

DES de Biologie Médicale  
Enseignement d'Immunologie



ET01 & ET02

## Introduction aux techniques immunologiques (Immunochimie)

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Phnom Penh  
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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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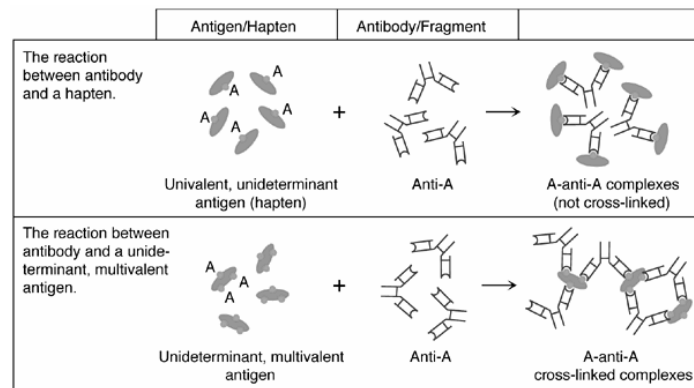
## Introduction aux techniques immunologiques (Immunochimie)

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## Réaction antigène/anticorps

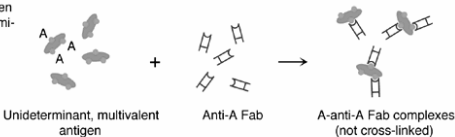


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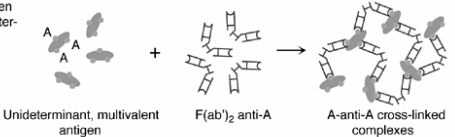
## Réaction antigène/anticorps

The reaction between Fab and a unideterminant, multivalent antigen.



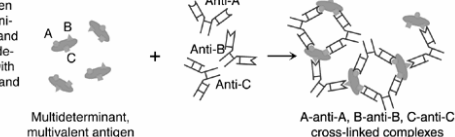
Unideterminant, multivalent antigen + Anti-A Fab → A-anti-A Fab complexes (not cross-linked)

The reaction between F(ab)<sub>2</sub> and a unideterminant, multivalent antigen.



Unideterminant, multivalent antigen + F(ab)<sub>2</sub> anti-A → A-anti-A cross-linked complexes

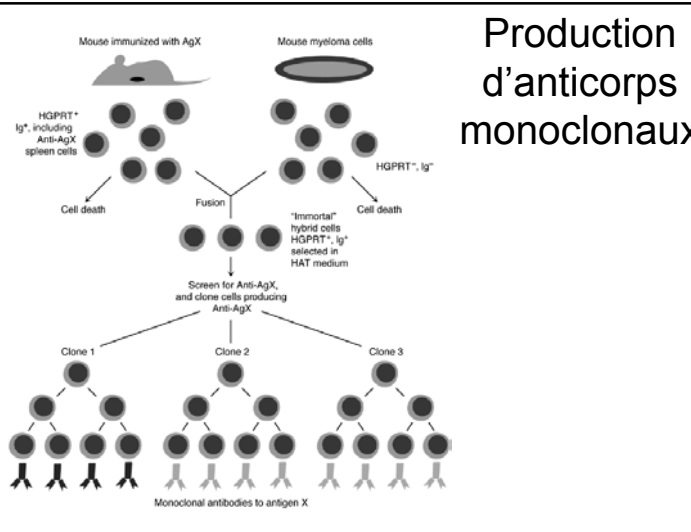
The reaction between antibodies to determinants A, B, and C, and a multivalent, multideterminant antigen with determinants A, B, and C.



