

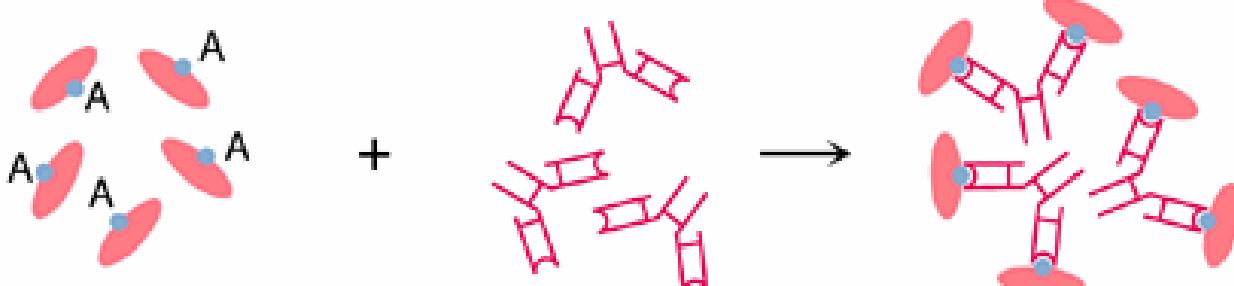
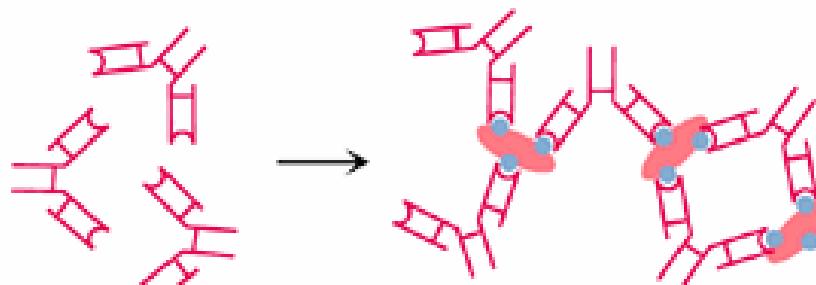
# Utilisation des anticorps *in vitro* principes et techniques

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Université Pierre et Marie Curie

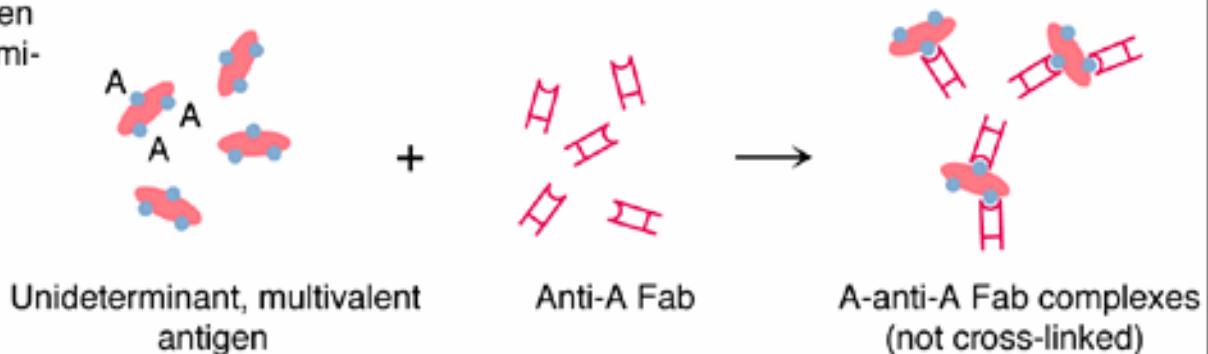
octobre 2005

# Réaction antigène/anticorps

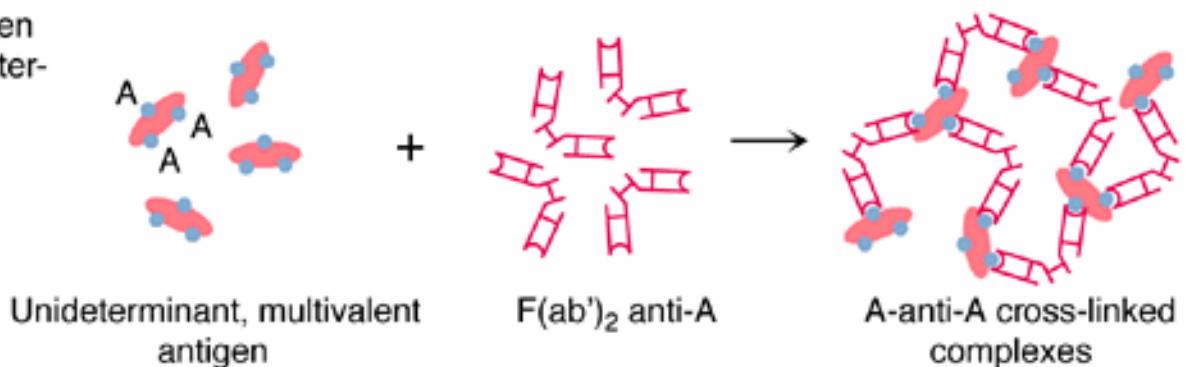
Antigen/Hapten	Antibody/Fragment	
The reaction between antibody and a hapten.	<p>The diagram illustrates the reaction between a univalent, unideterminant antigen (hapten) and anti-A antibody fragments. On the left, four red oval-shaped antigens, each with a blue dot representing a determinant, are shown. A plus sign follows. To the right, three Y-shaped anti-A antibody fragments are shown. An arrow points to the right, where the final state is depicted: three A-anti-A complexes, each consisting of one antigen molecule bound to a single Y-shaped antibody fragment. The complexes are not cross-linked to each other.</p>	 <p>A-anti-A complexes (not cross-linked)</p>
The reaction between antibody and a unideterminant, multivalent antigen.	<p>The diagram illustrates the reaction between a unideterminant, multivalent antigen and anti-A antibody fragments. On the left, four red oval-shaped antigens, each with a blue dot representing a determinant, are shown. A plus sign follows. To the right, three Y-shaped anti-A antibody fragments are shown. An arrow points to the right, where the final state is depicted: three A-anti-A cross-linked complexes. These complexes consist of two antigen molecules bound to a single Y-shaped antibody fragment, forming a ring-like structure where the antibody fragments are linked together by their bases.</p>	 <p>A-anti-A cross-linked complexes</p>

# Réaction antigène/anticorps

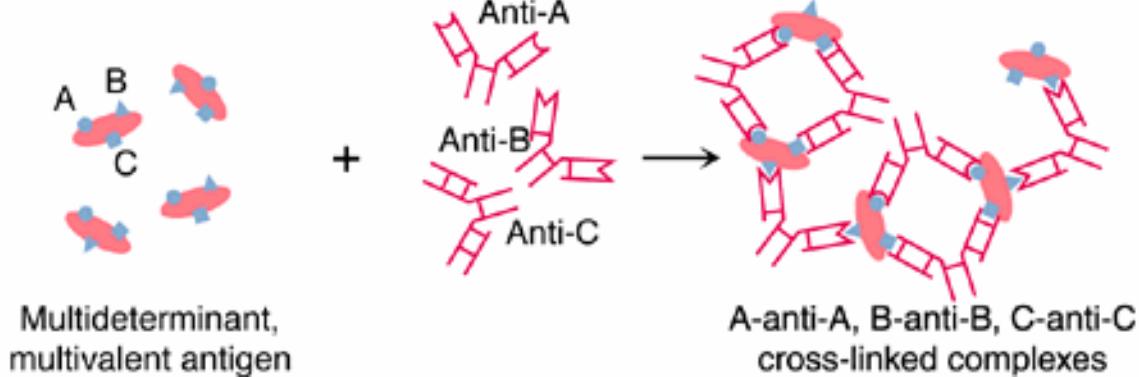
The reaction between Fab and a unideterminant, multivalent antigen.



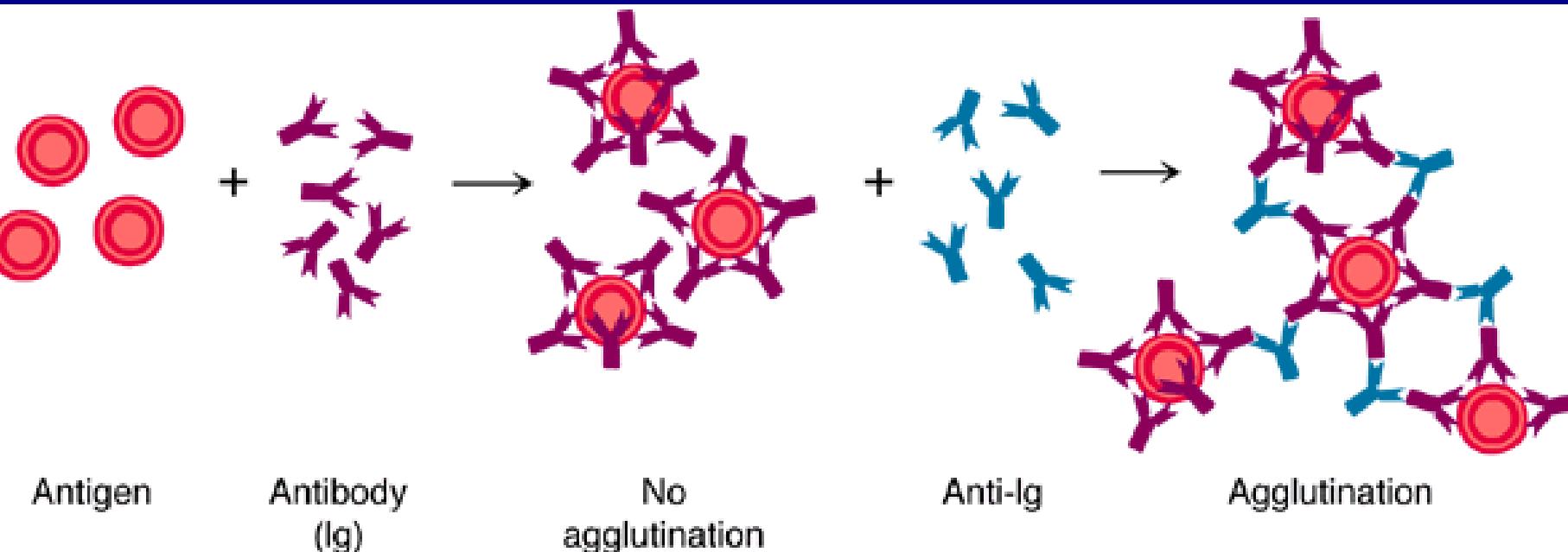
The reaction between  $F(ab')_2$  and a unideterminant, multivalent antigen.



