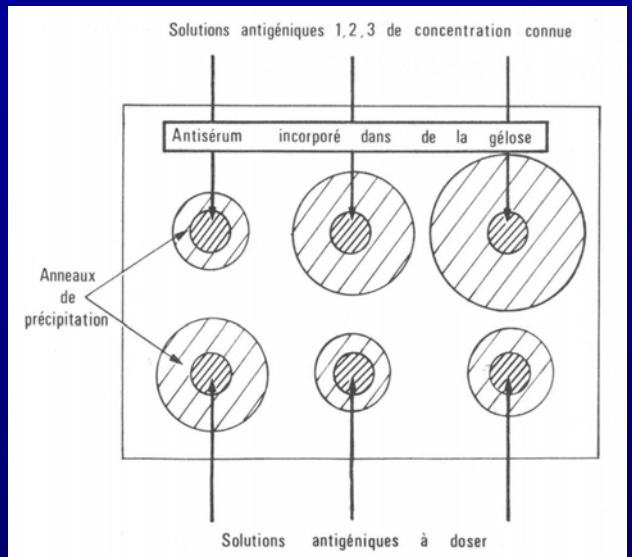
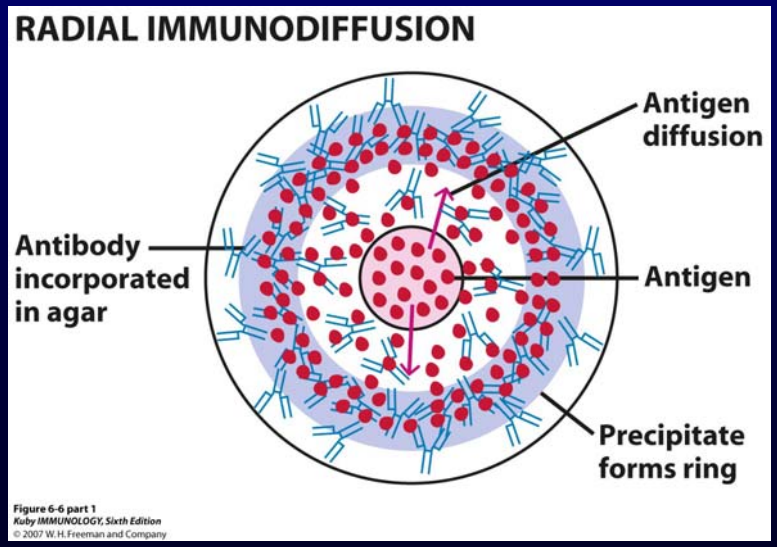
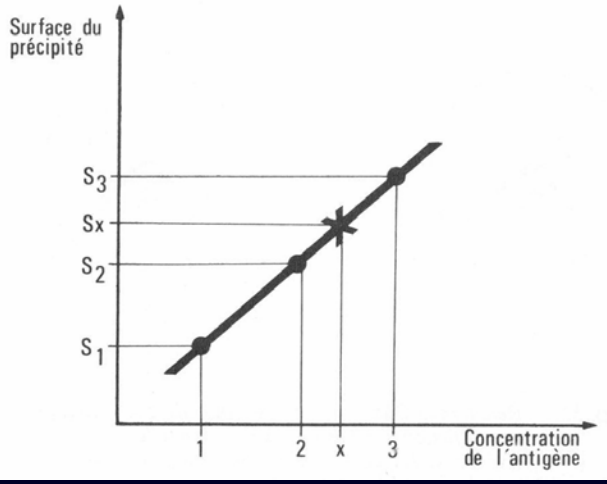


FIG. 31. – *Technique de Mancini : réaction de précipitation avec diffusion simple et bidimensionnelle.*

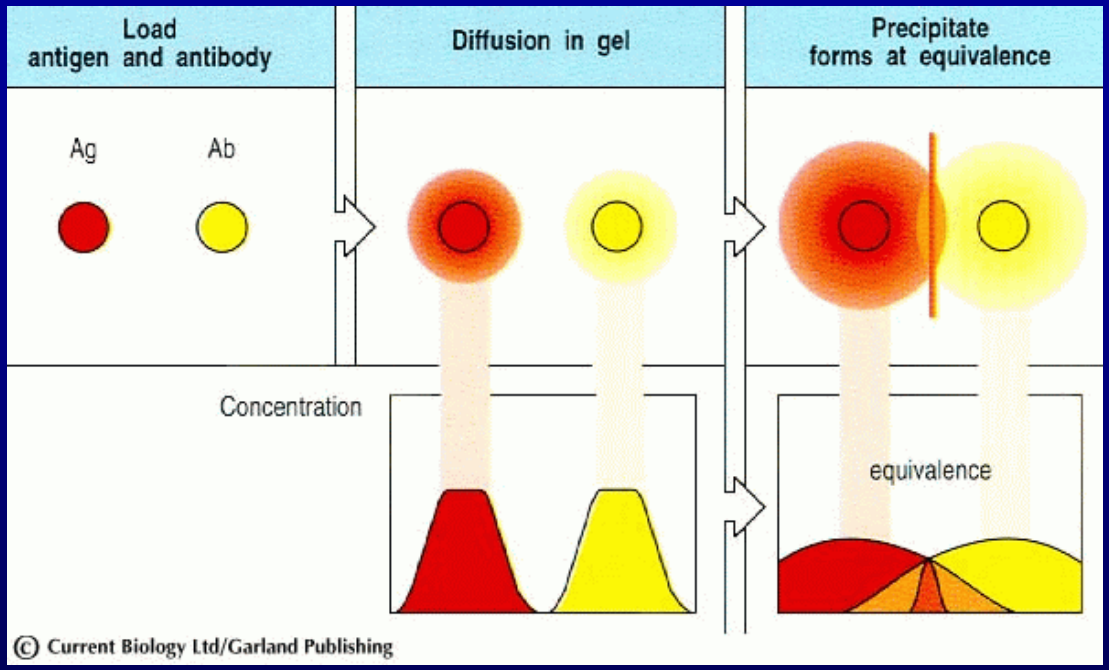


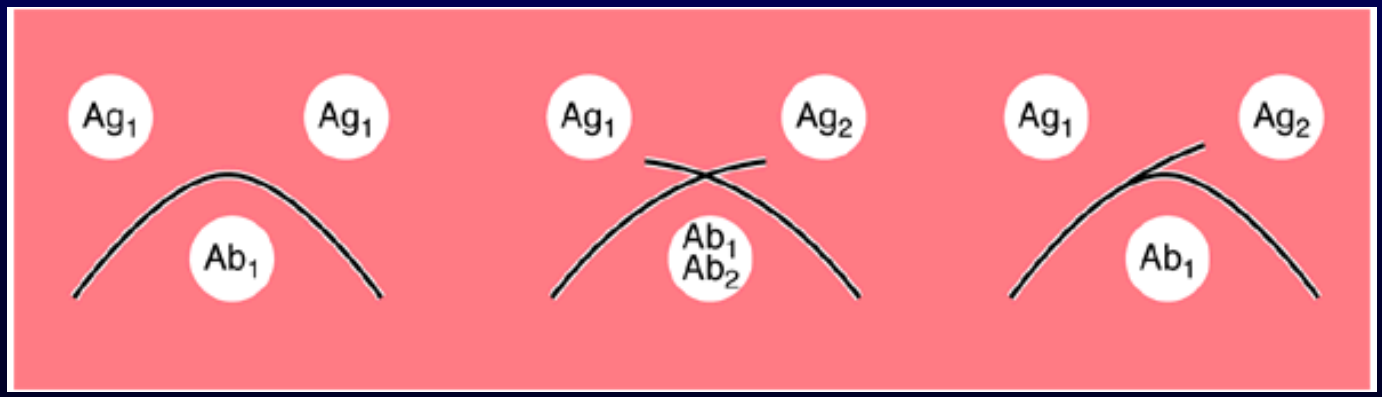


Précipitation en milieu solide

Technique d'Ouchterlony

Immunodiffusion double





Immunoélectrophorèse (Grabar/Williams)

Combine électrophorèse et immunodiffusion double:

1. Séparation électrophorétique d'une source d'antigènes selon leur charge
2. Immunodiffusion radiale des antigènes séparés
3. Immunodiffusion d'une source d'anticorps déposée dans une gouttière sur l'un ou l'autre des côtés des antigènes séparés
4. Formation éventuelle d'arcs de précipitation

→ Analyse qualitative

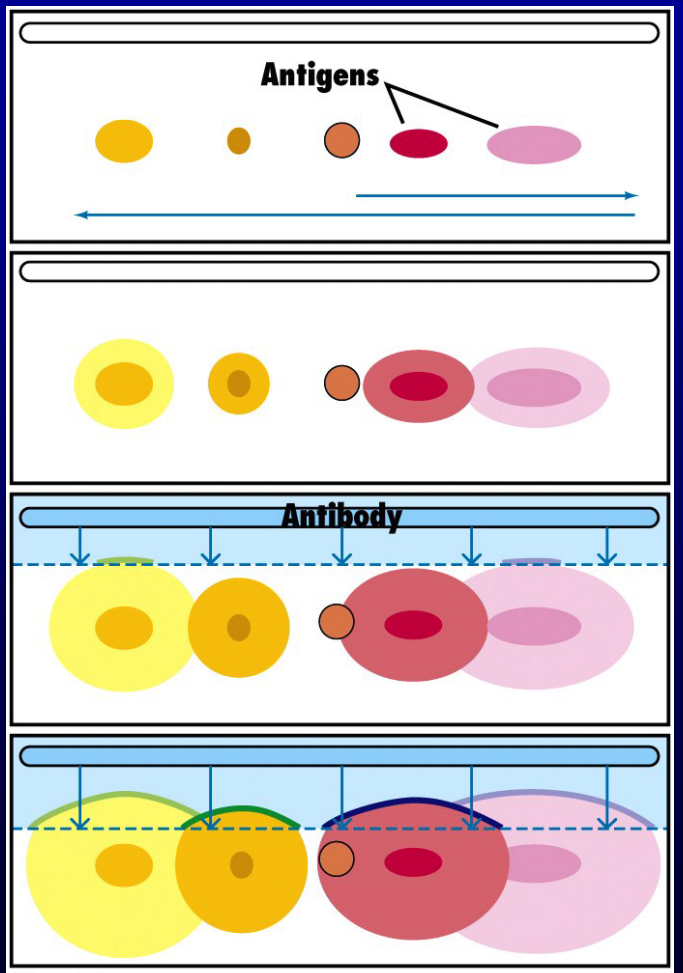


Figure 6-7
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Immunoélectrophorèse en fusée

Technique de Laurell:

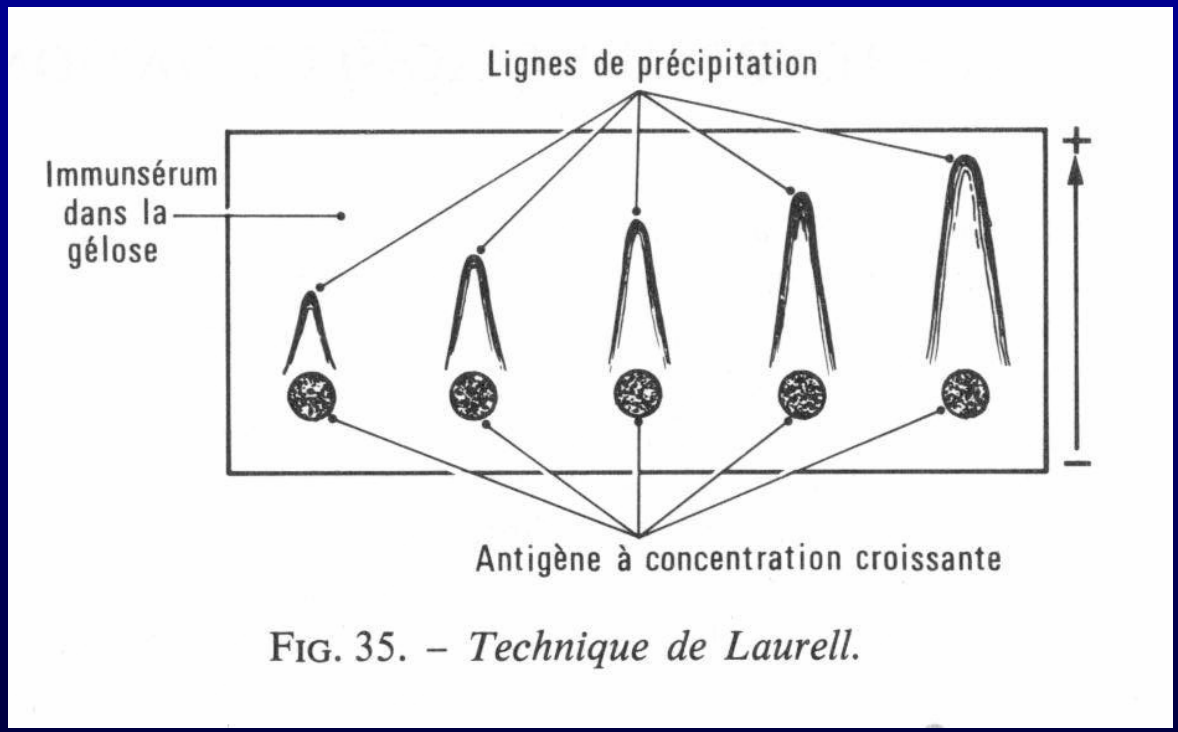

FIG. 35. – *Technique de Laurell.*

- Analyse quantitative
- Nécessité que l'Ag soit chargé négativement

Immunoélectrophorèse bidimensionnelle

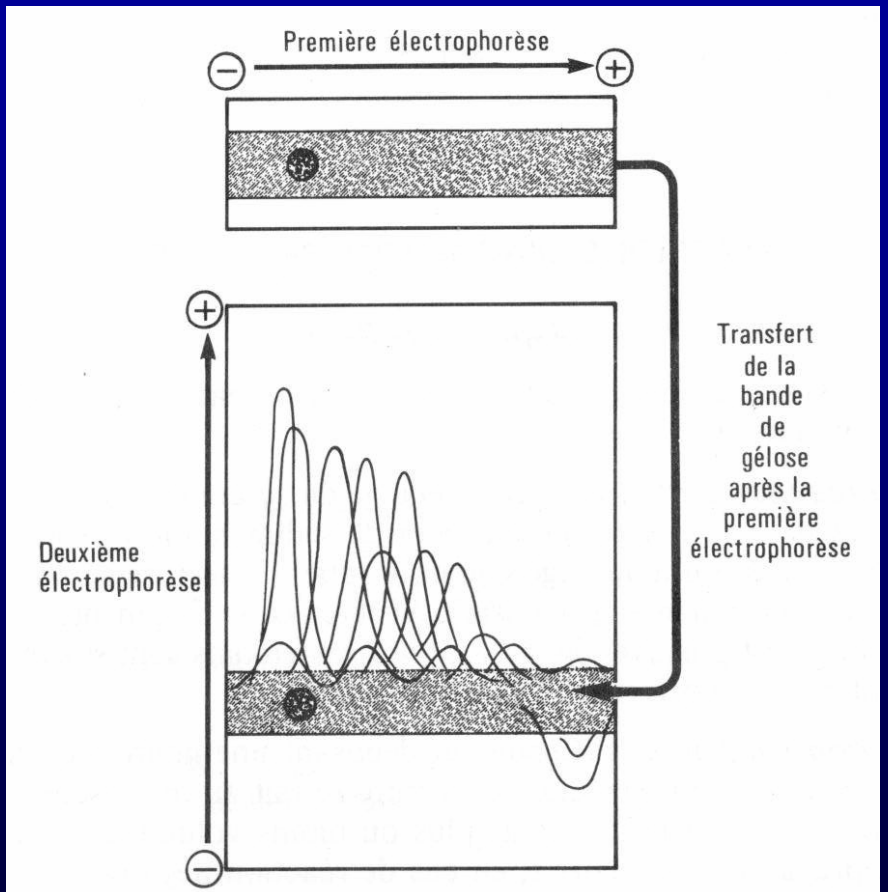

FIG. 36. – *Immuno-électrophorèse bidimensionnelle.*

Analyse qualitative & quantitative

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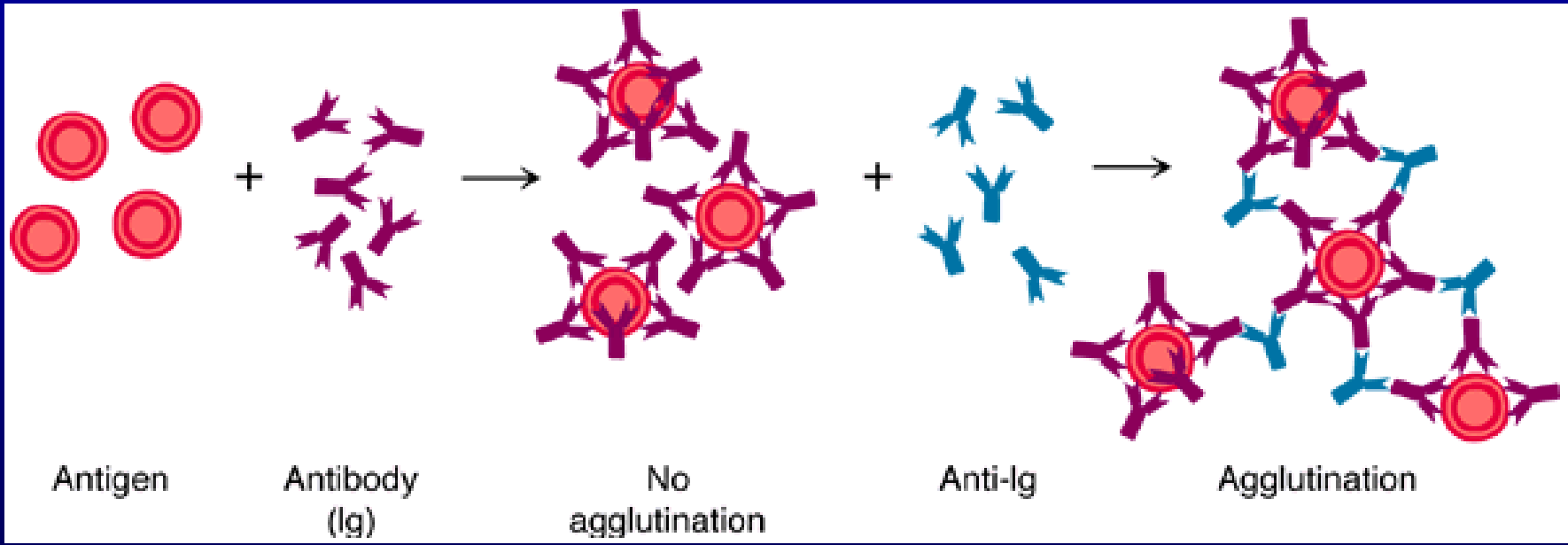
Réaction d'agglutination



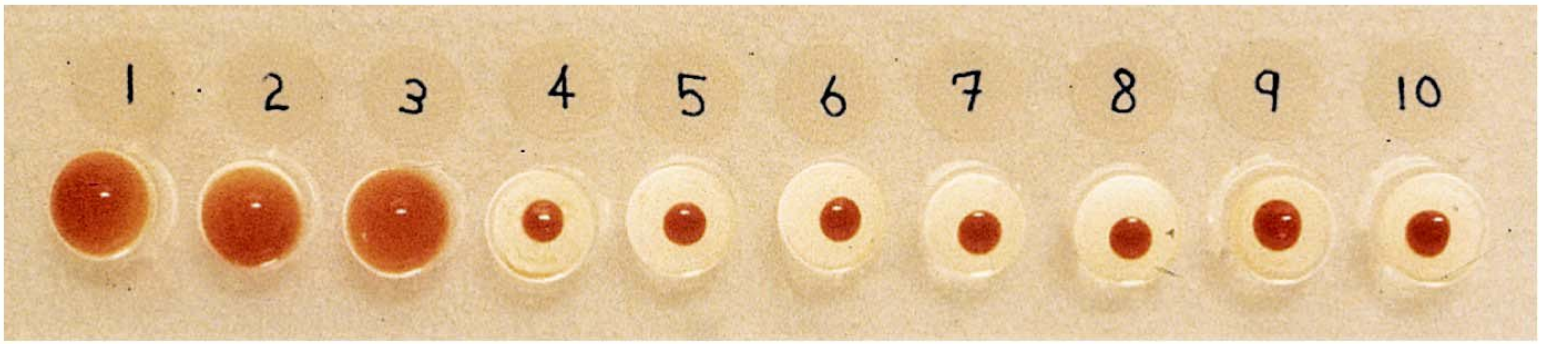


Figure 6-8
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Qualitatif: très sensible; Quantitatif: peu précis

Test de Coombs direct

Recherche d'anticorps anti-Rhésus chez le nouveau-né

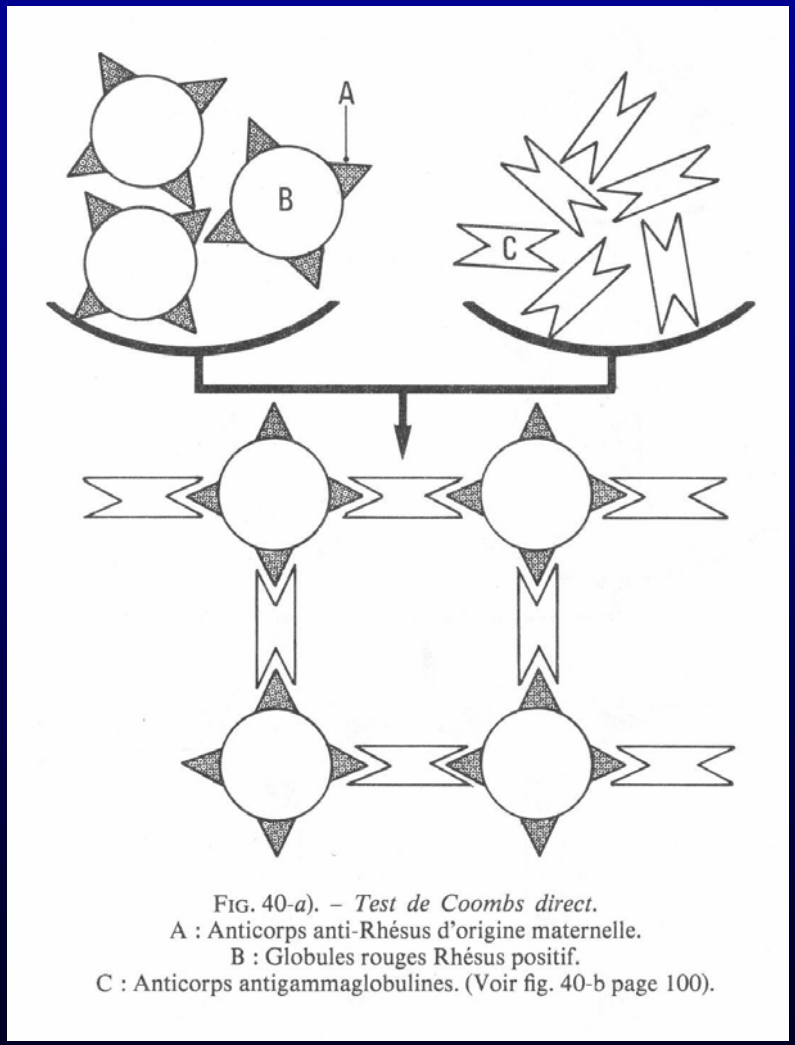

FIG. 40-*a*). – *Test de Coombs direct.*
A : Anticorps anti-Rhésus d'origine maternelle.
B : Globules rouges Rhésus positif.
C : Anticorps antigammaglobulines. (Voir fig. 40-b page 100).