Multideterminant, multivalent antigen + Anti-A, Anti-B, Anti-C → A-anti-A, B-anti-B, C-anti-C cross-linked complexes

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## Production d'anticorps monoclonaux



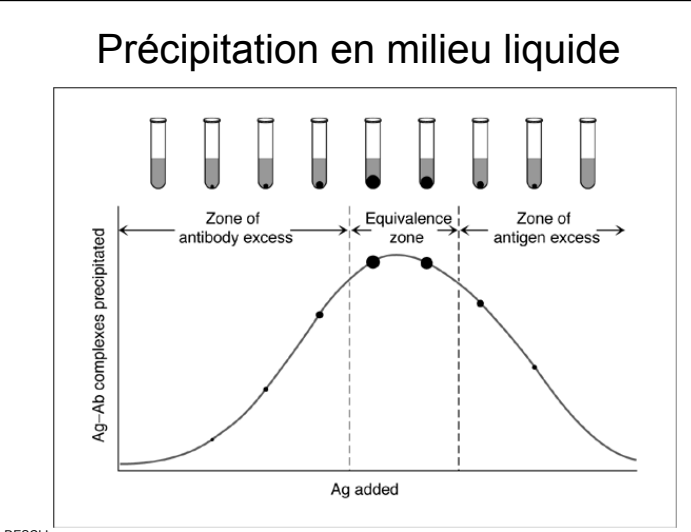
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## Précipitation en milieu liquide



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## Précipitation interfaciale

Technique du "ring test" (test qualitatif)

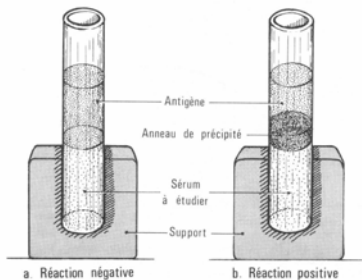


FIG. 24. – Méthode du disque ou de précipitation interfaciale.

## Diffusion unidimensionnelle

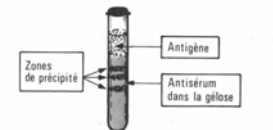


FIG. 29. – Technique de Oudin : réaction de précipitation avec diffusion simple unidimensionnelle.

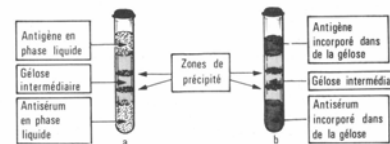


FIG. 30. – Réaction de précipitation avec diffusion double et unidimensionnelle. a) Technique de Preer. b) Technique d'Oakley-Fulthorpe.

## Diffusion simple bidimensionnelle

Technique de Mancini  
Immunodiffusion radiale

### RADIAL IMMUNODIFFUSION

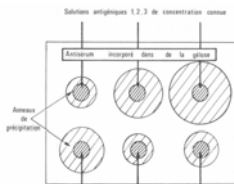
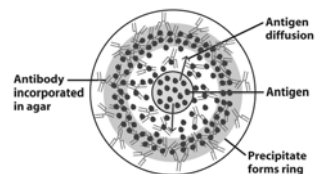
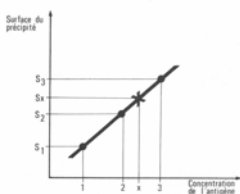


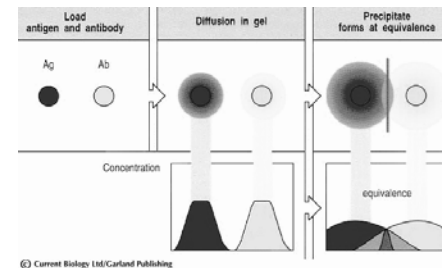
FIG. 31. – Technique de Mancini : réaction de précipitation avec diffusion simple et bidimensionnelle.



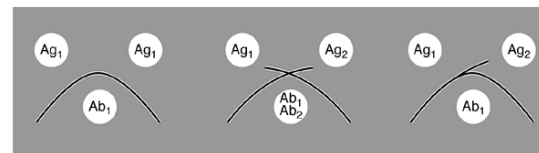
## Précipitation en milieu solide

Technique d'Ouchterlony

Immunodiffusion double



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## 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

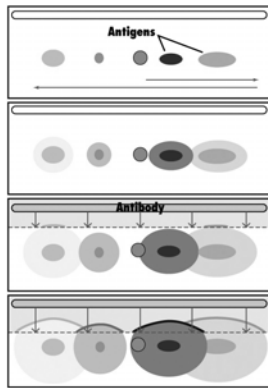


Figure 6-7  
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## Immunoélectrophorèse en fusée

Technique de Laurell:

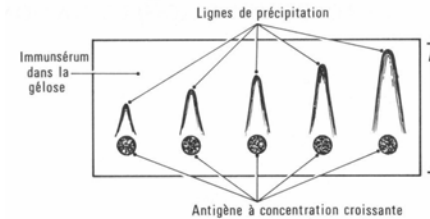


FIG. 35. – Technique de Laurell.