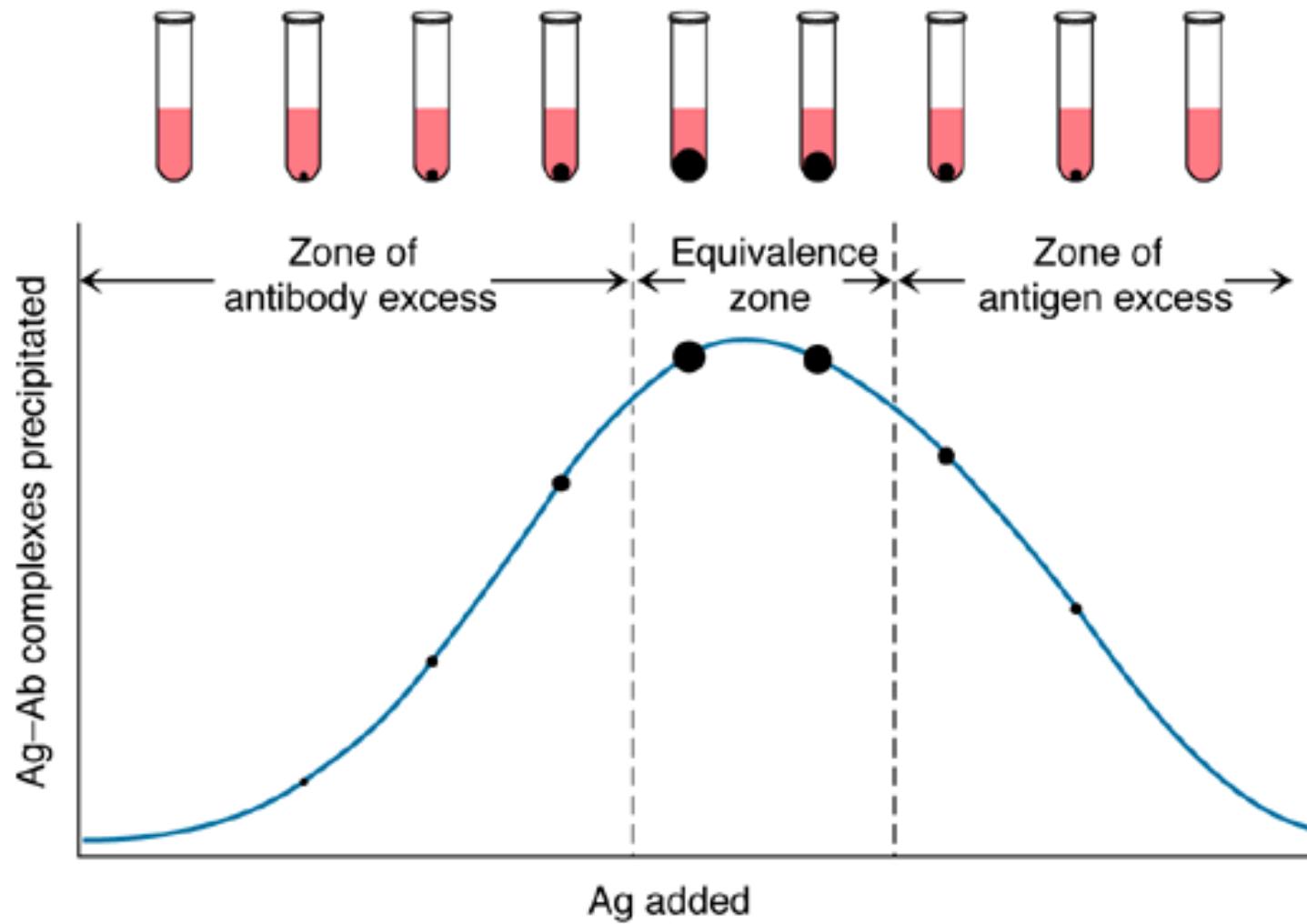
The reaction between antibodies to determinants A, B, and C, and a multivalent, multideterminant antigen with determinants A, B, and C.



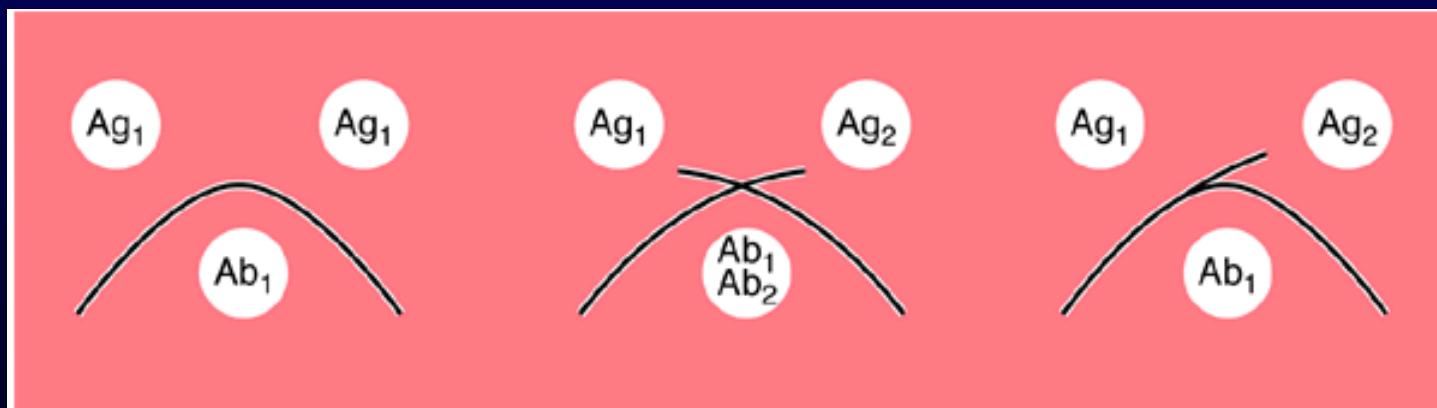
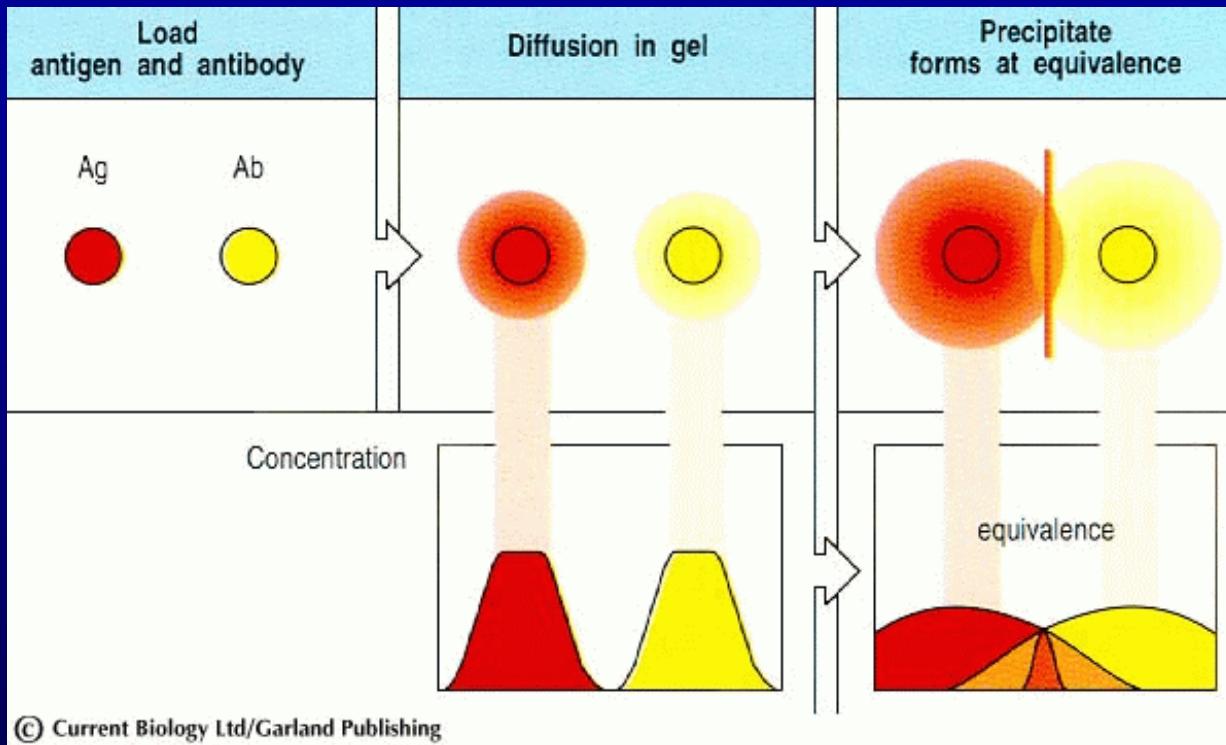
# Réaction d'agglutination