Test de Coombs indirect

Recherche d'anticorps anti-Rhésus chez la mère

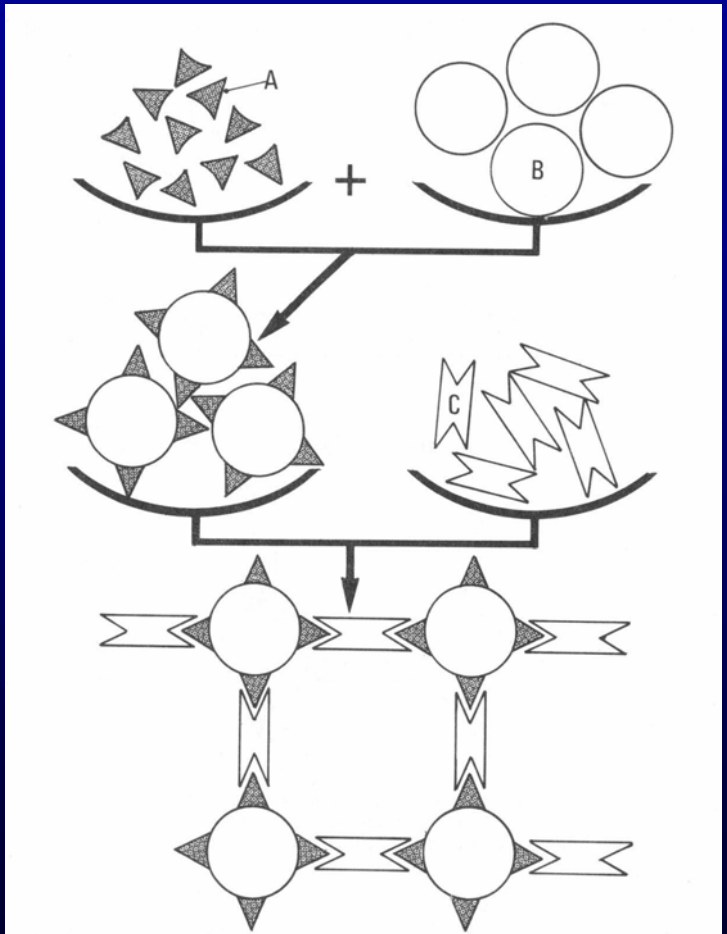

FIG. 40-*b*). – *Test de Coombs indirect.*
A : Anticorps anti-Rhésus.
B : Globules rouges Rhésus positif.
C : Anticorps antigammaglobulines.

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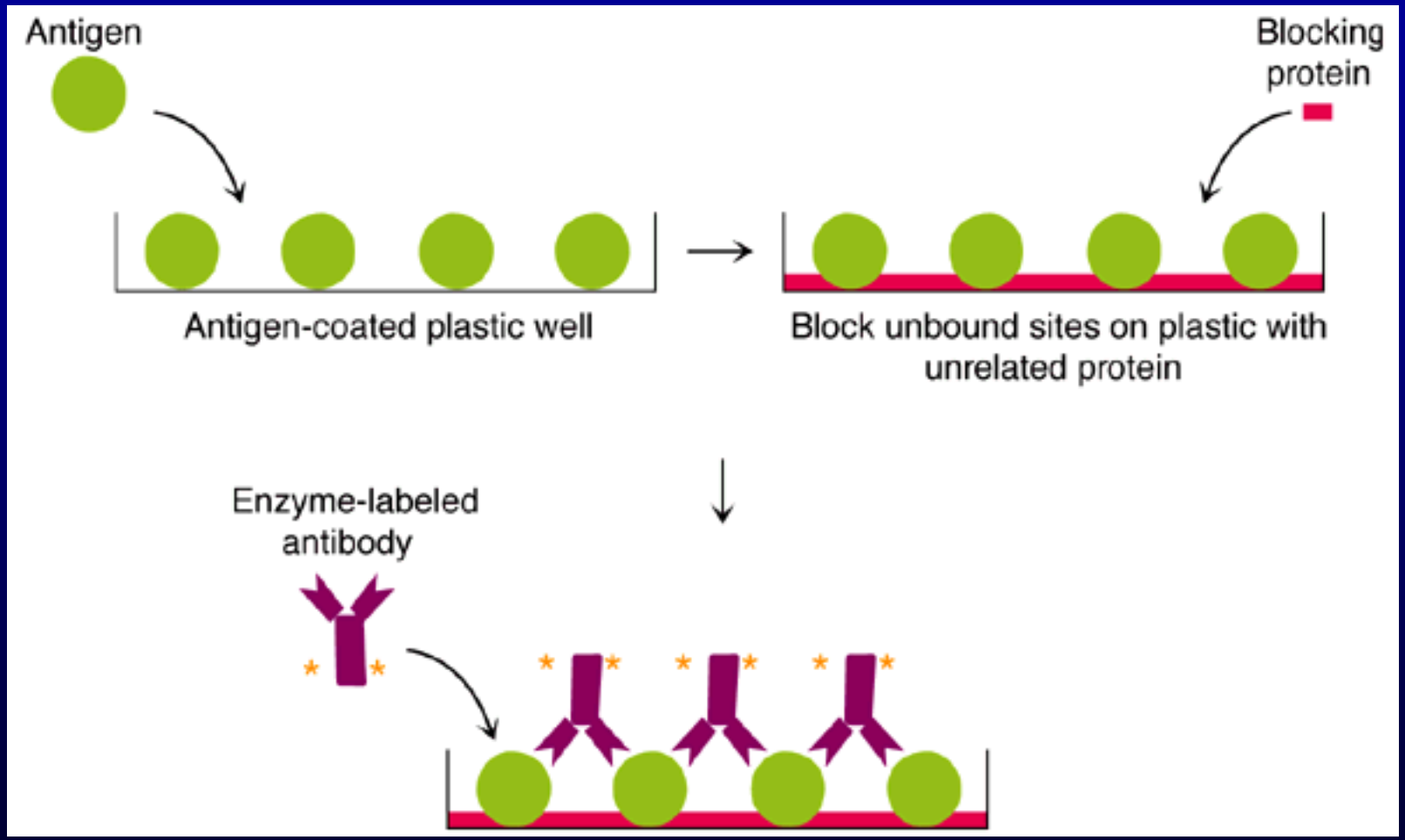
RIA/ELISA



RadioImmunoAssay/Enzyme-linked ImmunoSorbentAssay

ELISA indirect

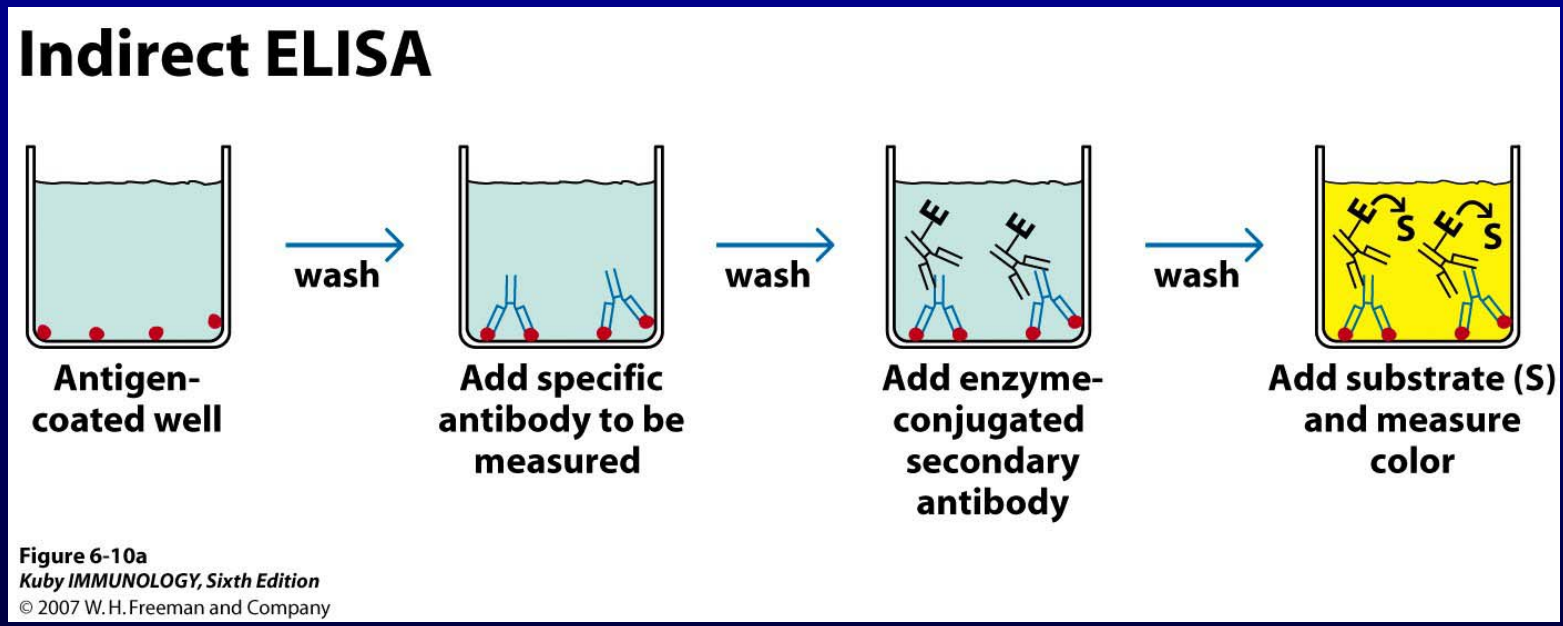


Figure 6-10a
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ELISA sandwich

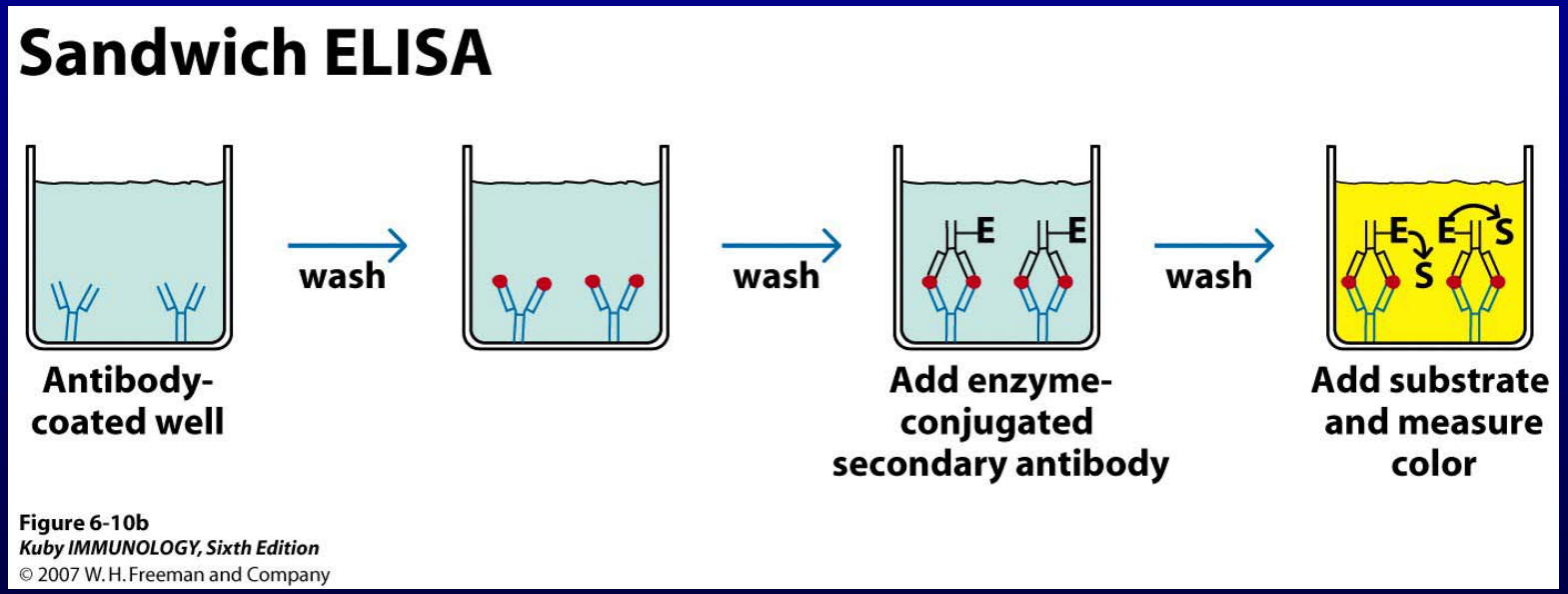

Figure 6-10b
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ELISA compétitif

