

Manufacturing of plasma Therapeutic proteins

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Summary

- Presentation of LFB
- Plasma
- From plasma to medicinal products
- Ethanol Fractionation
- VIH and Prion Crisis
- LFB an innovative compagny
- Case study: improvment of polyclonal immunoglobulin manufacturing process
- Conclusions

WHO ARE WE?

- Laboratoire français du fractionnement et des biotechnologies
- Major player in French plasma field

LFB GROUP OVERVIEW

- Since it was created in 1994, LFB has been **developing**, **manufacturing** and **marketing** therapeutic proteins for patients with serious and often rare diseases.

- Today, LFB is among the **leading European bio-pharmaceutical companies** providing mainly hospital-based healthcare professionals with plasma-derived or recombinant medicinal products in three major therapeutic areas: **immunology**, **haemostasis**, and **intensive care**.

3

Therapeutic areas:

IMMUNOLOGY
HAEMOSTASIS
INTENSIVE CARE

15

Plasma-derived medicinal products

LFB GROUP OVERVIEW

(2018 data)

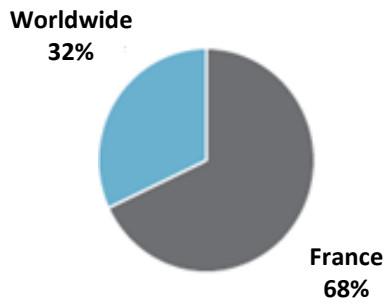
LFB is an historical leader on the French plasma medicines market

Key Figures

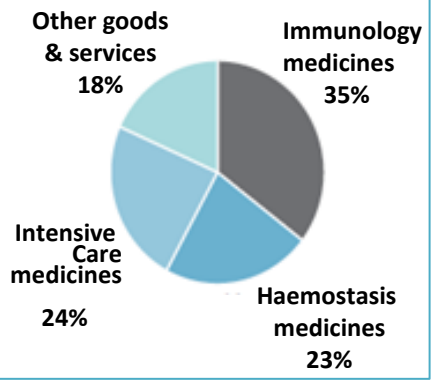
€400 M of turnover	**7TH** largest player worldwide in plasma derivatives	**15** medicines
2,300 employees, within 1,800 in France		**3** therapeutic areas

Turnover breakdown

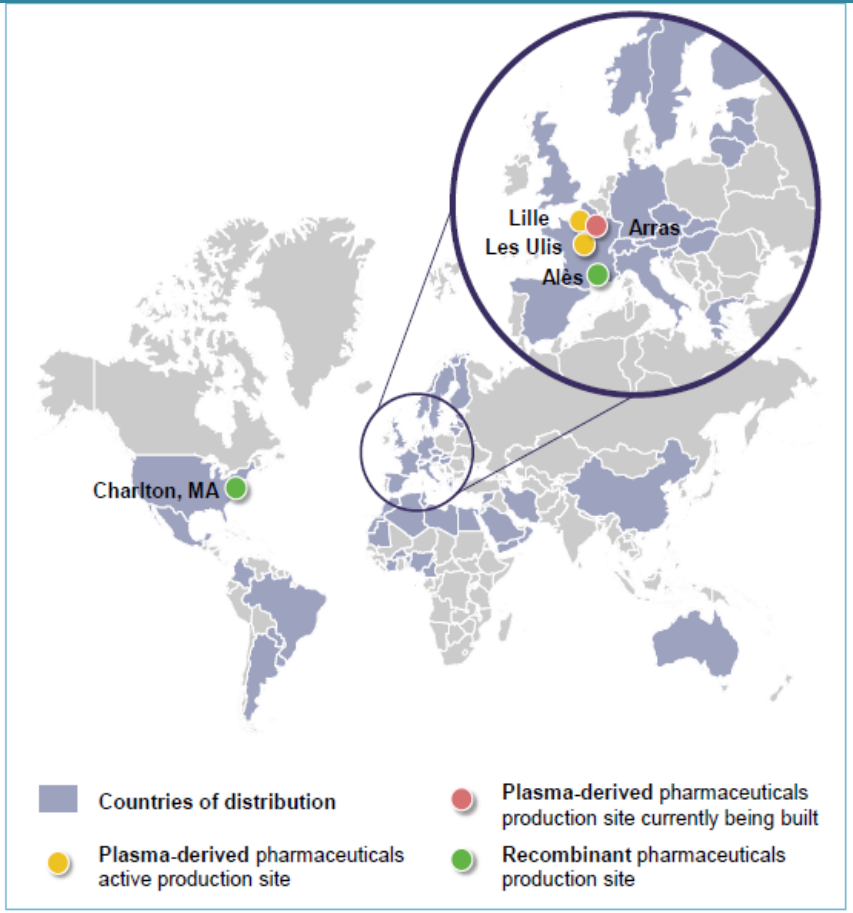
By geography



By therapeutic area



Geographical footprint



LFB MAJOR ACTOR OF THE FRENCH BLOOD CHAIN

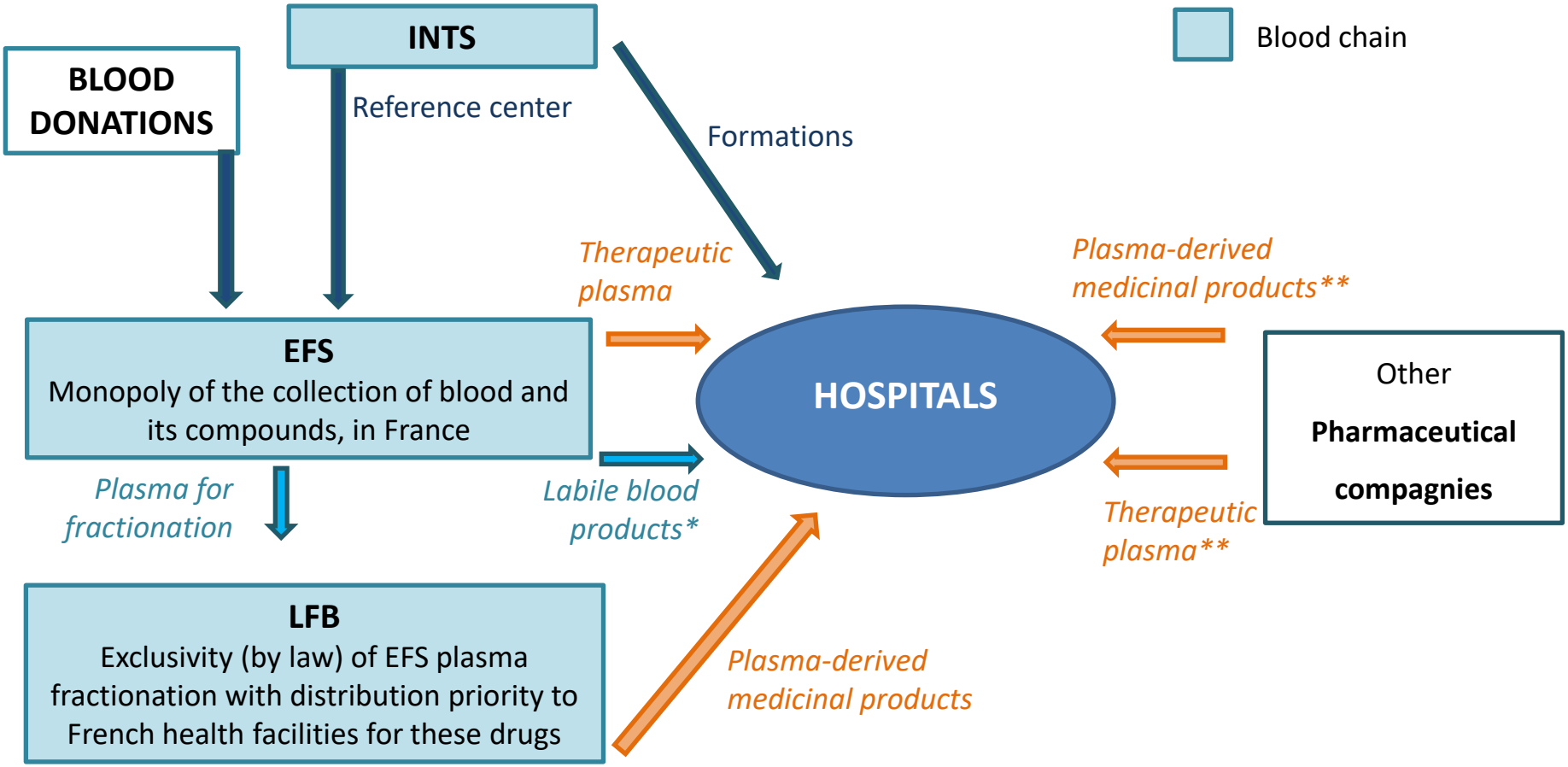




ORGANISATION OF BLOOD CHAIN IN FRANCE 1/2

- The French system for collecting blood and distributing blood products was deeply restructured during the 1990s.
- **In 1993**, the decision was taken to separate the missions between three operators:
  - L'agence Française du Sang, which later became l'Etablissemnt Français du Sang (EFS: French Blood Establishment),
  - The **Laboratoire français du fractionnement et des biotechnologies** (**LFB:** French Fractionation and Biotechnology Laboratory), created in 1994,
  - l'Institut national de la transfusion sanguine (INTS: National Institute of Blood Transfusion).
  - Safety of Plasma-derived medicinal products was the Agence du médicament responsibility. Agence du médicament became Afssaps, and finally Agence nationale de sécurité du médicament et des produits de santé (*ANSM*).