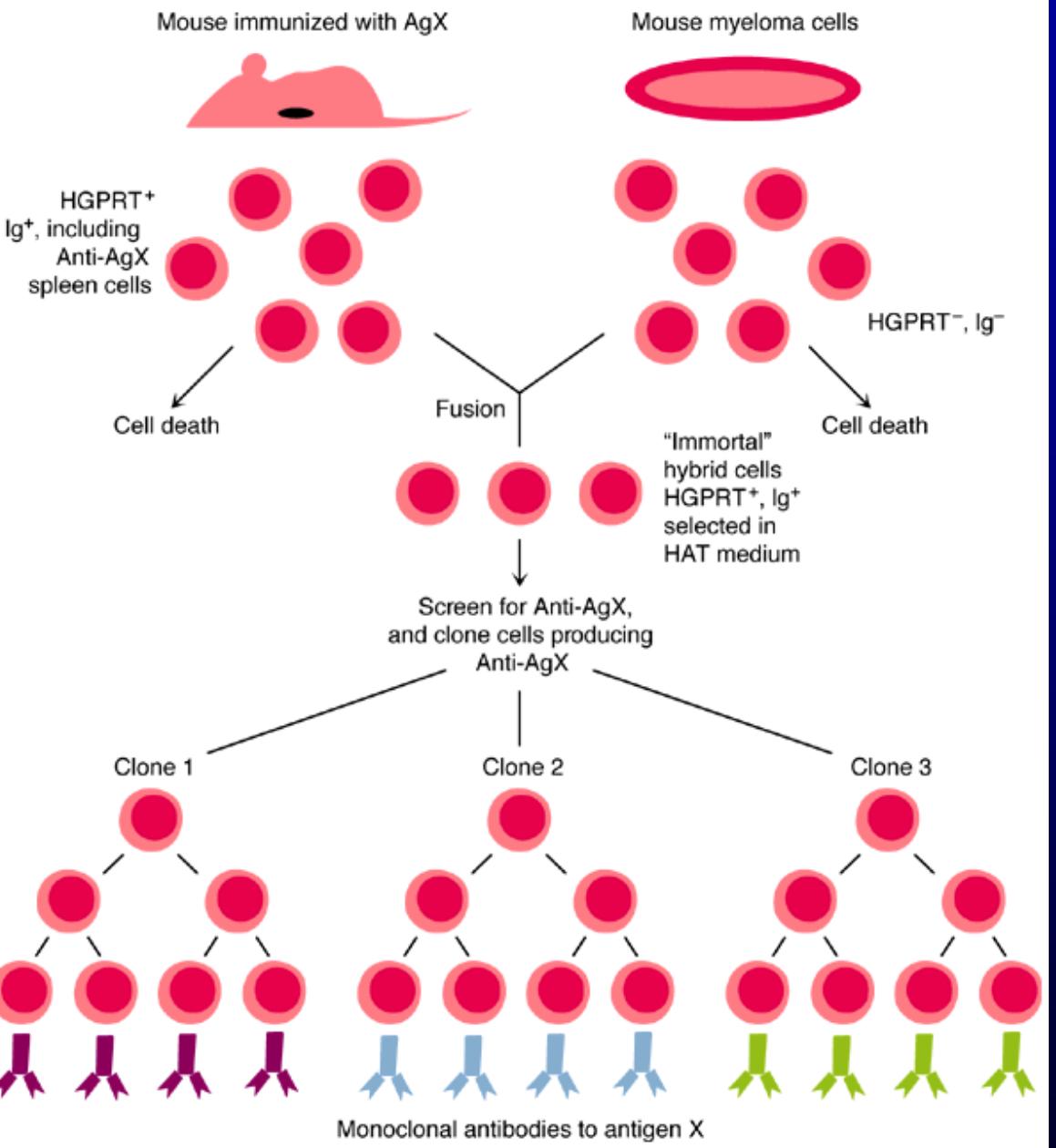
# Précipitation en milieu liquide



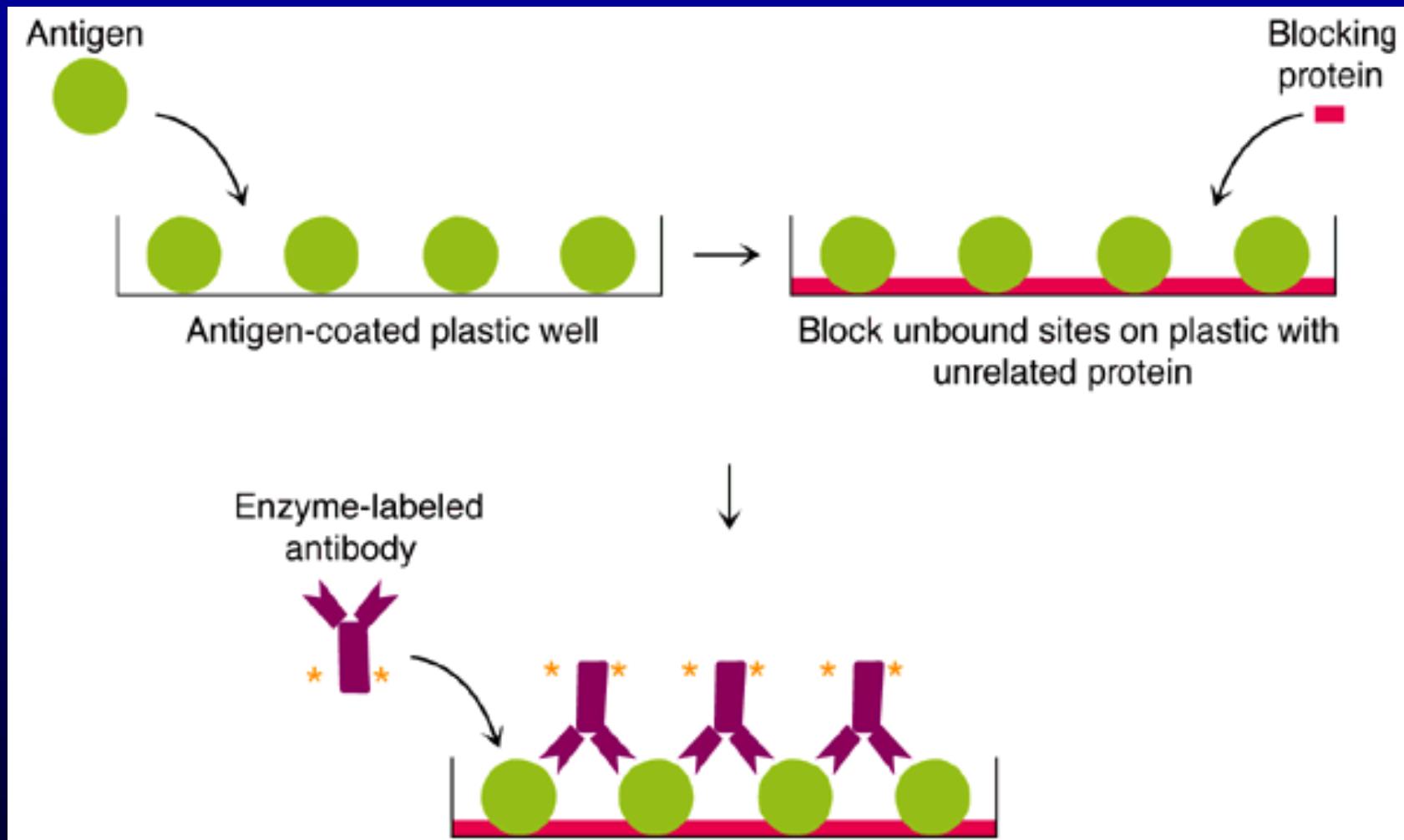
# Précipitation en milieu solide



# Production d'anticorps monoclonaux

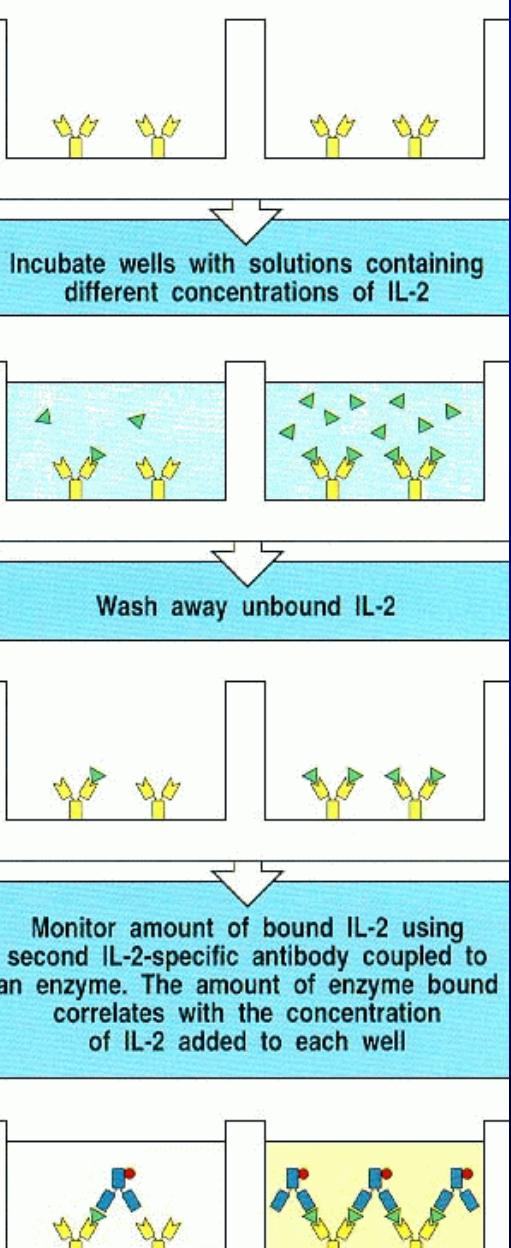


# RIA/ELISA

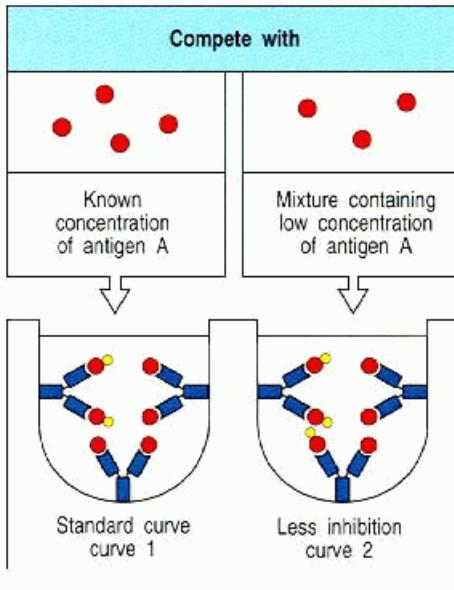
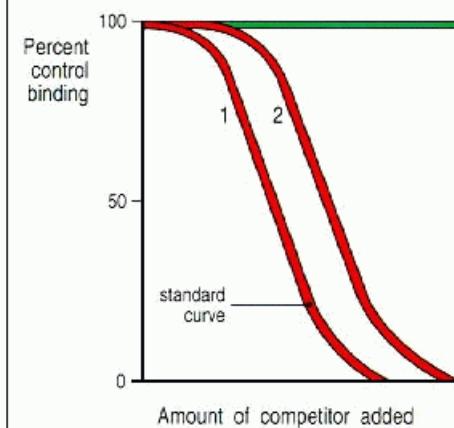
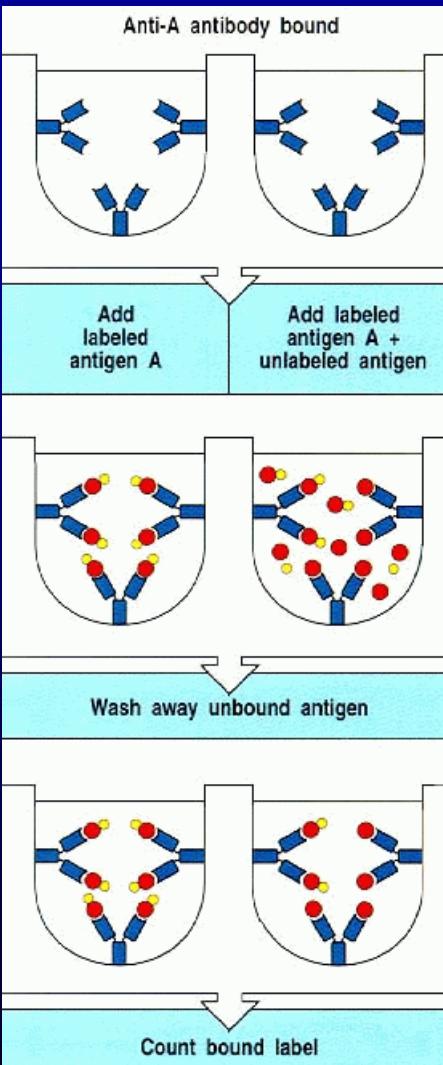