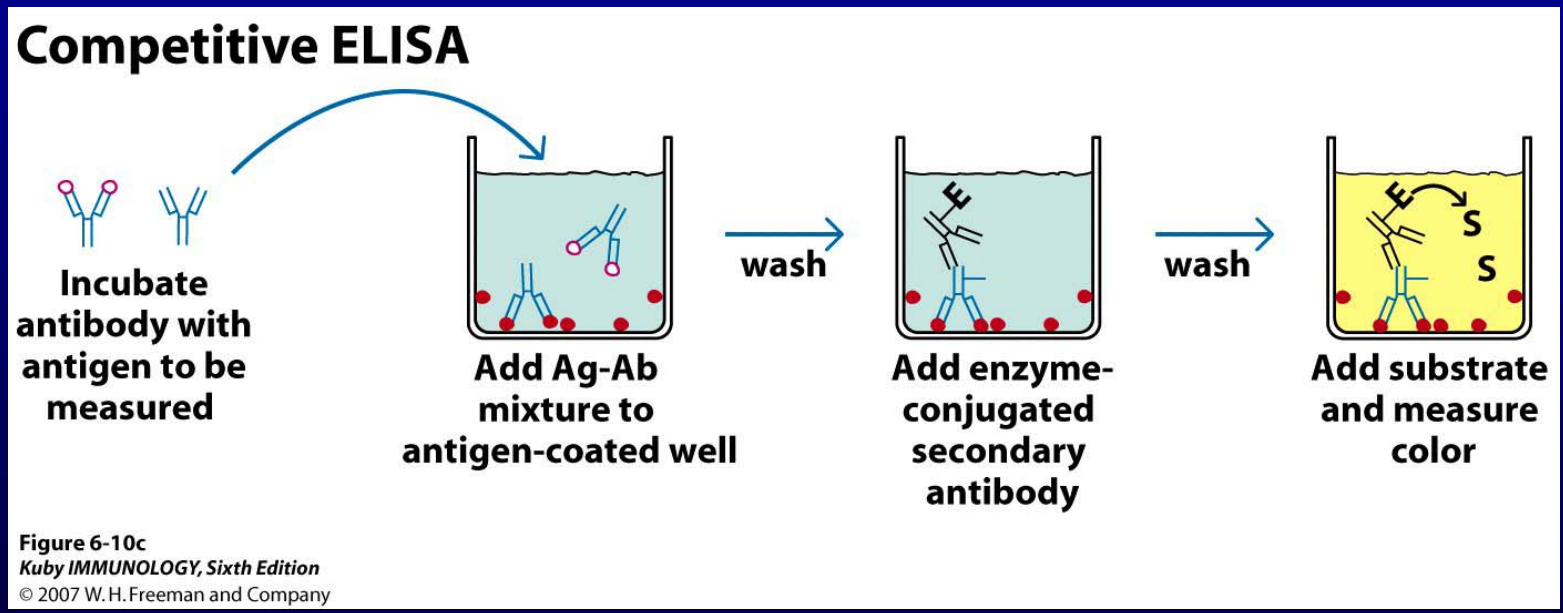

Figure 6-10c
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ELISpot

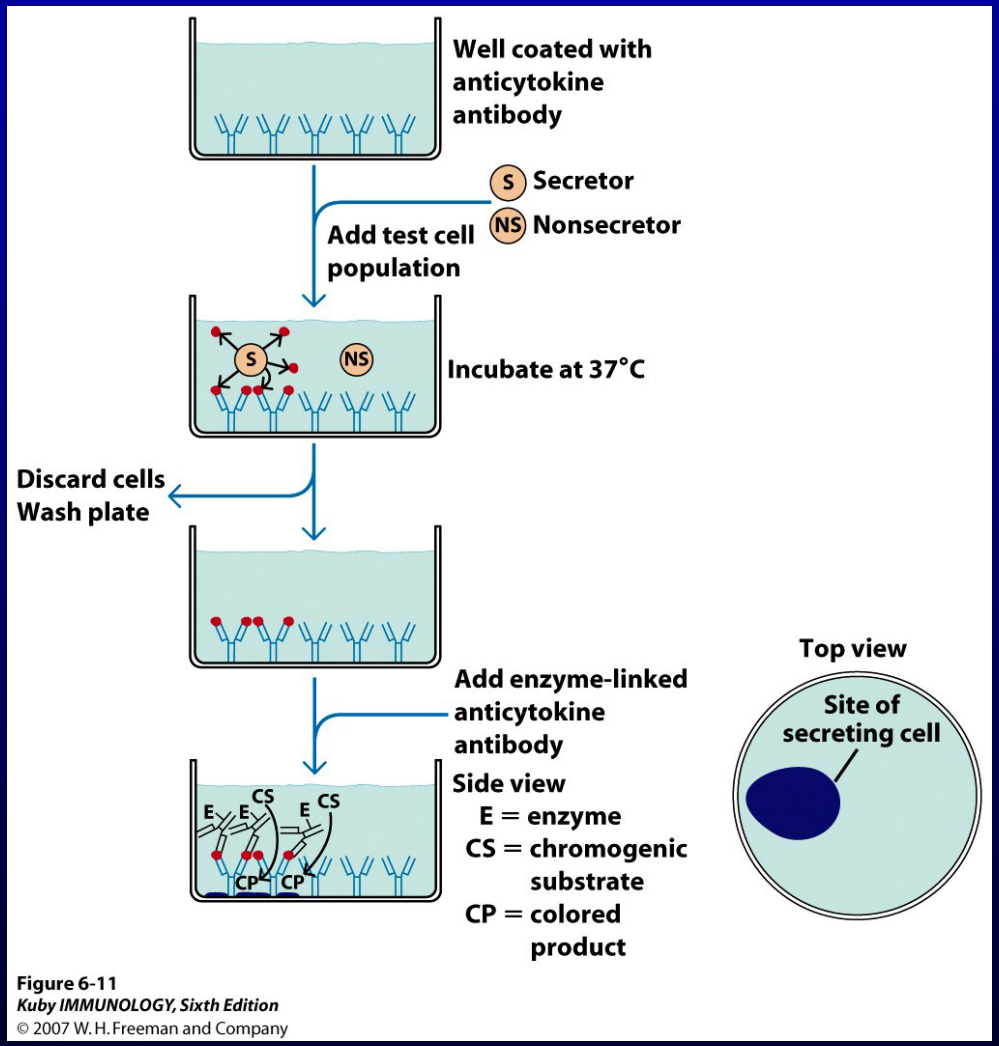

Figure 6-11
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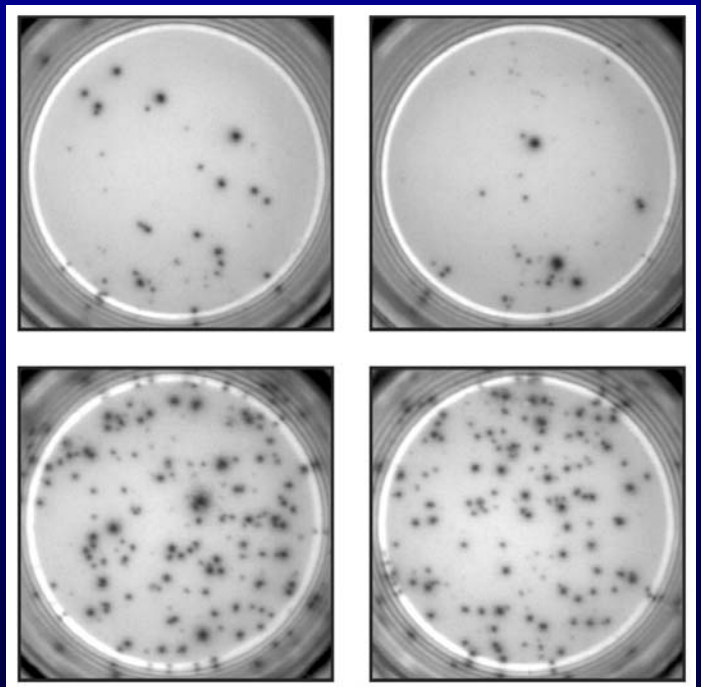

Figure A-29 part 3 of 3 Immunobiology, 6/e. (© Garland Science 2005)

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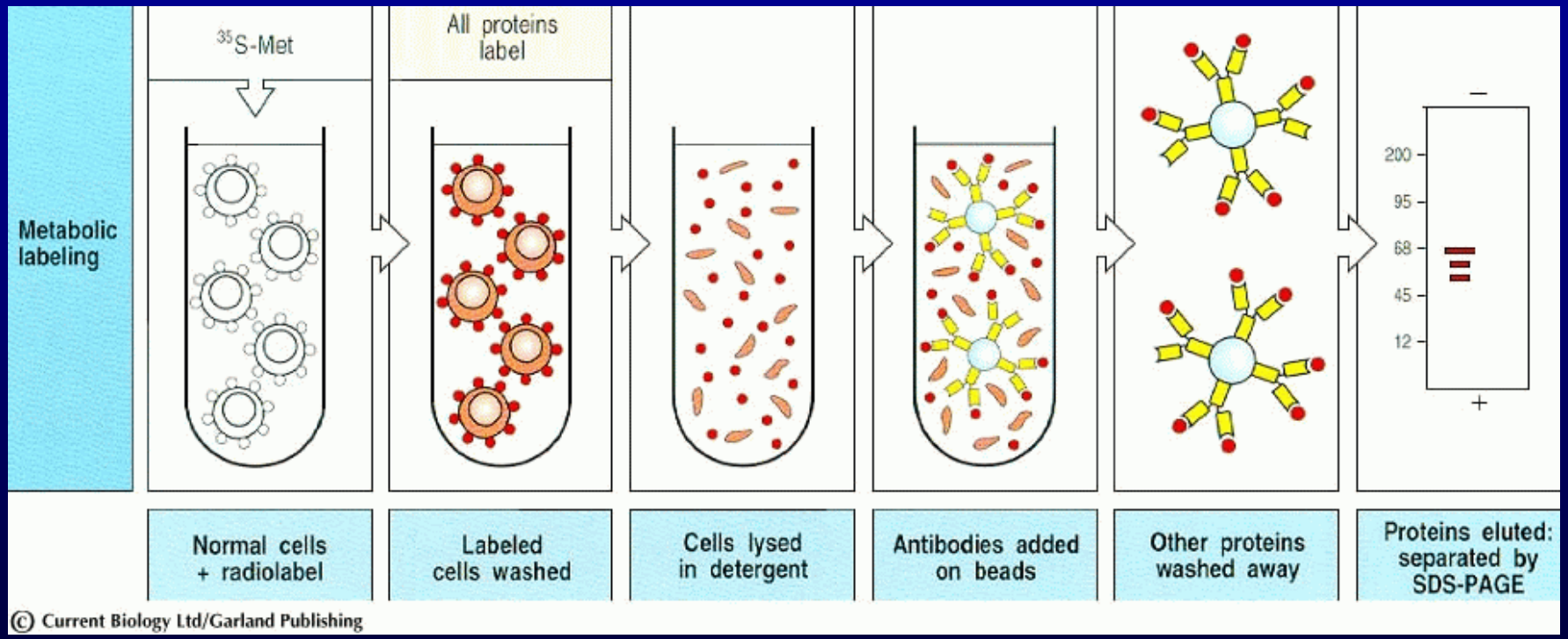
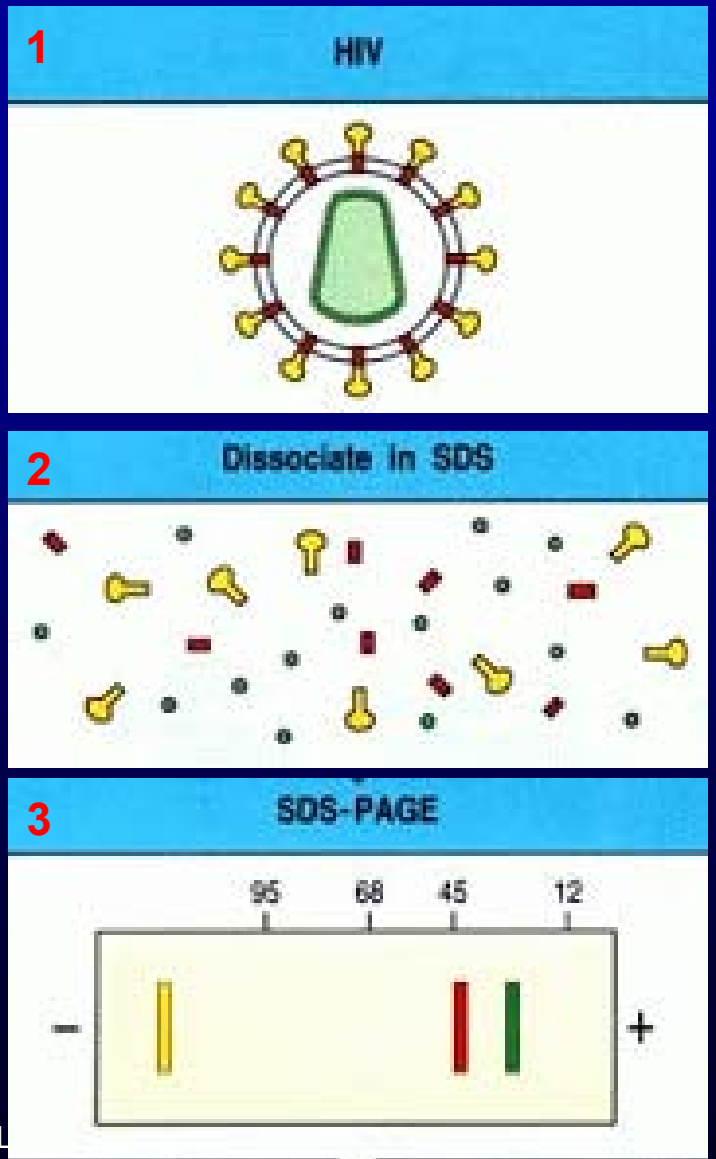
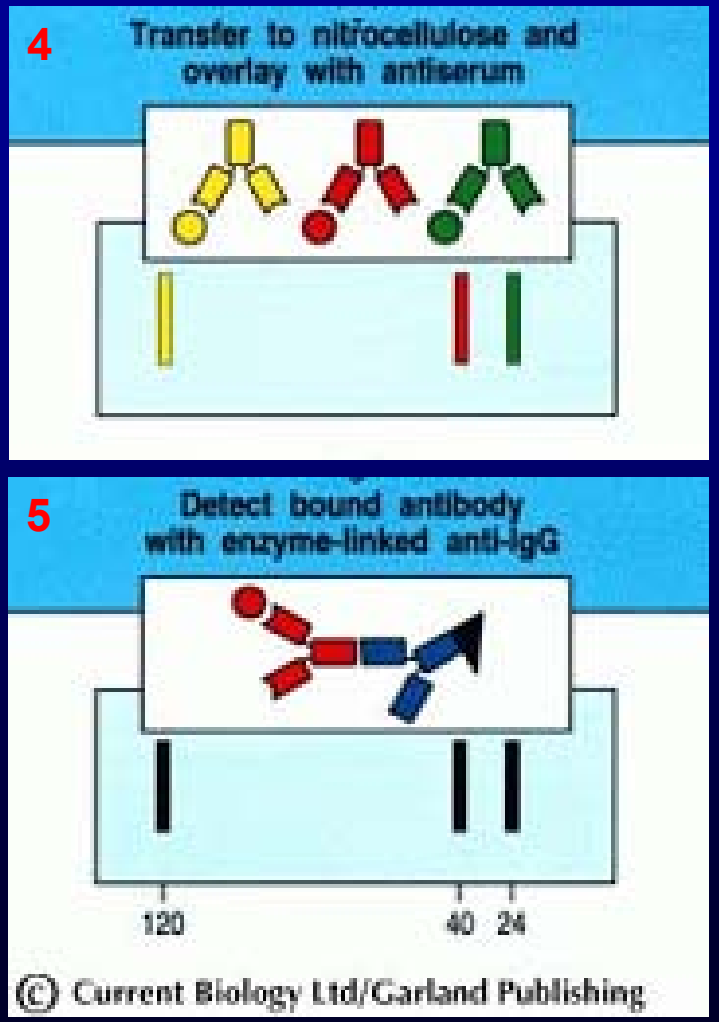
Immunoprécipitation