ORGANISATION OF BLOOD CHAIN IN FRANCE 2/2



*: Platelets, red blood cells, fresh frozen plasma

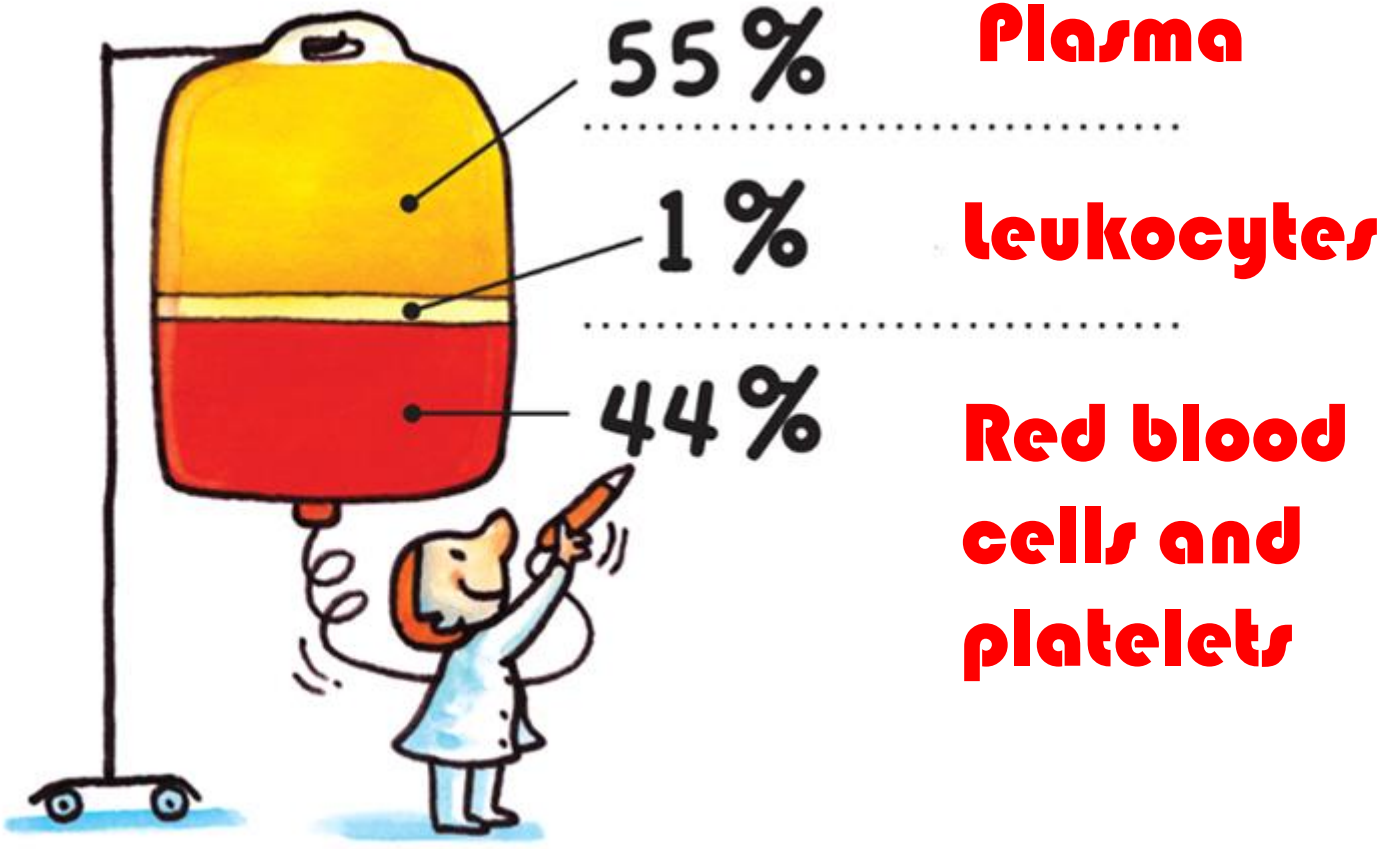
**: Exceptionally, an MA may, by way of derogation, be issued to a medicinal product prepared from blood or components of paid blood if the medicinal product provides an improvement in terms of efficacy or therapeutic safety or if equivalent medicinal products are not available. available in sufficient quantity to meet the health needs. In this case, the marketing authorization is issued for a period of two years

PLASMA





PLASMA 1/2



Composition of blood donation

PLASMA 2/2

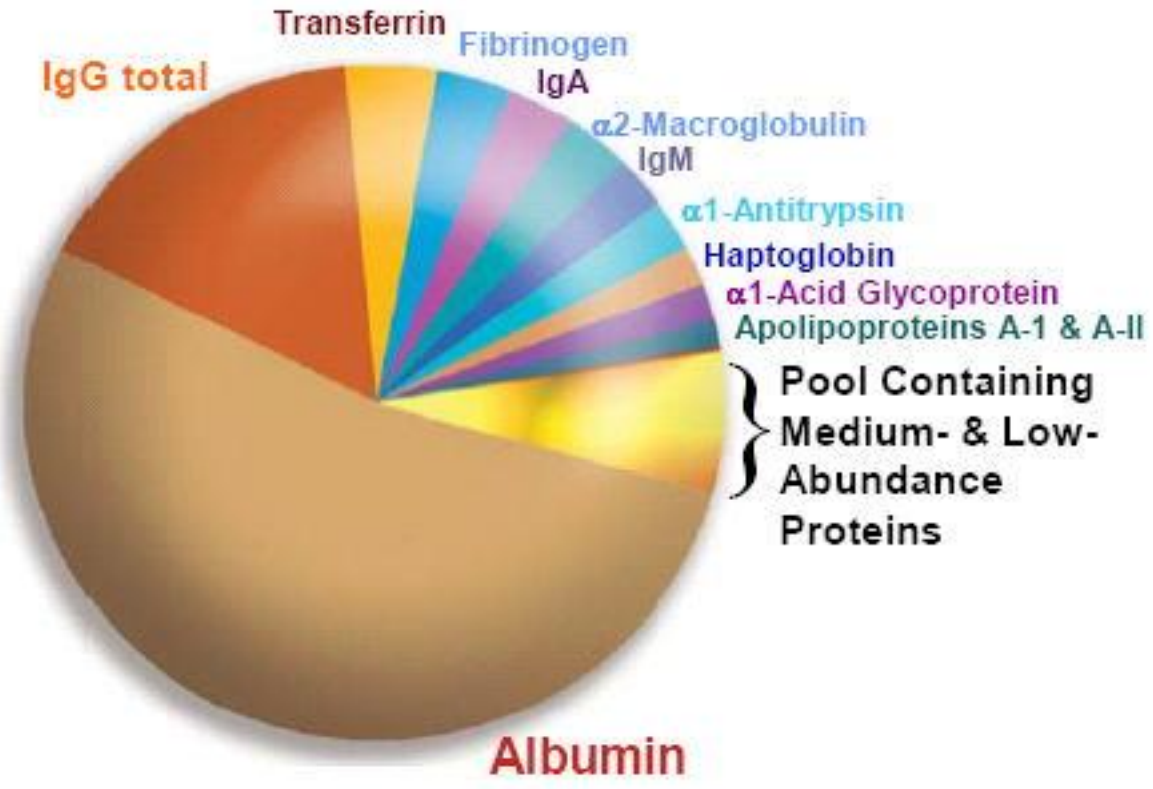
- The Plasma is formed by 90% water, salts, lipids and hormones,
- Plasma is mainly a protein-rich liquid, including albumin, its main protein; immunoglobulins; as well as coagulation factors and fibrinogen,
- Plasma performs several functions:
    - the transport of blood cells and nutrients
    - the regulation of water and mineral salts of the body
    - tissue irrigation
    - defense against infections
    - Blood coagulation

From plasma to medicinal products





LFB is fractionating annually 21 biological drugs from 1 million liters of plasma. LFB is intended to triple quantities in the coming years



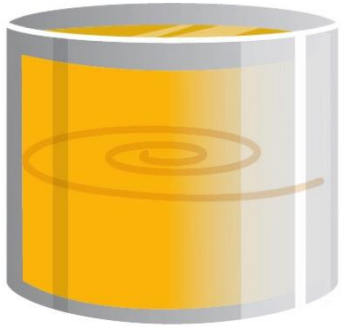
- From 1 million liters of plasma we have access to:
  - 35 tons of Albumin
  - 8 tons of Polyvalent Immunoglobulins (polyclonal antibodies)
  - 3 tons of Fibrinogen

CRYOSEPARATION

The CRYOSUPERNATANT is separated from the CRYOPRECIPITATE by centrifugation

CRYOSUPERNATANT

Light yellow liquid from which immunoglobulins, albumin and other proteins are extracted



CRYOPRECIPITATE

Light yellow paste from which Factor VIII and Von Willebrand Factor are extracted

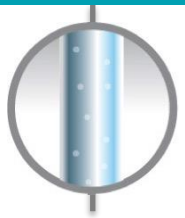
PURIFICATION

Each medicinal product has its own manufacturing process
Different techniques are used to isolate and purify the proteins

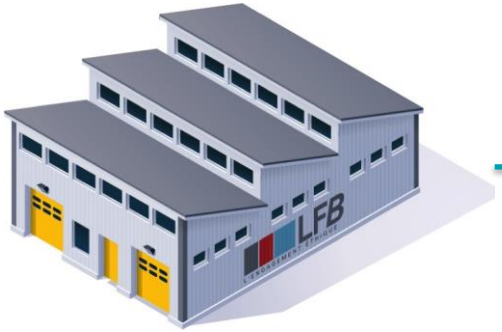
PRECIPITATIONS

Process to separate proteins by ethanol or caprylic precipitation

CHROMATOGRAPHIES



Process to separate proteins by chromatography gel



The medicinal products are purified and secured in the plants in Les Ulis and Lille (France).

PHARMACEUTICAL FORMULATION

The medicinal product is formulated, filled and then controlled again

Pharmaceutical release

Each year more than 1.2 million vials of medicinal products is released.

Most of them are freeze-dried

ETHANOL FRACTIONATION



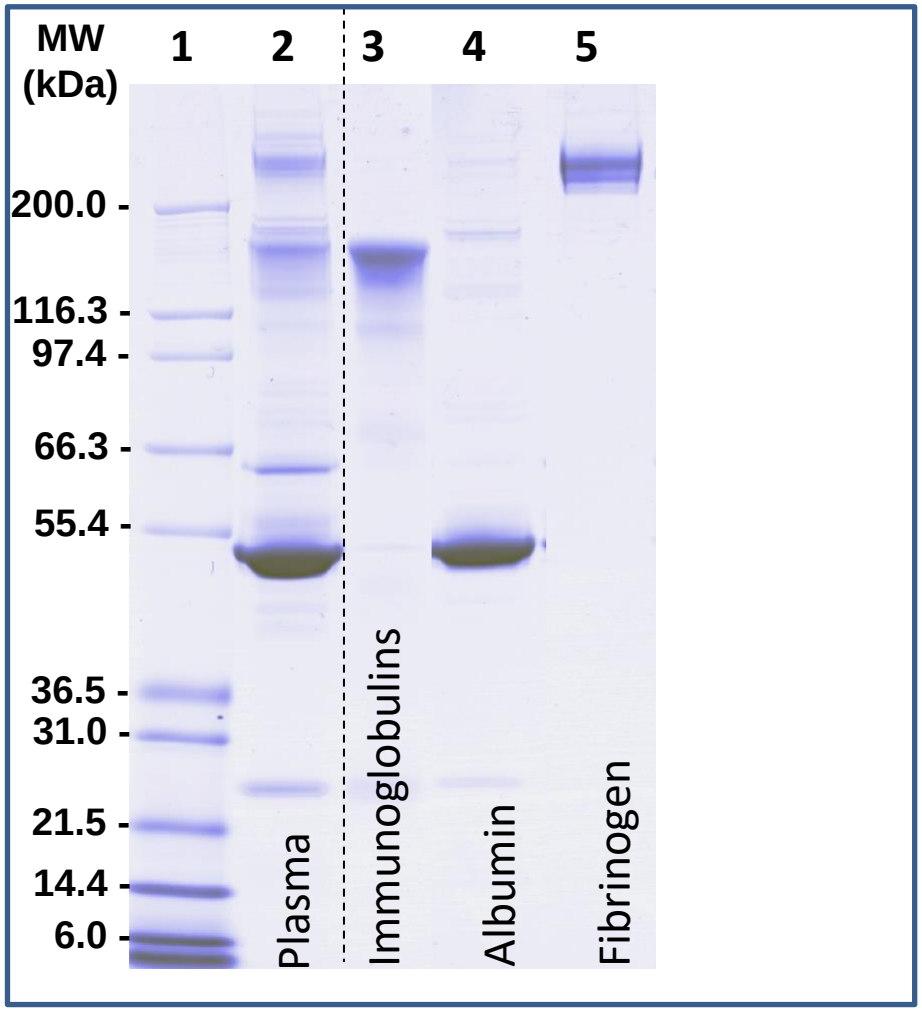


ETHANOL FRACTIONATION 1/2

A BIT OF HISTORY

- 1949: Cohn at al: Development of an albumin purification process based on differential solubility in ethanol between albumin and immunoglobulin.
- 1958: Biger Blombäck developed a Fibrinogen purification process with additive ethanolic precipitation steps
- Since the 80's chromatographic steps where introduced but only at the end or in the middle of purification processes (Ions exchange chromatography or Heparin/Gelatin affinity chromatography)

ETHANOL FRACTIONATION 2/2



VIH and PRION CRISIS





VIH and PRION CRISIS

- Viral and Prion safety of plasma-derived medicinal products is of paramount importance

- Between 1980 and 1990 plasma blood donations contaminated by the HIV were used. Public opinion was only really alerted in mid-1985, when the French Prime Minister announced the mandatory testing of blood donors from 1 August (order of 23 July 1985).

