

GEAI 2016



René Louis HUMBEL
Luxembourg

COMMENT RECHERCHER LES AUTOANTICORPS

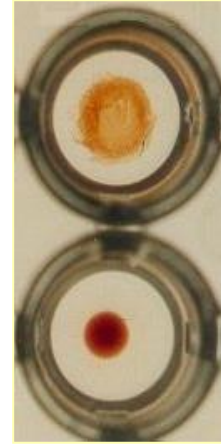
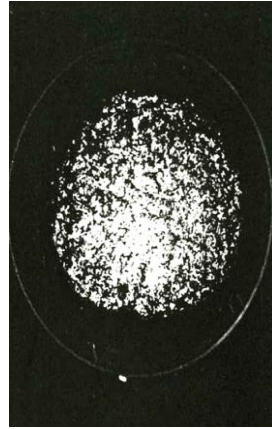
CHOIX ET PERFORMANCES DES METHODES

METHODES DE RECHERCHE DES AUTOANTICORPS

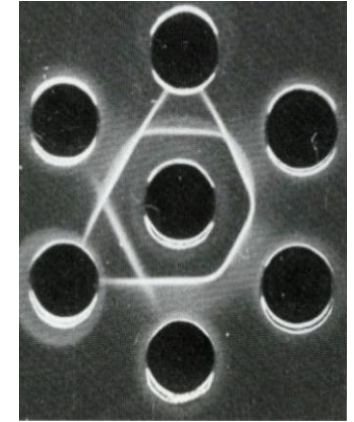
HEMOLYSE



AGGLUTINATION



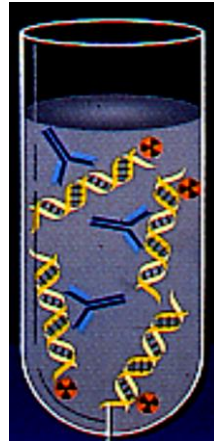
PRECIPITATION



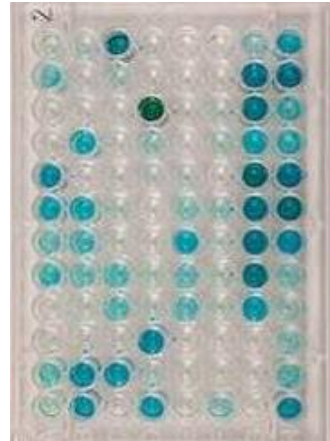
IMMUNOFLUORESCENCE



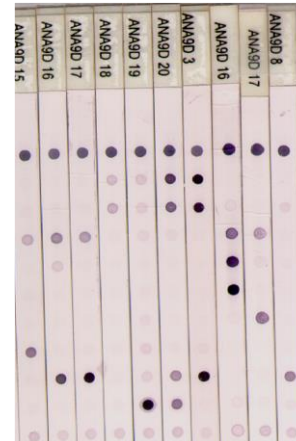
RIA



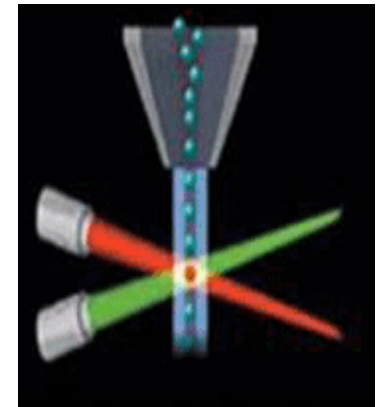
ELISA



DOT



LUMINEX



METHODES DE RECHERCHE DES AUTOANTICORPS

IMMUNOFLUORESCENCE



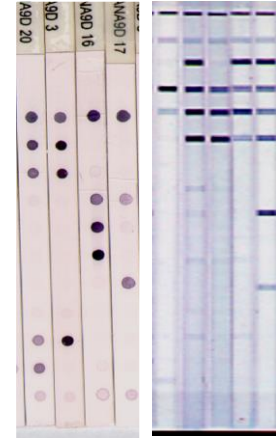
RIA



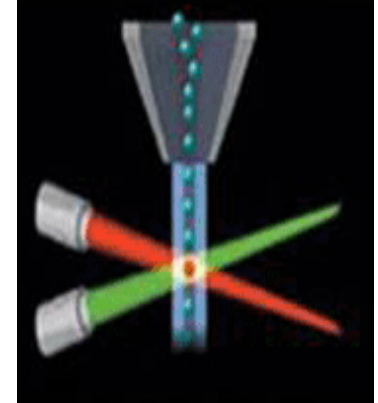
ELISA



DOT

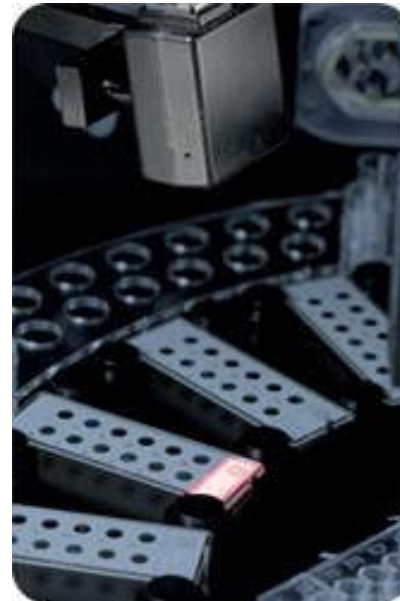


LUMINEX



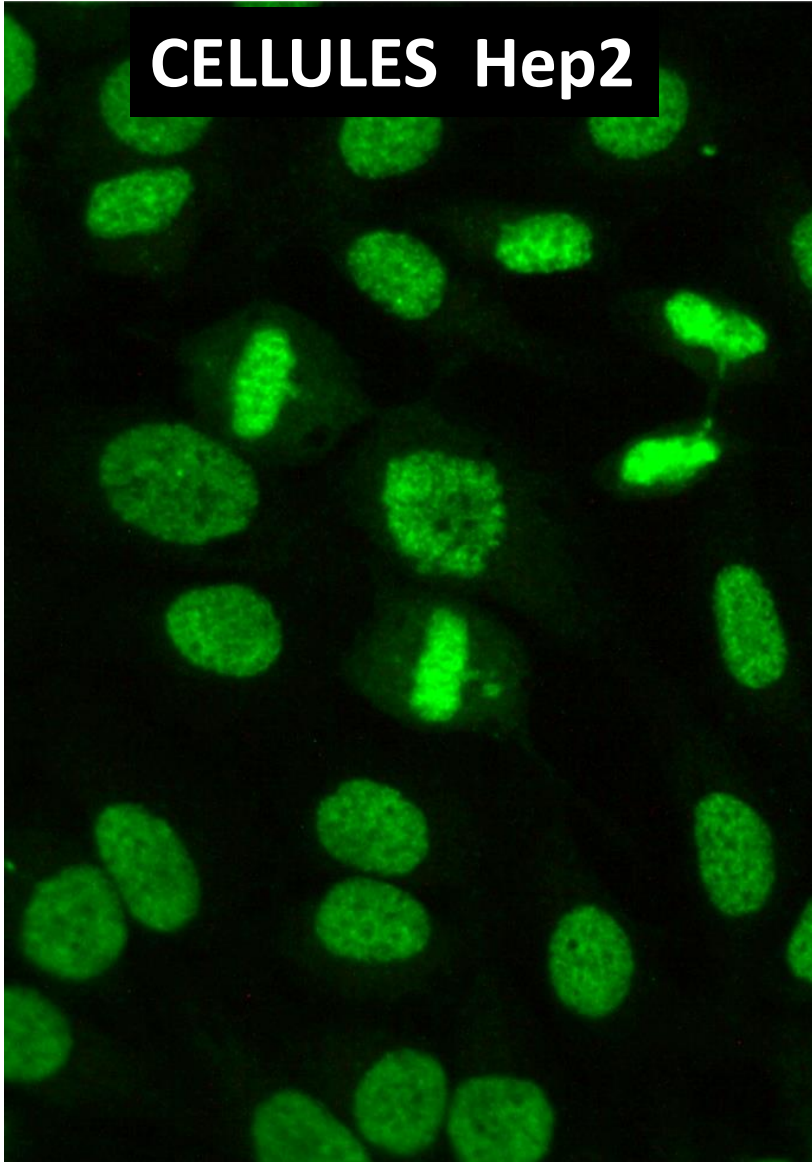
Culture Cell.HEp2
Etalements Cellules
Coupes d'organes

Cellules Transfectées

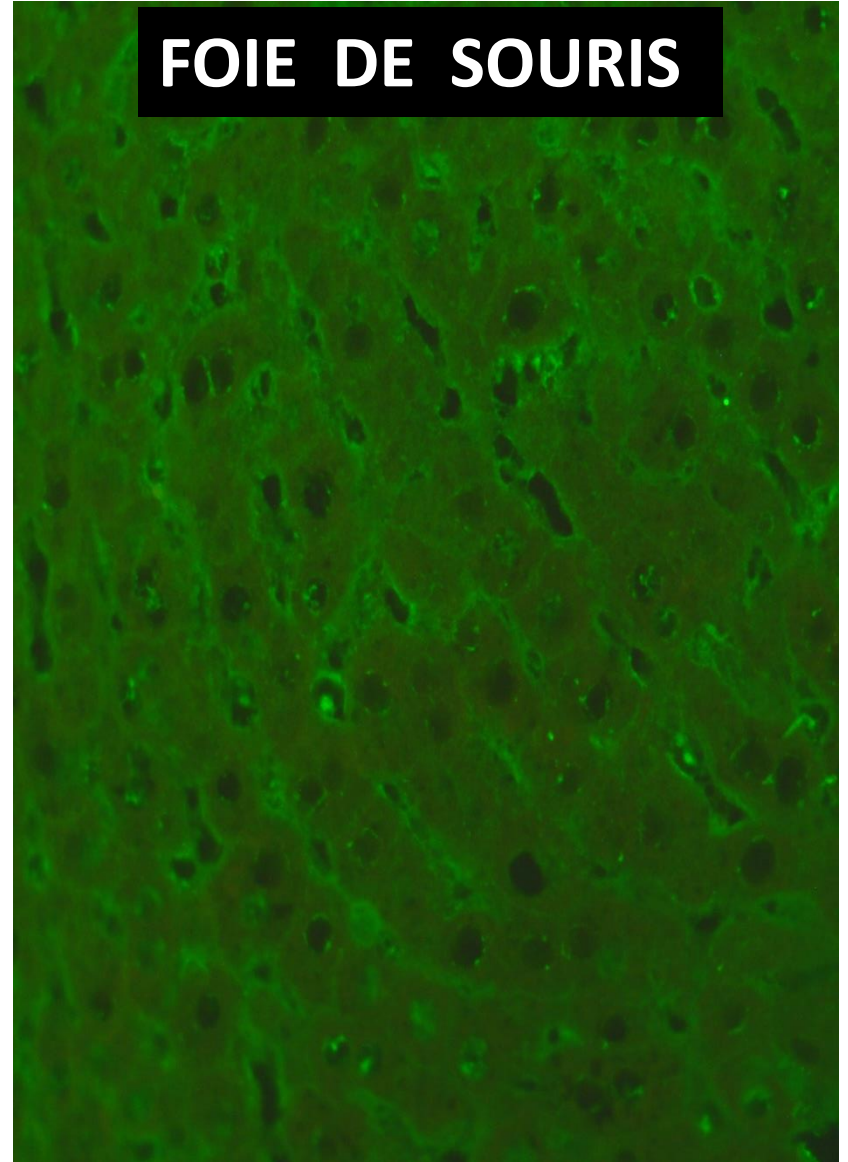


ANTICORPS ANTI-DFS 70

CELLULES Hep2

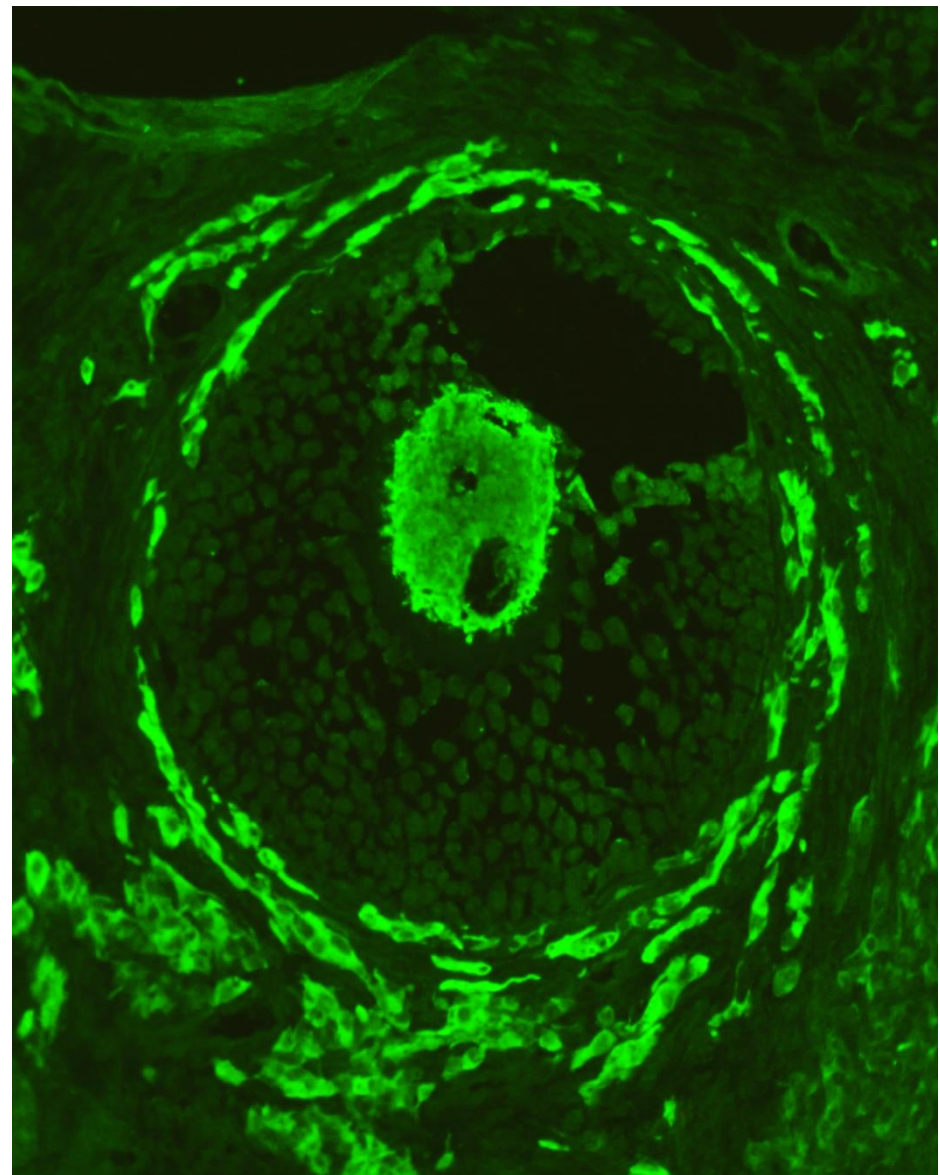
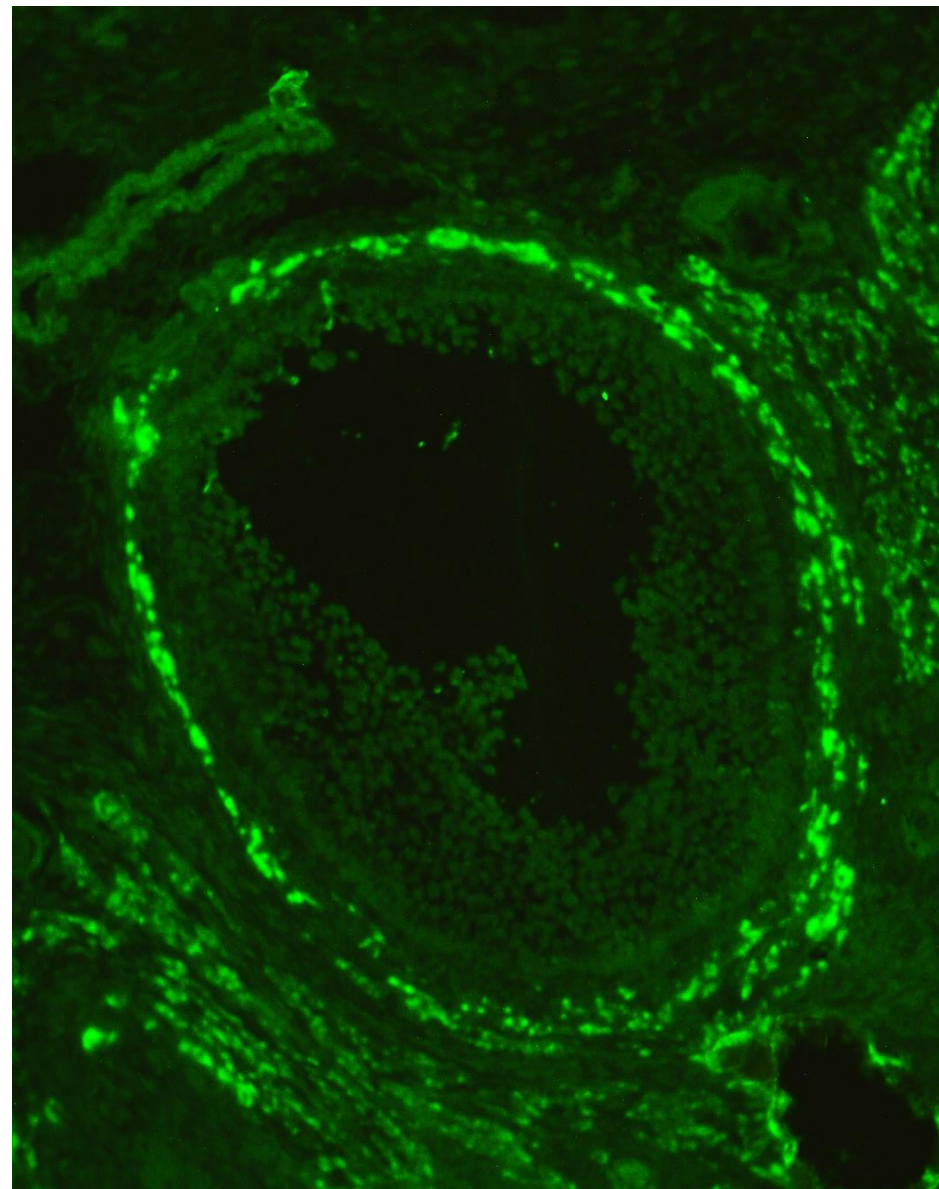


FOIE DE SOURIS



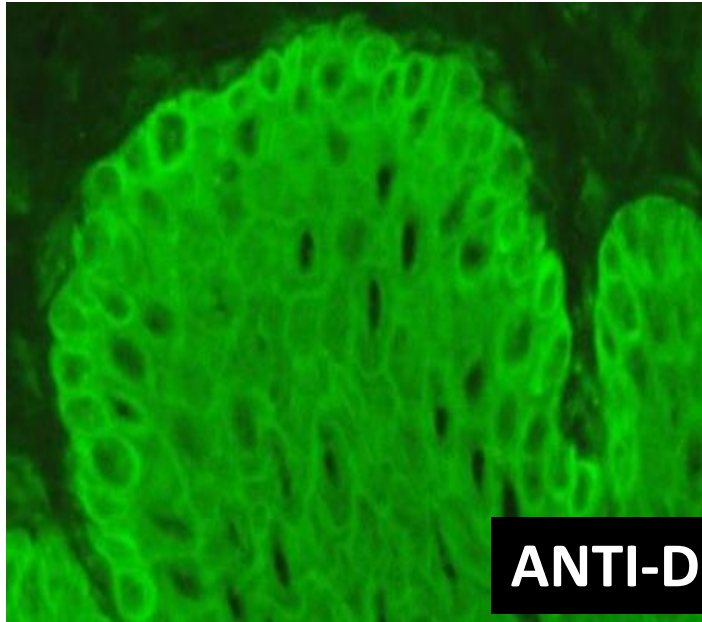
ANTICORPS ANTI - OVAIRES

OVAIRE DE SINGE

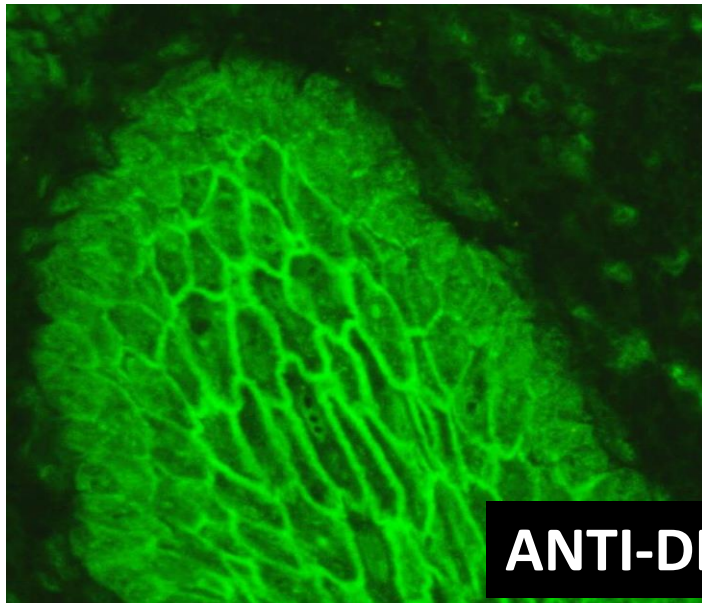
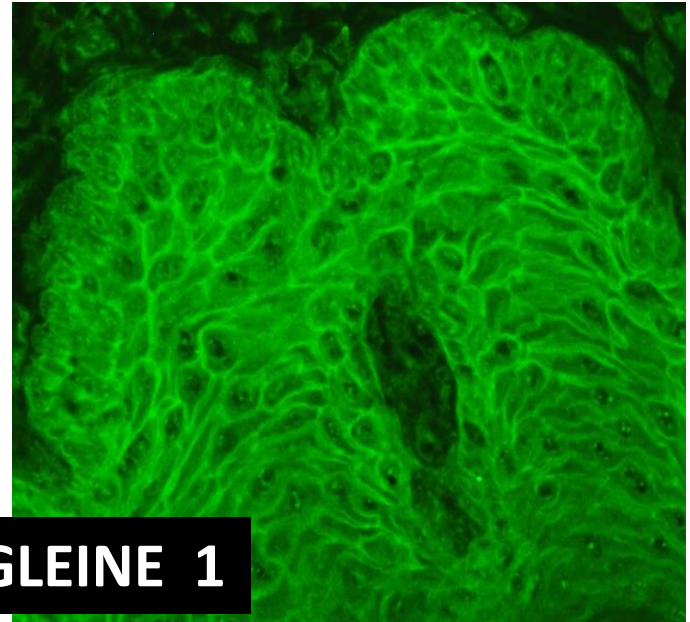