Western blot





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Immunofluorescence - principe

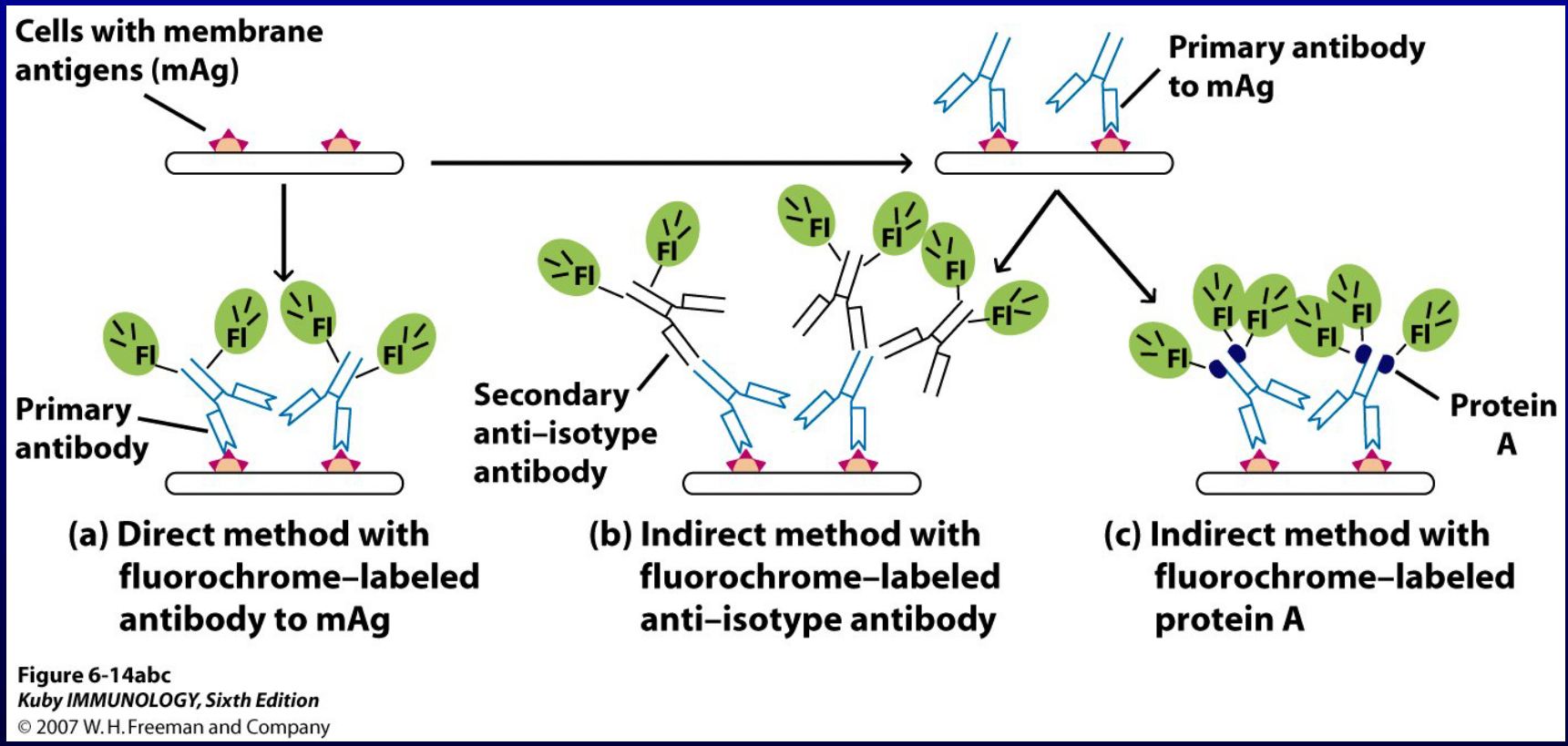

Figure 6-14abc
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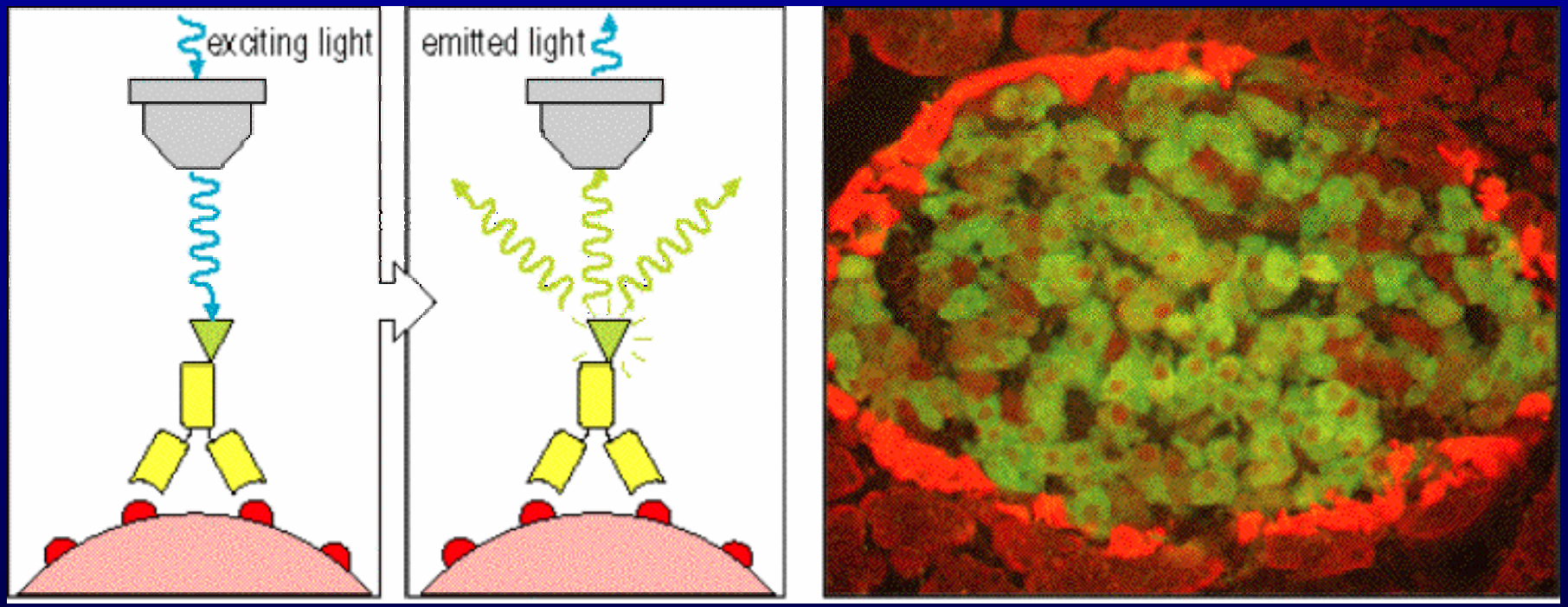
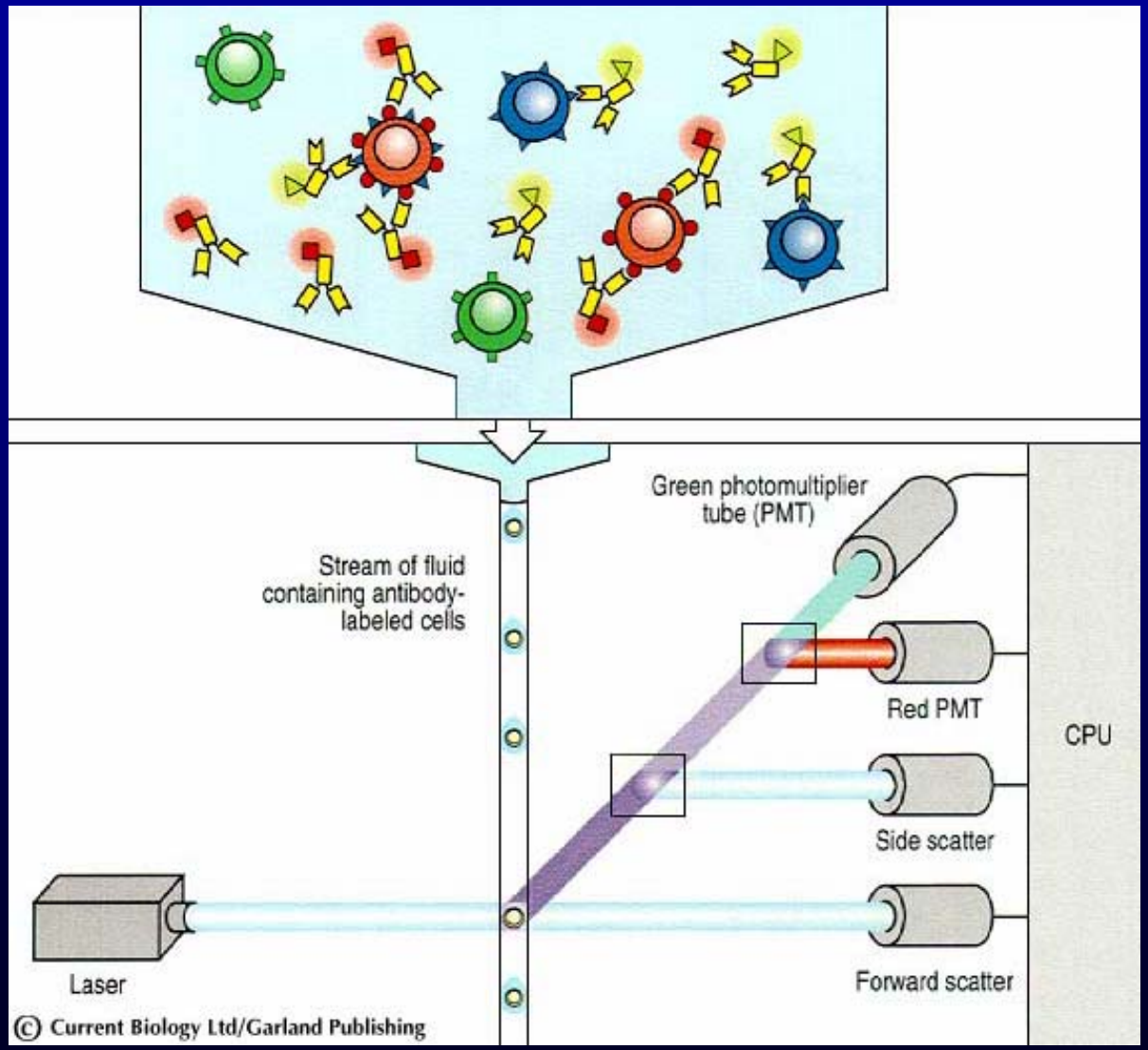
Immunofluorescence - exemple