RadioimmunoAssay/Enzyme-linked ImmunoSorbentAssay

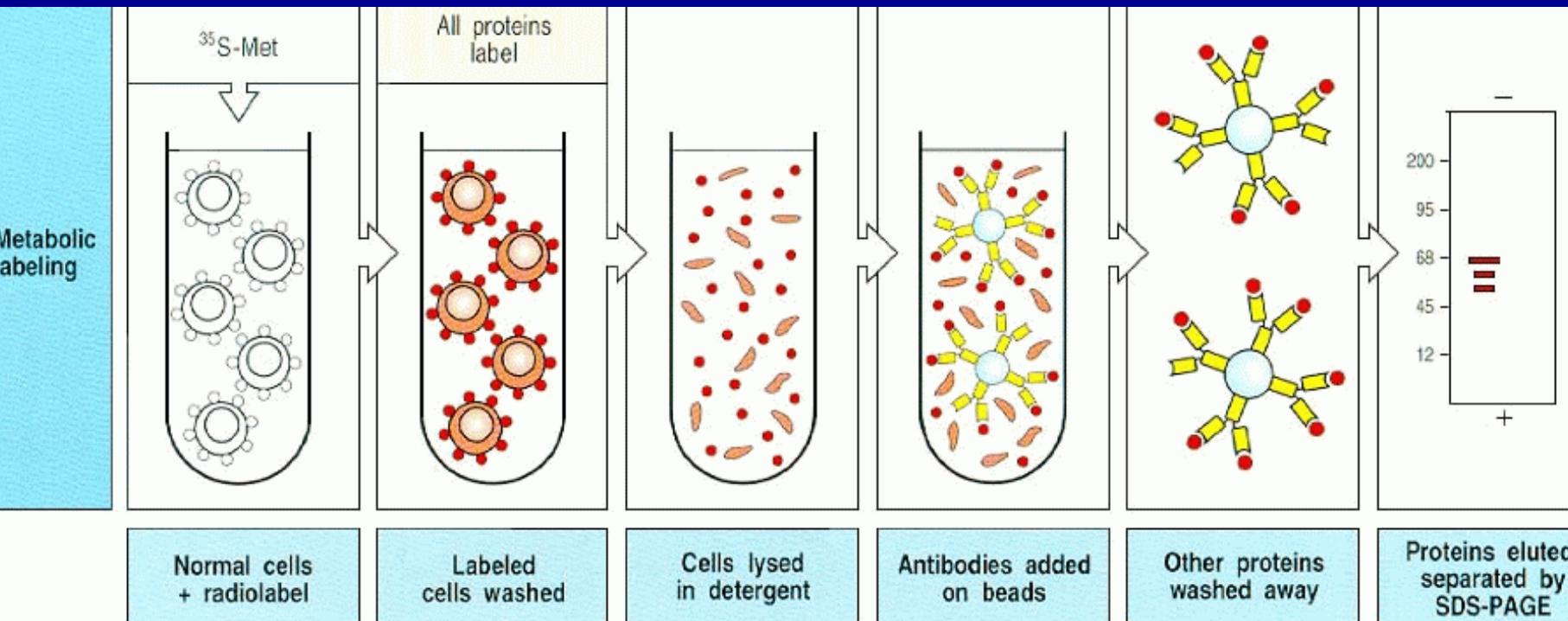
Coat plastic wells with antibody to IL-2



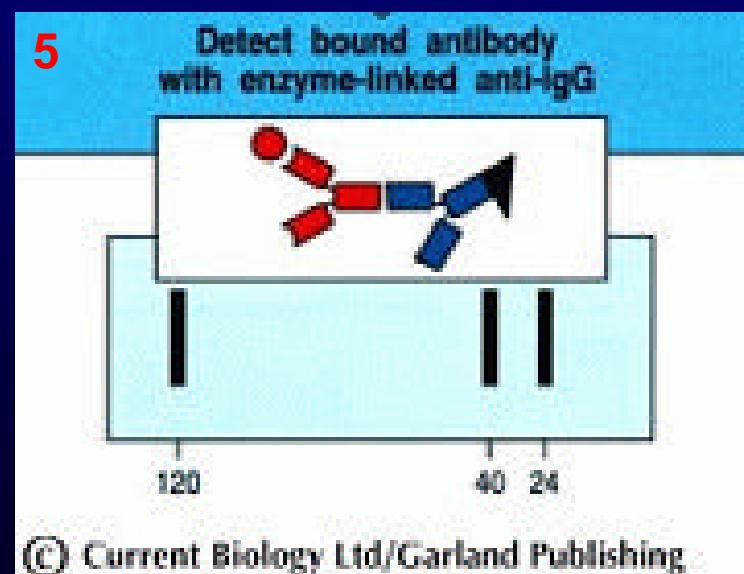
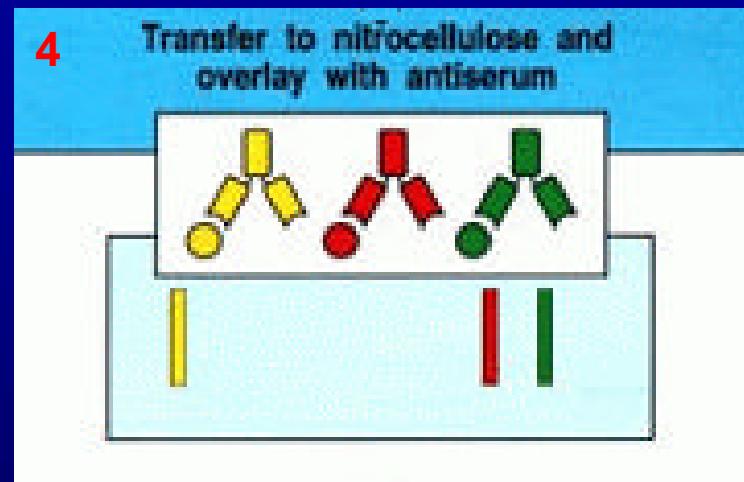
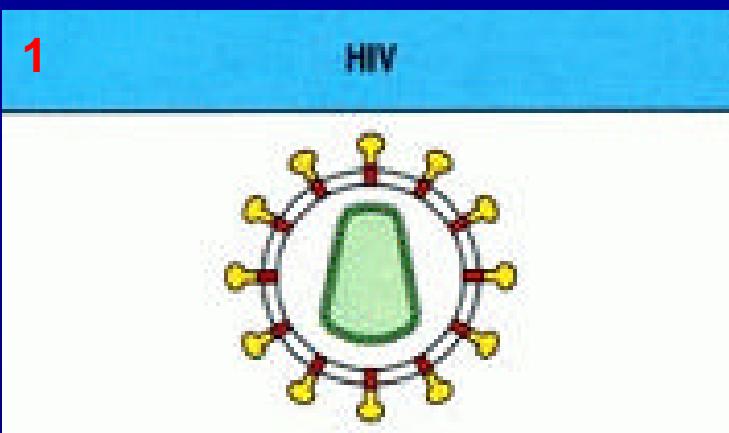
# Sandwich/Compétition



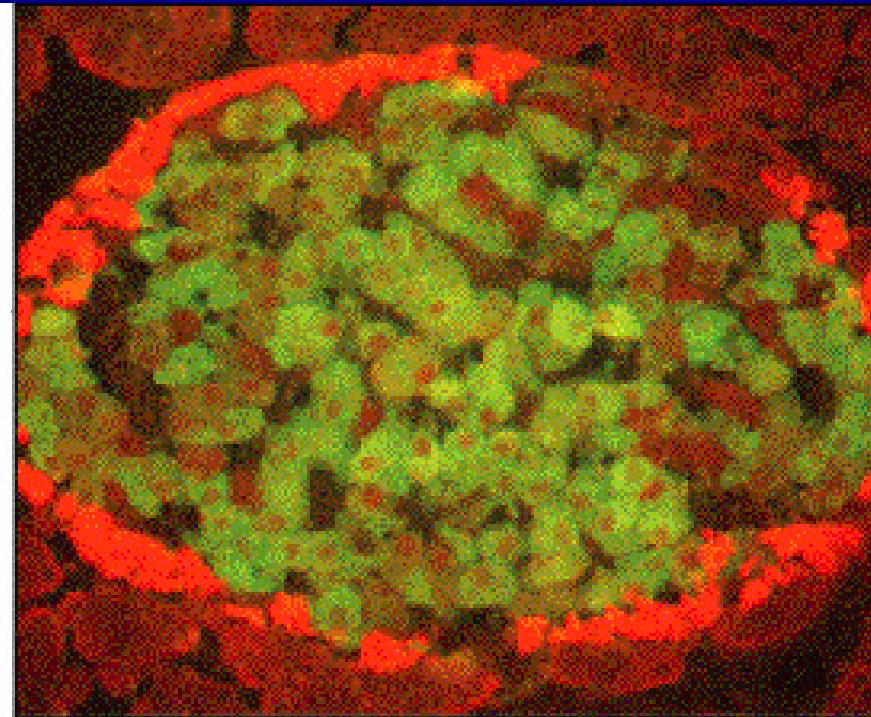
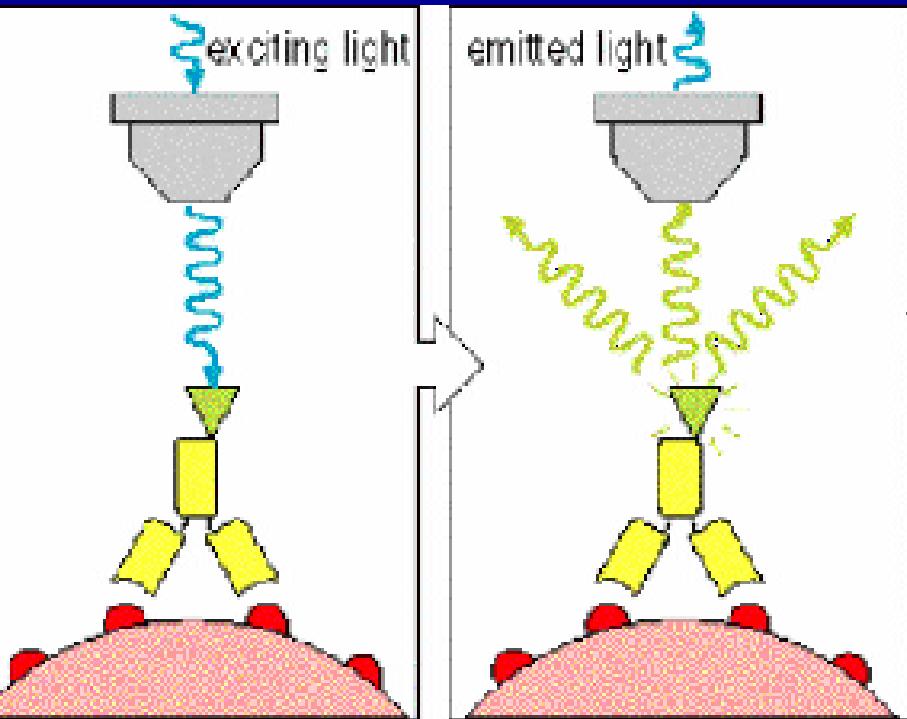
# Immunoprecipitation