ŒSOPHAGE DE SINGE

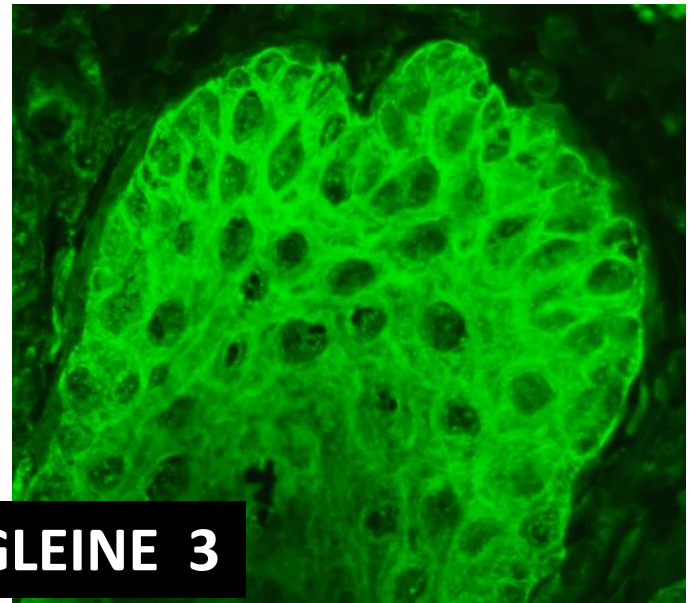
ŒSOPHAGE DE COBAYE



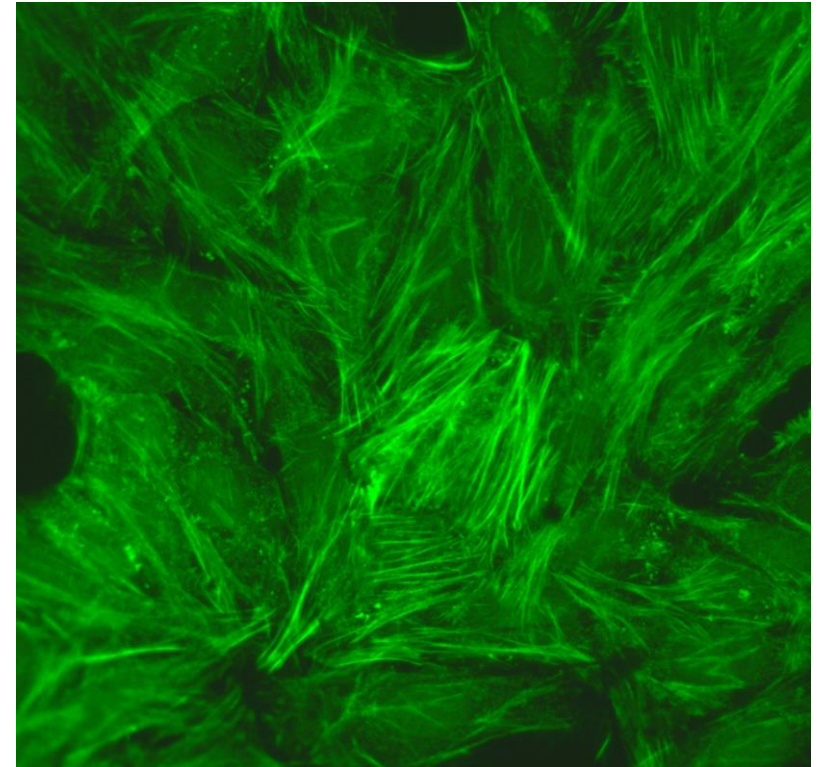
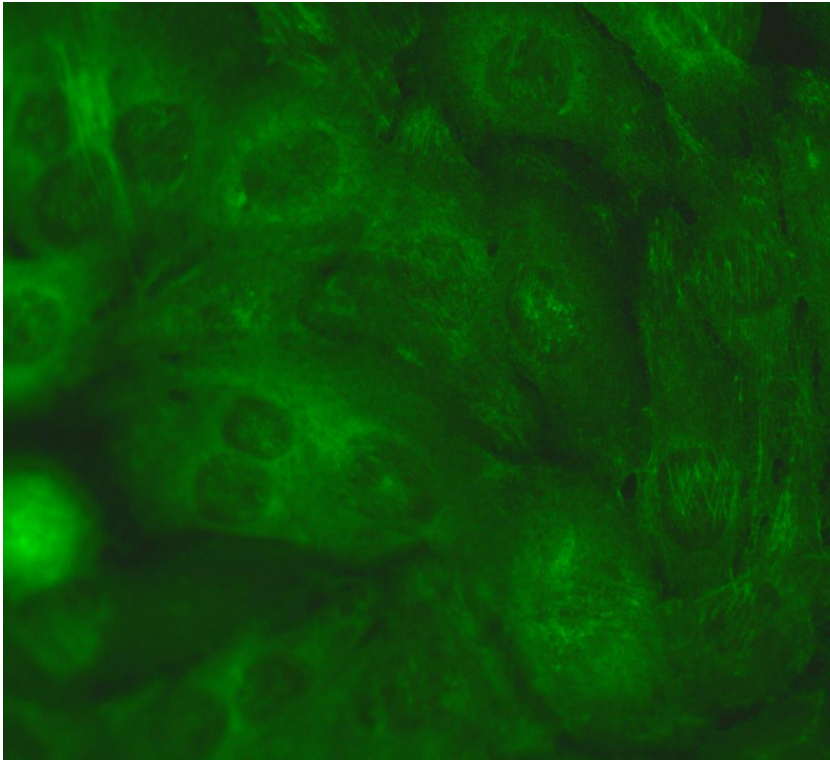
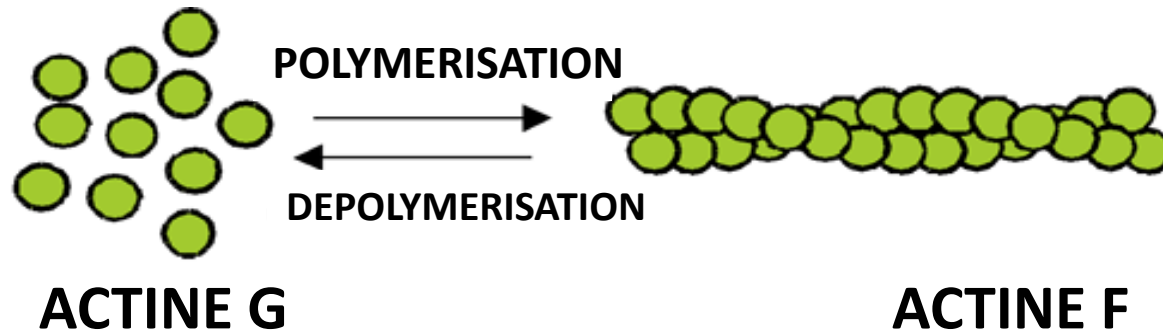
ANTI-DESMOGLEINE 1



ANTI-DESMOGLEINE 3



ANTICORPS ANTI-ACTINE



ANTICORPS ANTI-ACTINE

α ACTINE

MUSCLE CARDIAQUE

β ACTINE

CEL.EPITHELIALES

γ ACTINE

MUSCLE LISSE

ESTOMAC SOURIS

γ 1 ACTINE

CELL. HEp2

β ACTINE

CELL. INTESTINALES

γ 2 ACTINE

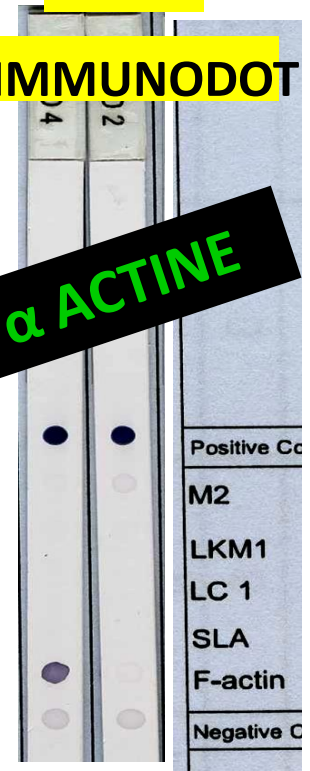
CELL. VSM47 AORTE

α ACTINE

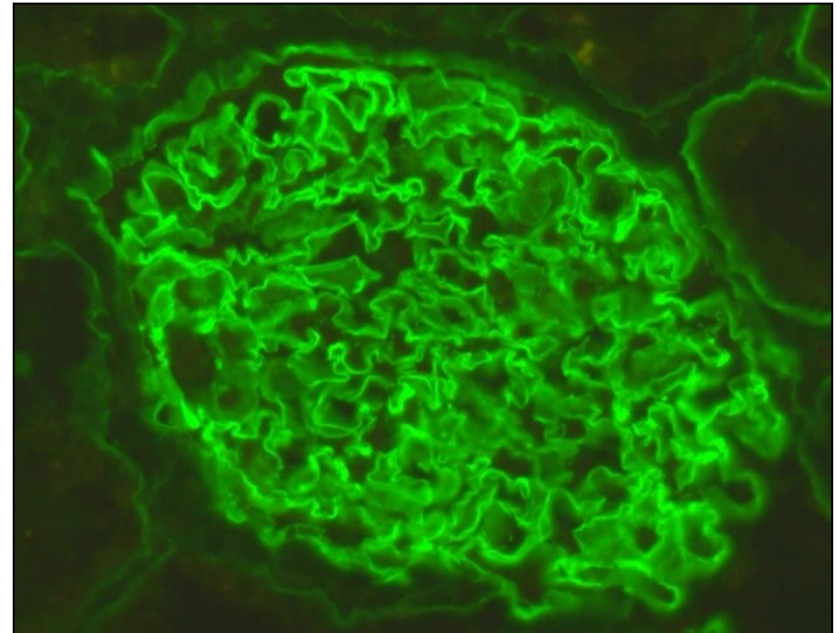
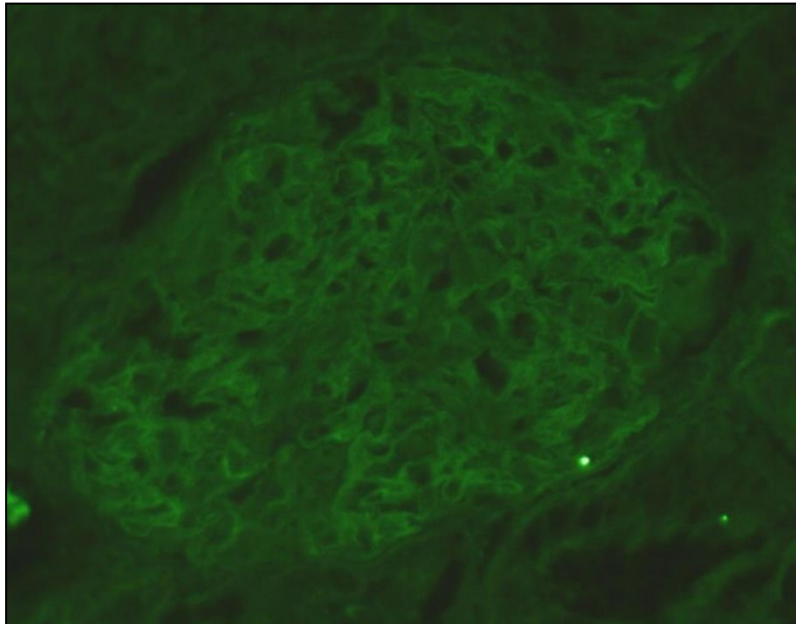
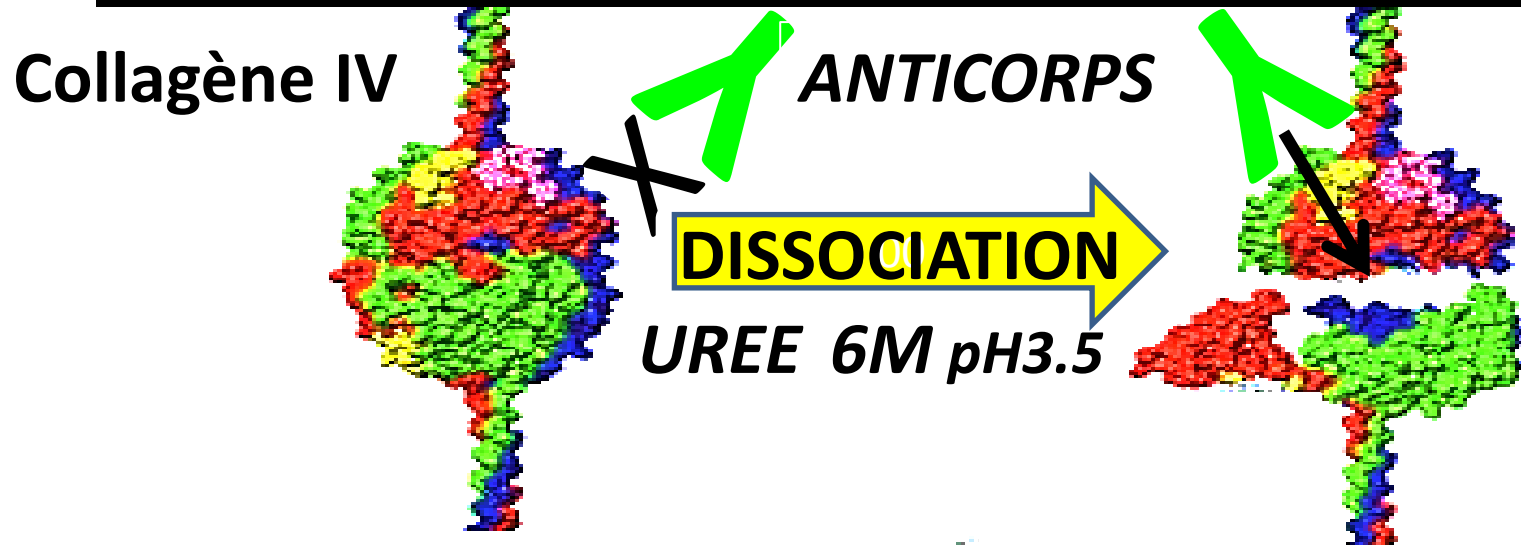
ELISA

IMMUNODOT

α ACTINE



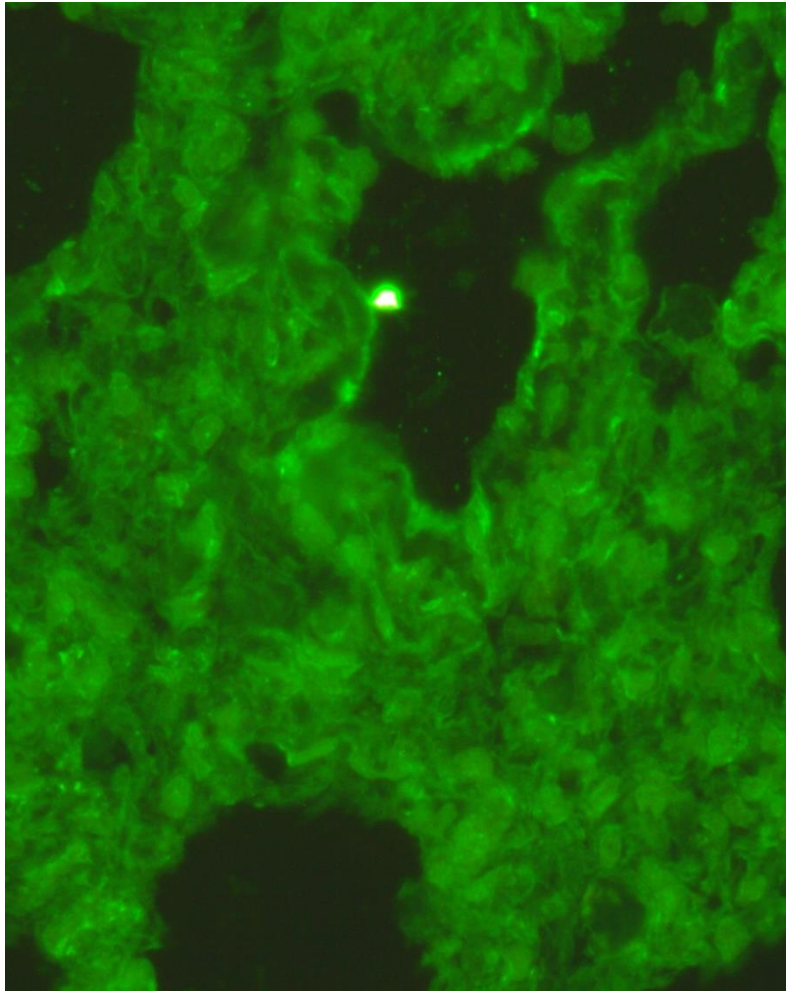
ANTICORPS ANTI - MEMBRANE BASALE GLOMERULAIRE