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

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## Immunoélectrophorèse bidimensionnelle

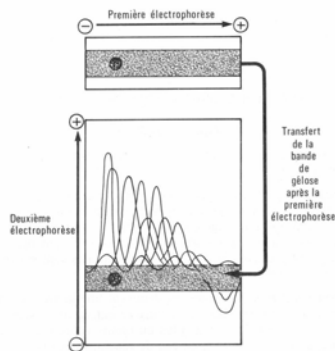


FIG. 36. – Immuno-électrophorèse bidimensionnelle.

Analyse qualitative & quantitative

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## Réaction d'agglutination

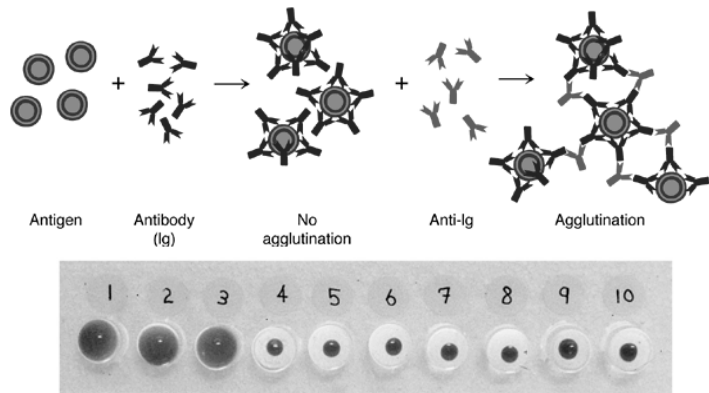


Figure 6-8  
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Qualitatif: très sensible; Quantitatif: peu précis

## Test de Coombs direct

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

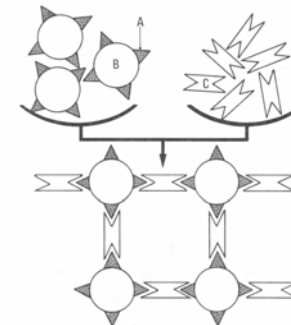


FIG. 40-a). – Test de Coombs direct.  
A : Anticorps anti-Rhésus d'origine maternelle.  
B : Globules rouges Rhésus positif.  
C : Anticorps antigammaglobulines. (Voir fig. 40-b page 100).