- The magnitude of the tragedy is known only in August 1986, with the publication of a report from the National Center for Blood Transfusion, which states that one in two hemophiliacs was contaminated, or nearly 2,000 people.

VIH and PRION CRISIS

- 10 years later, an epizootic of Bovine spongiform encephalopathy (BSE) affected the United Kingdom, and to a lesser extent in some other countries. Also known as "mad cow disease", BSE is a degenerative infection of the central nervous system of cattle. It is a fatal disease, caused by a particular type of molecular infectious agent (neither virus nor microbe), called a prion protein.

- Between 1986 and 2000, more than 190,000 animals were infected. This crisis has its origins in the use of animal meal in cattle feed, obtained from uneaten parts of cattle carcasses and dead animals.

- The disease crossed the species barrier and caused 231 human victims in 2006, affected by symptoms similar to Creutzfeldt-Jakob disease, a disease similar to BSE.

- Epidemic crisis seems to be behind us with no human death in France since 2015.

VIH and PRION CRYSIS

- In parallel to blood testing, heating of plasma products was shown to reduce HIV contamination and partially applied. The heated products were in turn abandoned in 1987 in favor of "solvent-detergent" treatment, because the heating process did not eliminate the hepatitis B and C viruses. In the early 1990s, the nanofiltration started to be introduced in blood products manufacturing, to also eliminate non-enveloped viruses.

VIH and PRION CRYSIS

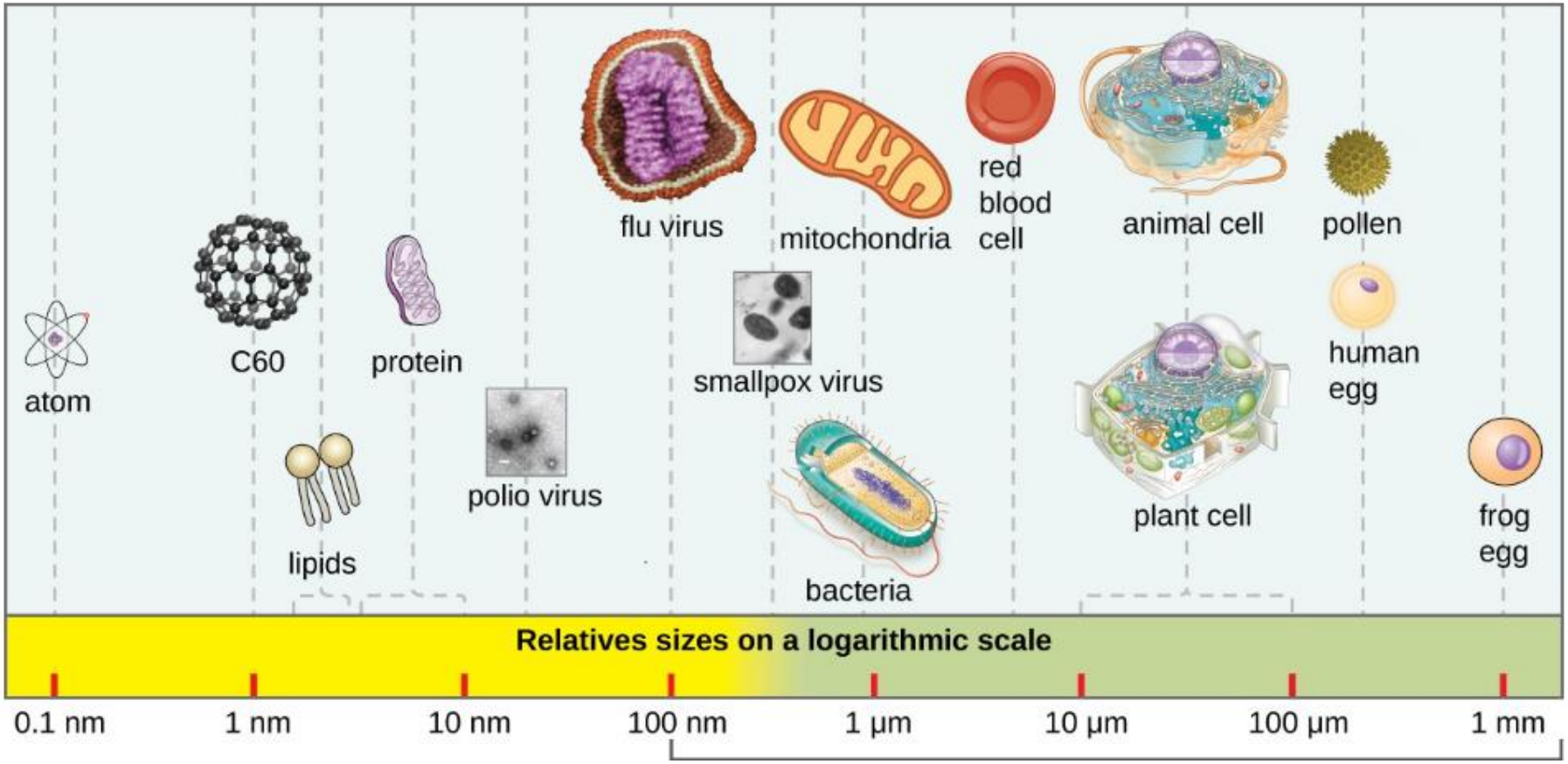
- Plasma production validation is described in an European guideline (EMA/CHMP/BWP/706271/2010 : Guideline on plasma-derived medicinal products).

- Virus inactivation/removal and Prion removal steps were deliberately introduced in manufacturing processes and are called “dedicated to viral/prion safety” and other steps were validated for the ability to contribute to viral/prion risk reduction and are called “contributive step”.

- LFB still takes care to emergent virus (SRAS, Dengue, West Nile) and the ability of its manufacturing processes to prevent patients infection.