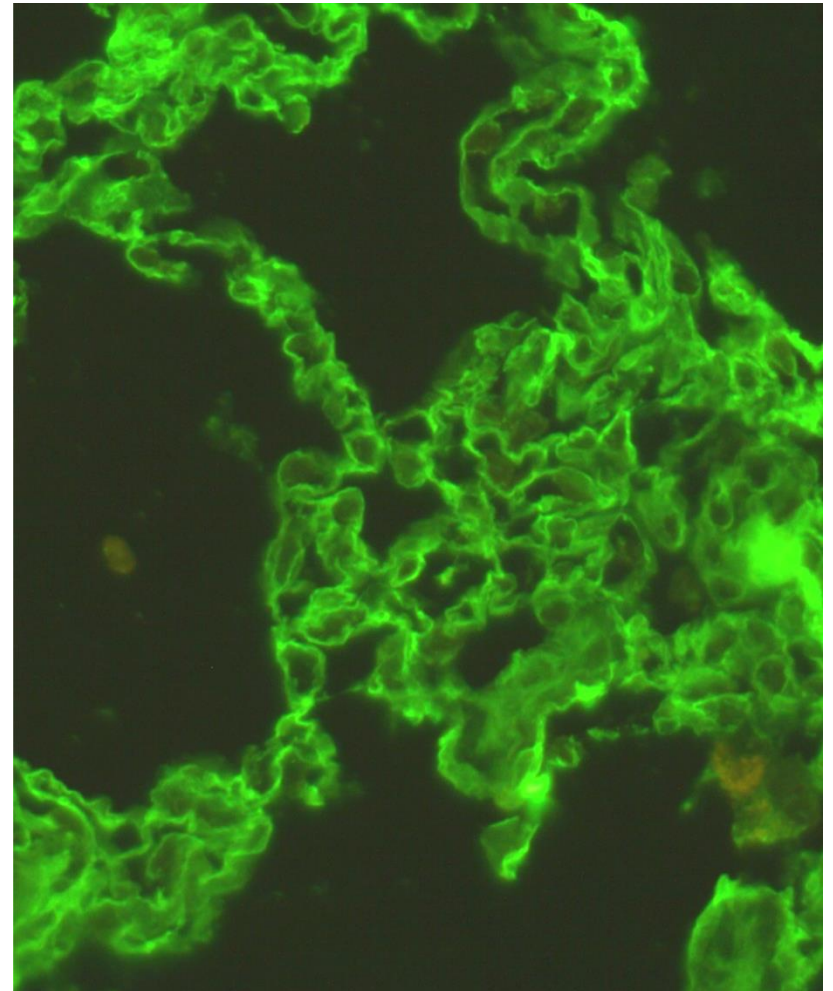
ANTICORPS ANTI - ALVEOLE PULMONAIRE

COUPES DE POUMON DE SINGE

Non Traitée



Traitement à l'urée pH 3.5



METHODES DE RECHERCHE DES AUTOANTICORPS

RIA



**ANTI-ADN
(Test de Farr)**

ANTI- GAD et ANTI-IA2

ANTI- RECEPTEUR TSH

ANTI- RECEPTEUR ACETYLCHOLINE

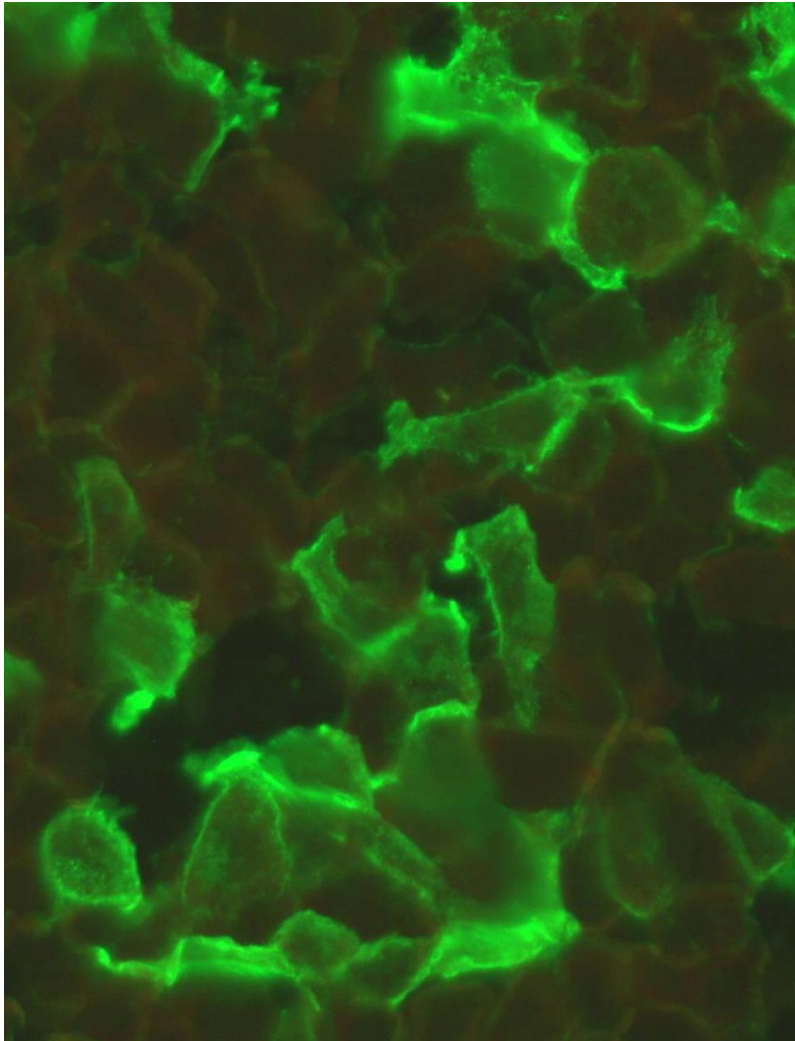
ANTI- Musk

ANTI- CANAUX CALCIQUES

ANTI- CANAUX POTASSIQUES

IF SUR CELLULES TRANSFECTES

ANTICORPS ANTI - PROTEINES MEMBRANAIRES



**RECEPTEURS GLUTAMATE
NMDAR , AMPAR**

**RECEPTEURS GABA A et B
VGKC-LGI1 - CASPR2
RECEPTEURS GLYCINE**

**AQUAPORINE 4
MOG**

***RECEPTEUR ACETYLCHOLINE
MUSK***

ANTICORPS ANTI-NUCLEAIRES

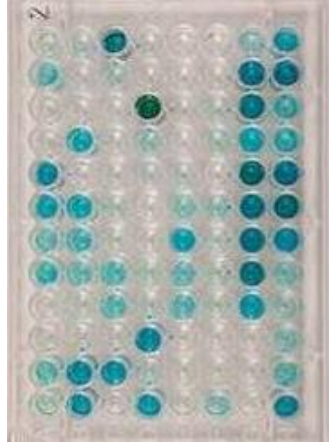
IMMUNOFLUORESCENCE



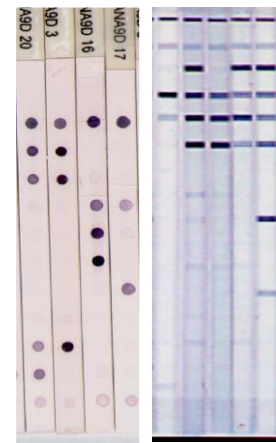
RIA



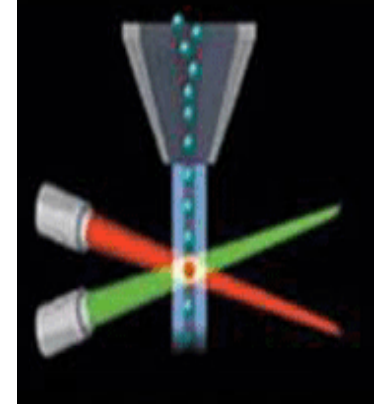
ELISA



DOT



LUMINEX



Cell.HEp2 : 65

40

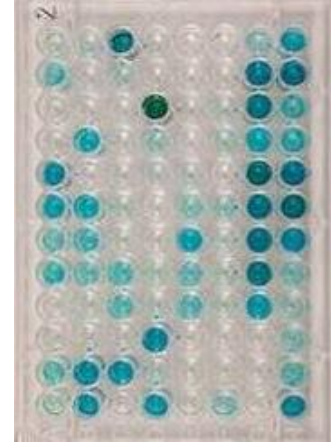
AUTOANTICORPS NON DETECTES EN IF

	ELISA	DOT	IF Cell.Transf.
SLA	+	+	+
Facteur Intrinsèque	+	+	+ (RIA)
Protéines Citrullinées	+	+	-
Gangliosides	+	+	-
Phospholipides	+	+	-
PLA2R	+	-	+
Cochlée (Po)	-	+	-
Rétine (Récovérine)	-	+	-
Récepteurs TSH	+	-	- (RIA)
Récepteurs Acétylcholine	+	-	+ (RIA)
Musk	+	-	+ (RIA)
Canaux Potassiques	-	-	+ (RIA)
Canaux Calciques	-	-	- (RIA)
MOG	-	-	+
Récepteurs Neurones	-	-	+

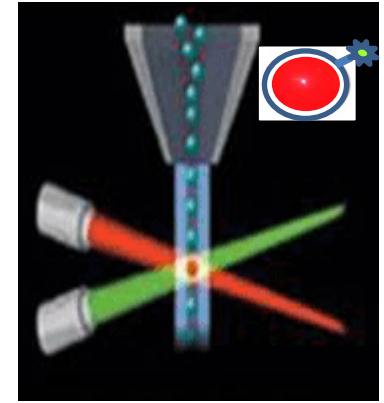
METHODES DE RECHERCHE DES AUTOANTICORPS

METHODES IMMUNOENZYMATIQUES EN PHASE SOLIDE

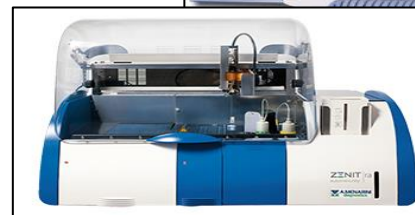
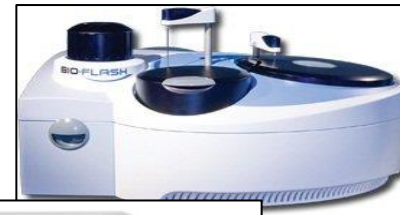
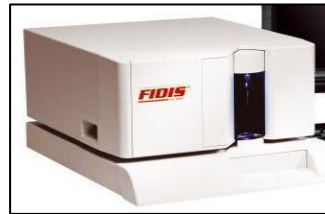
ELISA



LUMINEX

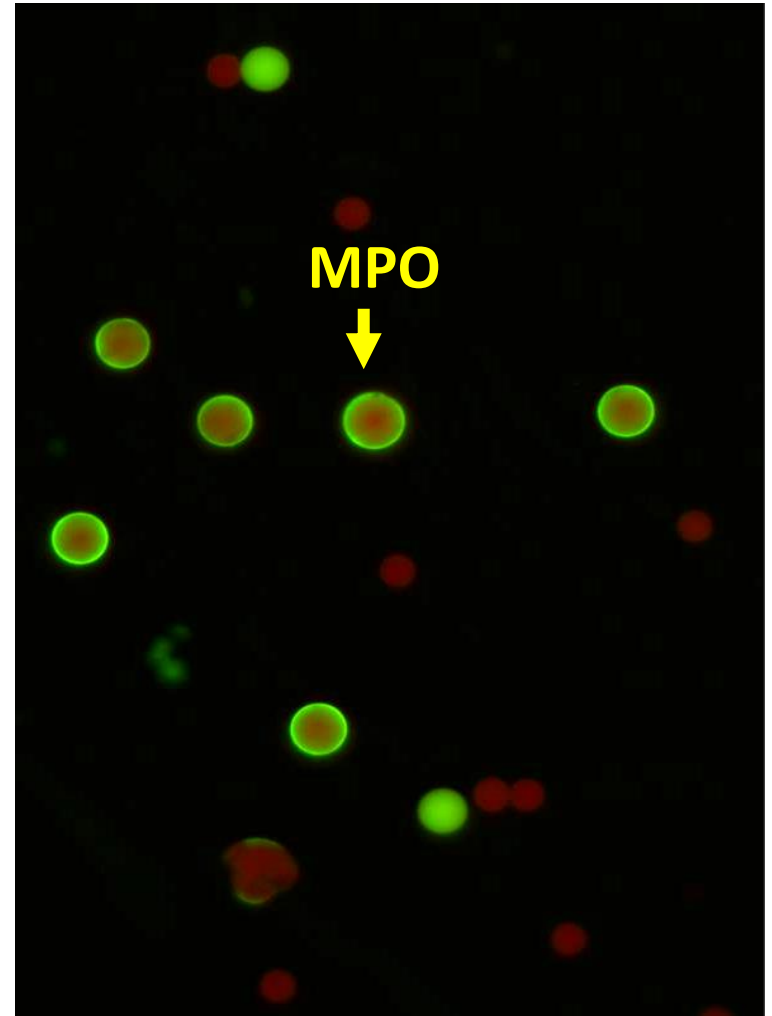
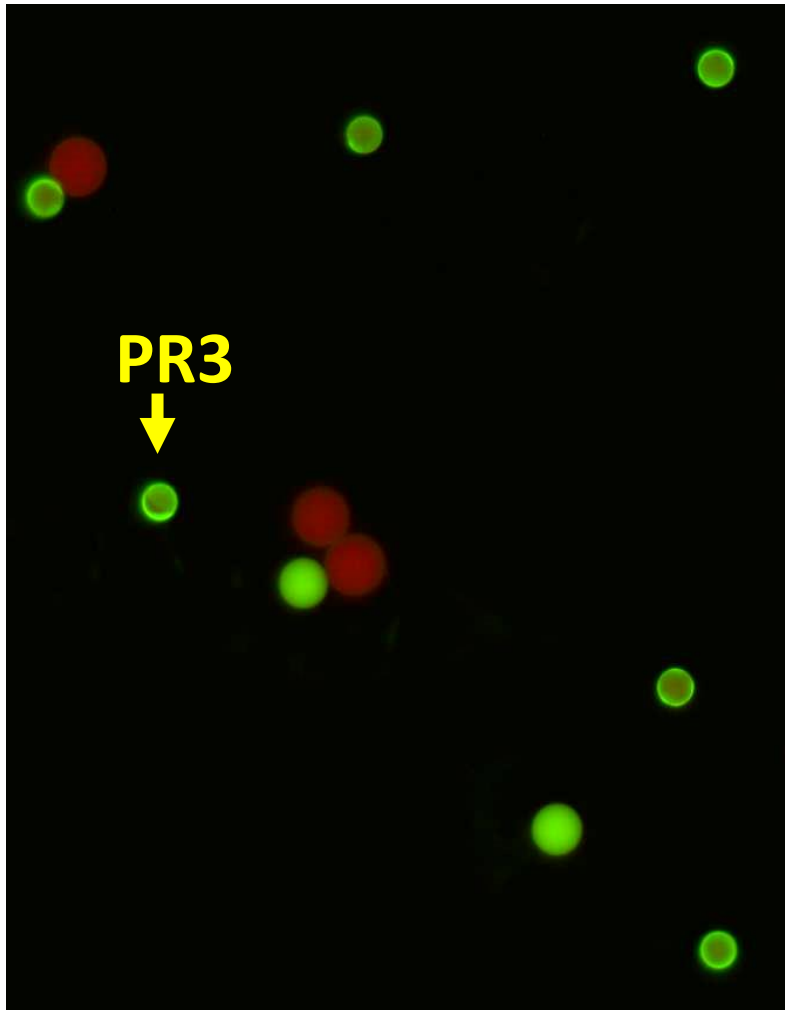


Spectrophotométrie
Fluorimétrie
Chimiluminescence



IMMUNOFLUORESCENCE SUR MICROBILLES

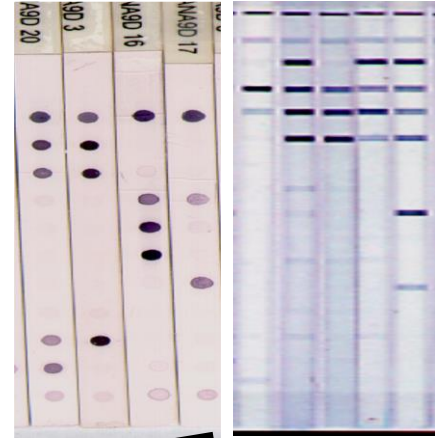
ANCA , ANA , MBG , Celiac ,



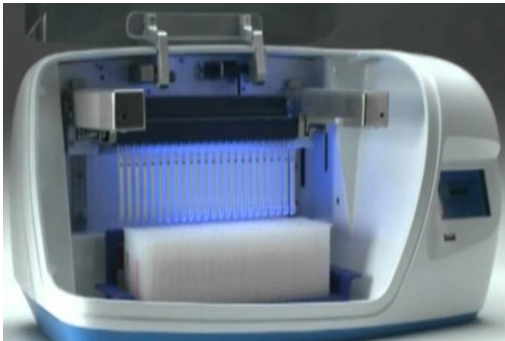
METHODES DE RECHERCHE DES AUTOANTICORPS

DOT

METHODES IMMUNOENZYMATIQUES EN PHASE SOLIDE



DETECTION SIMULTANEE DE PLUSIEURS ANTICORPS SPECIFIQUES



DOT PAS BLOT !!

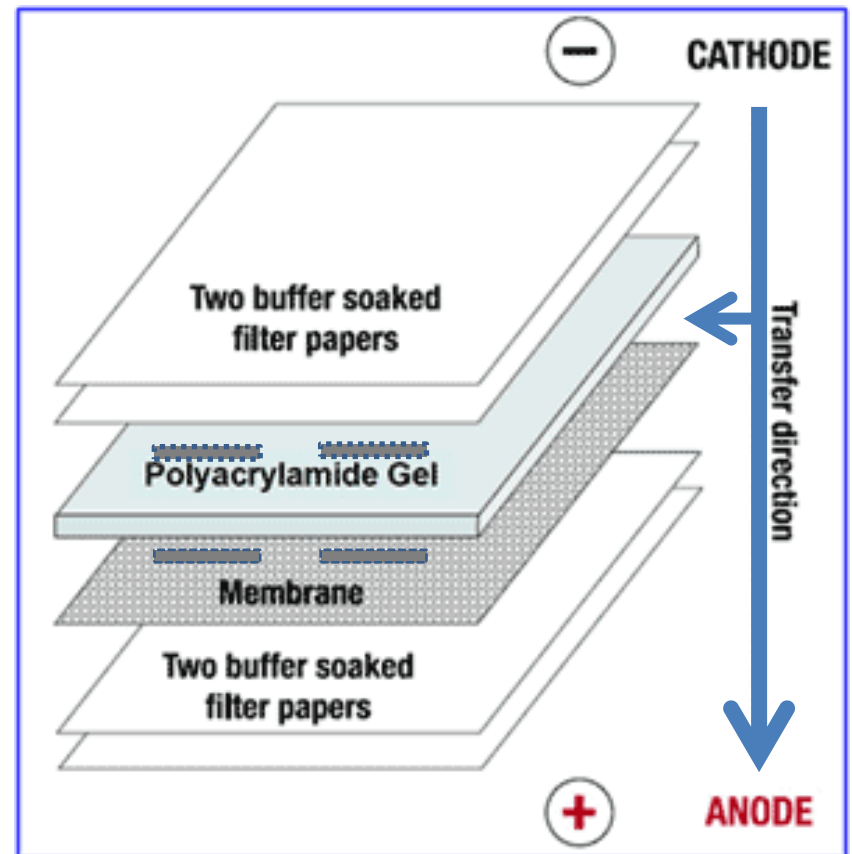
DOT

DEPOT DIRECT



BLOT

TRANSFERT



METHODES

ELISA

EliA

Alegria

QUANTA FLASH

BIOPLEX

MULTI LYTE

FIDIS

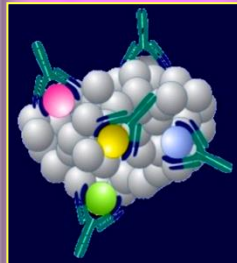
ZENIT

IMMUNOSTRIP

IMMUNODOT

MICROARRAY

REACTIFS

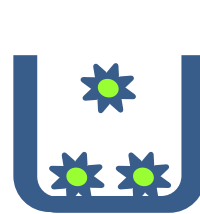


ANTIGENE

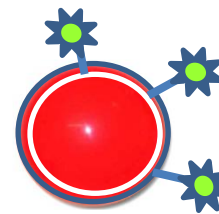


NATIF **RECOMBINANT** **SYNTHETIQUE**
Procaryote / Eucaryote

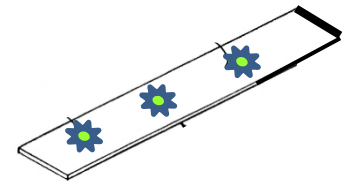
PRESENTATION



ELISA



LUMINEX



DOT

Quantité **Stabilité** **Dénaturation**
Orientation **Moléculaire**
Epitopes : Densité **Accessibilité**

METHODES

ELISA

EliA

Alegria

QUANTA FLASH

BIOPLEX

MULTI LYTE

FIDIS

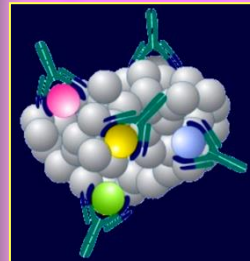
ZENIT

IMMUNOSTRIP

IMMUNODOT

MICROARRAY

REACTIFS



CONDITIONS REACTIONNELLES

Environnement moléculaire

TAMPON

Molarité, pH , Additifs

INCUBATION

Durée , Température



ANTIGLOBULINE

SELECTIVITE

SPECIFICITE

IgG (sous-classes)