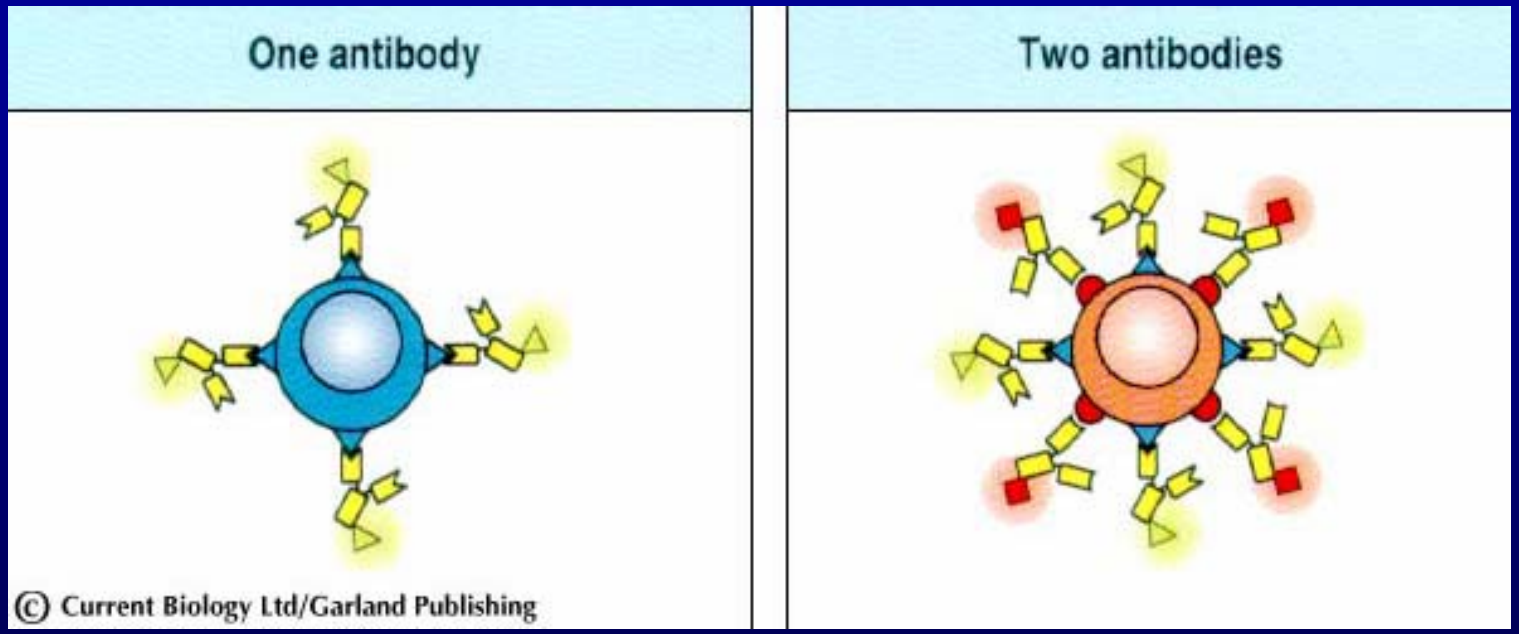


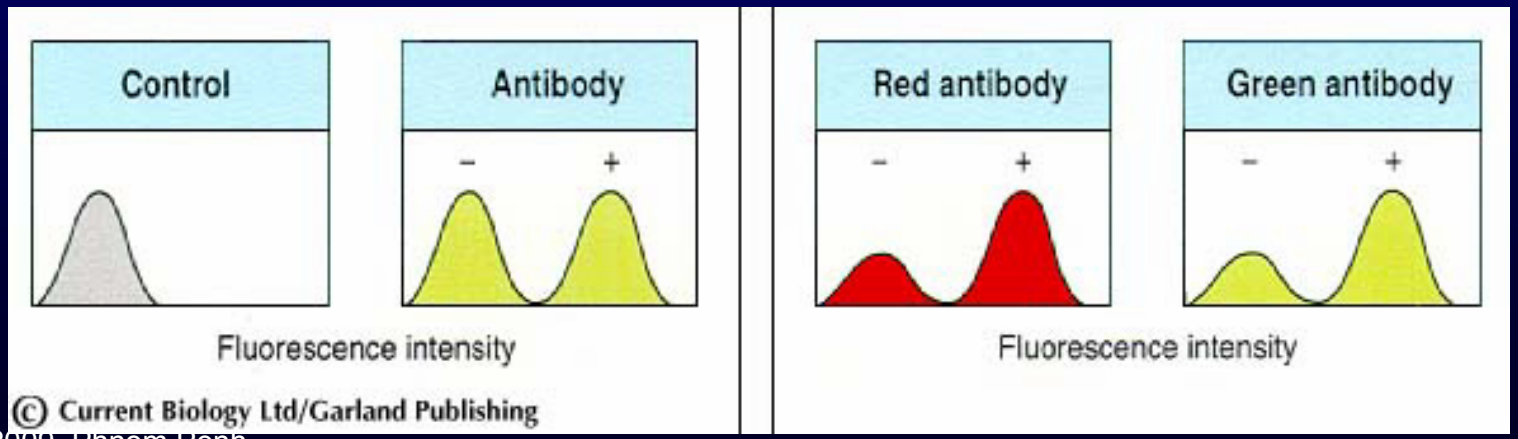
Coupe de pancréas:
Anticorps « vert » dirigé contre GAD (*glutamic acid decarboxylase*) marque les cellules β des îlots de Langerhans
Anticorps « orange » dirigé contre l'hormone glucagon marque les cellules α

Cytométrie de flux (1)

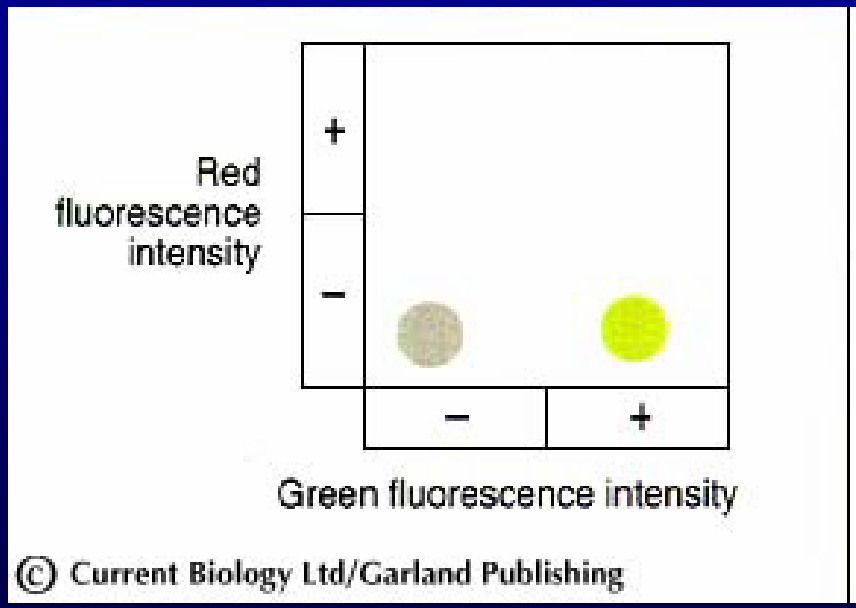


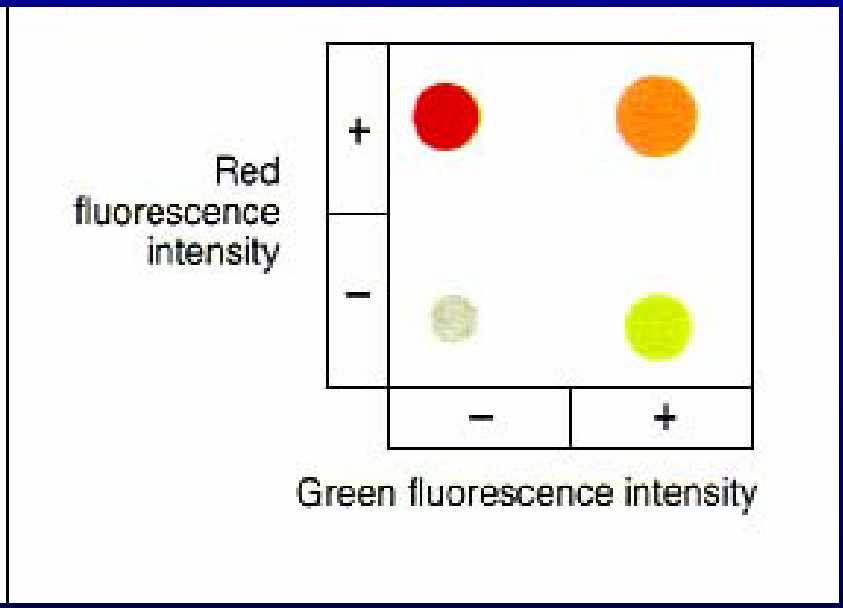
Cytométrie de flux (2)





Cytométrie de flux (3)





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8. Conclusion

Tri cellulaire par « panning »