VIRAL PRION SAFETY

- Size

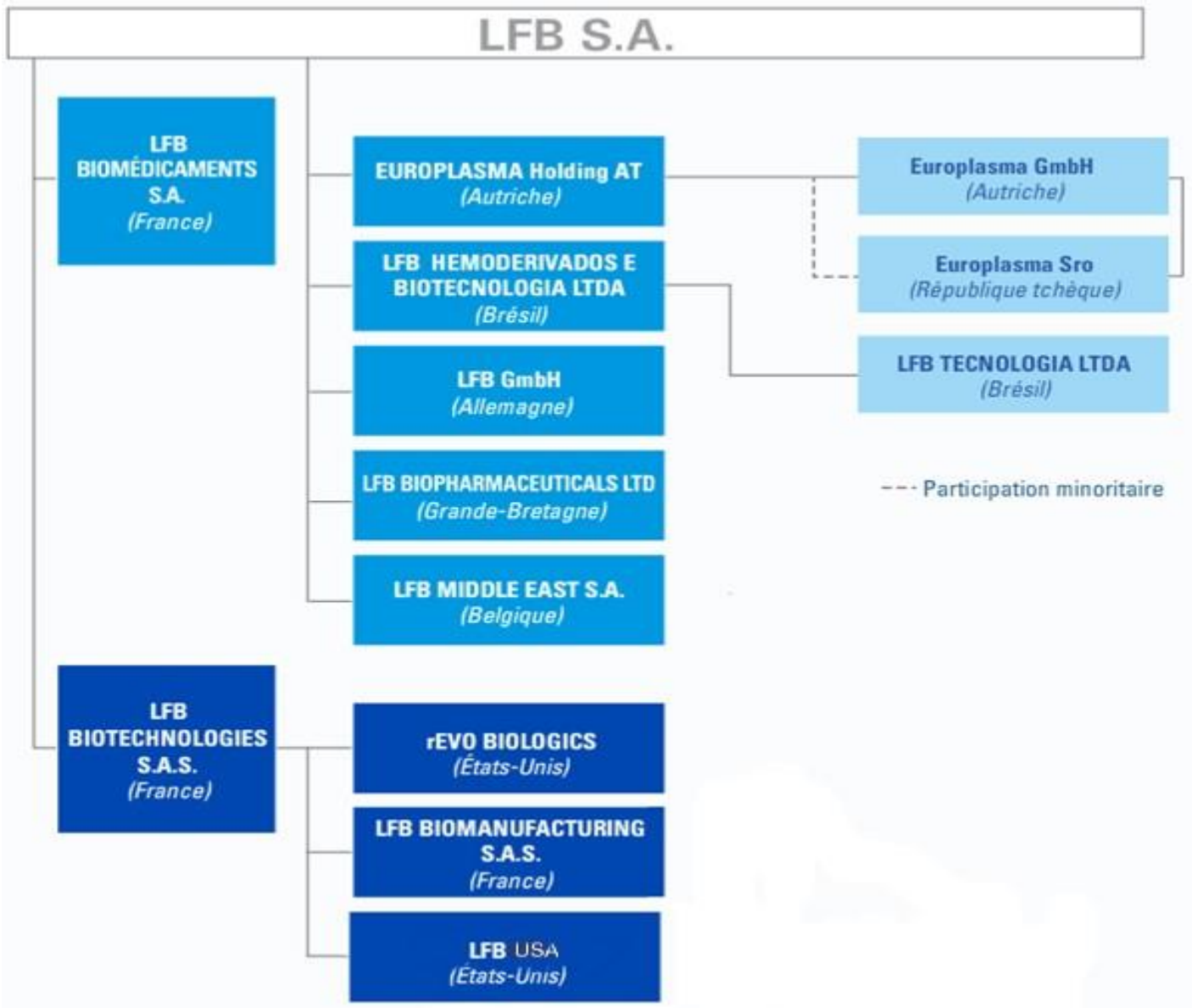


LFB AN INNOVATIVE COMPAGNY

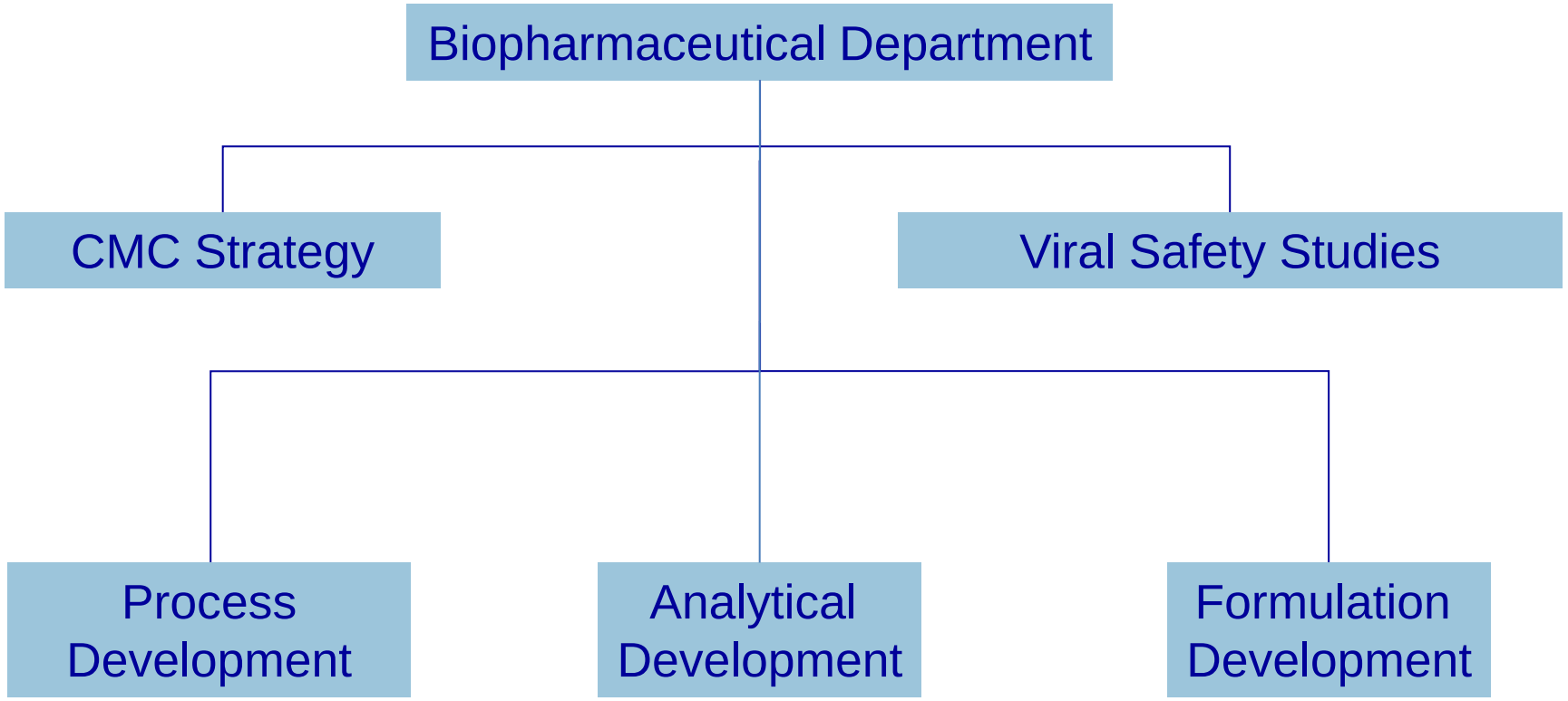




LFB BIOTECHNOLOGIES



BIOPHARMACEUTICAL DEPARTMENT



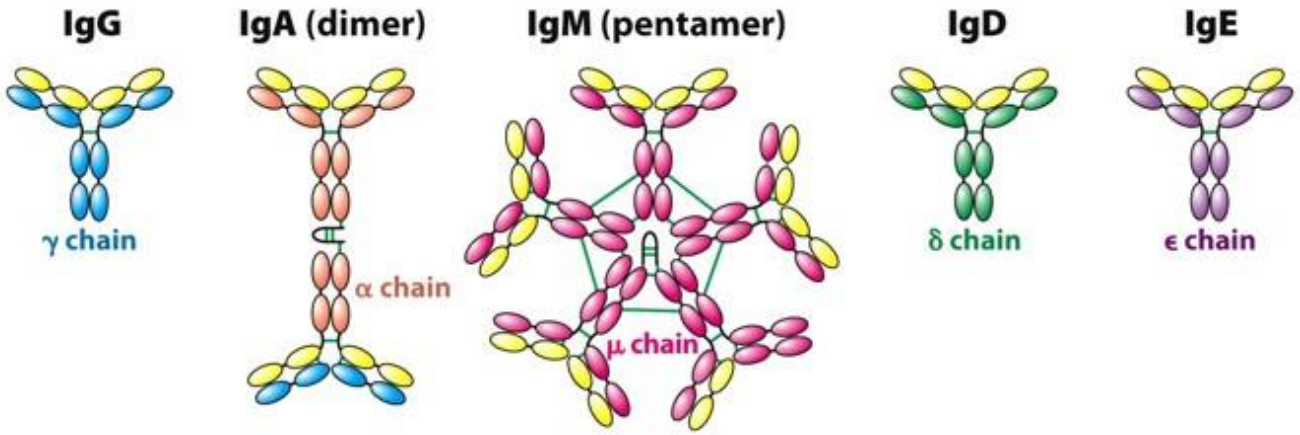


CASE STUDY IMPROVMENT OF POLYCLONAL IMMUNOGLOBULIN MANUFACTURING PROCESS


Polyclonal immunoglobulins

- Immunoglobulin (Ig) secreting cells occur in all lymphoid tissues, including the bone marrow (BM). Ig half life is between 7 and 9 days. Plasmatic concentration is ranging frome 8 to 12 g/L.

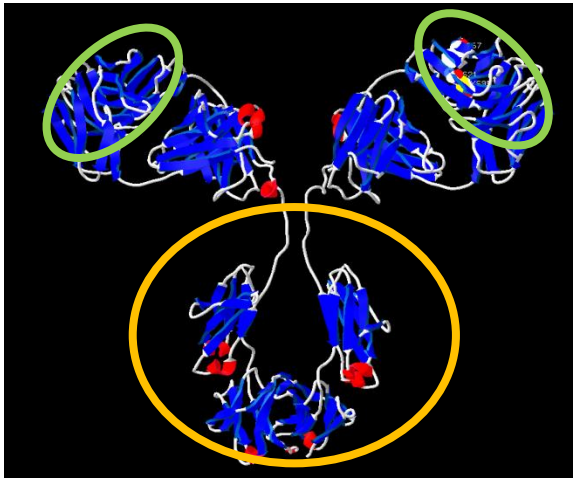
Polyvclonal immunoglobulins



- Immunoglobulins type G: 85% of immunoglobulins

Fab region– recognition of antigens => <u>polyclonal immunoglobulins</u>

Fc region– effector functions (phagocytosis, hemolysis, ...)

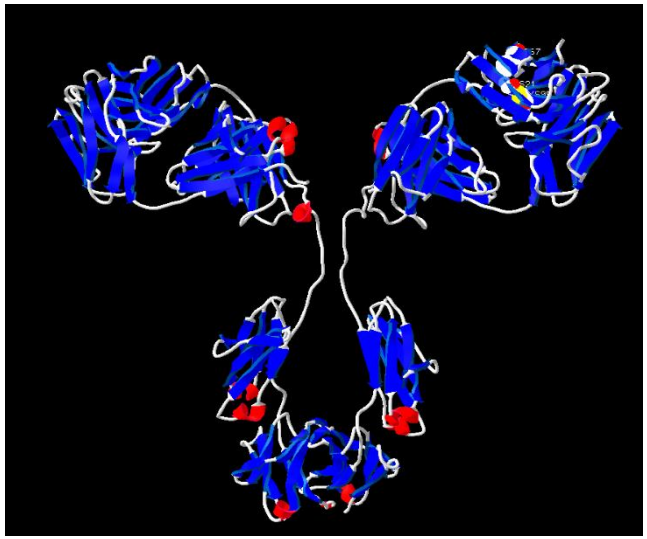


Polyclonal immunoglobulins: Therapeutic use

- Human immunoglobulin (Ig) is the most used blood product in the clinical practice.
- Immunoglobulins treatment in immune deficient patients is performed Intravenously (IVIG). Usually patients are receiving IVG at the hospital every 2 to 4 weeks.

Polyclonal immunoglobulins: Therapeutic use

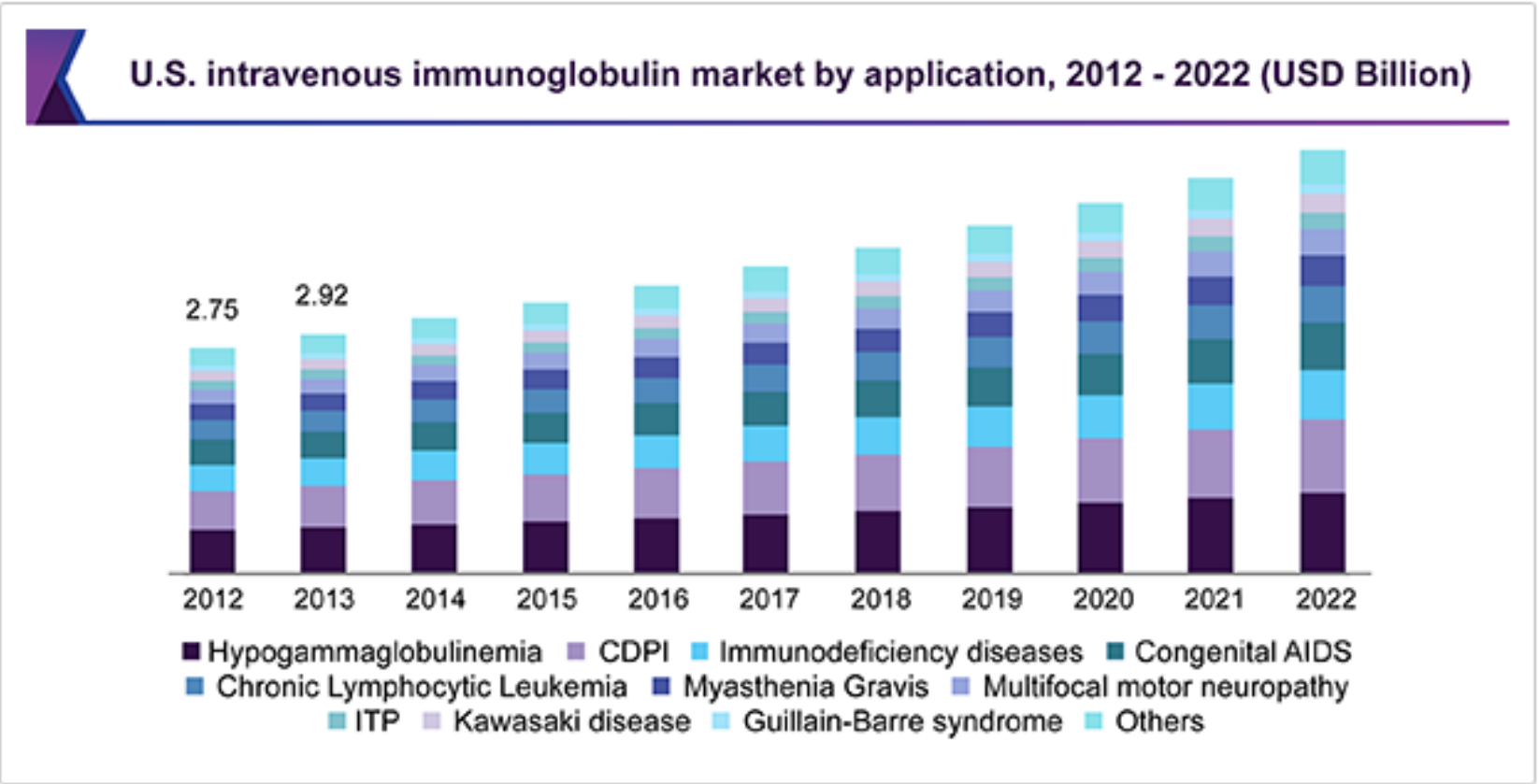
- Immunosubstitution properties :
  1. Primary immunodeficiencies :
     Associated to genetic defect
  2. Secondary immunodeficiencies :
     associated to AIDS or cancers treatments



- Immunomodulatory properties: ➔ Autoimmune diseases
  - Purpura Thrombocytopenic Idiopathic ➔ platelets
  - Retinochoroiditis of Birdshot ➔ Retina
  - Guillain-Barré Syndrome ➔ Myelin affected
  - Kawasaki Disease ➔ Blood vessel involvement
  - Multifocal Motor Neuropathy ➔ Myelin affected (Brain)