IgA, IgM, Protéine A

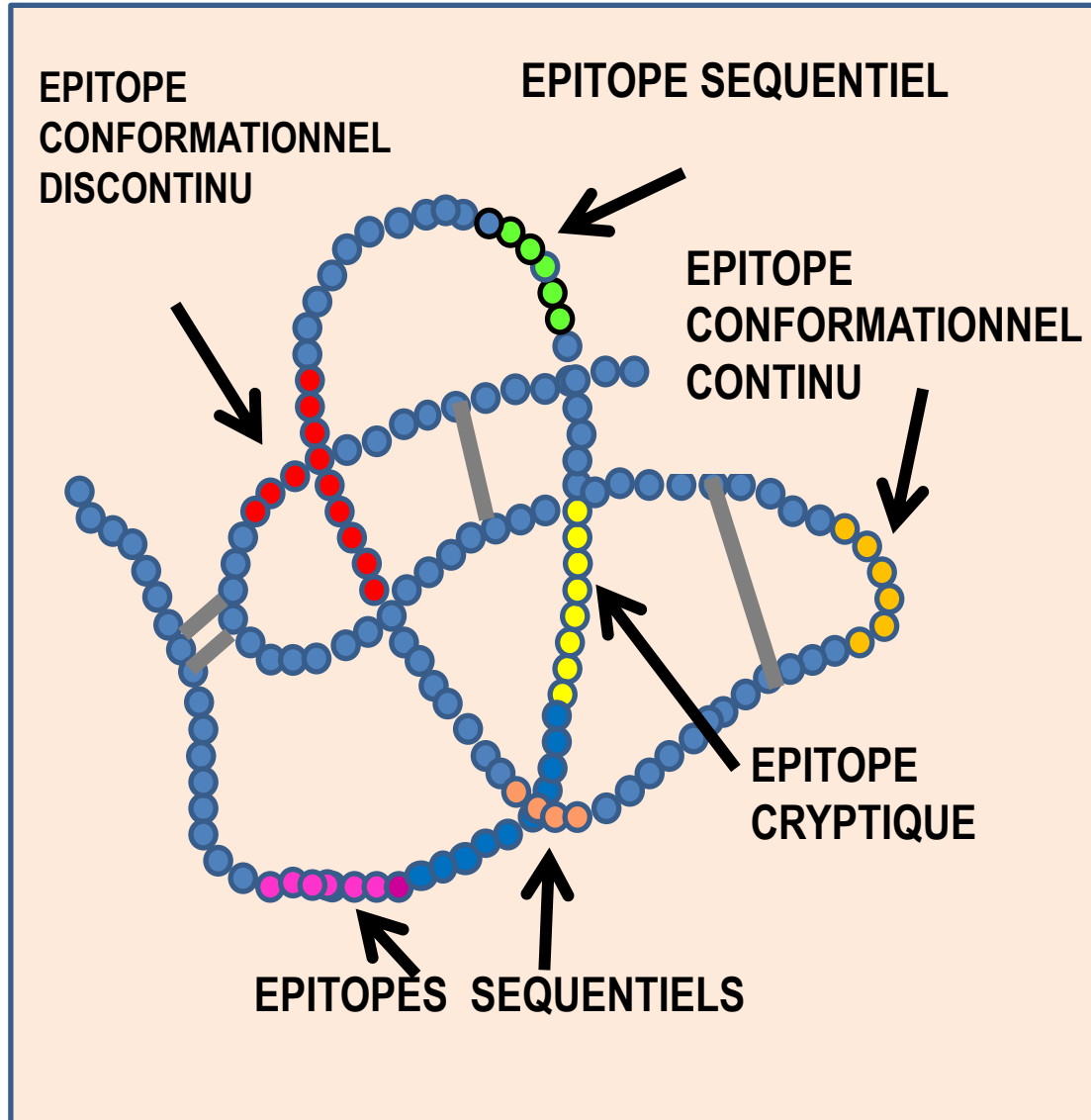
TYPE DE MESURE

Spectrophotométrie

Fluorimétrie

Chimiluminescence

ANTIGENE



ORIGINE

NATURE

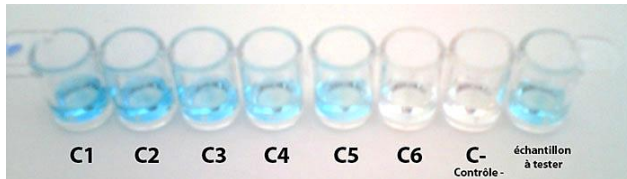
COMPOSITION

PRESENTATION

ANTICORPS ANTI-MYELINE / MAG

ELISA

MAG HUMAINE



BUHLMANN

IMMUNODOT

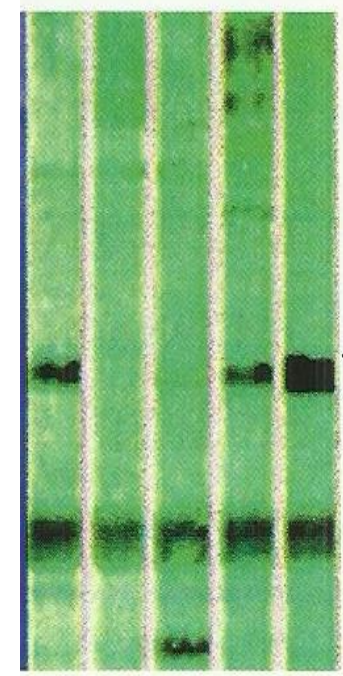
MAG BOVINE



RAVO

WESTERNBLOT

MAG BOVINE



IMMCO

QUANTA Lite™ Chromatin ELISA

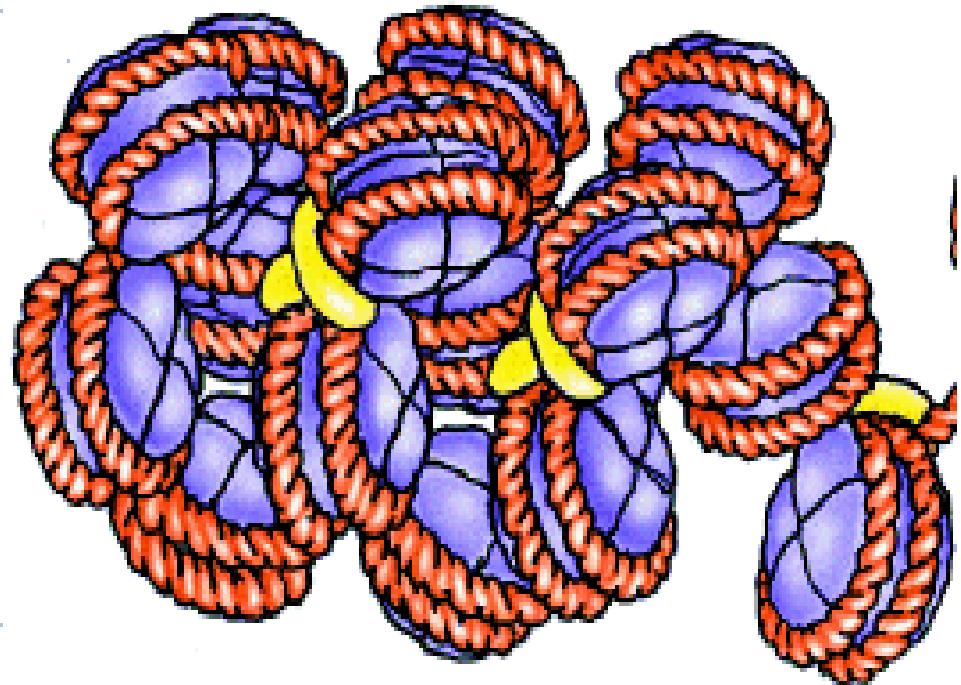
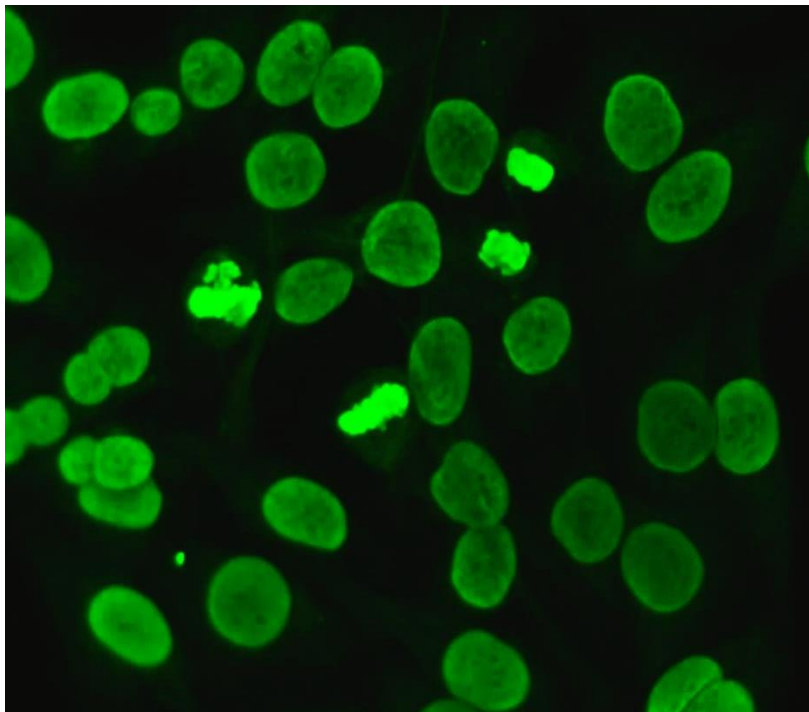
10

For *In Vitro* Diagnostic Use

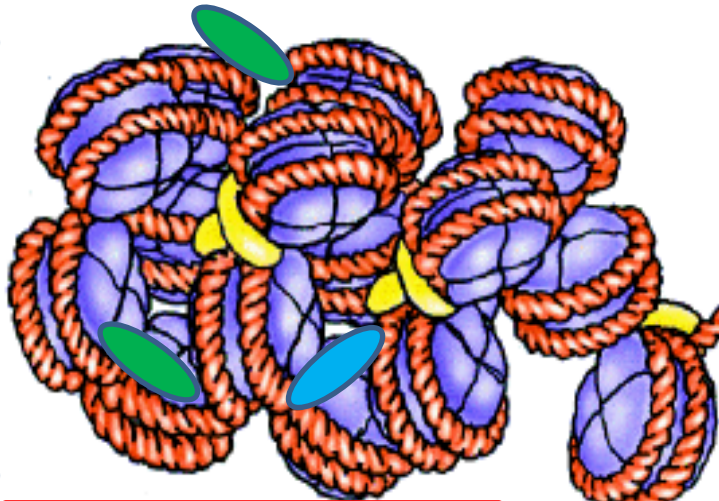
CLIA Complexity: High

Principles of the Procedure

Highly purified calf thymus chromatin consisting of DNA wrapped around the core histone (H2A-H2B-H3-H4)₂ octamer is bound to the wells of a microwell plate. Histone H1 and non-histone proteins have been removed from the chromatin during the purification process. Pre-diluted controls and diluted patient sera are added to separate wells

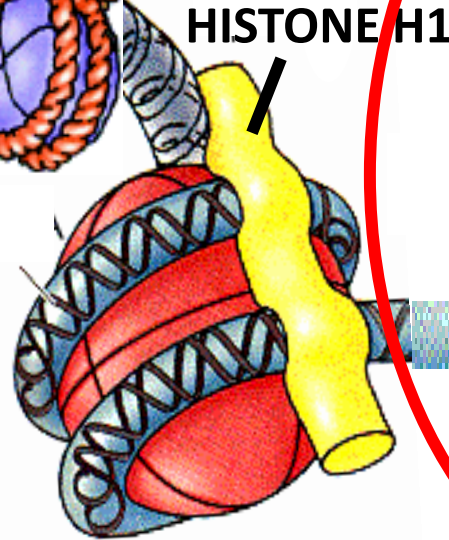


ANTICORPS ANTI-CHROMATINE = ANTI - NUCLEOSOME



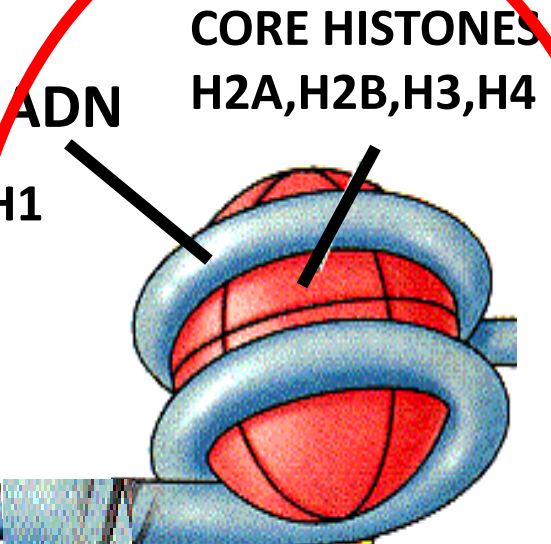
CHROMATINE

ADN
CORE HISTONES
HISTONE H1
PROTEINES ACIDES ,HMGB



CHROMATOSOME

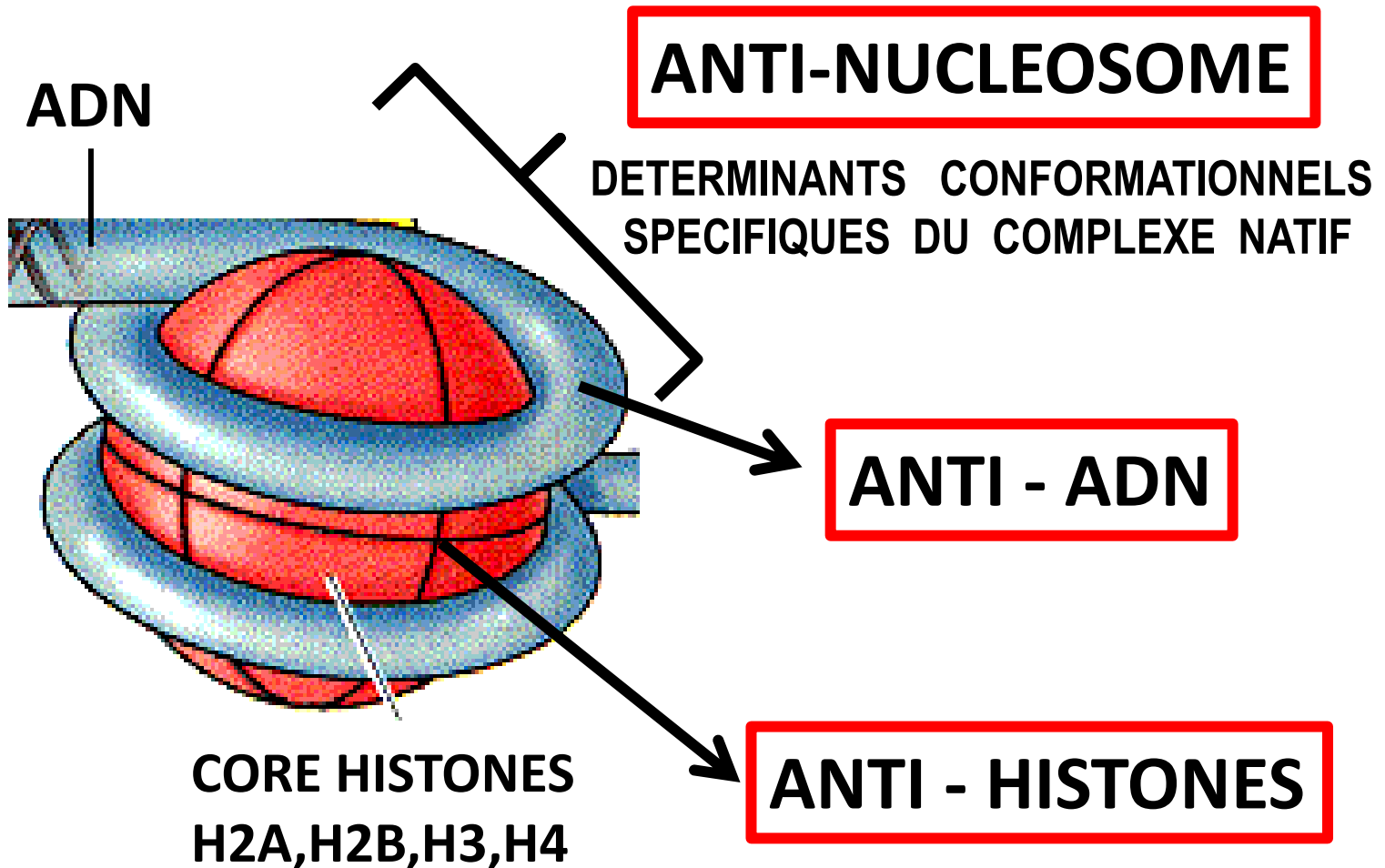
ADN - CORE HISTONES -
HISTONE H1



NUCLEOSOME

ADN - CORE HISTONES

ANTI - NUCLEOSOME



ANTICORPS ANTI-HISTONES



**HISTONES
?**

*INOVA
MENARINI
ZEUSS*

**HISTONES
H2A, H2B, H3, H4, H1**

*DTEK
EUROIMMUN
PhaDIA
AESKU*

HISTONE H1

BMD

QUANTA Lite® RNP ELISA

Uniquement pour "Diagnostics *In-Vitro*"

Complexité de CLIA: Haut

Application

QUANTA Lite® RNP est un test ELISA (Enzyme-linked Immunosorbent Assay) pour la détection semi-quantitative des anticorps anti-RNP dans le sérum humain. La recherche d'anticorps anti-RNP peut être utilisée conjointement aux signes cliniques et à d'autres tests de laboratoire pour faciliter le diagnostic du Lupus Erythémateux Disséminé (LED) et d'autres maladies du tissu conjonctif.

Principe du test

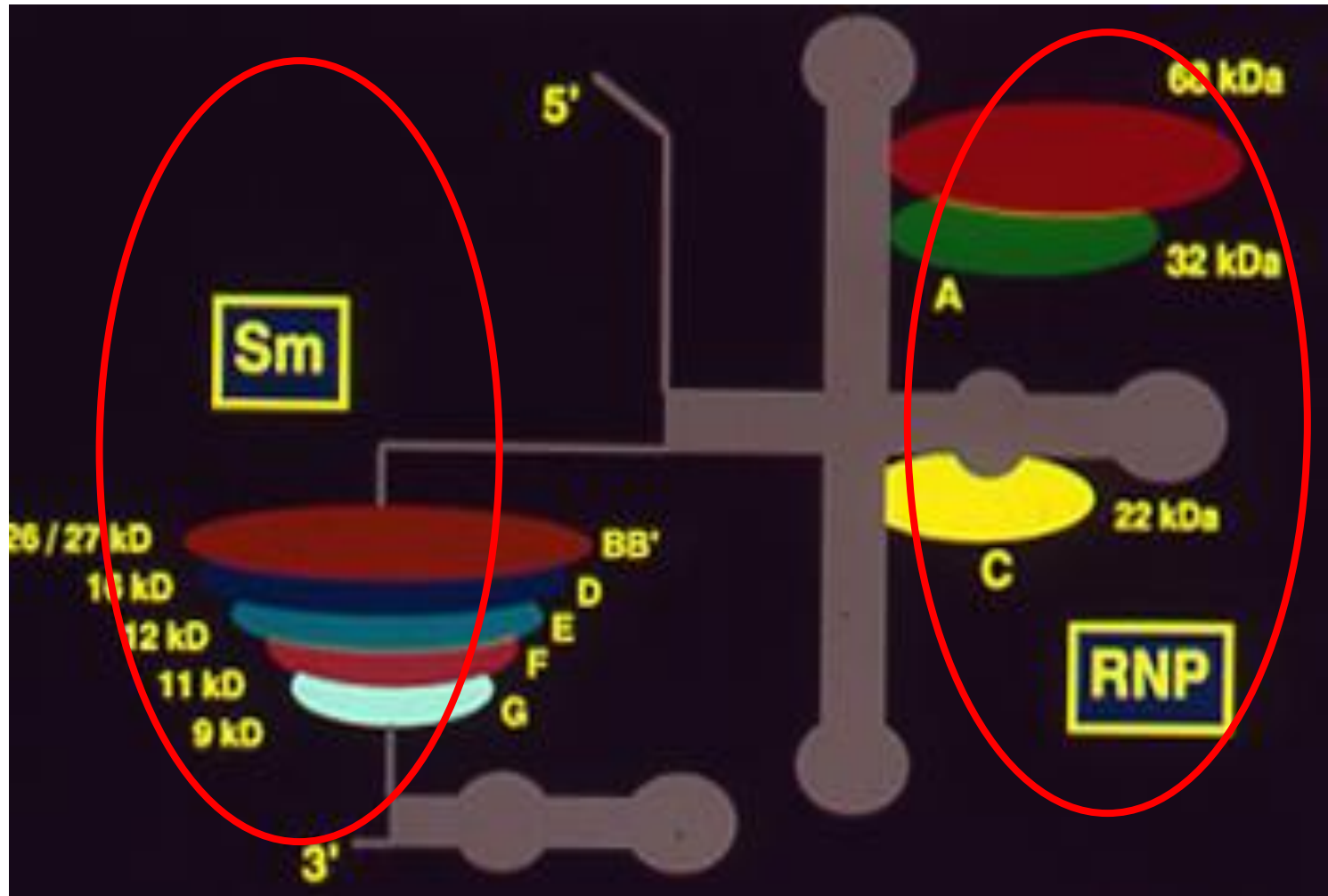
Le complexe antigène RNP/Sm extrait du thymus de veau et purifié est fixé sur les puits d'une plaque de microtitration

Informations concernant le test

RNP mais aussi ceux en commun avec l'antigène Sm. Etant donné que l'antigène RNP fixé sur les puits de ce test présente aussi les épitopes qu'il partage avec l'antigène Sm, la réponse du sérum au regard de l'antigène Sm doit aussi être prise en considération.