## Test de Coombs indirect

Recherche d'anticorps anti-Rhésus chez la mère

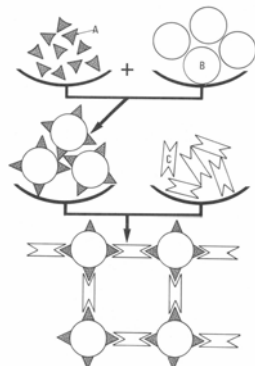
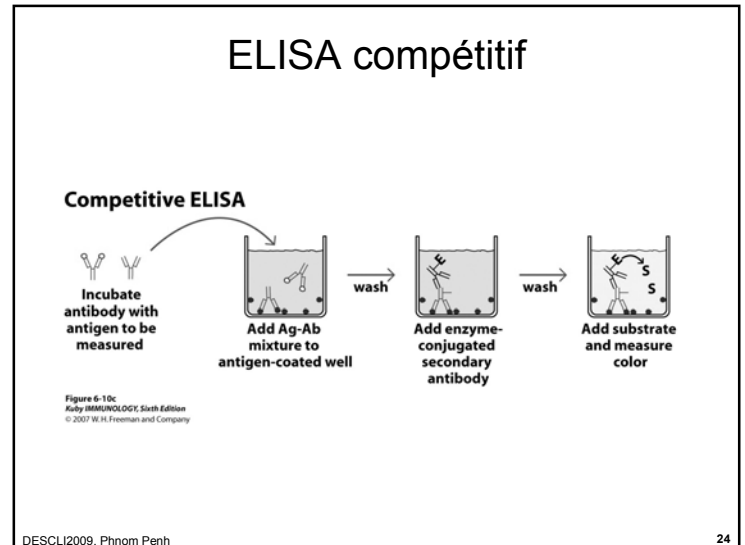
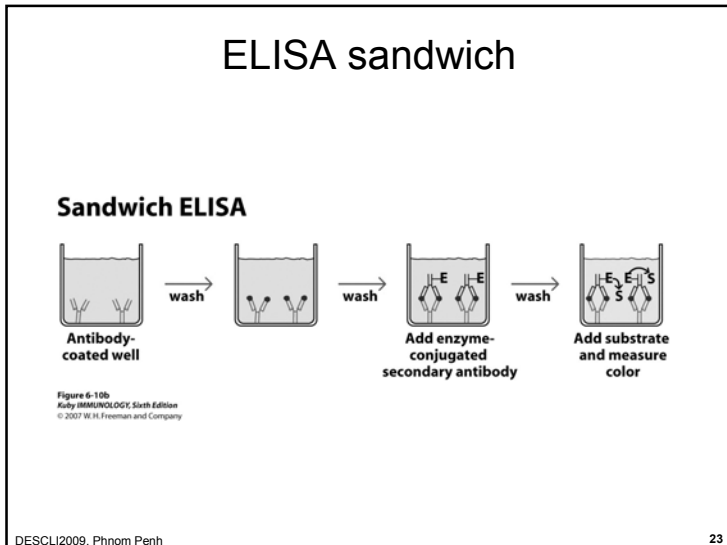
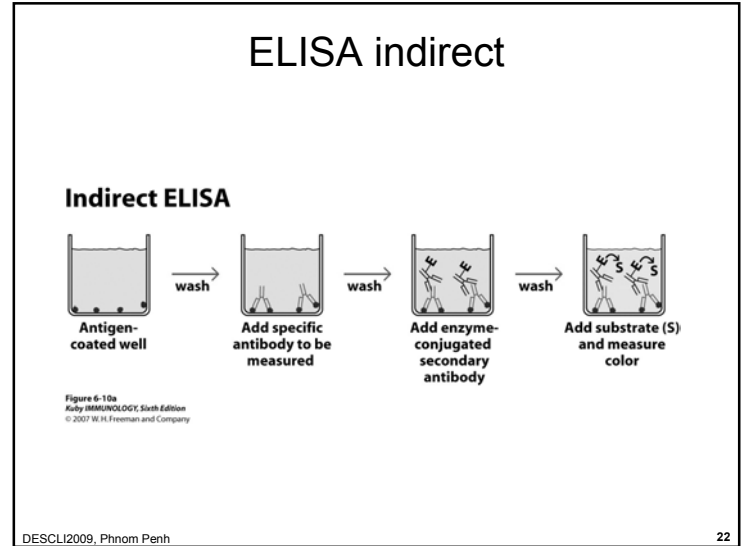
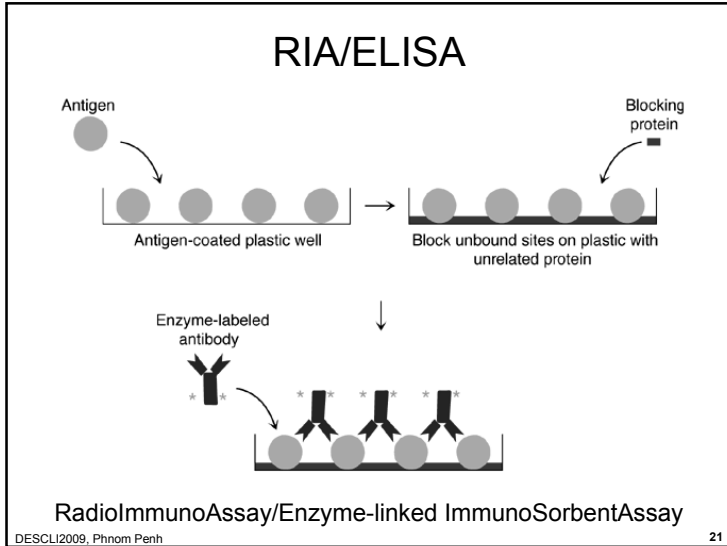


FIG. 40-b). – Test de Coombs indirect.  
A : Anticorps anti-Rhésus.  
B : Globules rouges Rhésus positif.  
C : Anticorps antigammaglobulines.