POLYCLONAL IMMUNOGLOBULINS MARKET

- 5-10% Yearly increase of IVIG need (Europe/US)



Intravenous Immunoglobulin (IVIG) Market Size, Share & Trend Analysis Report By Application (Hypogammaglobulinemia, CIDP, Congenital AIDS), By Route of Administration, And Segment Forecasts, 2012 - 2022



TEGELINE: IVIG LYOPHILIZED PRODUCT

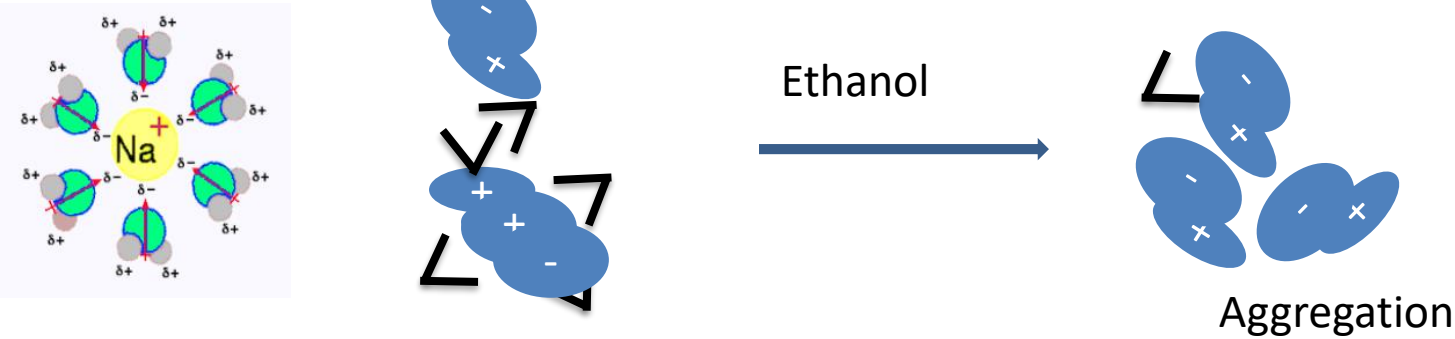

TEGELINE

- TEGELINE obtained the Marketing Authorisation in France in September 1996.
- TGELINE is a lyophilized product that is reconstituted in water before use.
- 0.2 up to 2 g/kg patient per days.

- The manufacturing process is only based on precipitation

TEGELINE: Precipitation

- Precipitations :
  - Lose of protein Solubility
  - Protein charges (or absence of charges) are hidden by water molecules and ions allowing protein to stay freely in solution.
  - By increasing ethanol concentration we will decrease water molecules concentration. Ethanol is a neutral molecule unable to shade charges.



- Factors affecting precipitation rate :
  - Size of the protein
  - Charge of the protein
  - Concentration in precipitating agent
  - Concentration in proteins
  - Solution : pH, conductivity/osmolality, temperature



CLAYRIG: NEW IVIG


CLAYRIG: Context

- Strong increase in global immunoglobulin (IVIG) consumption
- Insufficient quantities available => Patients deprived of care when shortage period are occurring
- Contingencies on Europe and France market because products available to the highest bidder (preferred US market because more expensive)

➔ **Objectif :**
Urgent need to improve the efficiency of extraction of immunoglobulins from the plasma used

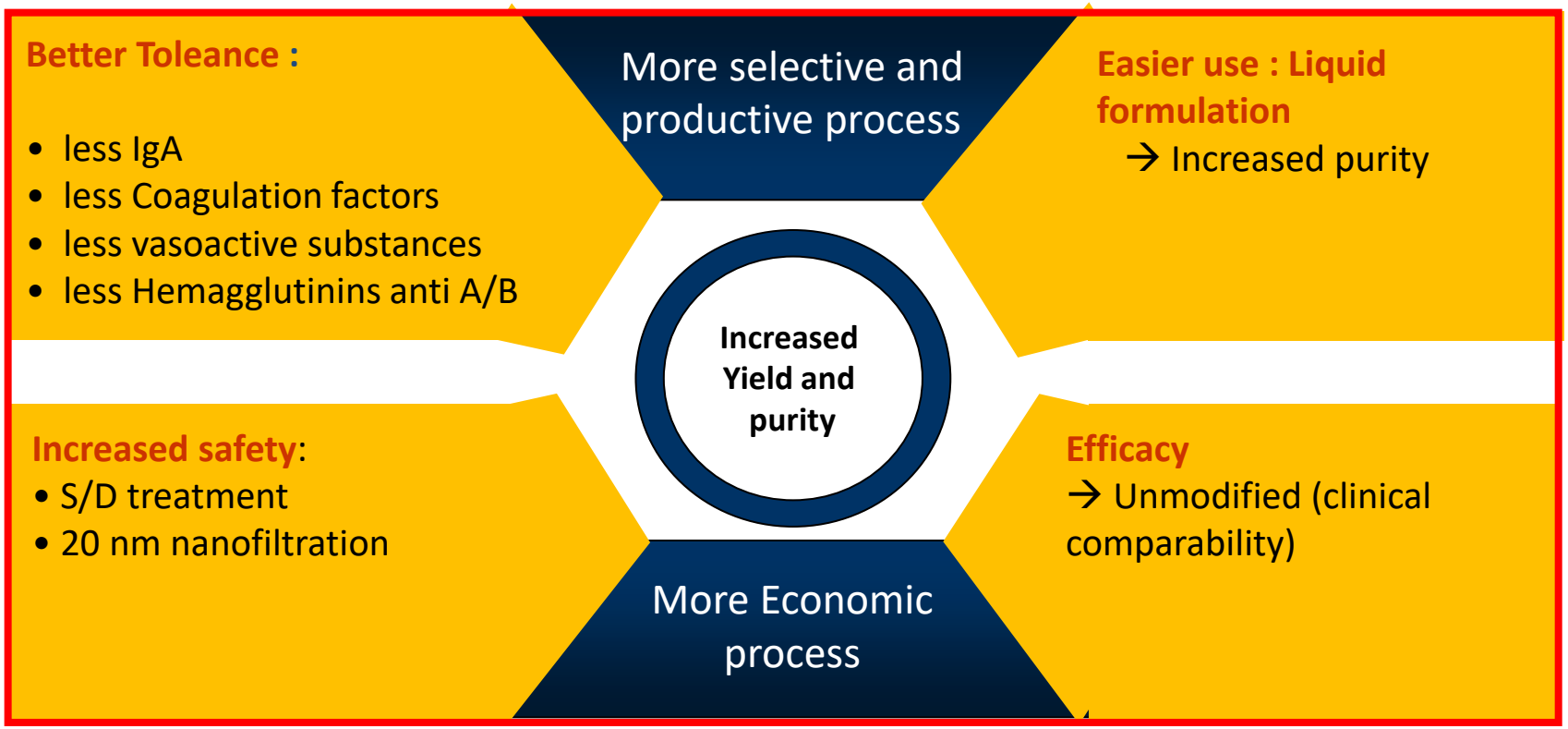
CLAIRYG: Context

- Side effects related to the use of immunoglobulins injected intravenously (IVIG) are reported worldwide:
  - Few cases of hypotension related to IVIG administration
  - Haemolytic anemia in some A, B or AB patients
  - Thrombotic Incidents Linked to Traces of Activated Coagulation Factors (FXIa and FXIIa)
  - Renal intolerance in some patients with renal insufficiency

➔ **Objectif :**

Need to increase the purity of the product and reduce / eliminate the contaminants responsible for reported intolerances

CLAIRYG: Summary of specifications



CLAIRYG: Improvement of precipitation yield

- IVIG Processes were all based on fractionation with ethanol
- This is a low extraction efficiency process (20 to 30% from plasma)
- Stages of "maturing" of the product sometimes necessary to obtain compliant products
- No innovation for more than 50 years on ethanolic precipitation

➔ **Development of caprylic precipitation**

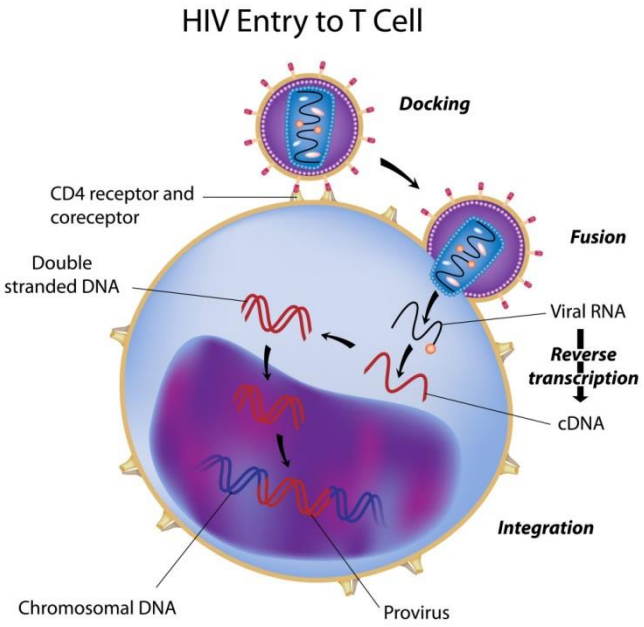
- Addition of caprylic acid conducts to precipitation of contaminants but not immunoglobulins.
- Precipitate is removed by filtration, Ig are going through filters.

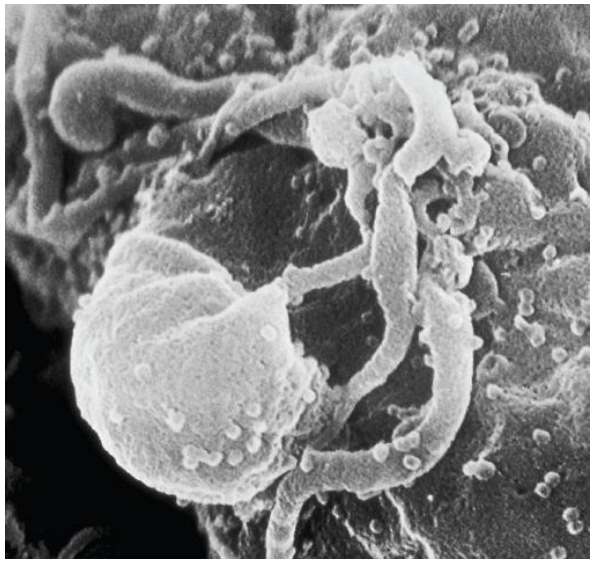
CLAIRYG: S/D treatment and IgA removal

- The addition of the viral inactivation step by Solvent/Detergent treatment required removal of solvent and detergent
- An ions Exchange chromatography was developed to remove IgA and by the way removing solvent and detergent

CLAIRYG: S/D treatment and IgA removal

- Solvent/Detergent treatment:
  - Solvent and Detergent (Triton/TnBp) are added in the intermediate of production.
  - Solvent and detergent will solubilize the phospholipid envelope of enveloped viruses (HIV for example). By this way the virus lose its ability to infect cells: it is inactivated.