# Western blot



# Immunofluorescence

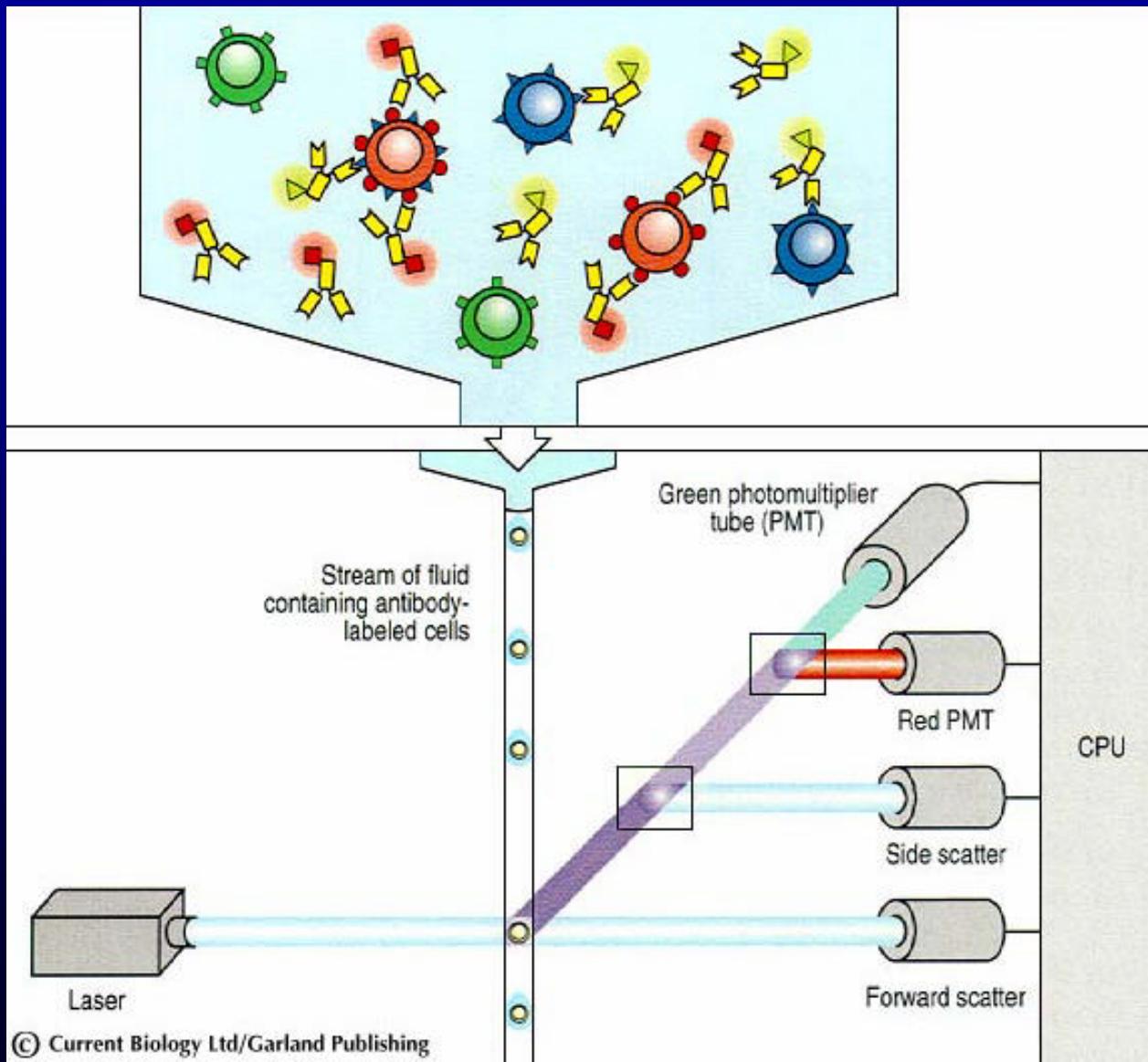


## Coupe de pancréas:

Anticorps « vert » dirigé contre GAD (*glutamic acid decarboxylase*)  
marque les cellules  $\beta$  des îlots de Langerhans

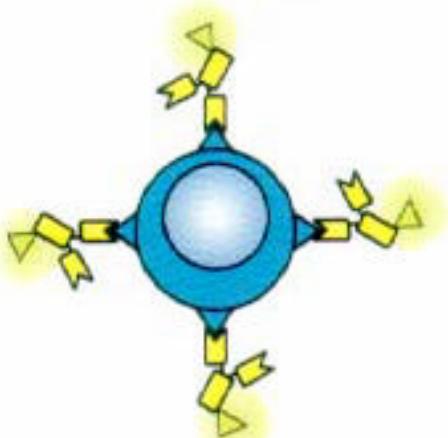
Anticorps « orange » dirigé contre l'hormone glucagon marque les  
cellules  $\alpha$

# Cytométrie de flux (1)

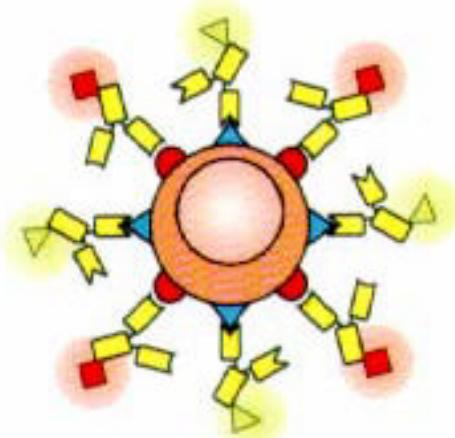


# Cytométrie de flux (2)

One antibody

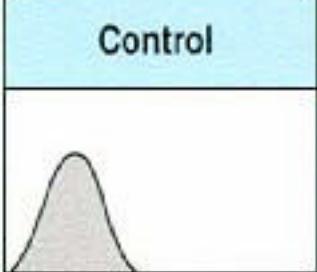


Two antibodies

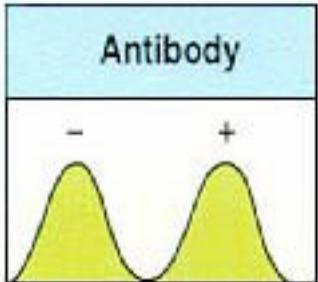


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Control

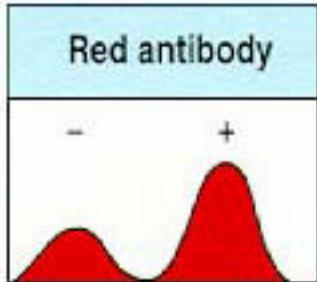


Antibody

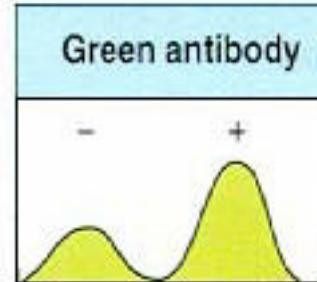


Fluorescence intensity

Red antibody



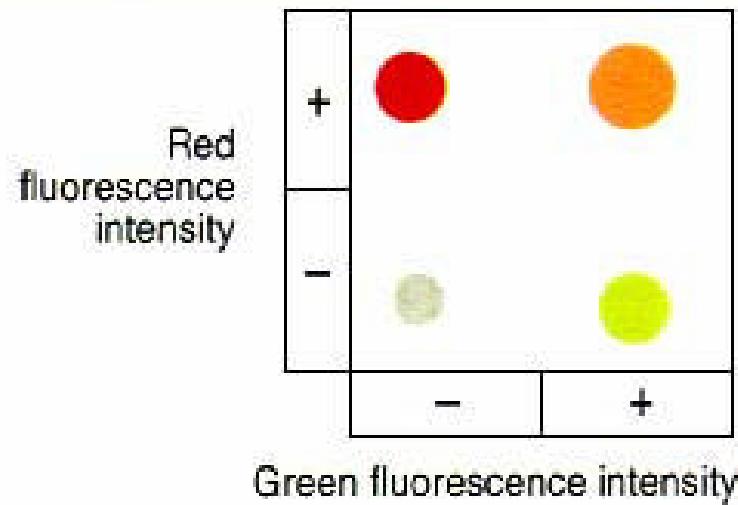
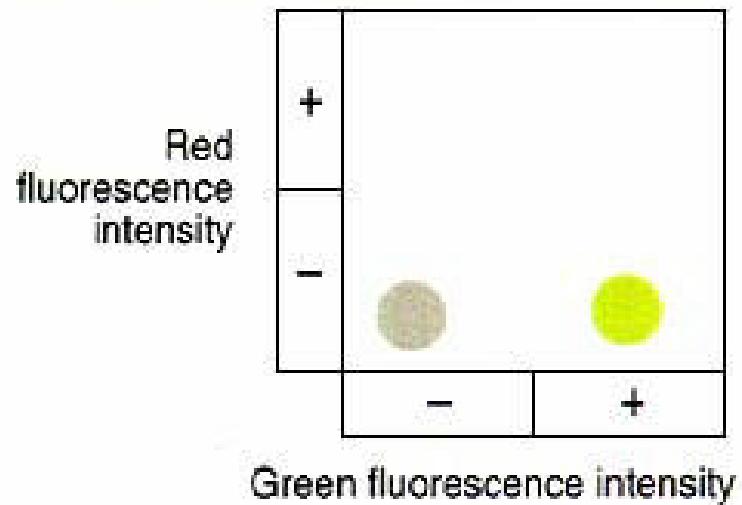
Green antibody