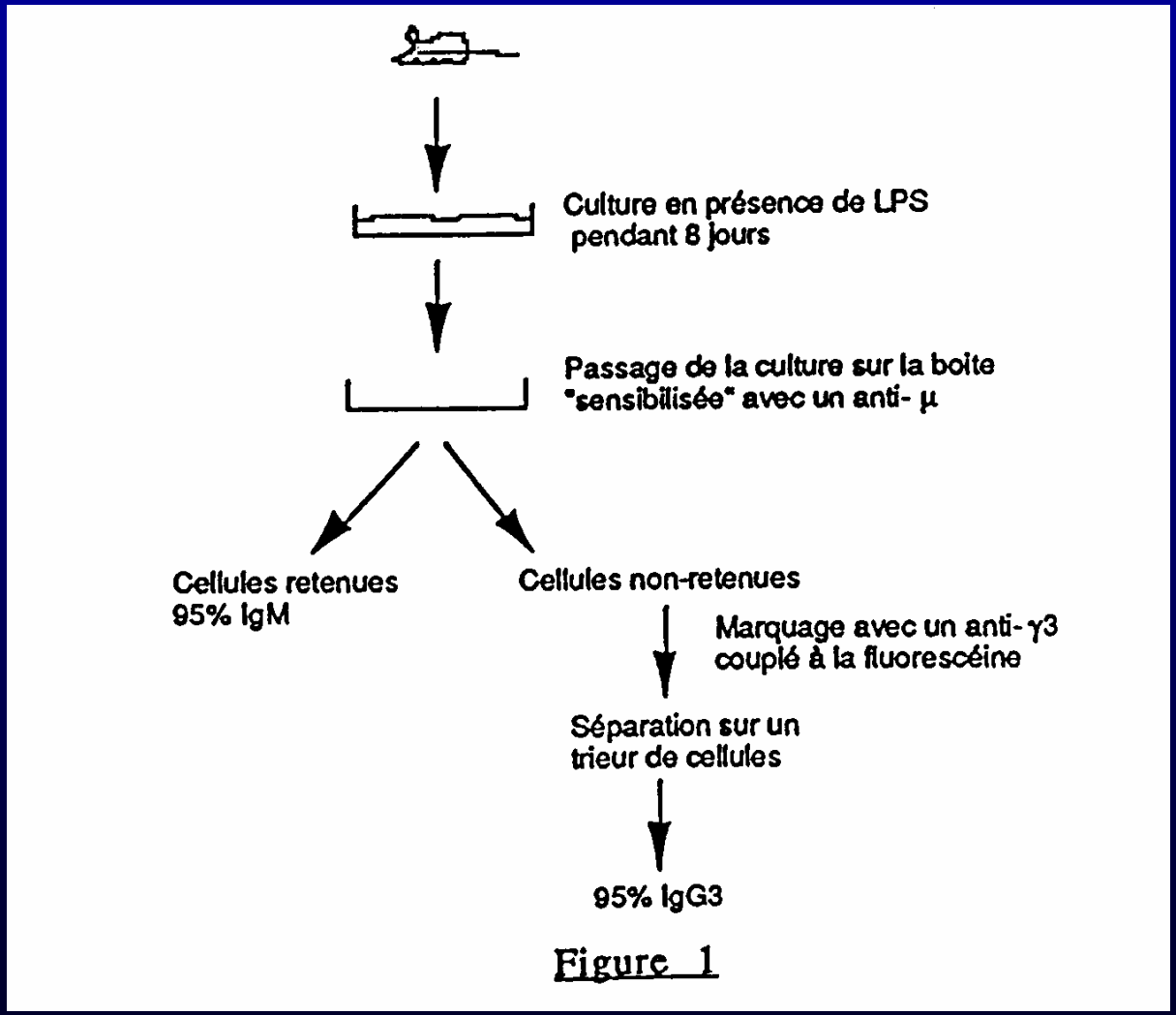
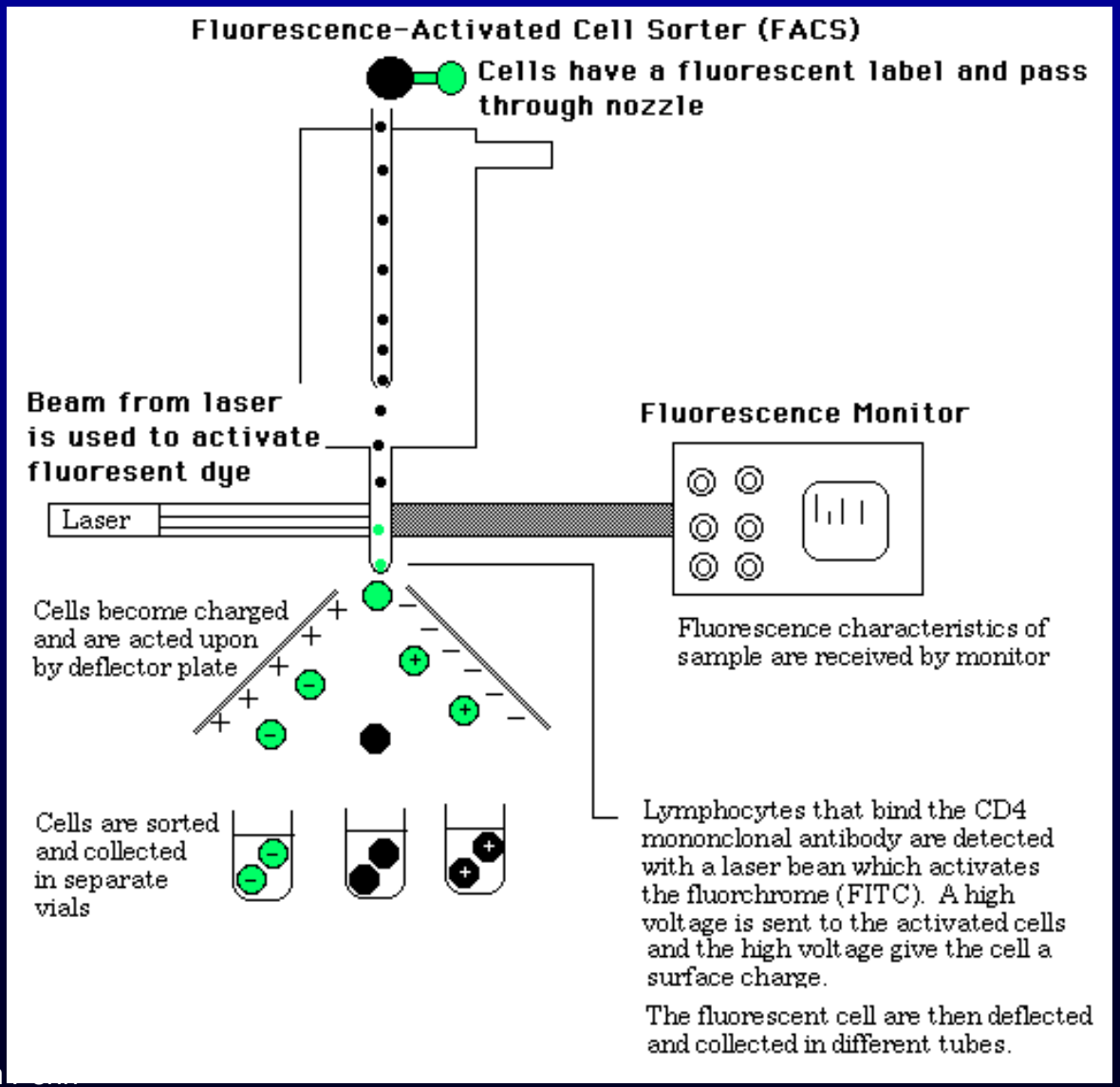
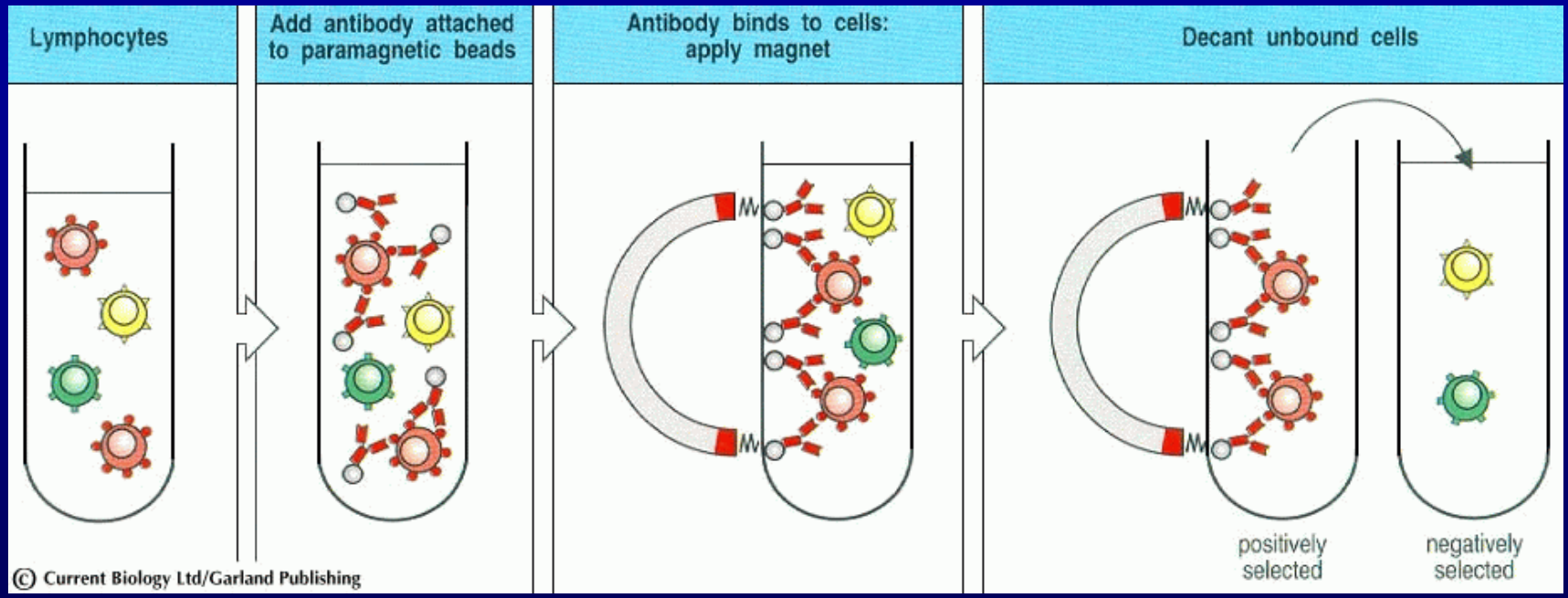


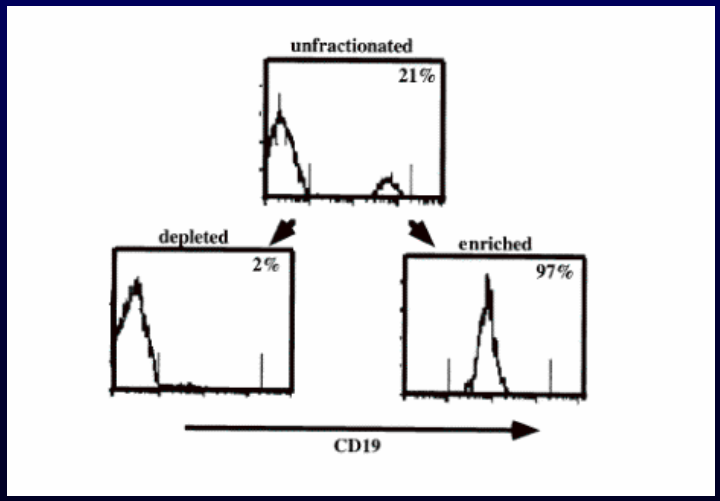
Figure 1

Tri cellulaire par cytométrie



Tri cellulaire magnétique





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Sensibilité des techniques de détection