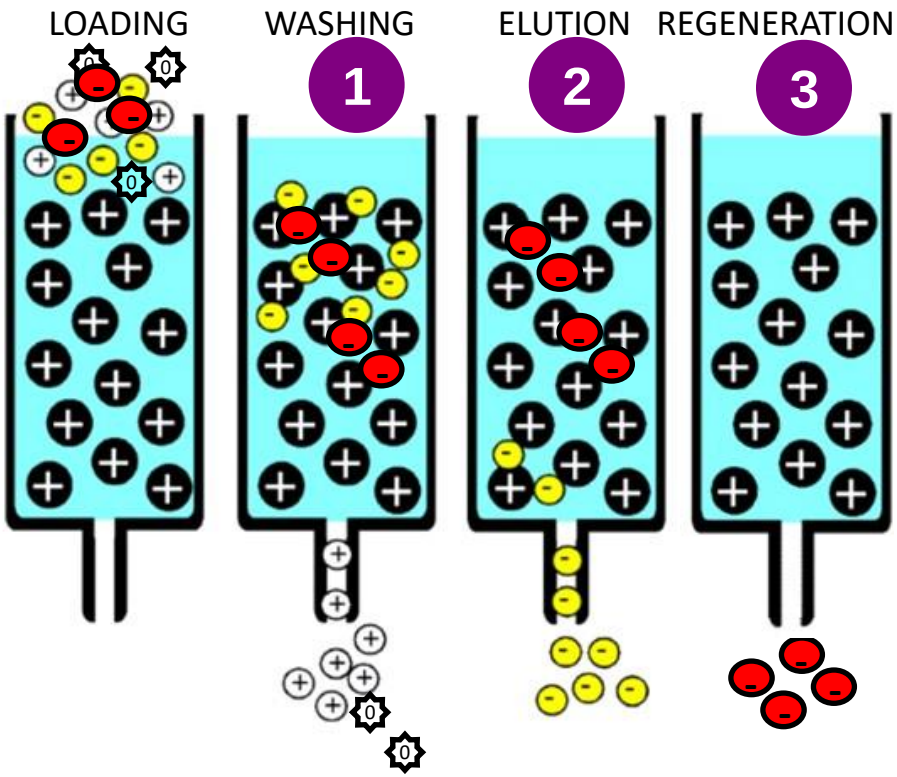


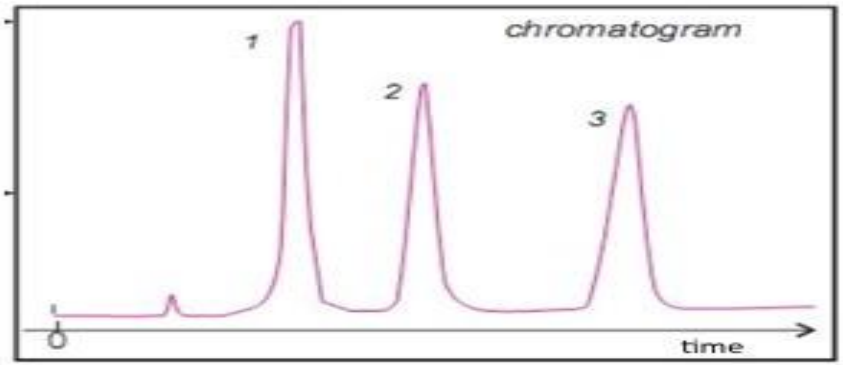


The enveloped human immunodeficiency virus uses spikes made of glycoproteins embedded in its envelope to bind to host cells (credit a "micrograph": modification of work by NIAID; credit b "micrograph": modification of work by Centers for Disease Control and Prevention)

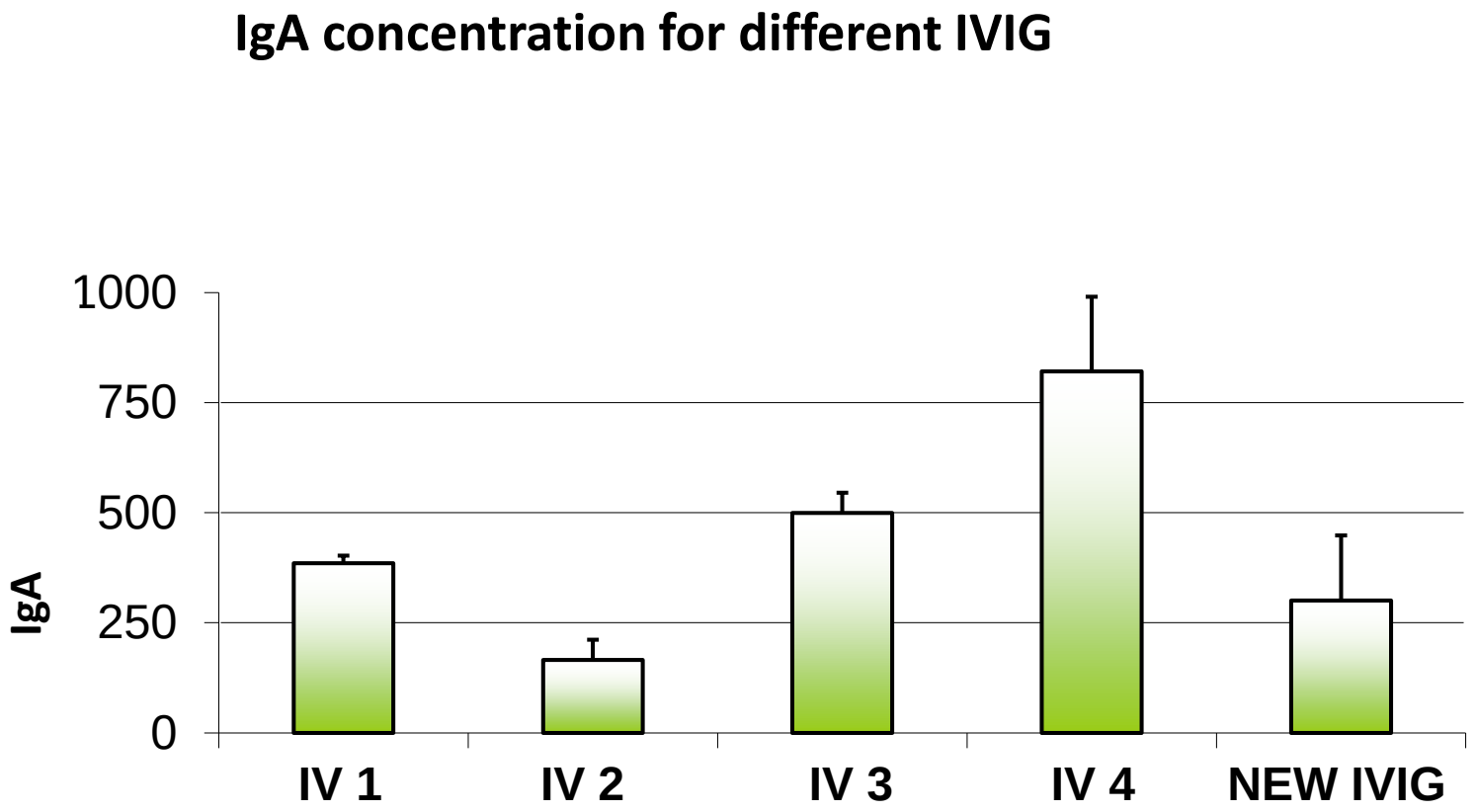
CLAIRYG: S/D treatment and IgA removal

- Ions Exchange chromatography:
  - At pH 9 IVIG are negatively charged.
  - IgA have a stronger charge compared to IgG
  - IgG are eluted before IgA
  - Elution is performed by charge displacement with buffers containing increasing quantities of negative and positive ions (NaCl : Sodium Chloride)





CLAIRYG: S/D treatment and IgA removal



CLAIRYG: Hemagglutinins anti A/B

- Blood groups are defined by a system called the "ABO system".
  - Type A blood group has A antigens on its red blood cells and anti-B antibodies in its plasma.
  - Type AB blood group has two antigens A and B, but no antibodies.

    Receiving blood from the wrong ABO system can put the life of the recipient at risk because the antibodies of a person with, for example, a blood group A, will attack the antigens of a person with blood group B and vice versa.

- Plasma donations are a pool of all groups, as a consequence plasma contains Anti-A and Anti-B antibodies that has to be removed.

CLAIRYG: Hemagglutinins anti A/B

- Development of a purification step dedicated to the capture of anti-A and anti-B antibodies in presence of IgG:
  - The new IVIG was the first manufacturing process integration such a step : 4 years were spent from development to industrialization
  - The technology use is an home made affinity chromatography

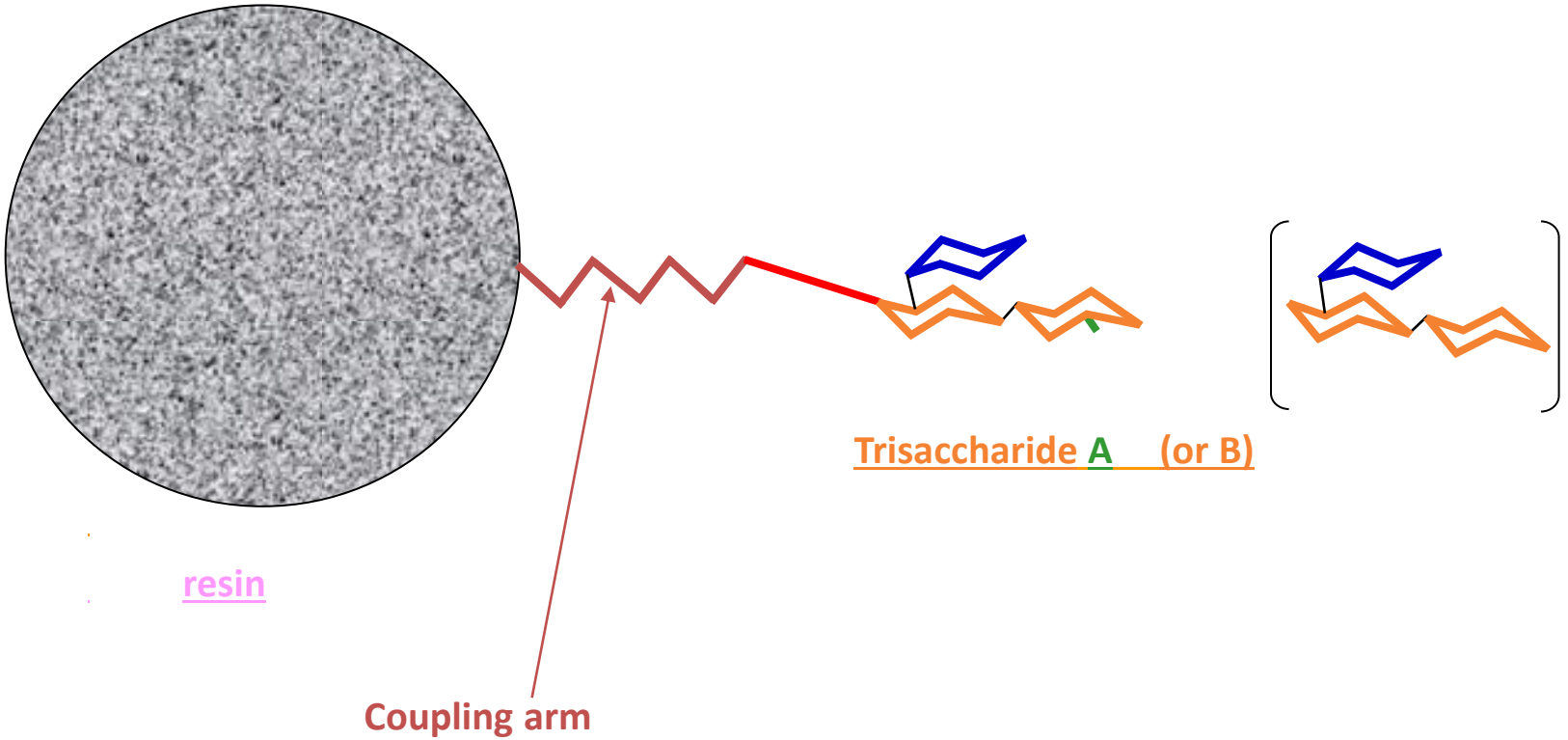
CLAIRYG: Hemagglutinins anti A/B

- Design of the Hemagglutinins anti A and anti B affinity chromatography:
  - Constructing sugar mimicking hemagglutinins A and hemagglutinins B
  - Grafting of sugars on a chromatography resin => two different resins who will bind antibodies against A and B serotypes.
  - IgG will go through the column
  - A and B resins will be mixed 50/50 in the chromatography column