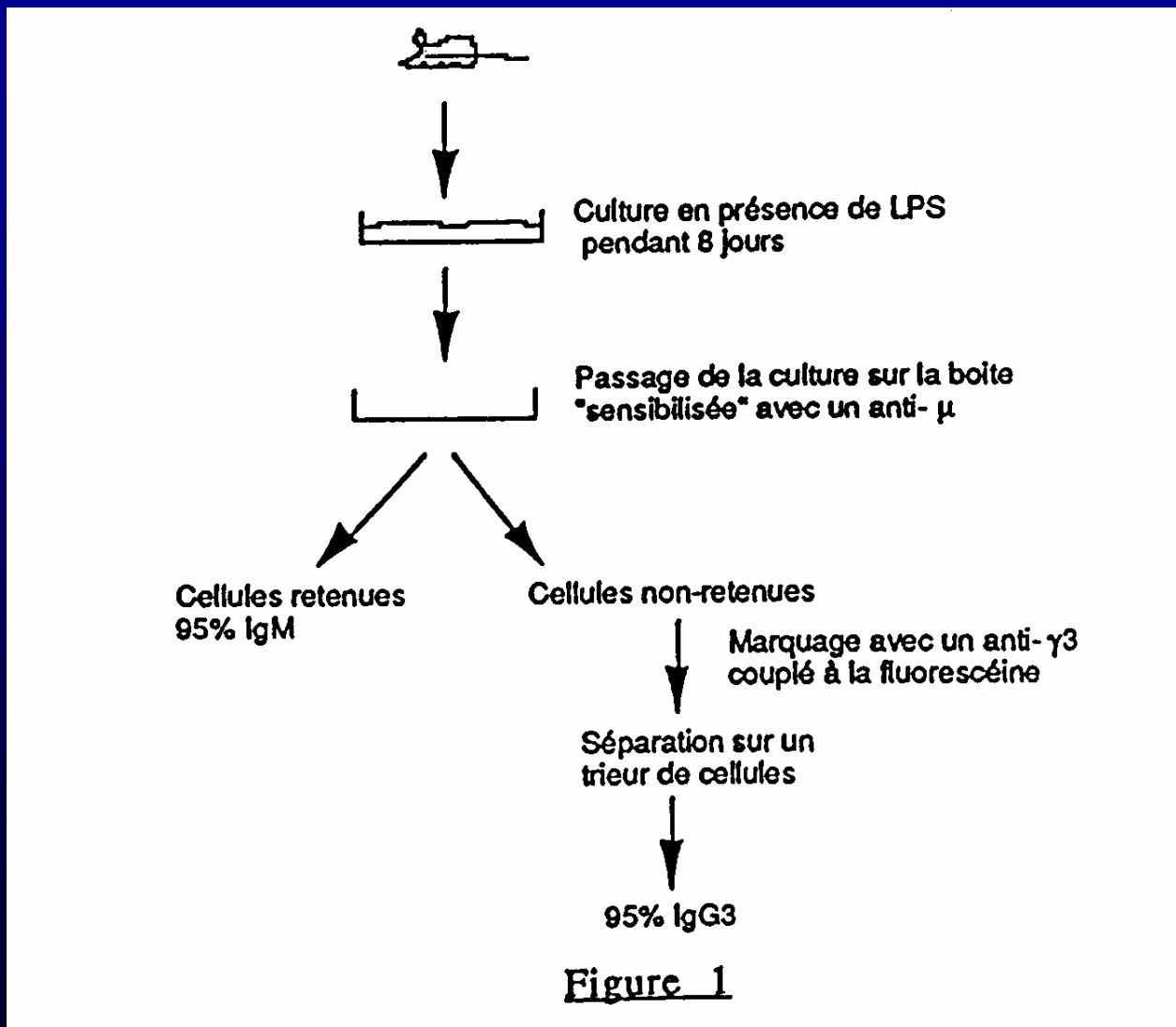
Fluorescence intensity

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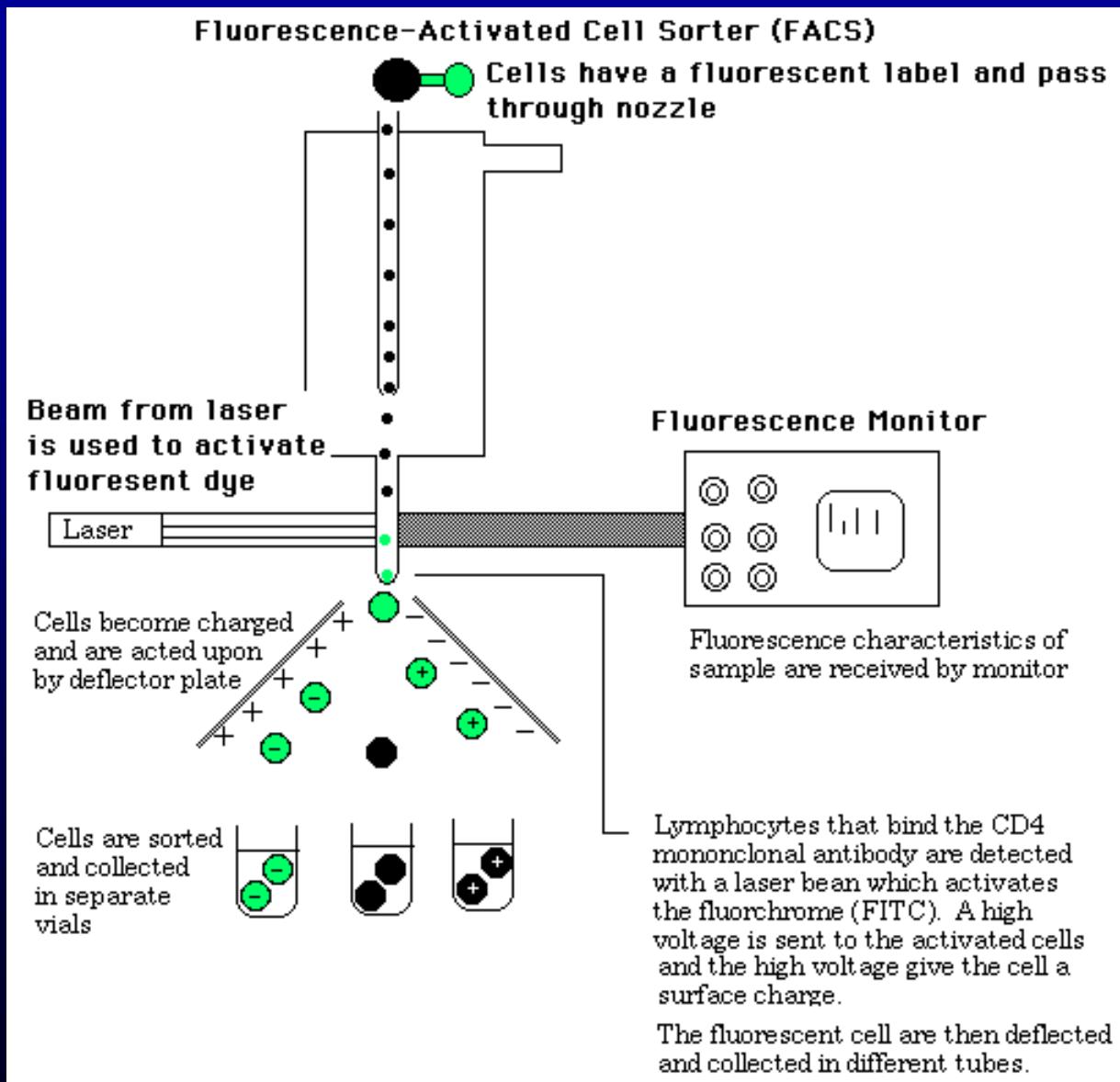
# Cytométrie de flux (3)



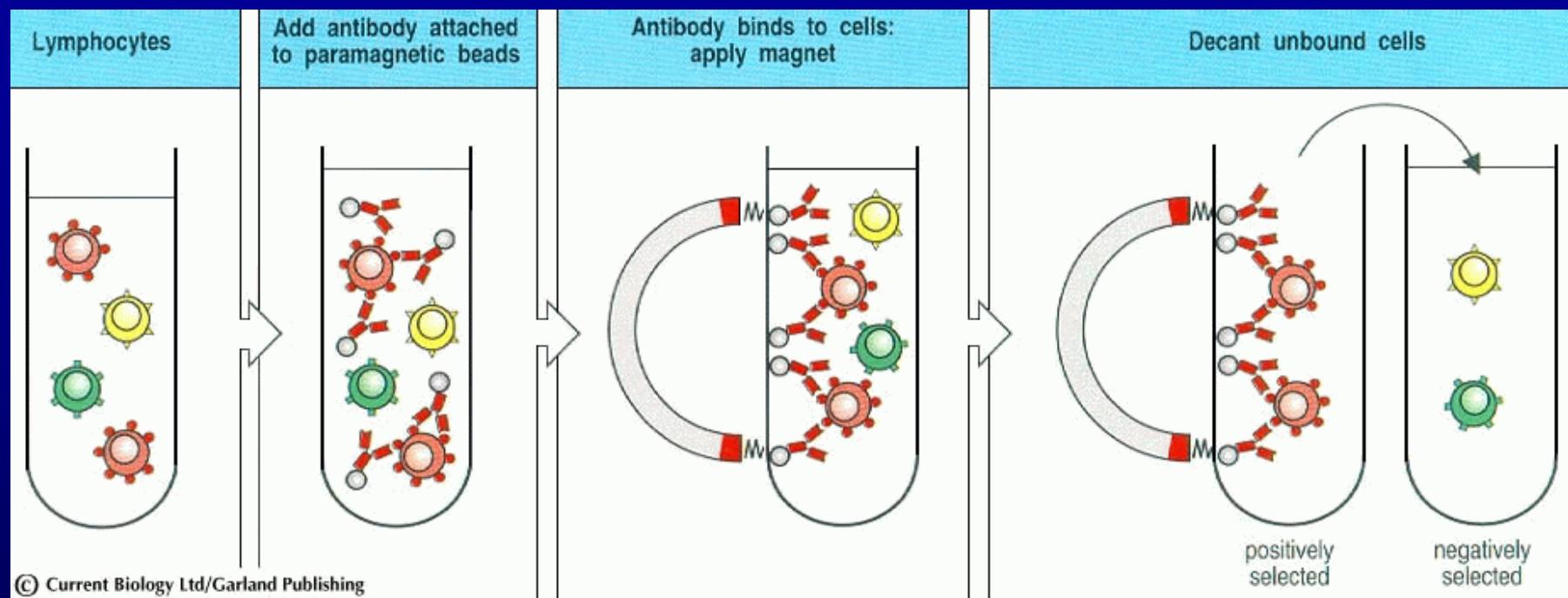
# Tri cellulaire par « panning »



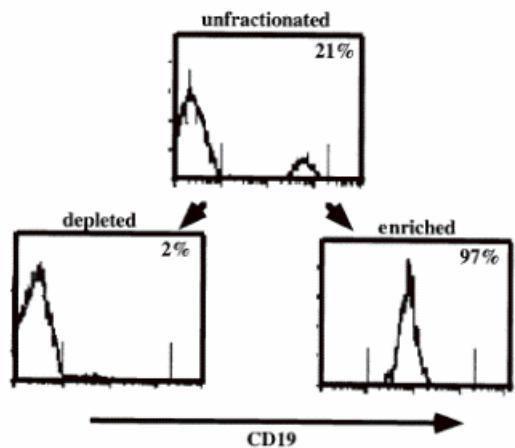
# Tri cellulaire par cytométrie



# Tri cellulaire magnétique



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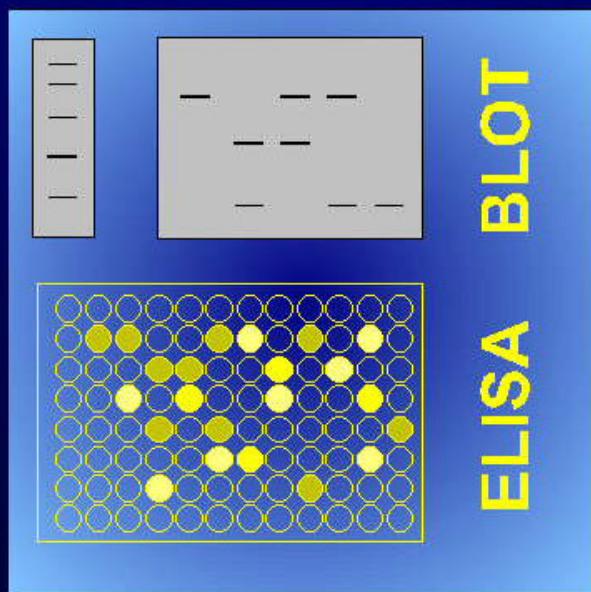