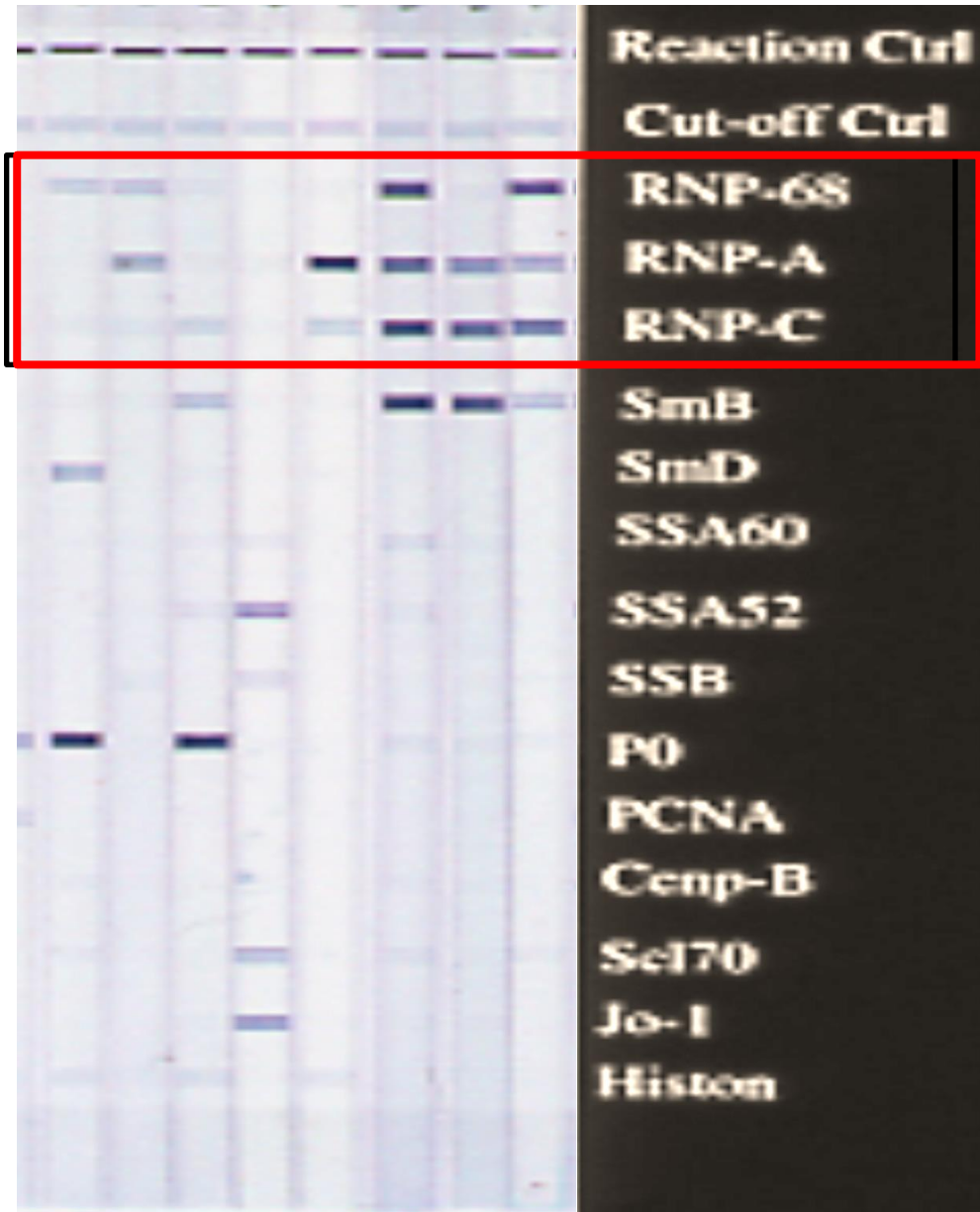
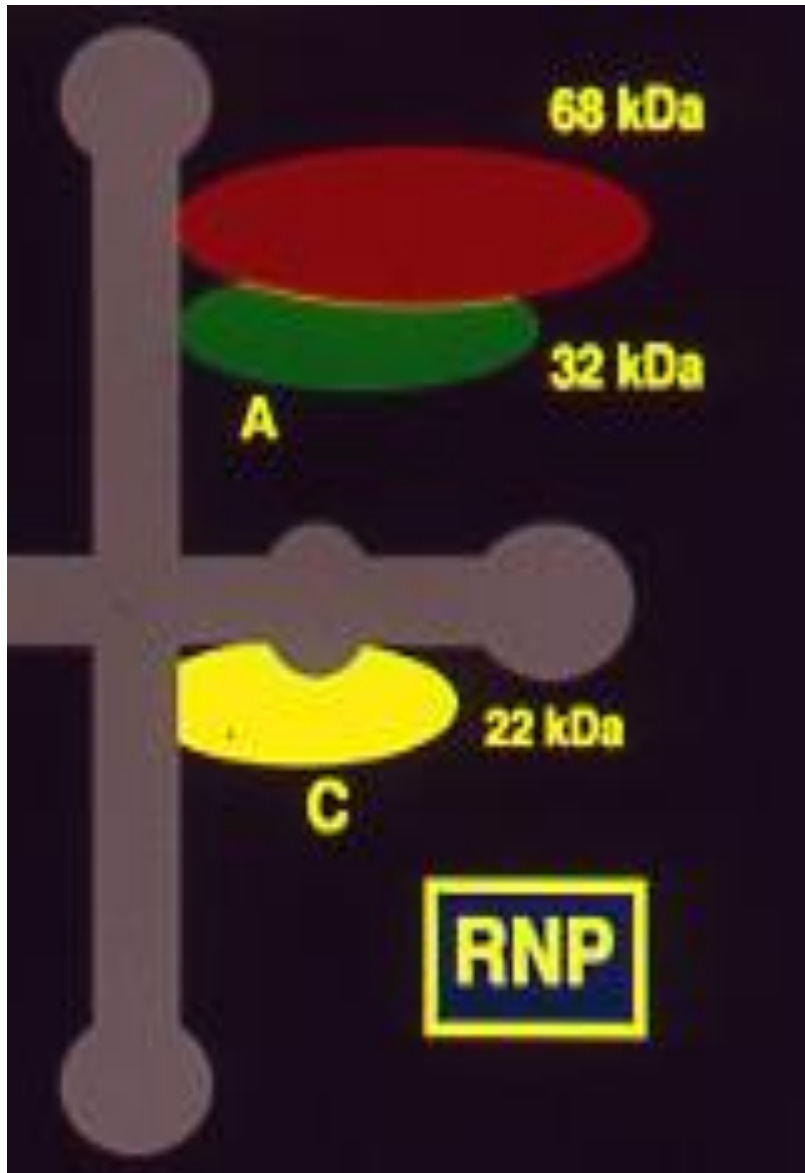
L'antigène RNP portent des épitopes spécifiques de

U1 RNP ANTI-Sm/RNP

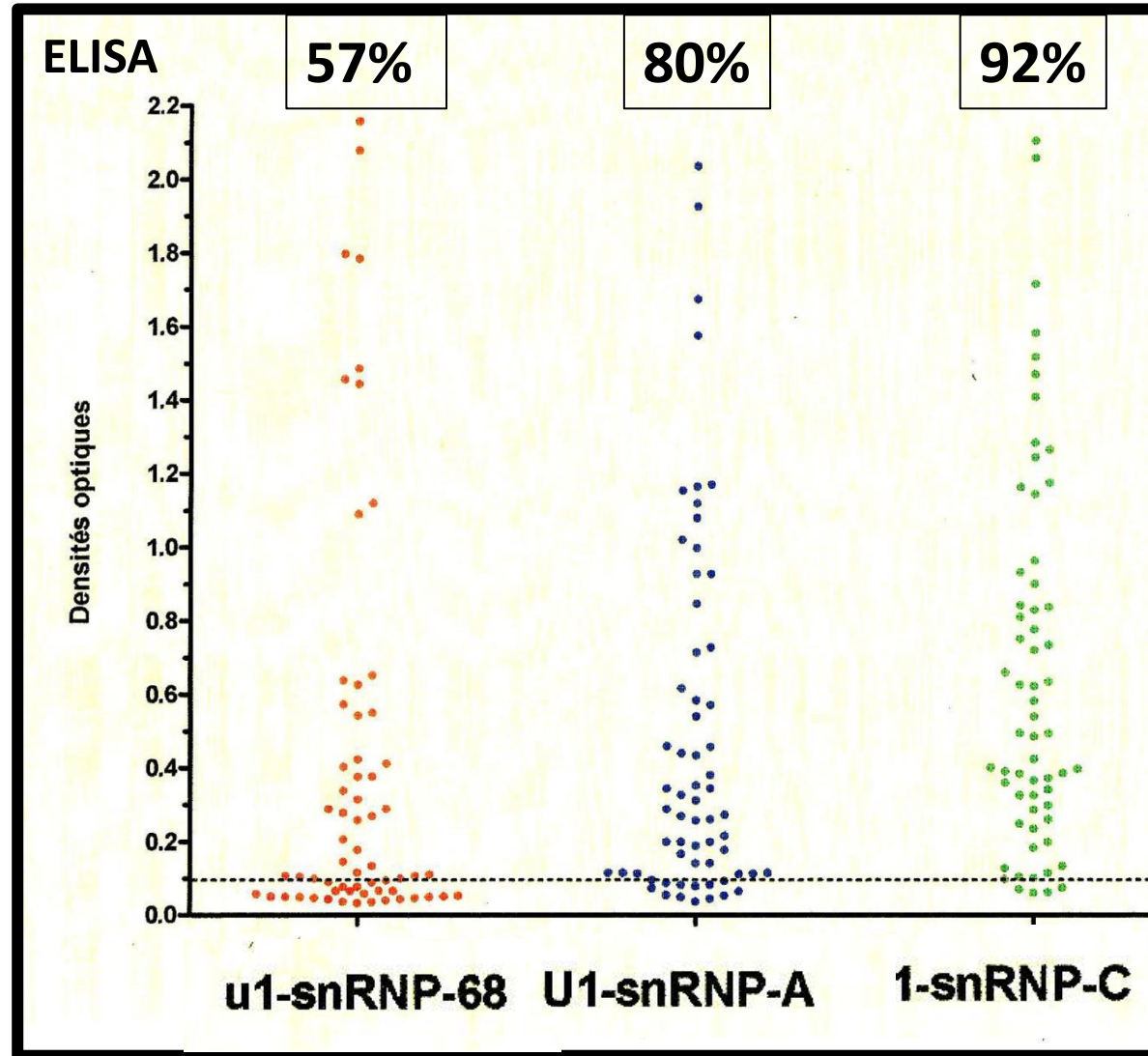
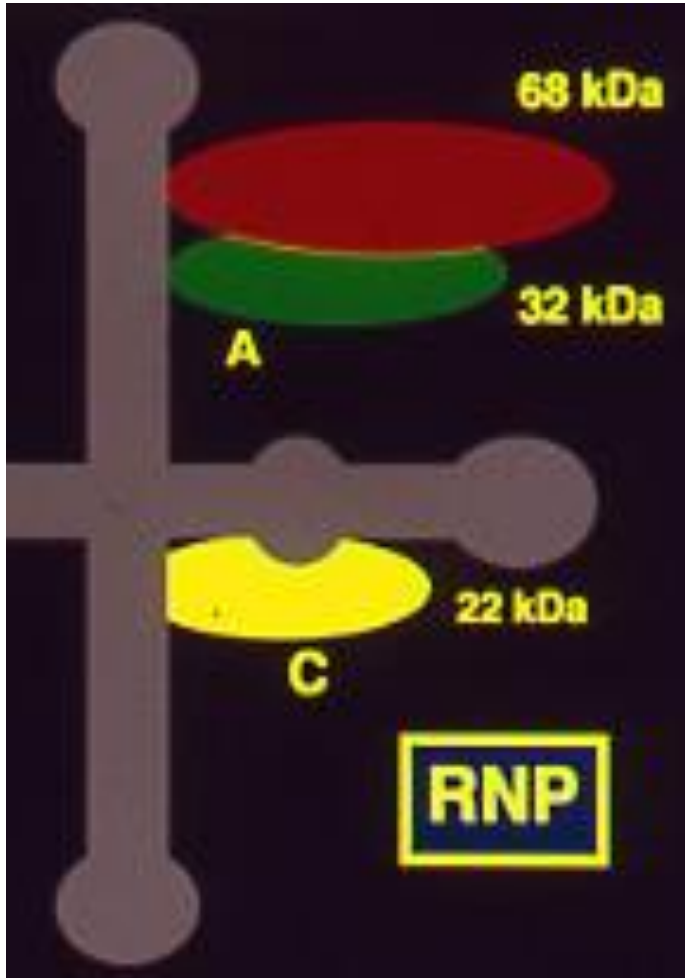


COMPLEXE ARN + PROTEINES

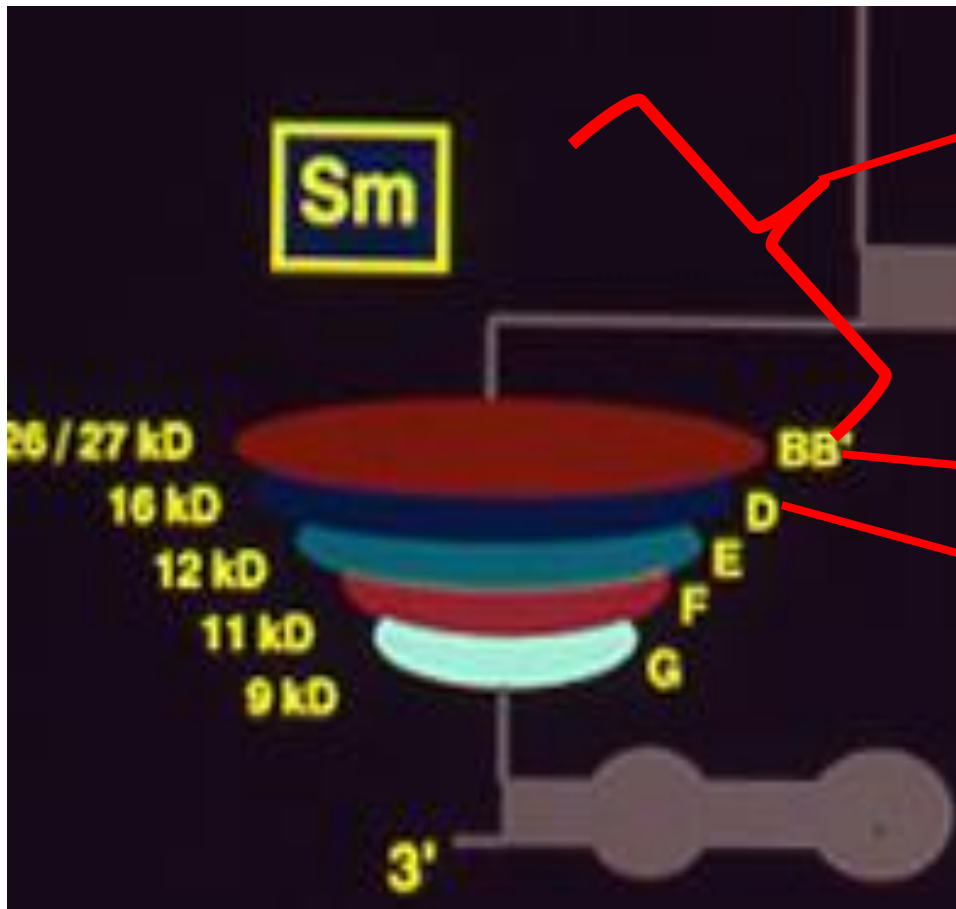
ANTICORPS ANTI- RNP



ANTICORPS ANTI-RNP



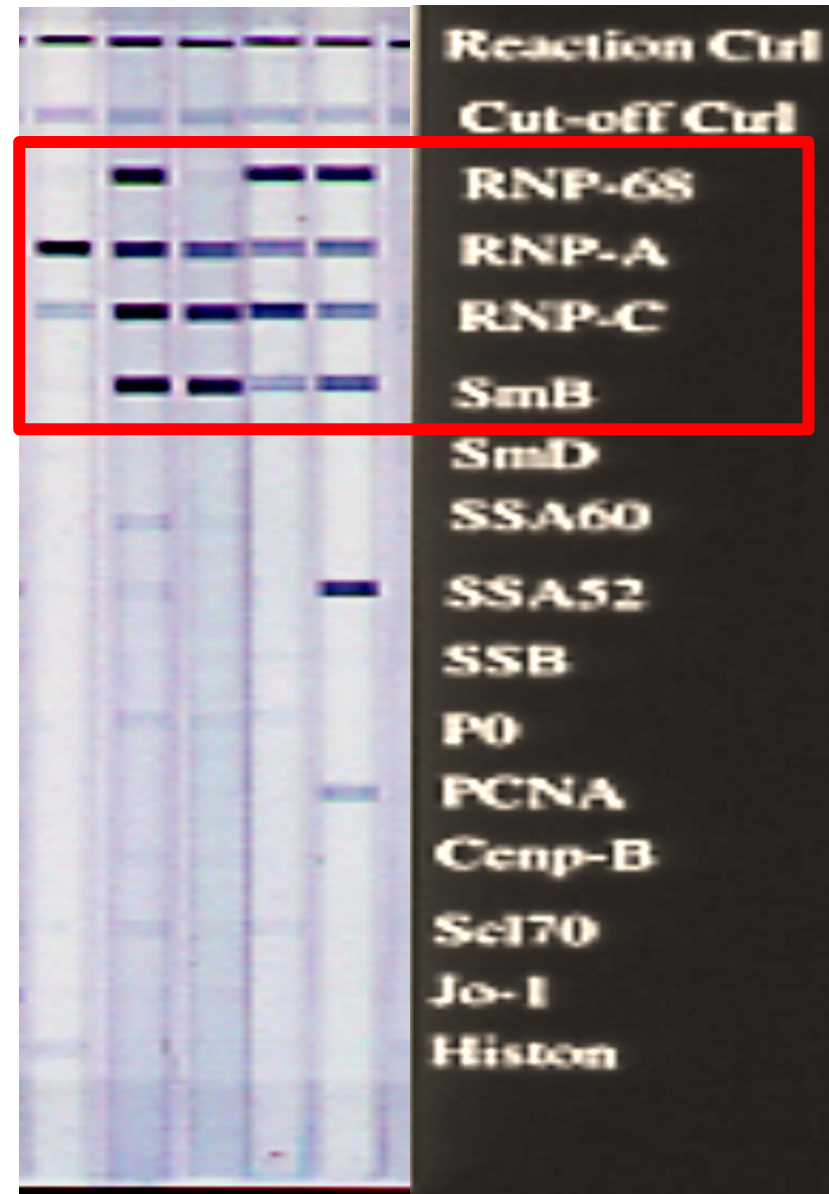
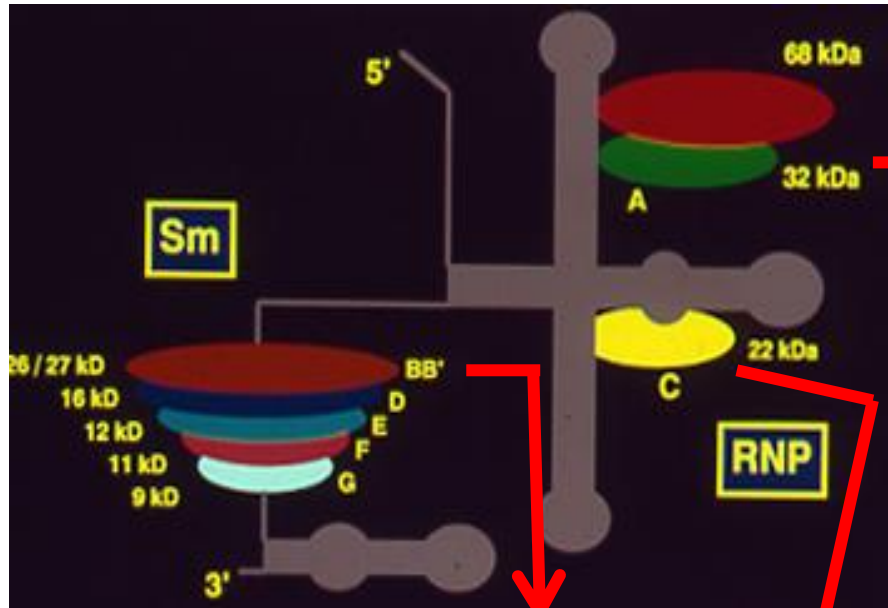
ANTICORPS ANTI - Sm



**COMPLEXE NATIF
PURIFIE**

**PROTEINES B/B
PROTEINES D**

ANTICORPS ANTI-SmB/B'



Sm B/B'