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## ELISpot

**Well coated with anticytokine antibody**

➊ Secretor  
➋ Nonsecretor

**Add test cell population**

**Incubate at 37°C**

**Discard cells**  
**Wash plate**

**Add enzyme-linked anticytokine antibody**

**Side view**  
E = enzyme  
CS = chromogenic substrate  
CP = colored product

**Top view**  
Site of secreting cell

Figure 6-11  
Atlas of Immunology, Sixth Edition  
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Figure 8-29 part 1 of 3 Immunobiology, 6e © Garland Science 2005

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## Immunoprécipitation

**Metabolic labeling**

<sup>35</sup>S-Met

All proteins label

Normal cells + radiolabel

Labeled cells washed

Cells lysed in detergent

Antibodies added on beads

Other proteins washed away

Proteins eluted: separated by SDS-PAGE

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## Western blot

- 1 **HIV**
- 2 **Dissociate in SDS**
- 3 **SDS-PAGE**
- 4 **Transfer to nitrocellulose and overlay with antiserum**
- 5 **Detect bound antibody with enzyme-linked anti-IgG**

95 68 45 12

120 40 24

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## Immunofluorescence - principe

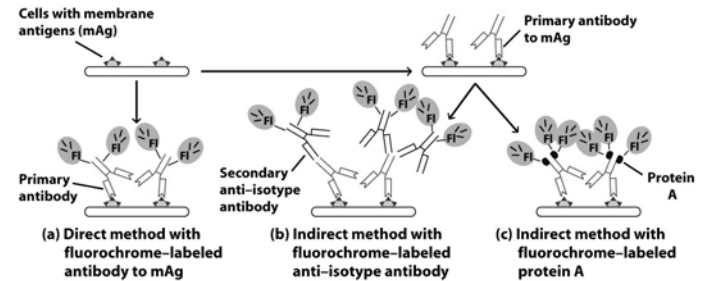
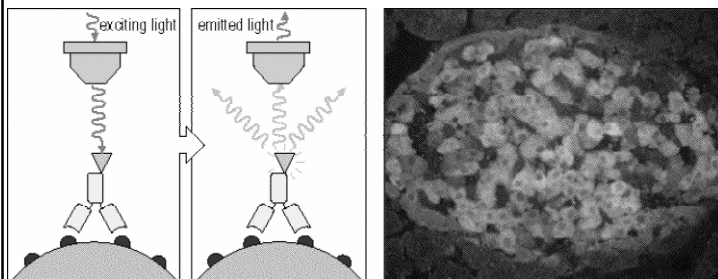


Figure 6-14abc  
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## Immunofluorescence - exemple



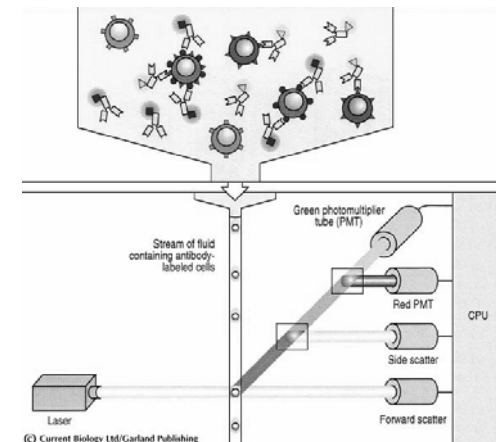
### Coupe de pancréas:

Anticorps « vert » dirigé contre GAD (*glutamic acid decarboxylase*) marque les cellules  $\beta$  des îlots de Langerhans  
Anticorps « orange » dirigé contre l'hormone glucagon marque les cellules  $\alpha$