# Cartographie épitopique (1)

## "Classical" epitope mapping

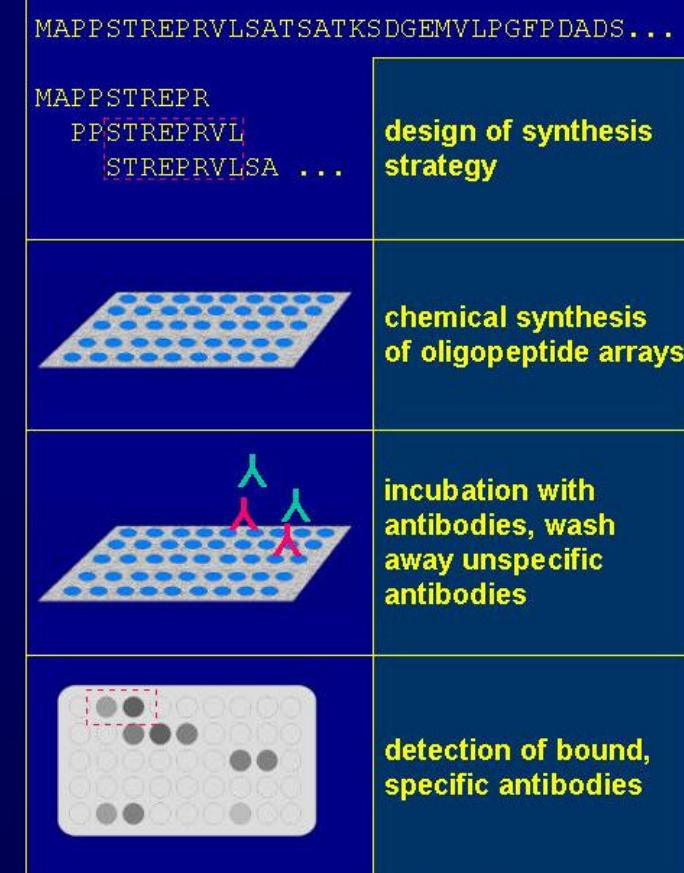
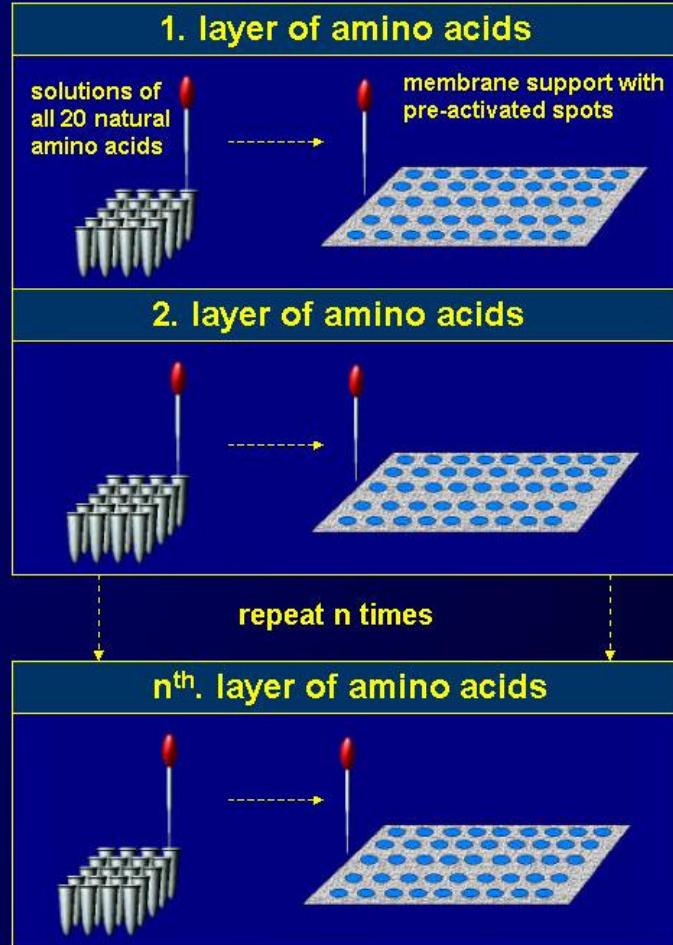


cDNA fragments  
are expressed as  
recombinant proteins

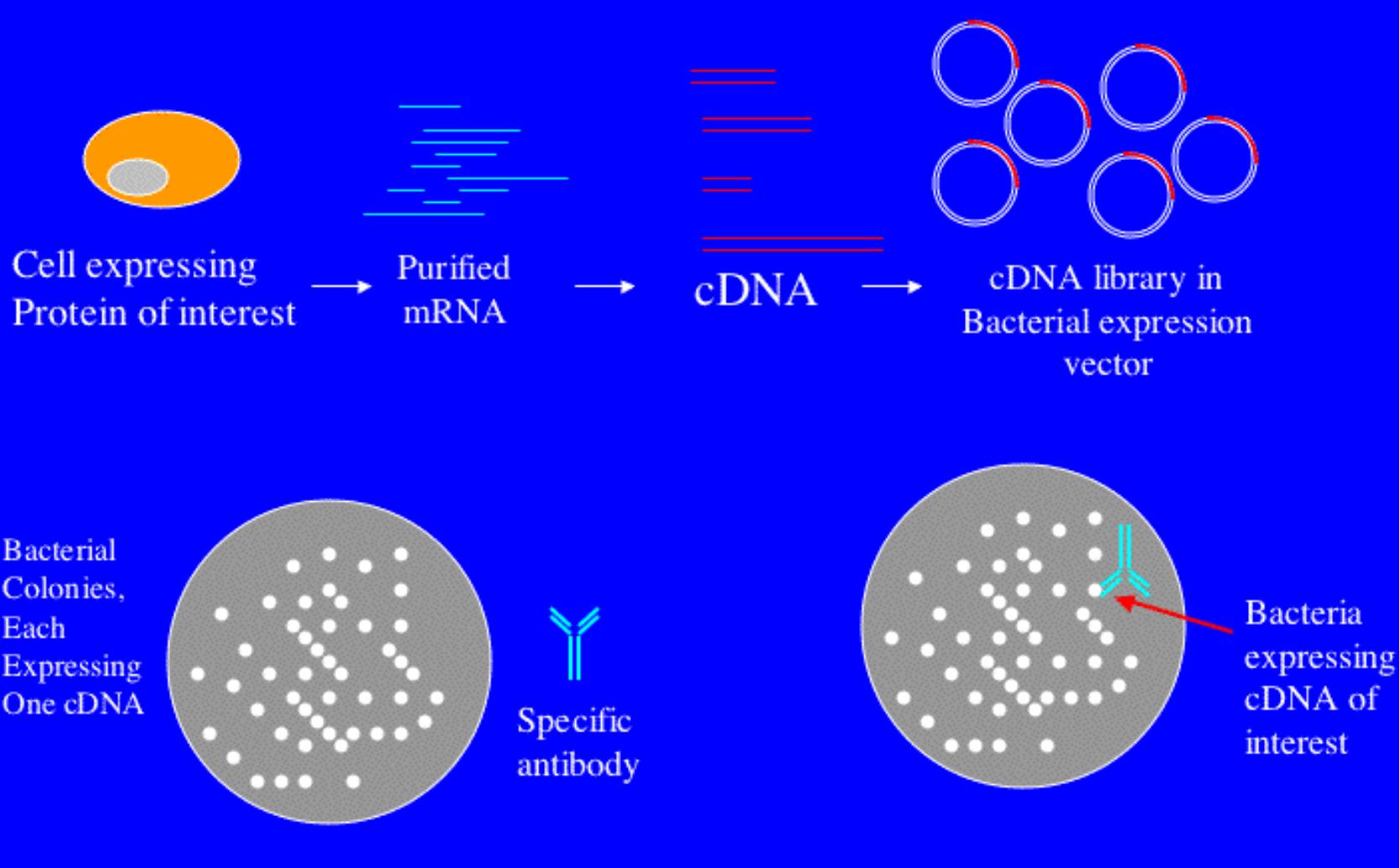


Identification of  
immunoreactive  
recombinant fragments  
by ELISA or Westernblot

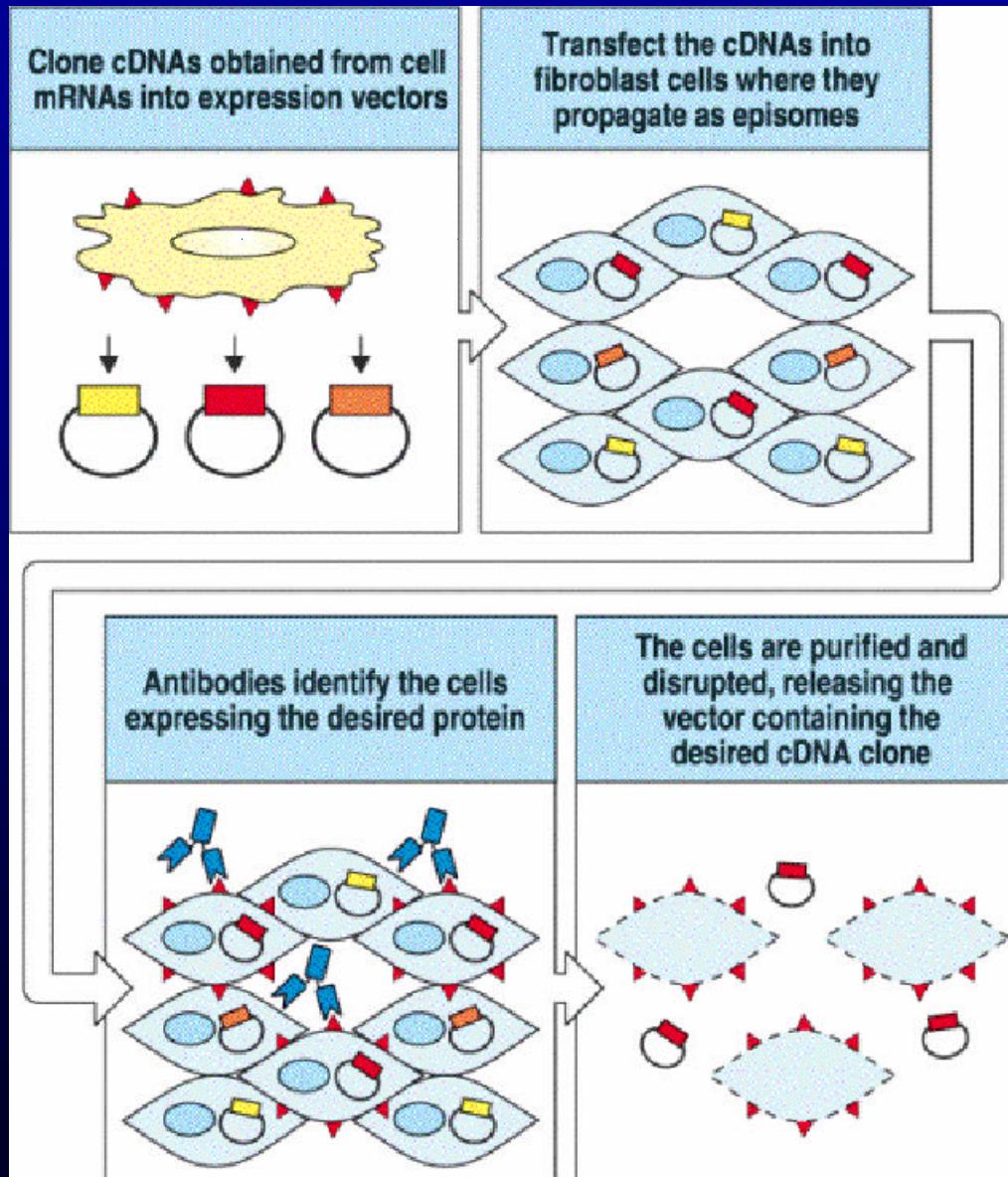
# Cartographie épitopique (2)



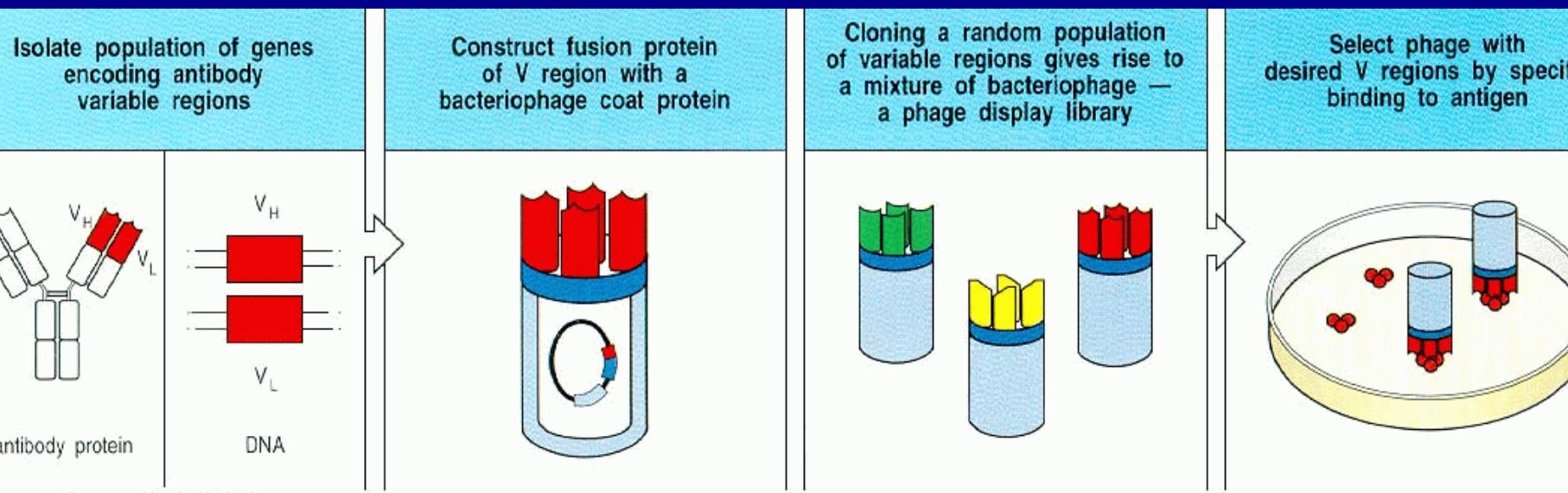
# Clonage et criblage (1)