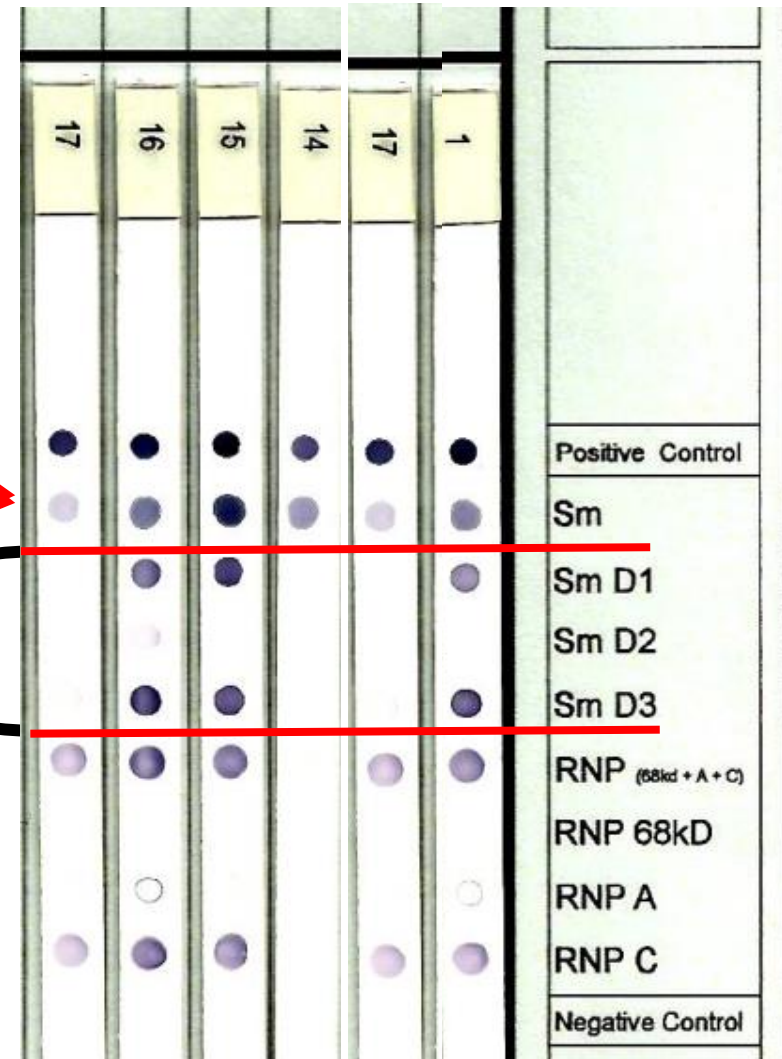
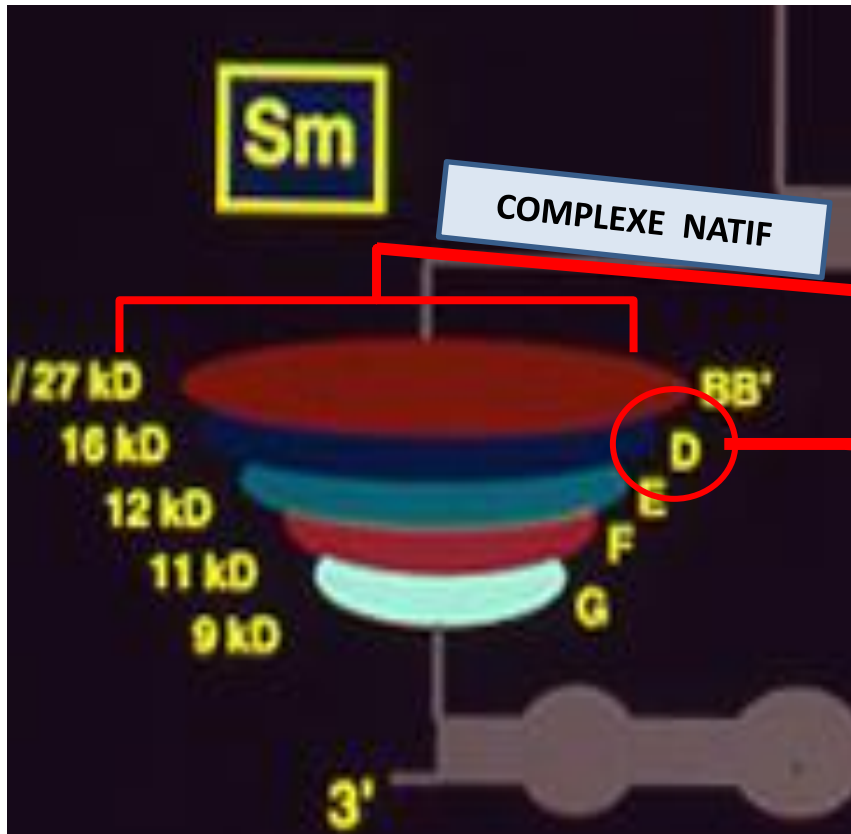
RNP C



RNP A



ANTICORPS ANTI-Sm



ANTICORPS ANTI - SSA

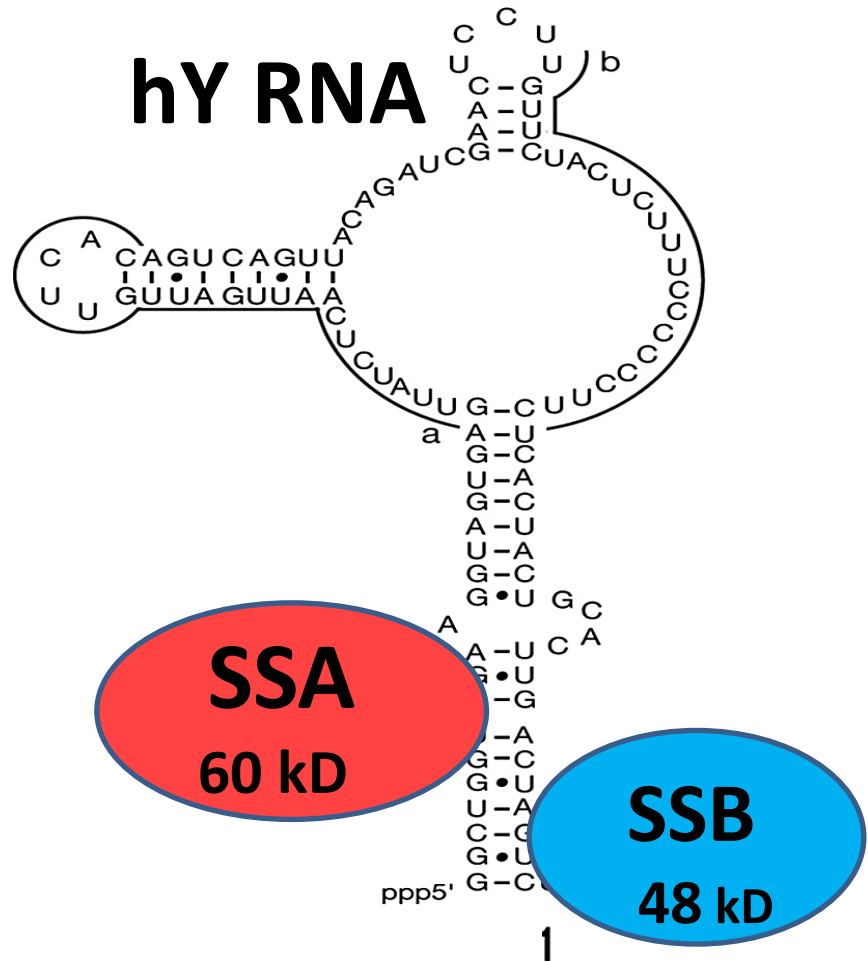
Ro52 ?

Ro52 ?

Proteine 52kDa

TRIM21

~~SSA52~~



1

QUANTA Lite® SS-A ELISA

Principles of the Procedure

Purified SS-A antigen is bound to the wells of a polystyrene microwell plate under conditions that will preserve the antigen in its native state. It has been reported that autoantibodies to both parts of the SS-A antigen, SS-A 60 and SS-A 52 are diagnostically important.⁸ Pre-diluted controls and diluted patient sera are added to separate wells, allowing any

Ro

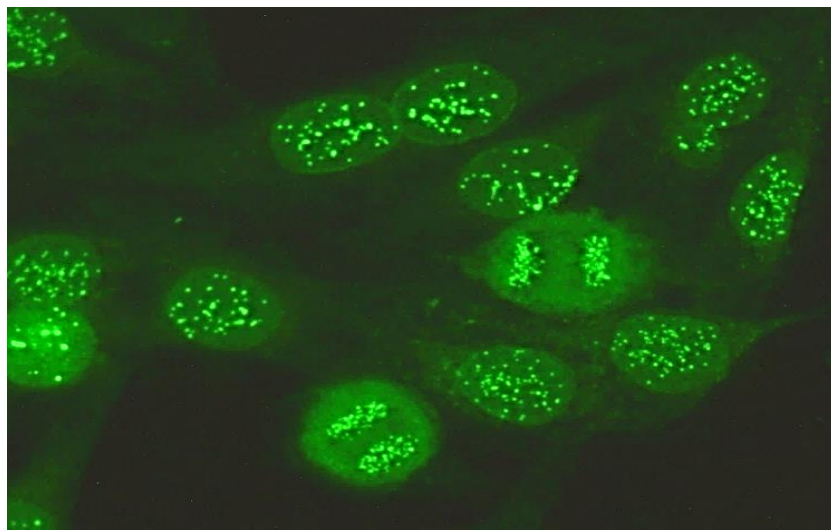
FLUOROENZYMEIMMUNOASSAY FOR ANTI RO ANTIBODIES

FOR IN VITRO DIAGNOSTIC USE

DIRECTIONS FOR USE

CONTENTS

The EliA Ro Wells are coated with human recombinant SS-A/Ro (60 kDa, 52 kDa) proteins. If present in the patient's specimen, antibodies to Ro bind to their specific antigen. After washing away non-bound antibodies, enzyme-labeled antibodies against human IgG antibodies (EliA IgG Conjugate) are added to form an antibody-conjugate complex. After incubation, non-

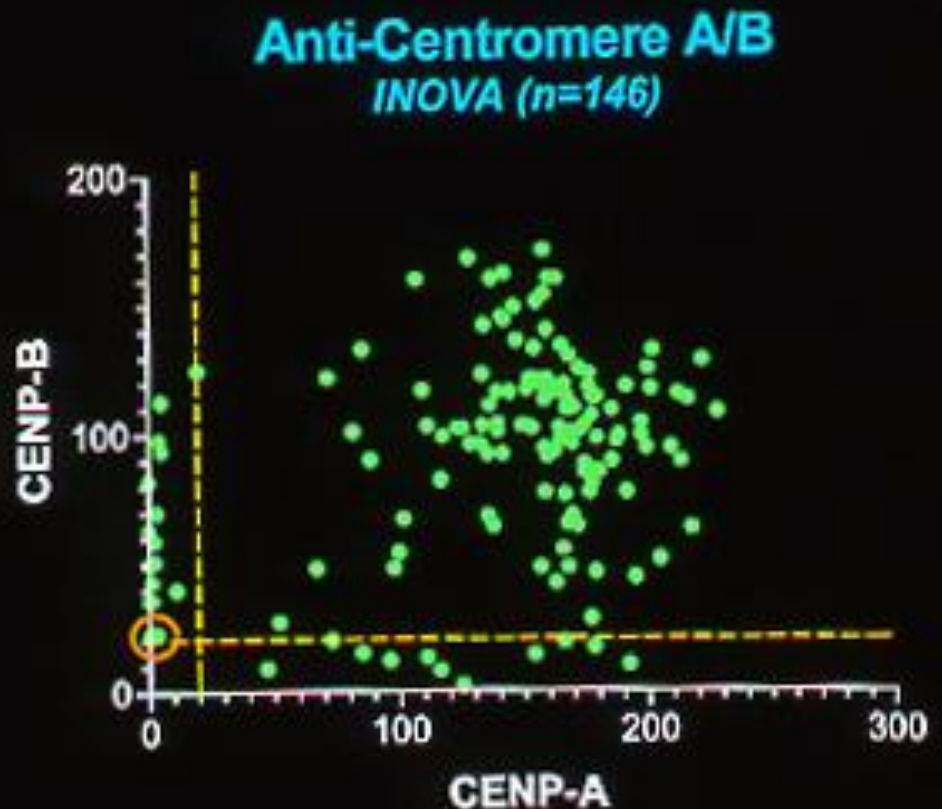


ANTICORPS ANTI-CENTROMERE

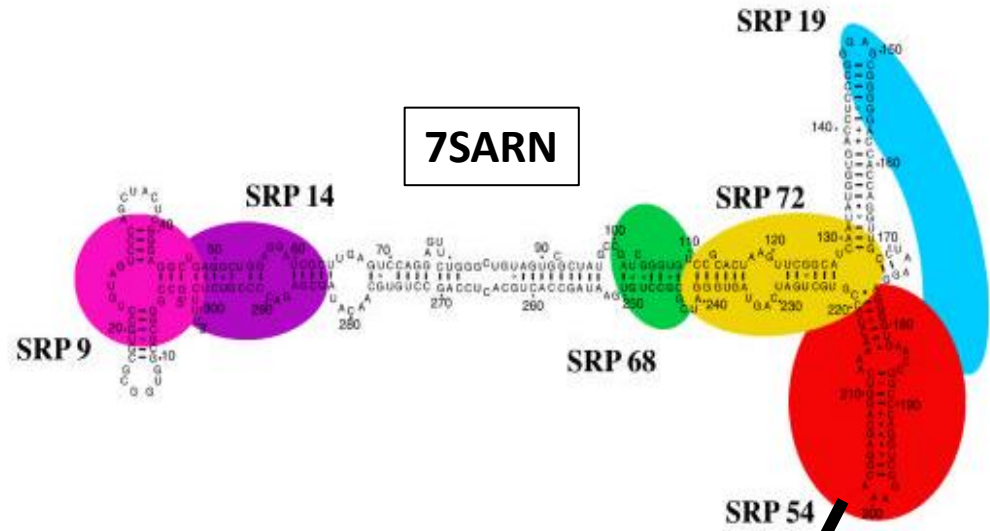
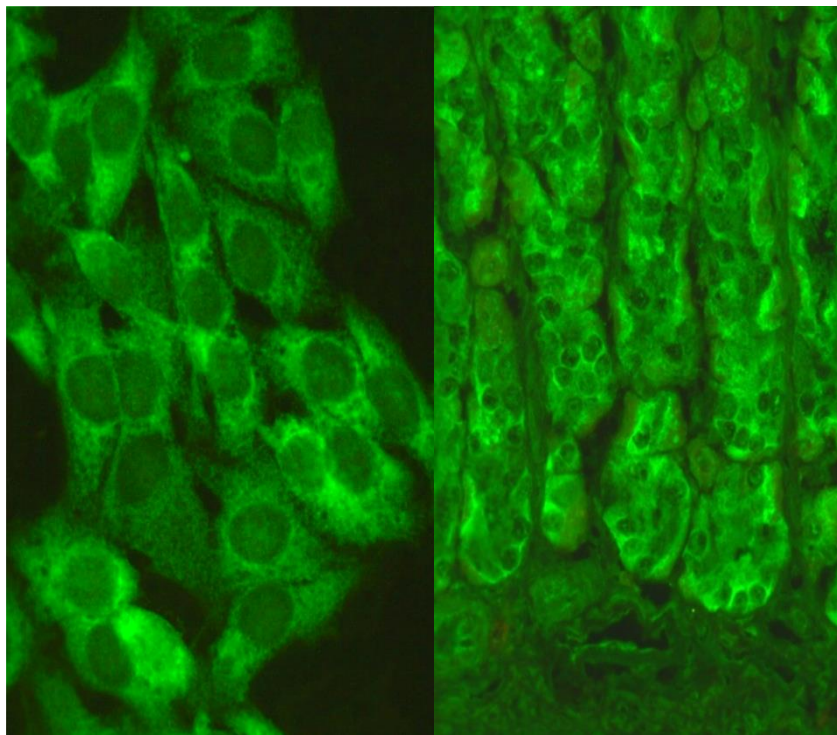


PROTEINES

CENP-A
CENP-B
CENP-C
CENP-D
CENP-G
CENP-H



ANTI-SRP



ANTI - MITOCHONDRIE

ELISA M2 · PDC

DOT M

ELISA

ANTI-PDC E2

80 - 90 %

isolés

20 %

ANTI-BCOADC

50 - 60 %

isolés

10 %

OGDC E2

10 - 20 %

isolés.

0.5 %

MIT3

85 - 95 %

OGDC E2

OGDC E2

on

Positive Control

M2/nPDC

M2/OGDC-E2

M2/BCOADC-E2

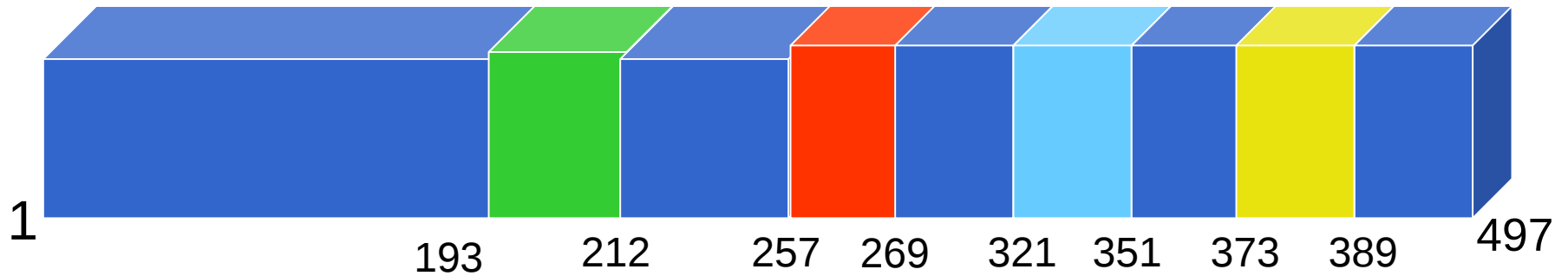
M2/PDC-E2

Negative Control

IgG - IgM

ANTICORPS ANTI-LKM1

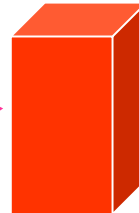
CYTOCHROME P450IID6



PROTEINE PLEINE LONGUEUR

D.TEK, ORGENTEC , EUROIMMUN

**PEPTIDE
SYNTHETIQUE**



*MBL , INOVA , IMMCO
MENARINI*

ANTICORPS ANTI-PROTEINES CITRULLINEES

ACPA

PROTEINES

VIM.MUTEE (MCV)

ENOLASE (CEP1)

MBP (Sa)

FIBRINOGENE

COLLAGENE I et II

HISTONES

PEPTIDES

CCP 1

CCP 2

CCP 3

Hs CCP (MCV)