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## Cytométrie de flux (1)



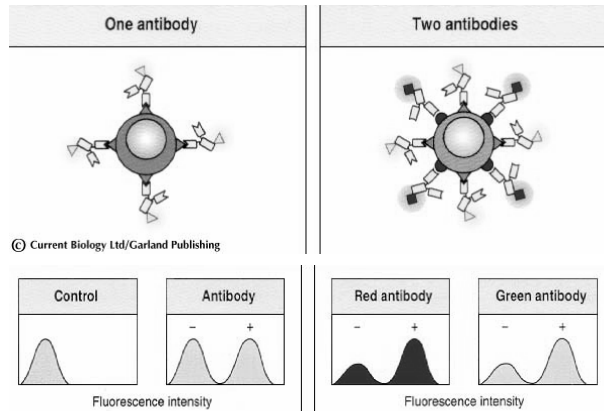
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## Cytométrie de flux (2)



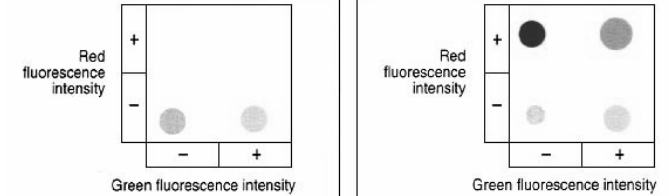
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## Cytométrie de flux (3)



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## Tri cellulaire par « panning »

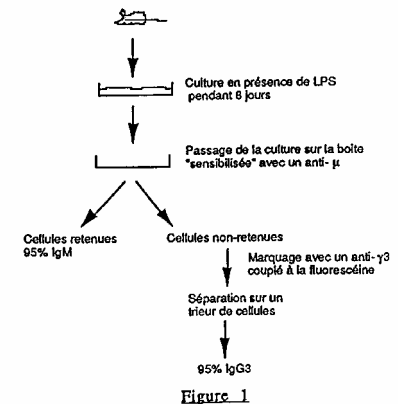
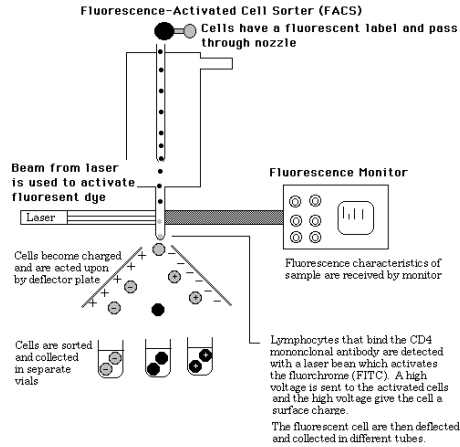


Figure 1

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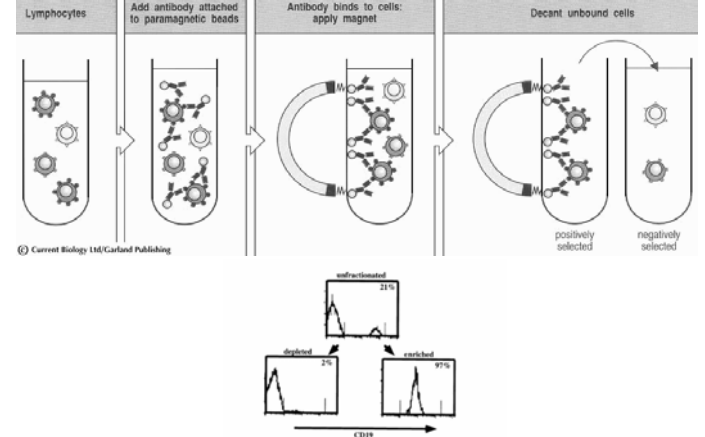
## Tri cellulaire par cytométrie



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## Tri cellulaire magnétique



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## Sensibilité des techniques de détection

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
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