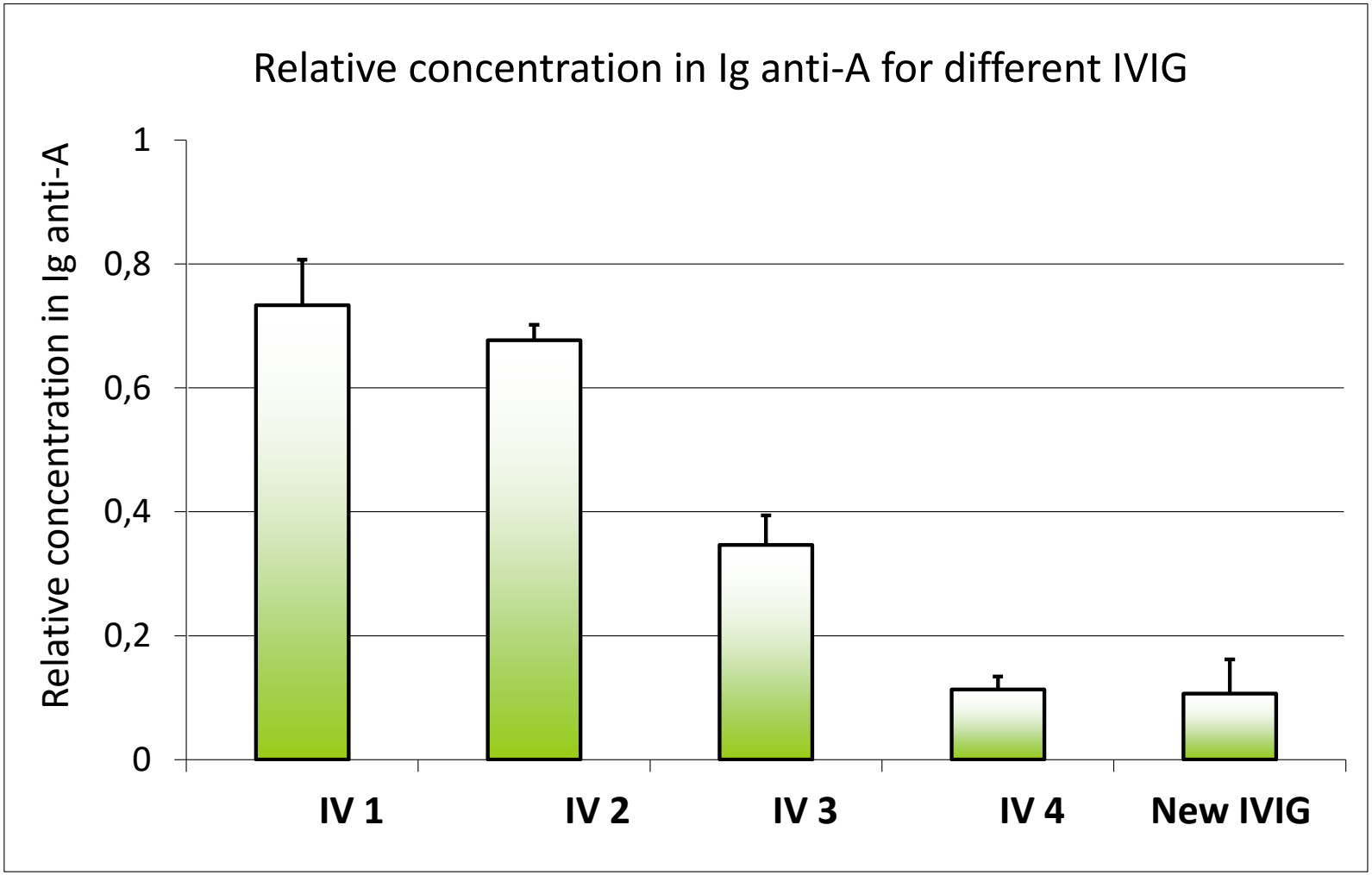
CLAIRYG: Hemagglutinins anti A/B

Grafting of ligand on chromatography resin

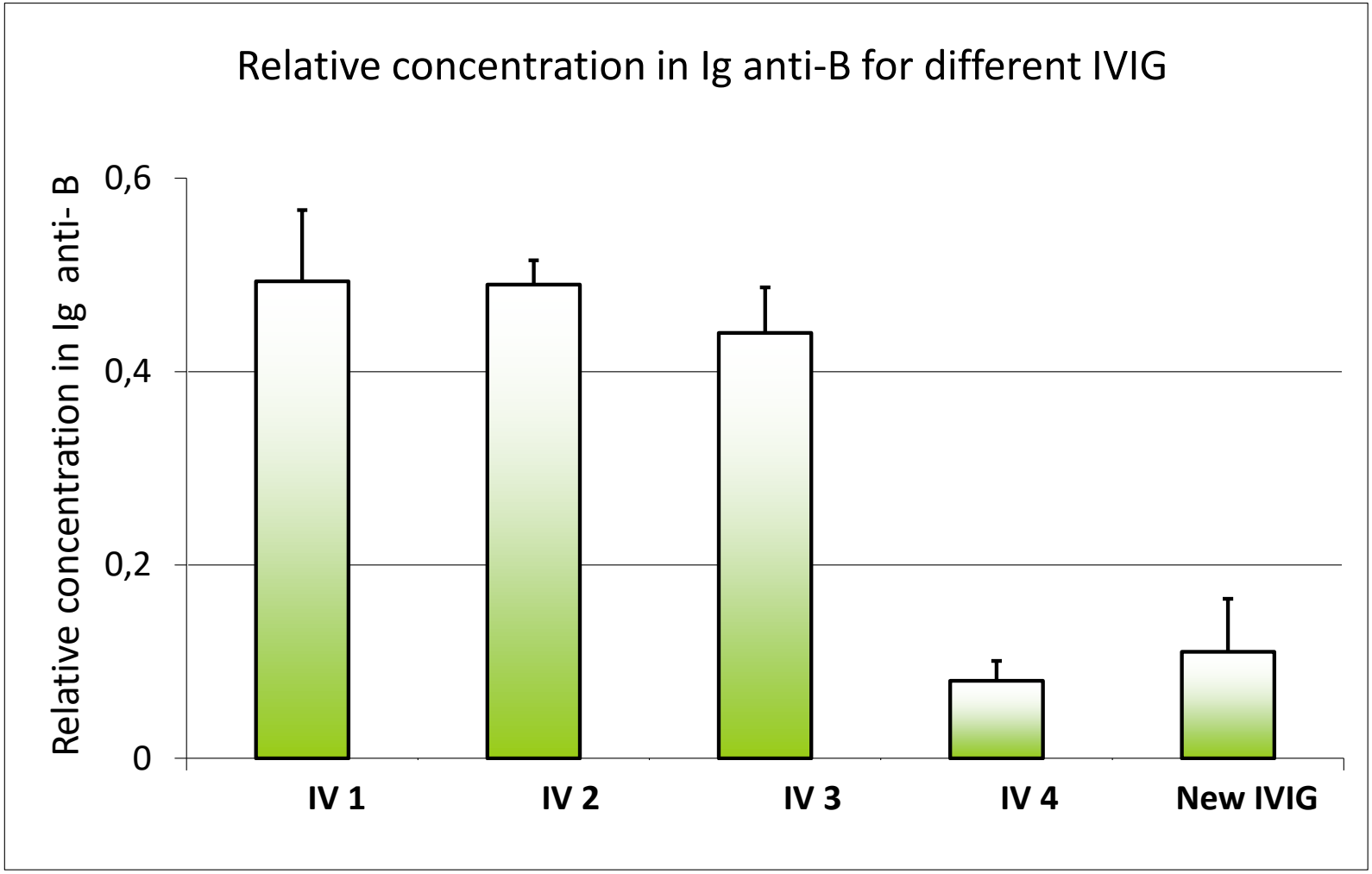
➔ **Affinity resin type A (ou B)**



CLAIRYG: Hemagglutinins anti A/B



CLAIRYG: Hemagglutinins anti A/B



CLAIRYG: Nanofiltration 20 nm

- In addition of the S/D treatment that was not present in Tegeline process the nanofiltration 35 nm was replaced by a 20 nm nanofiltration with the aim to eliminate viruses of size bellow 20 nm (Parvoviruses) and to get a full removal of larges viruses as HIV

CLAIRYG: Formulation

- Formulation development

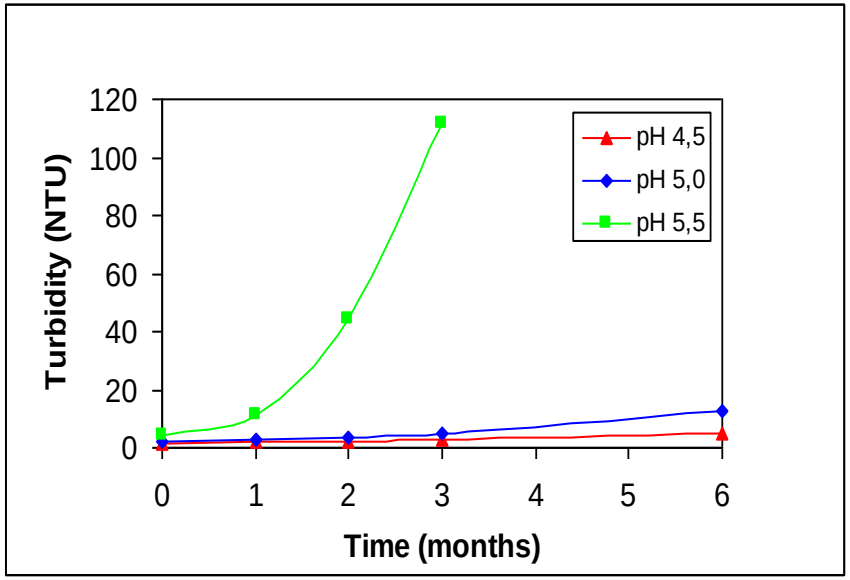
Formulation Number	Component 1	Component 2	Component 3	Analytical results		
				Heat Stress	Agitation stress	Oxydation stress
1	-	-	-			
3	+	-	-			
4	-	+	-			
5	+	+	-			
28	+	-	+			
29	-	+	+			
30	+	+	+			