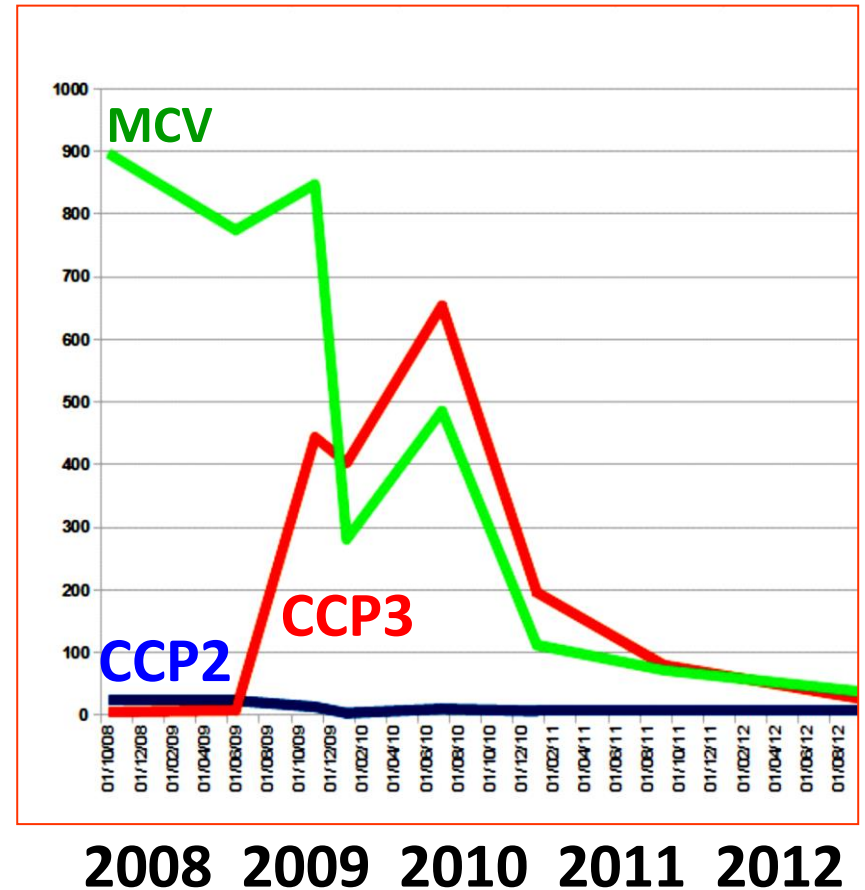
EBNA-1,2

ANTI-CARBAMYLATED PROTEIN / CARP

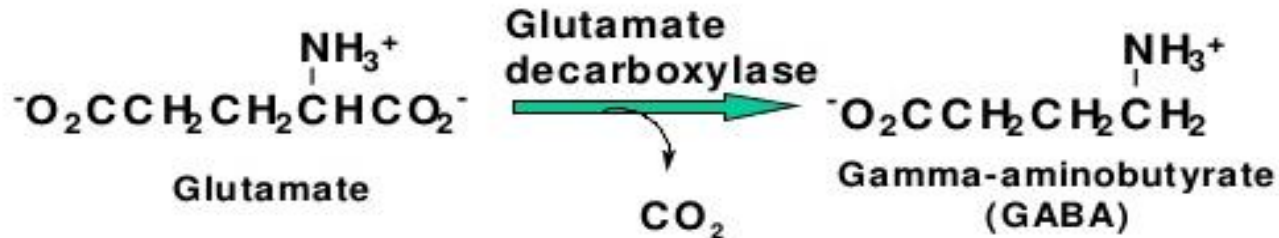
LYSINE → HOMOCITRULLINE

ACPA

CCP2	CCP3	MCV
360	450	277
7	440	775
280	385	14
32	165	22
30	65	485
32	165	22



ANTICORPS ANTI - GAD

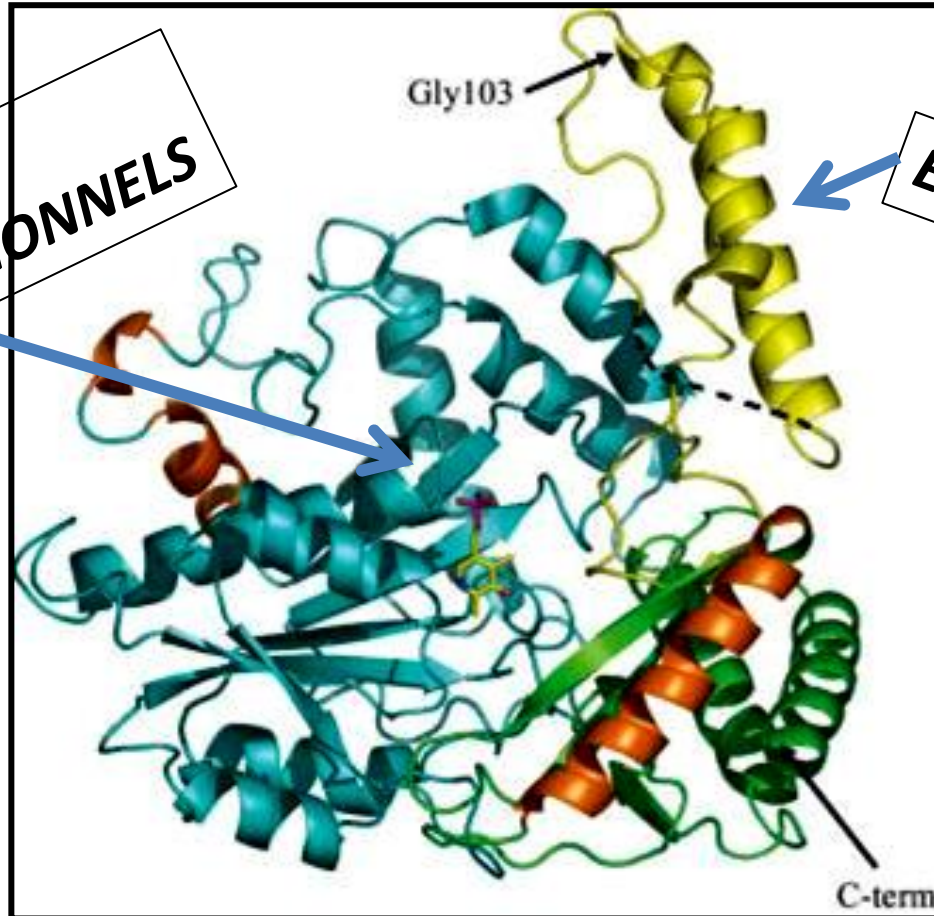


PANCREAS

**EPITOPES
CONFORMATIONNELS**

DIABETE

Autoimmun



CERVEAU

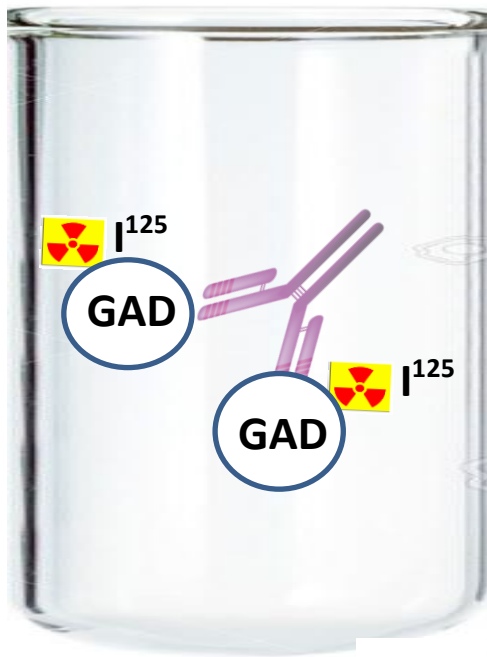
EPITOPES LINEAIRES

**MALADIES
NEUROLOGIQUES**

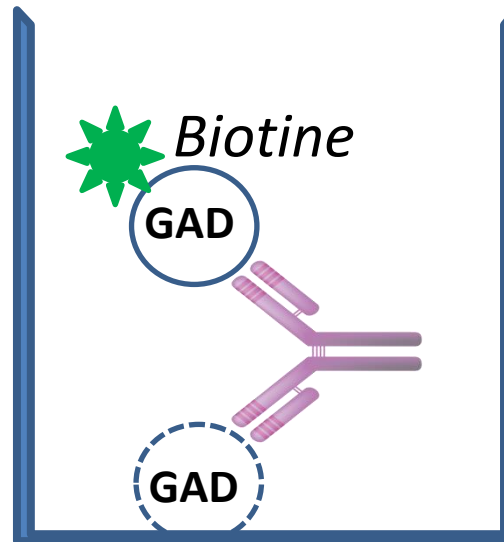
M.Homme-Raide
Encéphalite
Ataxie
Epilepsie

ANTICORPS ANTI - GAD

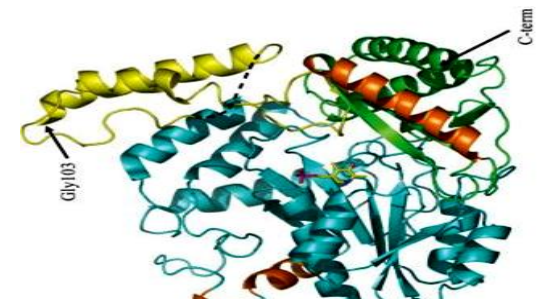
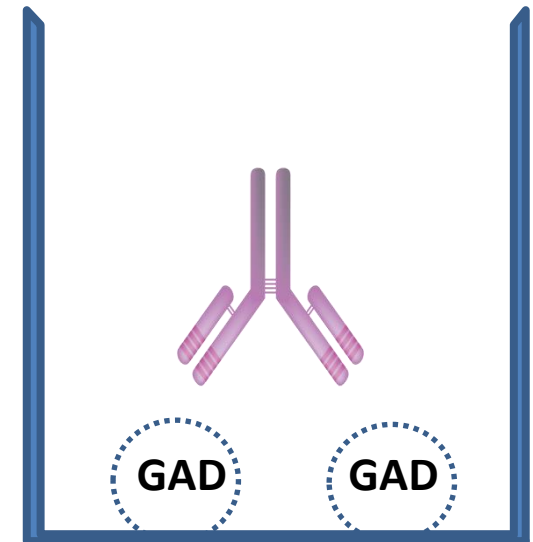
RIA



ELISA SANDWICH

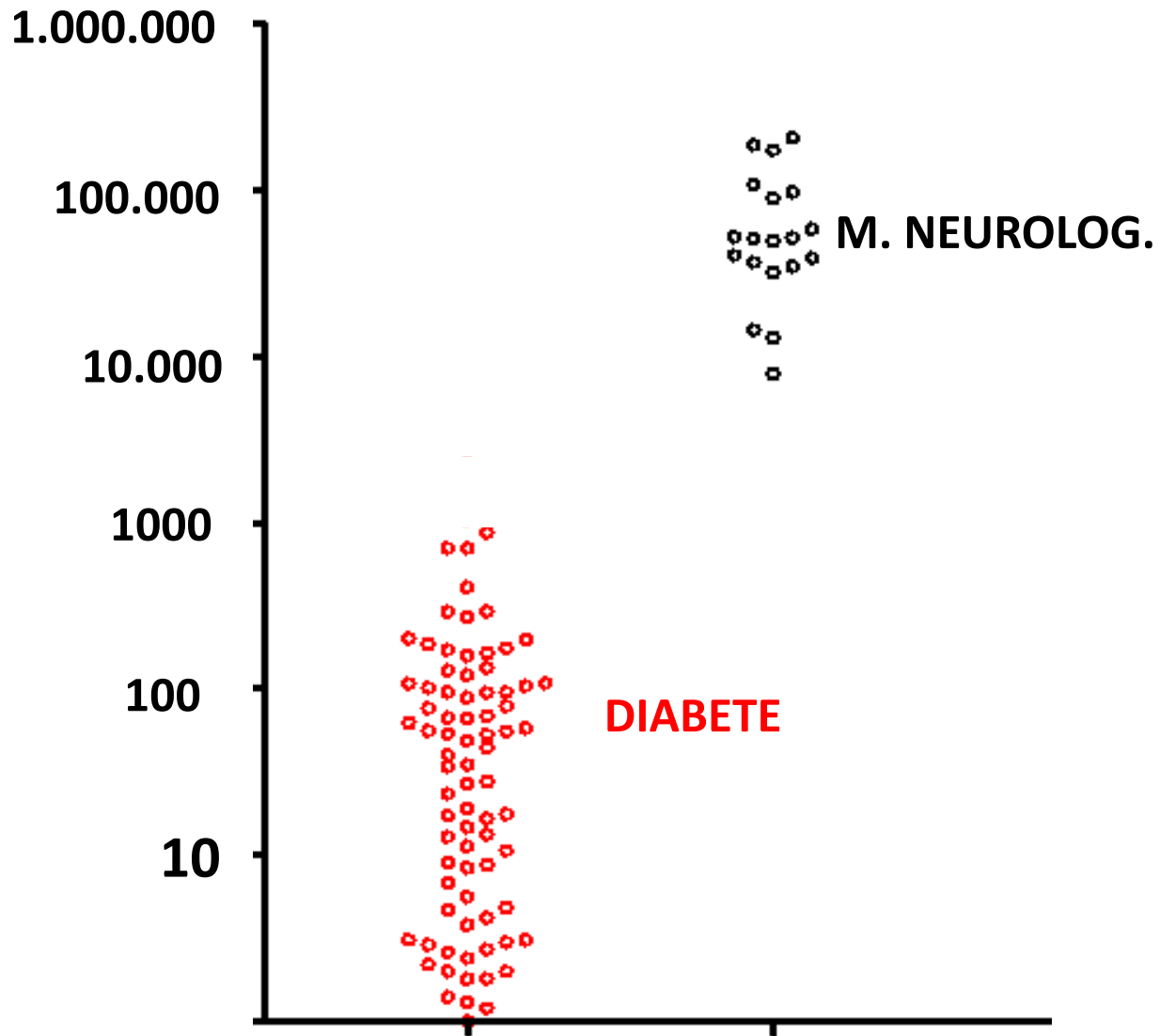


ELISA DIRECT IMMUNODOT



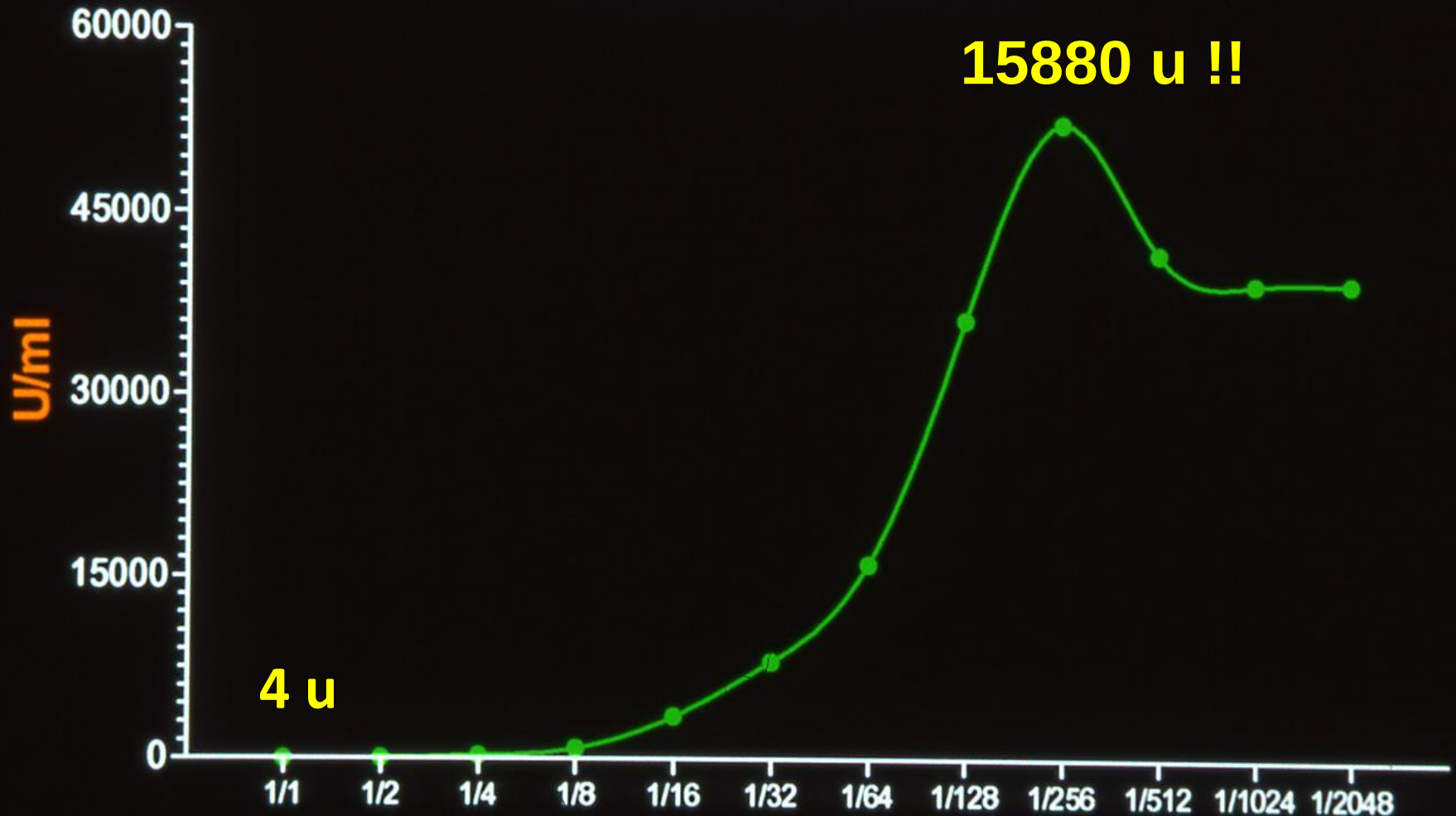
ANTICORPS ANTI-GAD 65

RIA



Anti-GAD65 (RIA)

Mr.Por. Stiff Man Syndrome

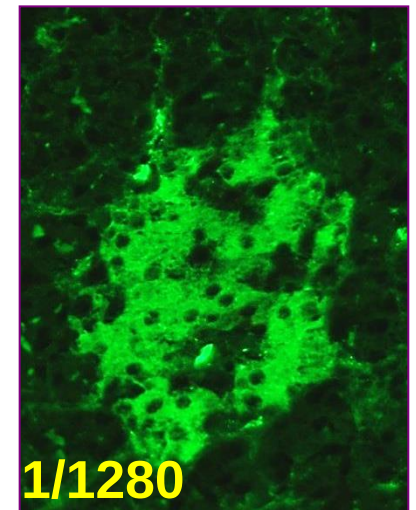
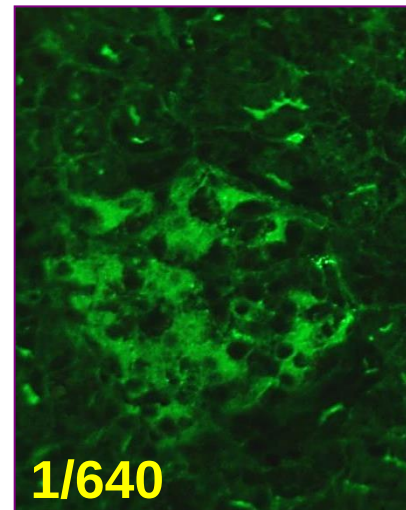
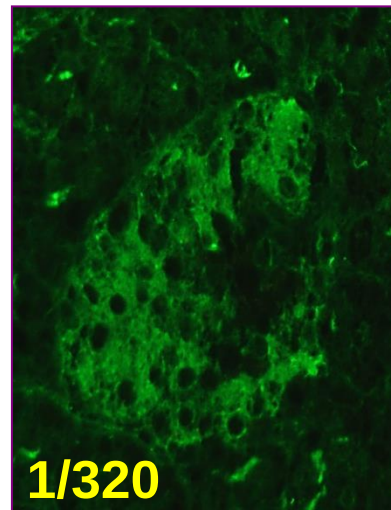
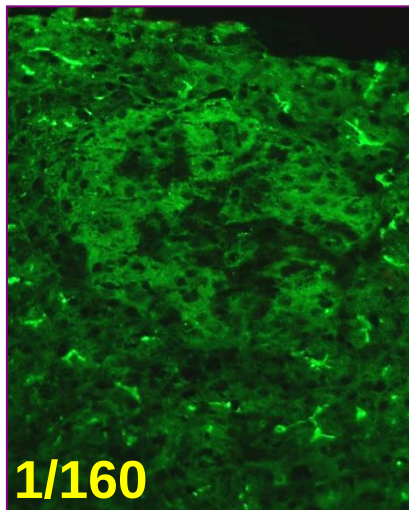
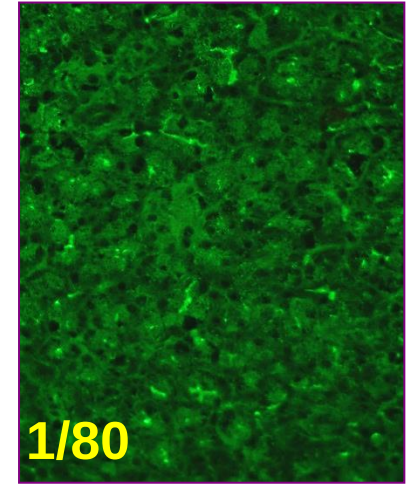
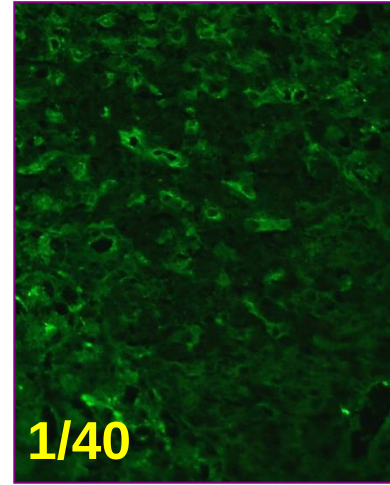
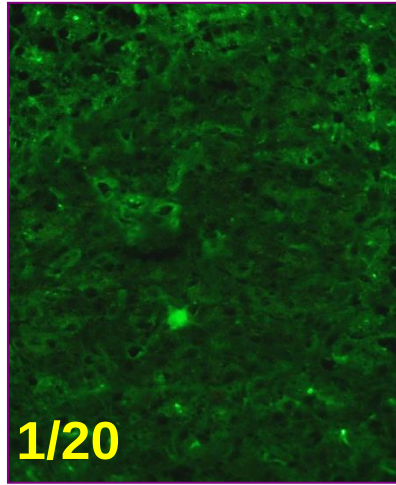
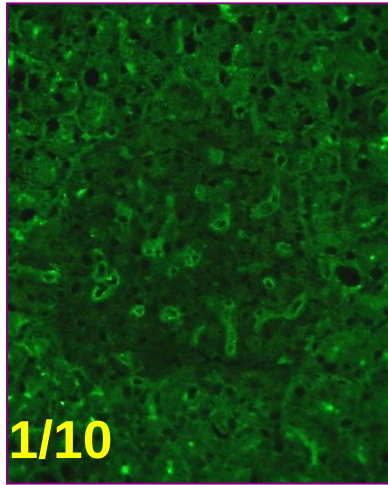