# Clonage et criblage (2)

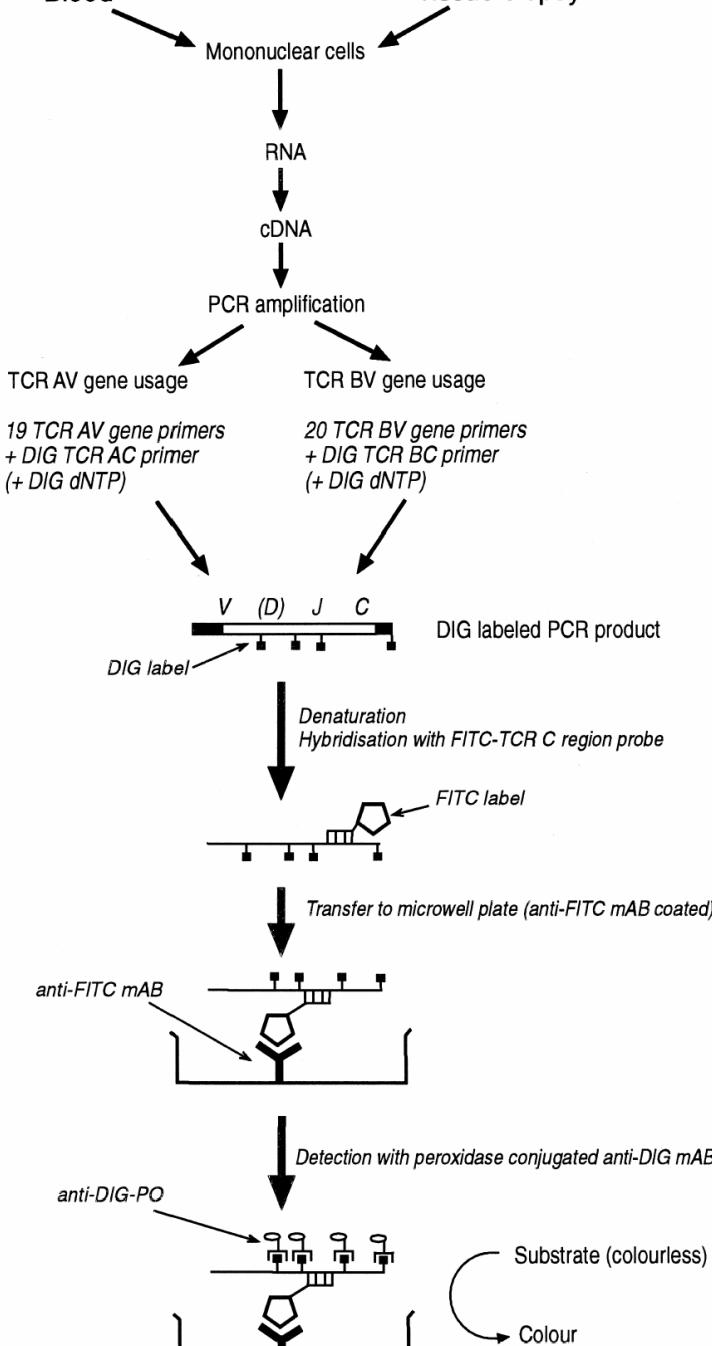


# Clonage et criblage (3)



# PCR-ELISA

PCR

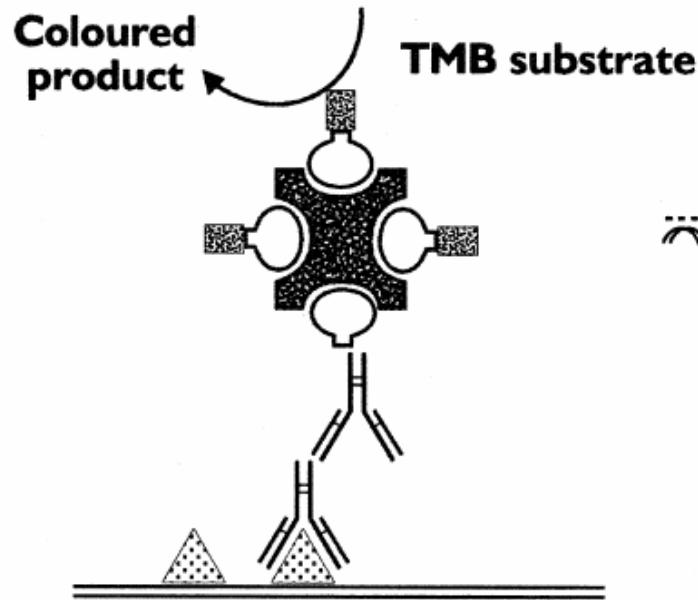


ELISA

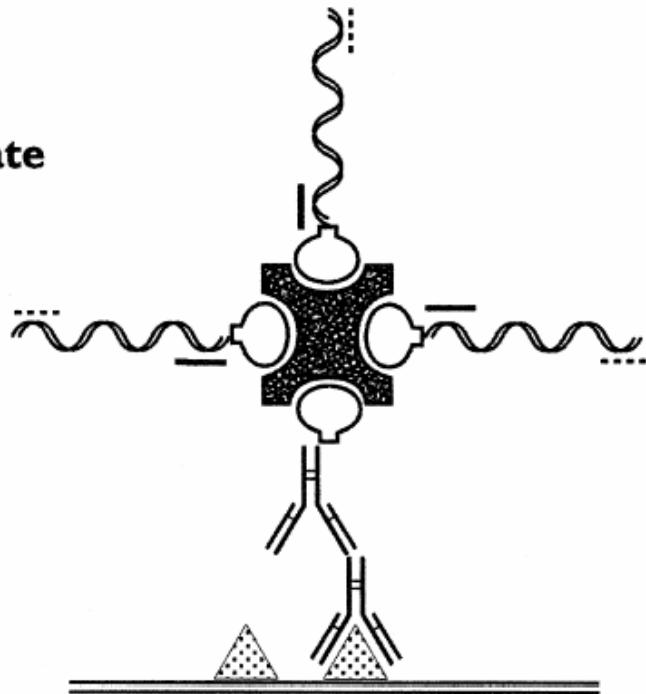
A. VanderBorgh et al. (1999)  
J. Immunol. Methods 223:47.

# Immuno-PCR

## ELISA



## IMMUNO-PCR



Key:



Biotin



Antigen



NeutrAvidin



Horseradish peroxidase

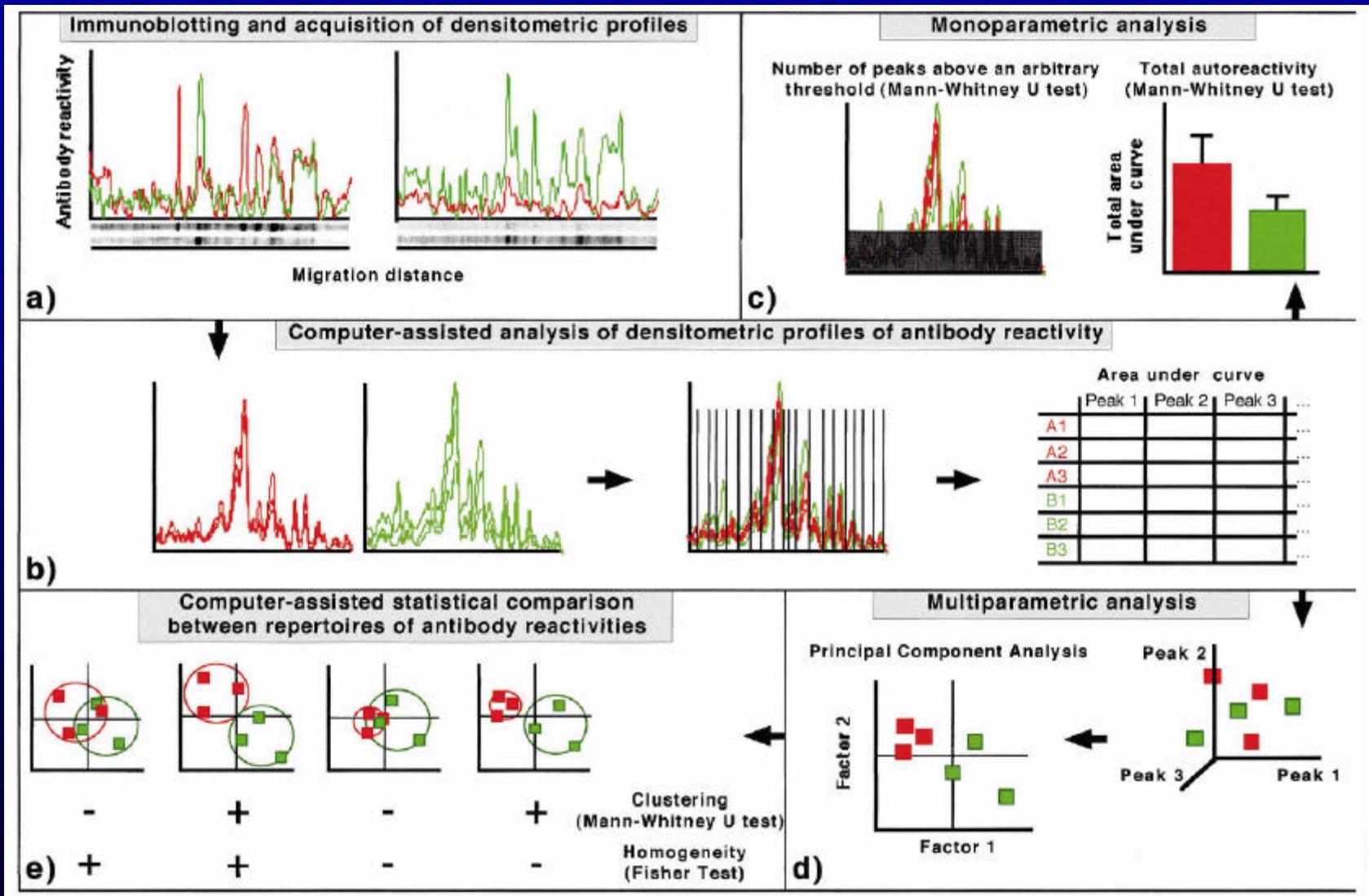


Forward primer



Reverse primer

# Panama-blot



# 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.