TABLE 6-3 Sensitivity of various immunoassays

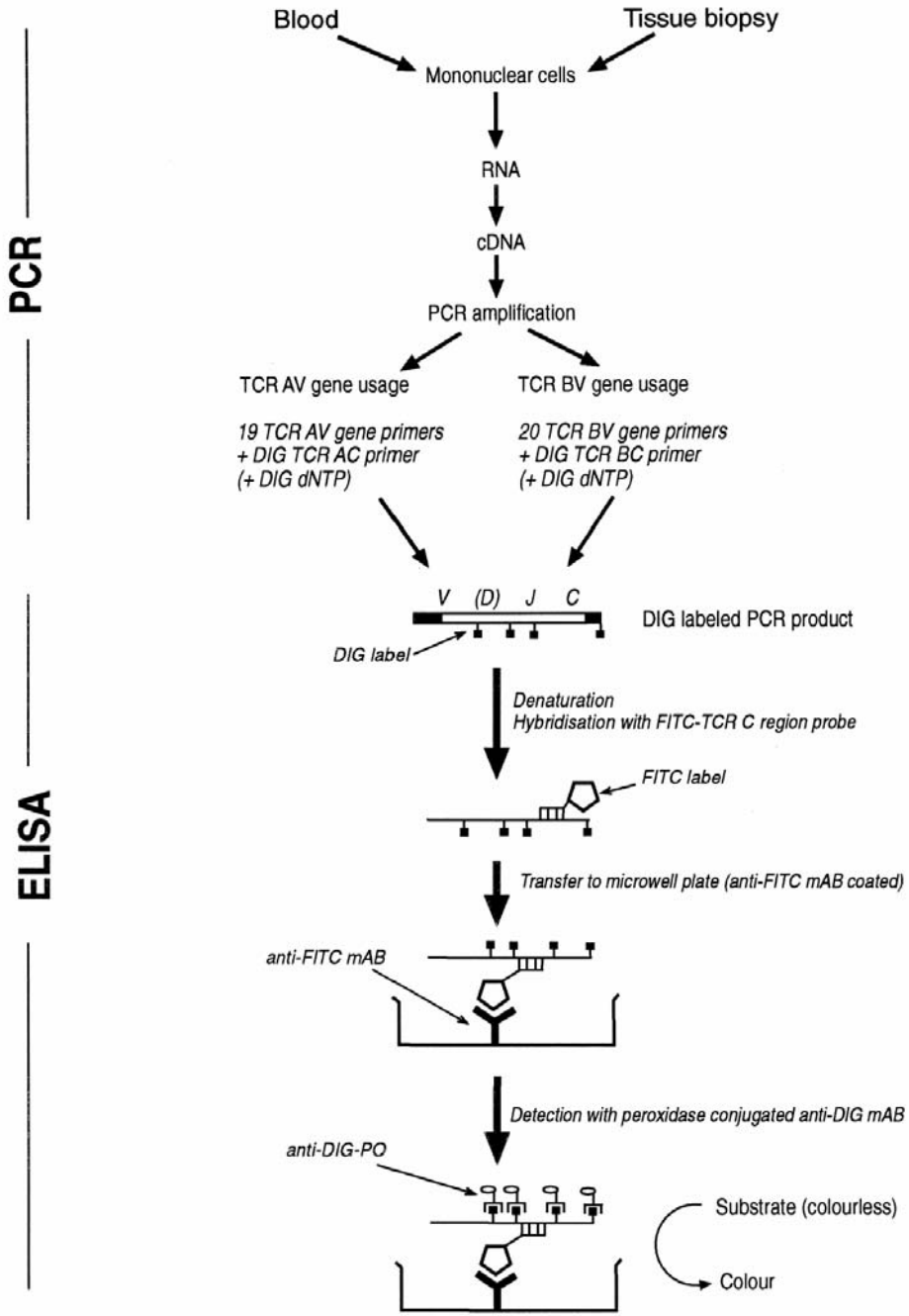
Assay	Sensitivity* (μg antibody/ml)
Precipitation reaction in fluids	20–200
Precipitation reactions in gels	
Mancini radial immunodiffusion	10–50
Ouchterlony double immunodiffusion	20–200
Immunoelectrophoresis	20–200
Rocket electrophoresis	2
Agglutination reactions	
Direct	0.3
Passive agglutination	0.006–0.06
Agglutination inhibition	0.006–0.06
Radioimmunoassay (RIA)	0.0006–0.006
Enzyme-linked immunosorbent assay (ELISA)	~0.0001–0.01
ELISA using chemiluminescence	~0.00001–0.01†
Immunofluorescence	1.0
Flow cytometry	0.006–0.06

*The sensitivity depends on the affinity of the antibody used for the assay as well as the epitope density and distribution on the antigen.
†Note that the sensitivity of chemiluminescence-based ELISA assays can be made to match that of RIA.

SOURCE: Updated and adapted from N. R. Rose et al., eds., 1997, *Manual of Clinical Laboratory Immunology,* 5th ed., American Society for Microbiology, Washington, DC.

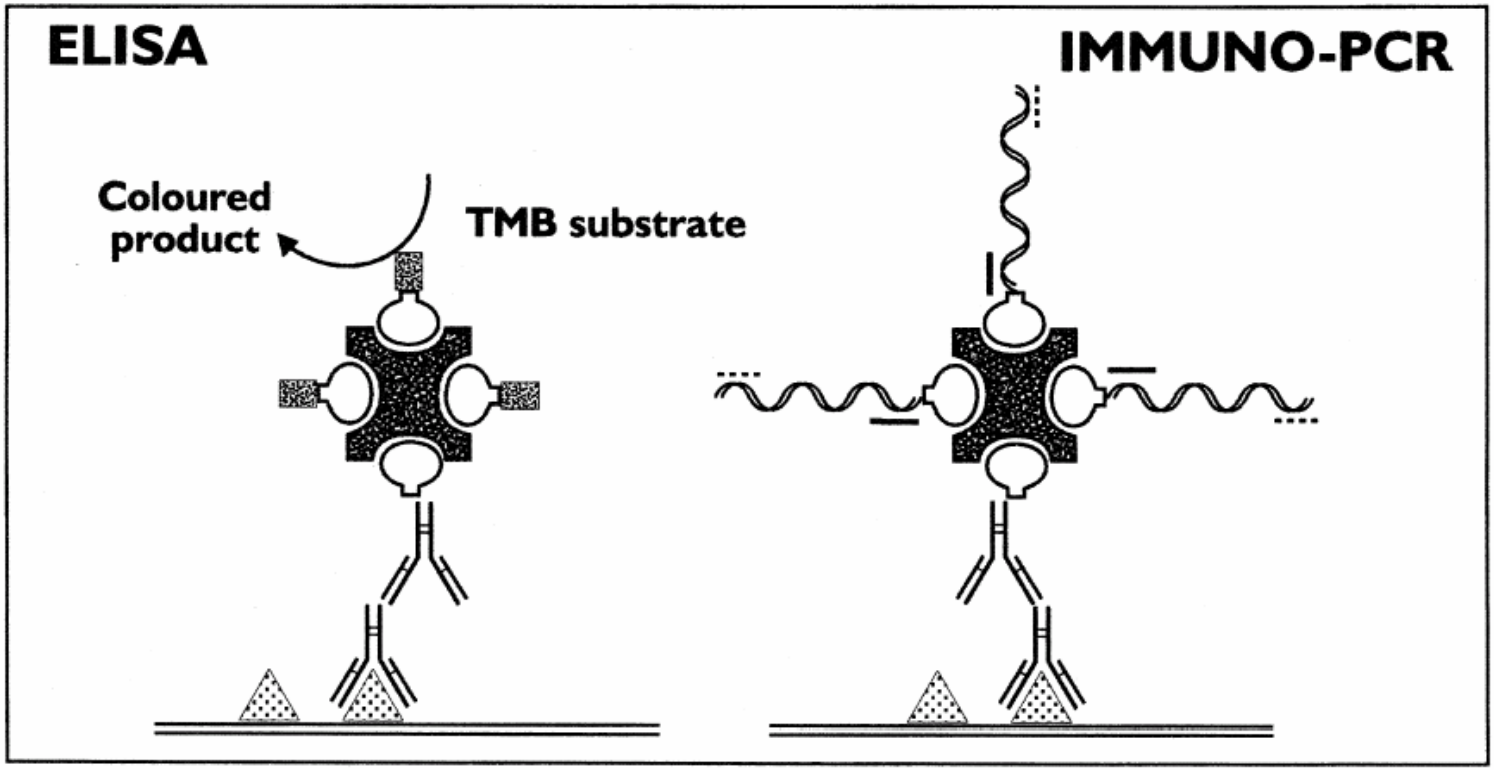
Table 6-3
Kuby IMMUNOLOGY, Sixth Edition

PCR-ELISA



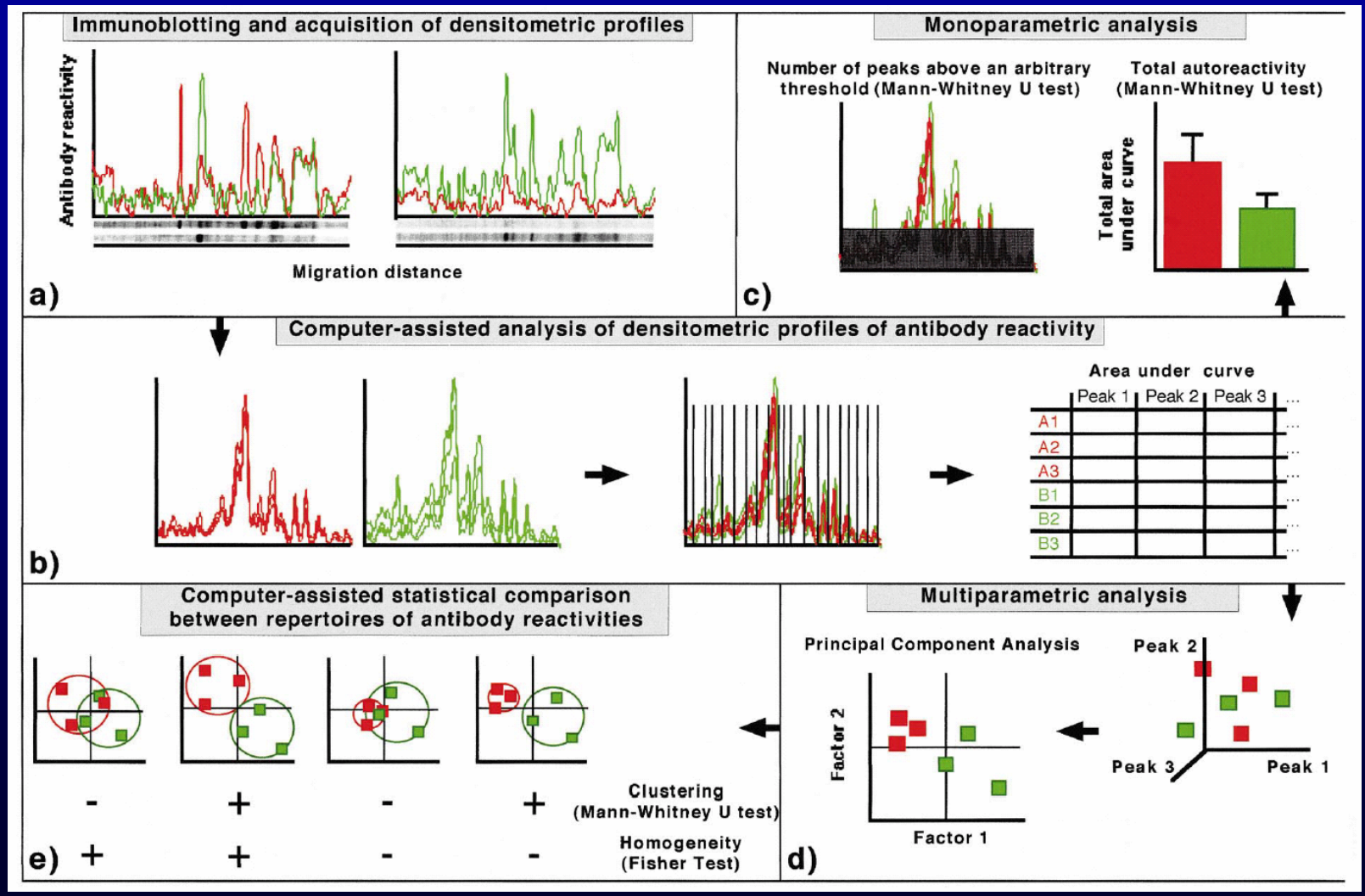
A. VanderBorght *et al.* (1999) *J.Immunol.Methods* 223:47.

Immuno-PCR



M. C. Case *et al.* (1999) *J.Immunol.Methods* 223:93.

Panama-blot



D. Stahl *et al.* (2000) *J.Immunol.Methods* 240:1.

Immunoproteomics

Francisco J. Quintana, Peter H. Hagedorn, Gad Elizur, Yifat Merbl, Eytan Domany, and Irun R. Cohen.
Functional immunomics: Microarray analysis of IgG autoantibody repertoires predicts the future response of mice to induced diabetes.
PNAS 101:14615-14621, 2004.