Dilutions du Sérum

ANTI- ILOTS : PANCREAS SINGE

Mr.Por. Stiff Man Syndrome

ANTI-GAD : 15880 u. (< 5)



ANTICORPS ANTI - GAD

IMMUNODOT

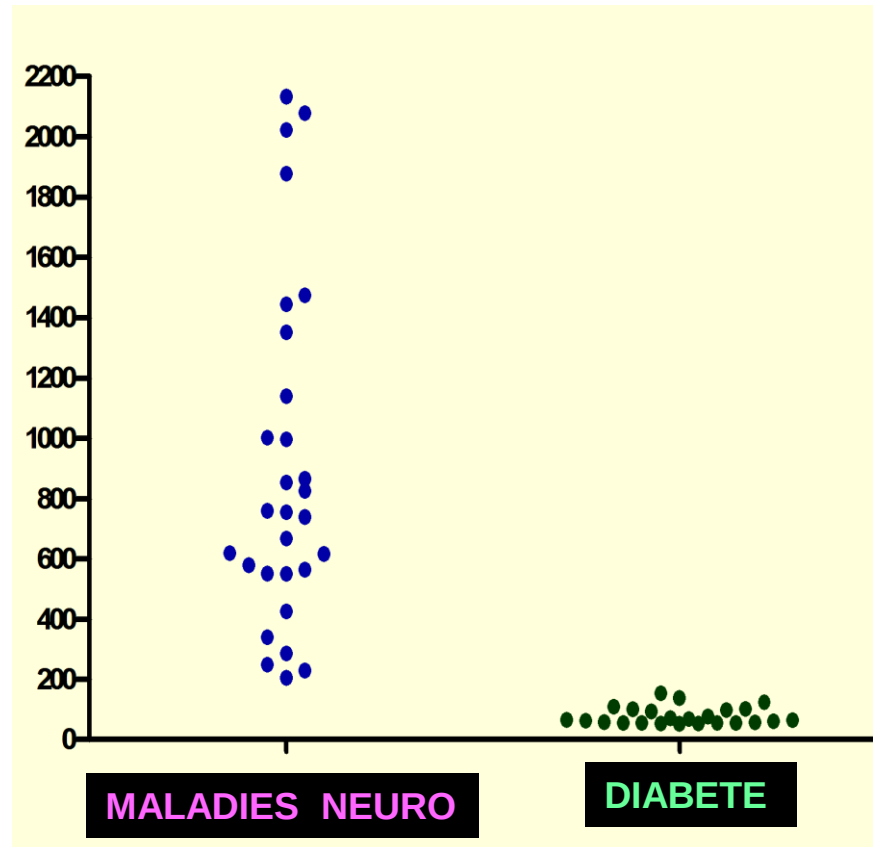
MALADIES NEUROLOGIQUES



DIABETE TYPE I

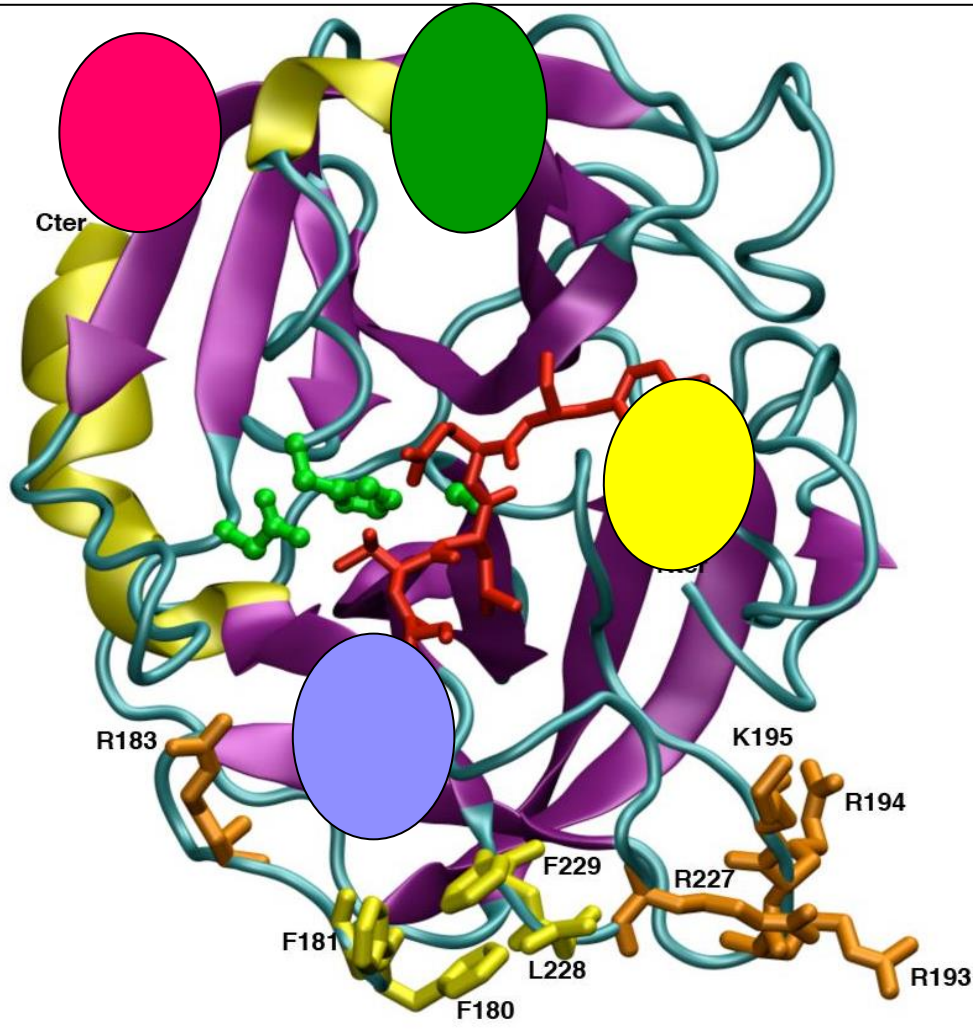


ELISA DIRECT

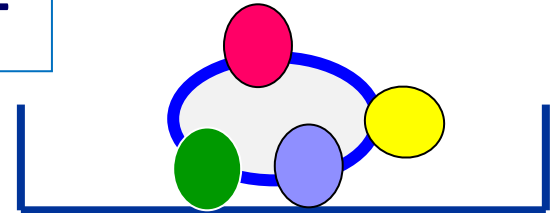


ANTICORPS ANTI-PR3

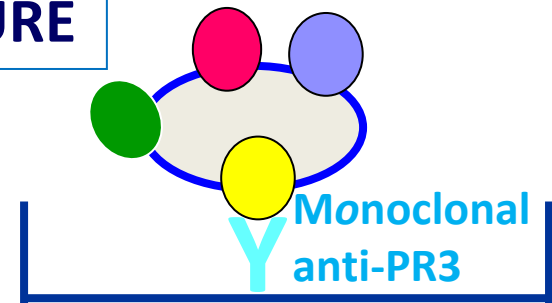
ELISA



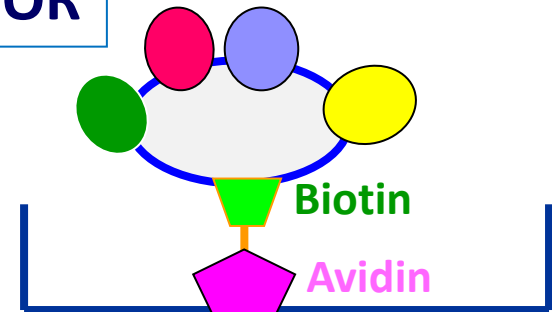
DIRECT

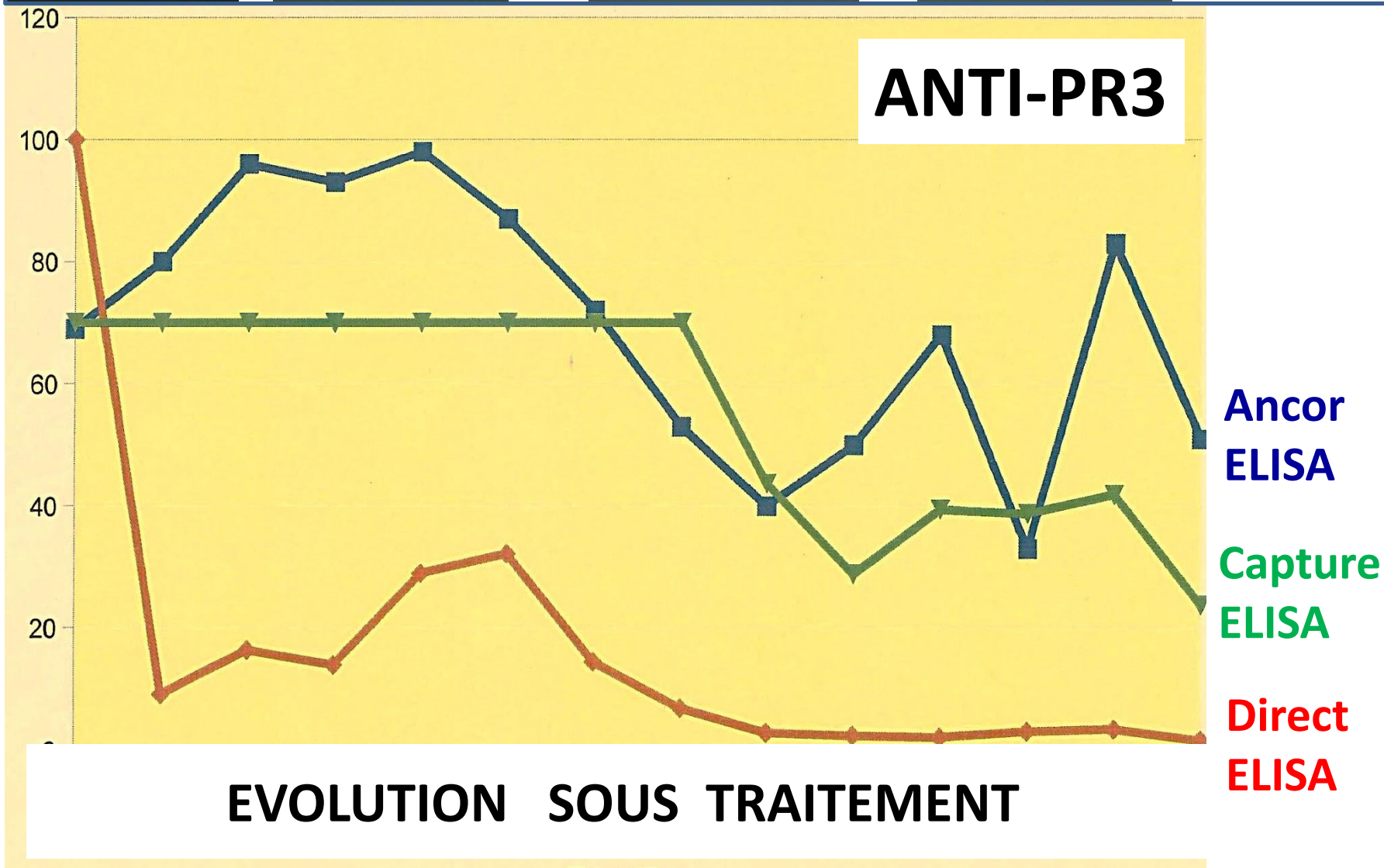
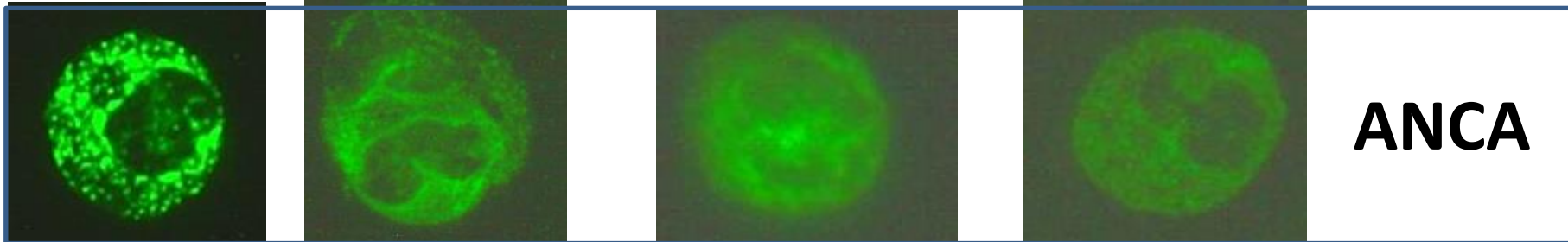


CAPTURE

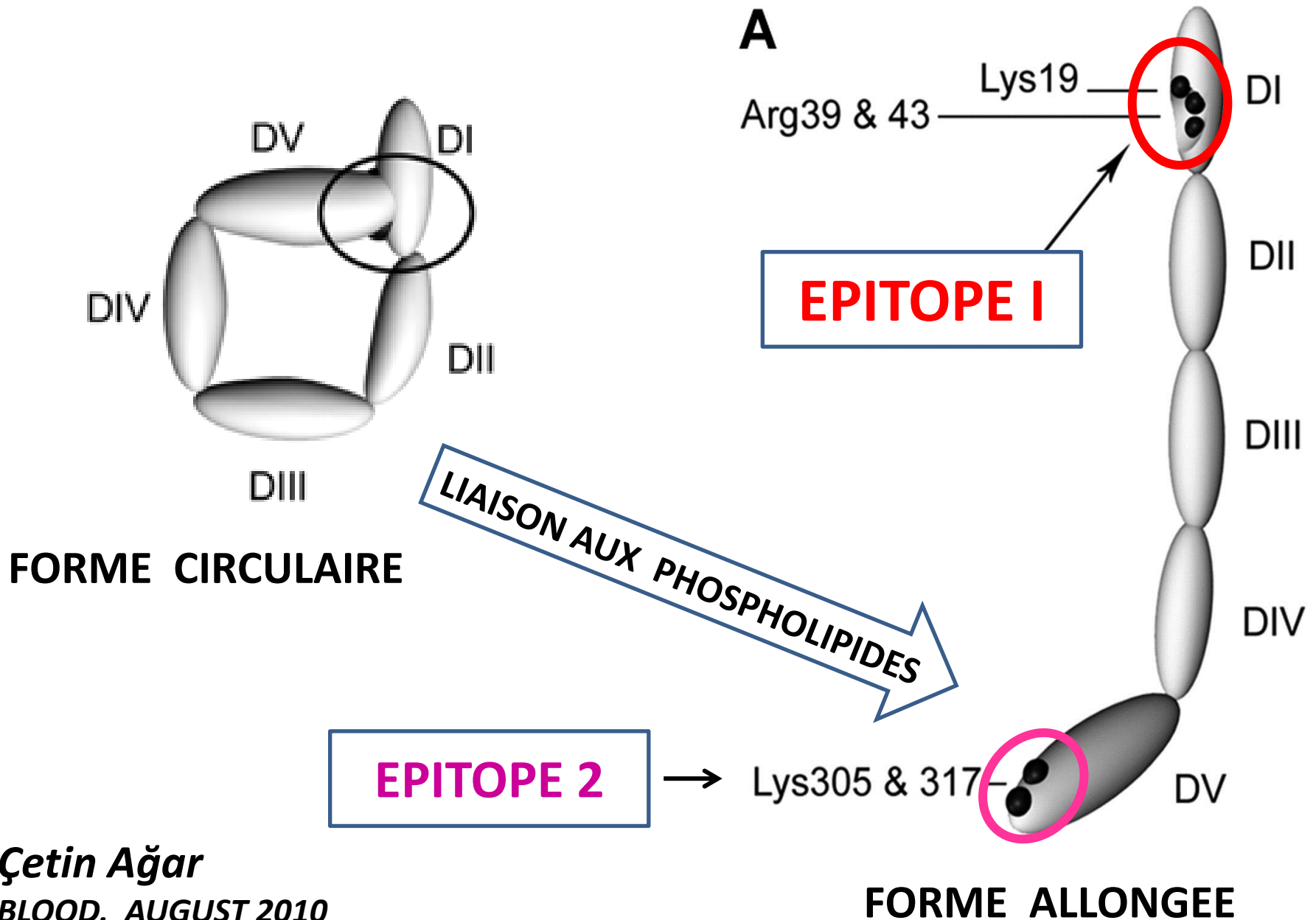


ANCHOR





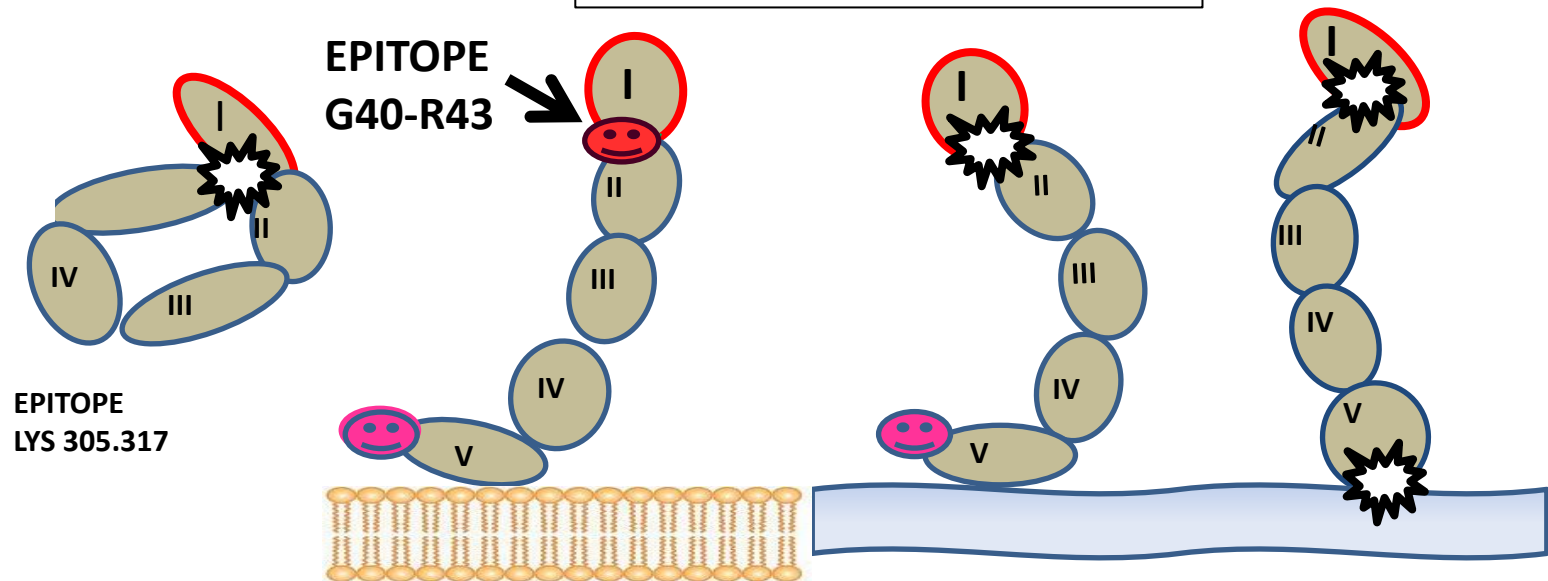
ANTI - β 2 GLYCOPROTEINE 1



Çetin Ağar

BLOOD, AUGUST 2010

β2 - GLYCOPROTEINE I



Variability in Exposure of Epitope G40-R43 of Domain I in Commercial

ANTI-β2GP1 D1

D1 recombinant (aa 1-64)

Leonie Pelkmans¹

Denis Wahl^{4,5}, Vittorio Pengo⁶, Bas de Laat^{1,2,3,7}

Stéphane Regnault⁵,

PLoS ONE August 2013, 8

¹Cardiovascular Research Institute Maastricht, Maastricht University Medical Centre, Maastricht, The Netherlands, ²Synapse BV, Maastricht, The Netherlands, ³Department of Clinical Chemistry and Haematology, University Medical Centre Utrecht, Utrecht, The Netherlands, ⁴Vascular Medicine Division, Regional Competence Centre for Rare Vascular Diseases, CHU de Nancy, Nancy, France, ⁵INSERM U1116, Université de Lorraine, Nancy, France, ⁶Clinical Cardiology, Department of Cardiothoracic and Vascular Sciences, Thrombosis Centre, University of Padua, Padua, Italy, ⁷Sanquin Research, Amsterdam, The Netherlands

ANTICORPS : NEO - EPITOPE tTG/DGP

MALADIE COELIAQUE