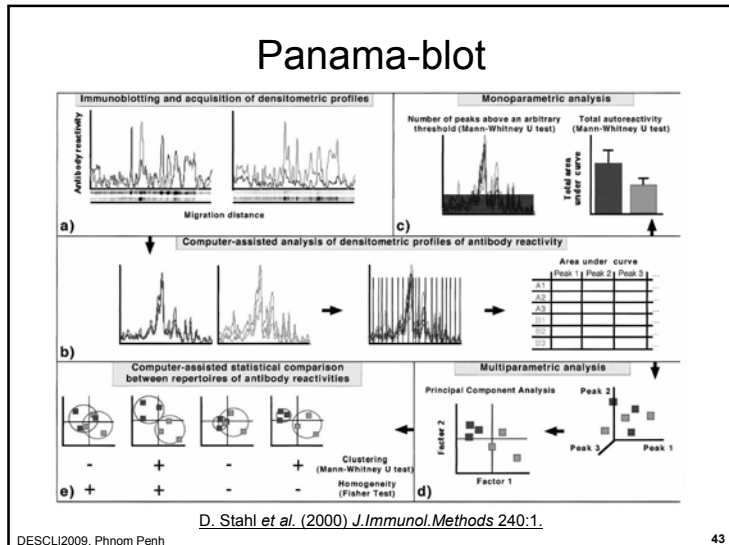
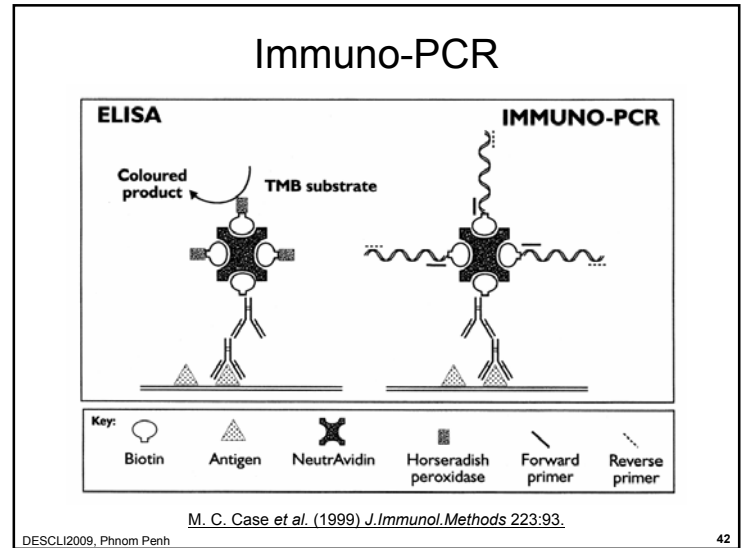
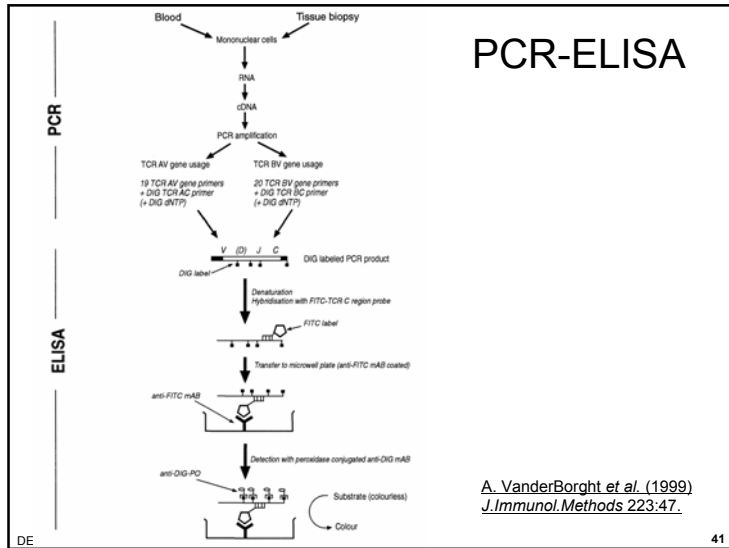
\*The sensitivity depends on the affinity of the antibody used for the assay as well as the epitope density and distribution on the antigen.  
†Note that the sensitivity of chemiluminescence-based ELISA assays can be made to match that of RIA.

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  
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### 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.

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