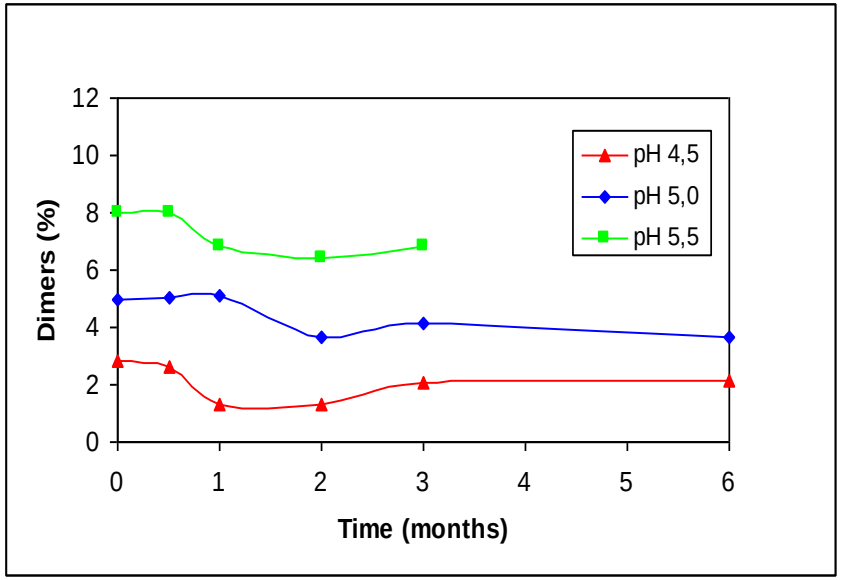
	Strong instability
	Low instability
	Good stability

=> Selection of formulation components for liquid state

CLAIRYG: Formulation

- **Influence of pH on IVIG solution turbidity and dimerisation at 40 °C**

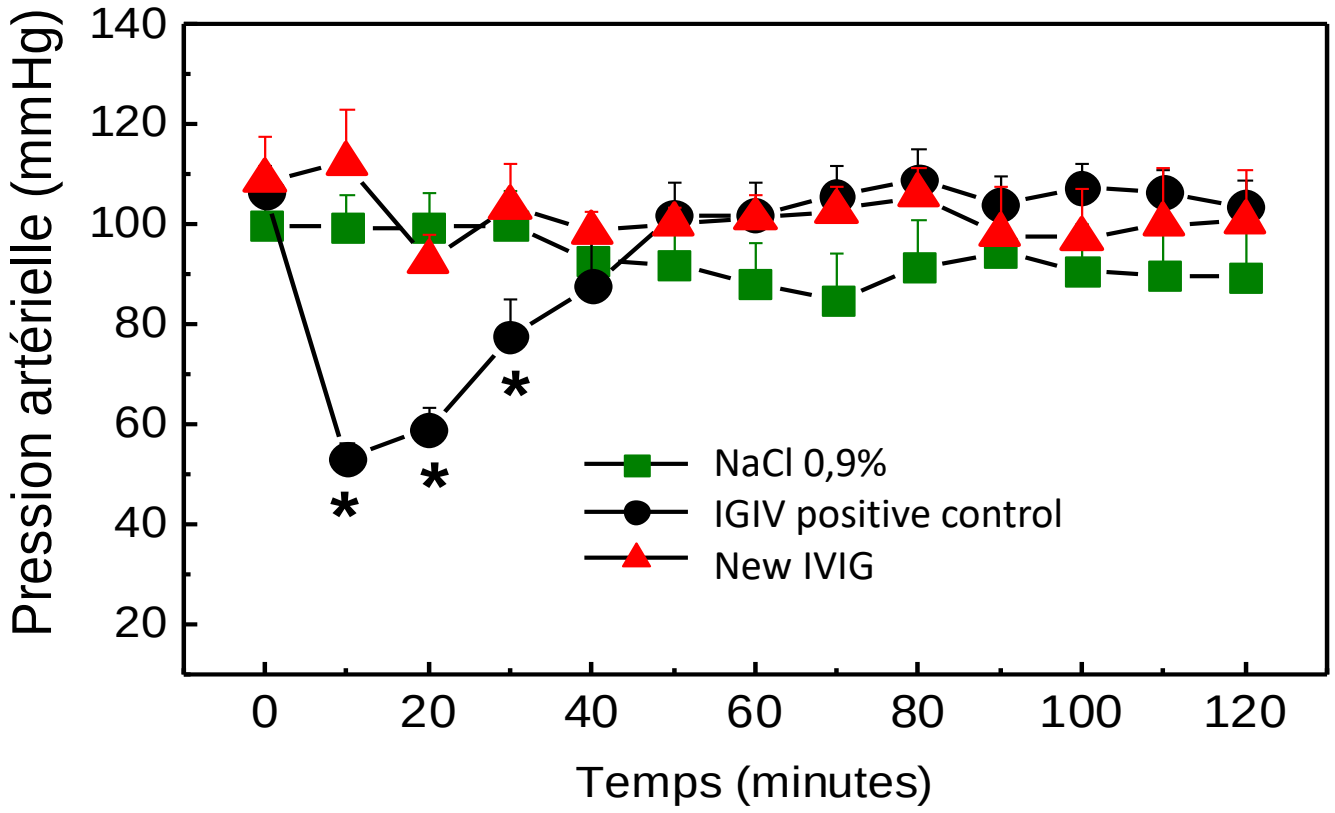




➔ **A strong acidic pH was selected**

CLAIRYG: Formulation

- Hypotensive effect test (On rats)

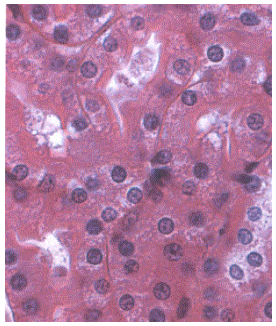


➔ **Good tolerence**

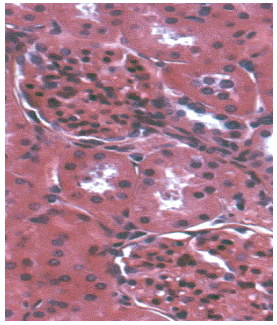
CLAIRYG: Formulation

- Verification of the renal tolerance of the formulated product
  - Test in normal rats after single intravenous administration

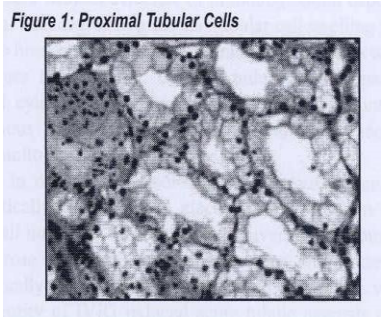
➞ The histological study revealed no renal dysfunction, in particular:
No modification of tubular epithelial cells, no necrosis.
No osmotic nephrosis
Absence of glomerular or vascular lesions
No difference between the groups treated by the new IVIG and control groups



Glomerular cells
after treatment



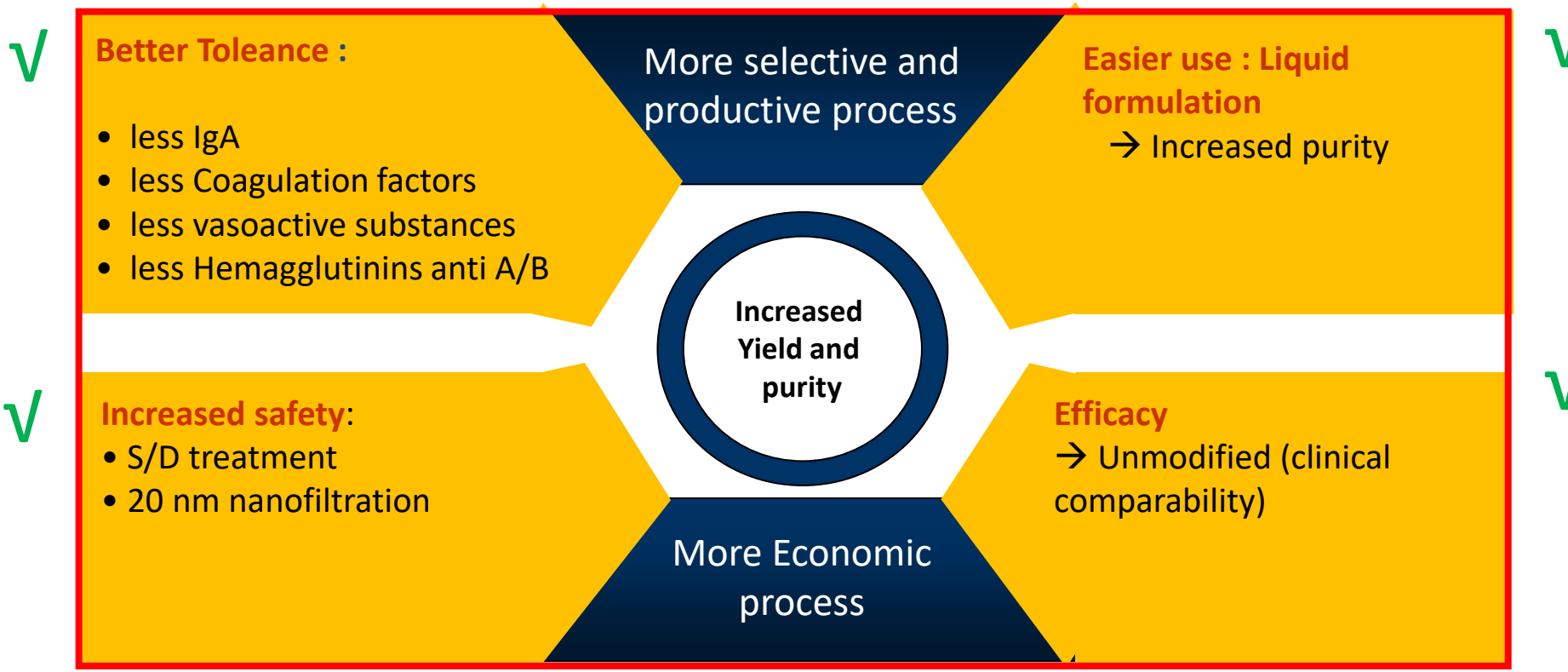
Tubular cells after treatment



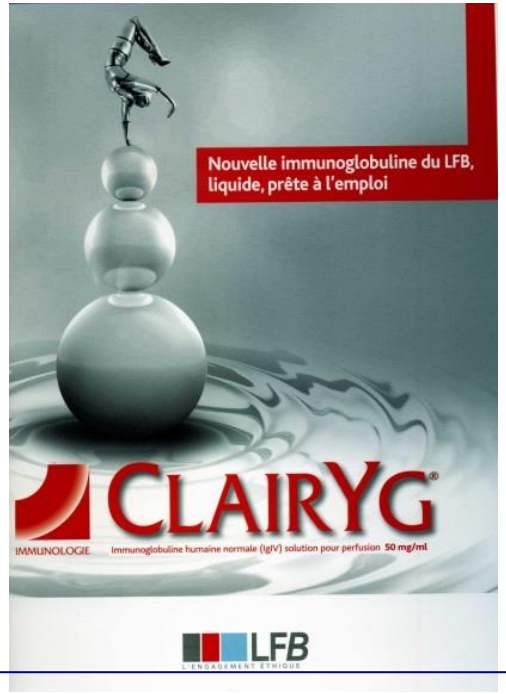
Osmotic nephrosis
induced by IGIV

➔ **Good renal tolerance**

CLAIRYG: Summary of specifications



France and European Approval



→ ClairYg approval in France in August 2010

→ European approval since January 2016

→ Today 15 countries in Europe and Mexico.

→ Coming next USA.



Conclusions


Conclusions

- LFB was able to face two strong biological safety crises and potential undesirable effects and put in place high development strategies based on product specification focused on patient needs.
- By the use of modern affinity technologies we put on the market in France and Europe a competitive modern IVIG, with an increased purity and tolerability