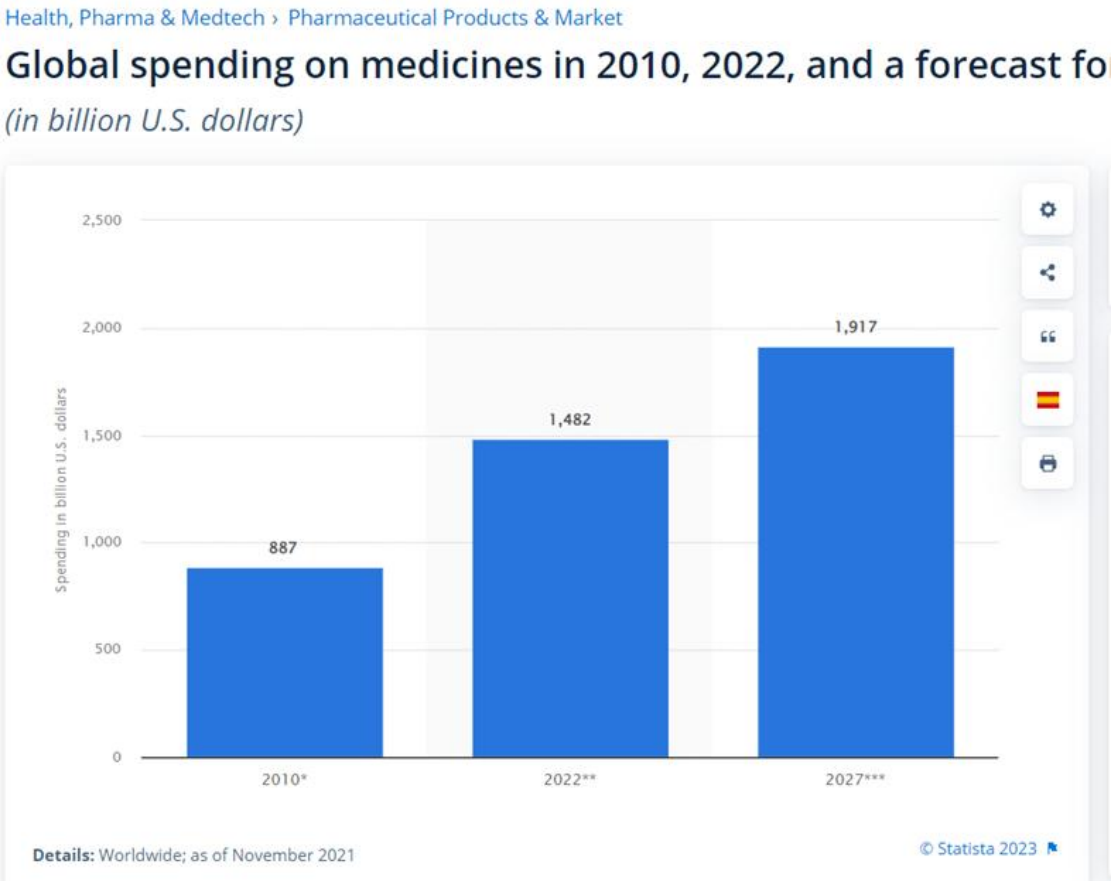
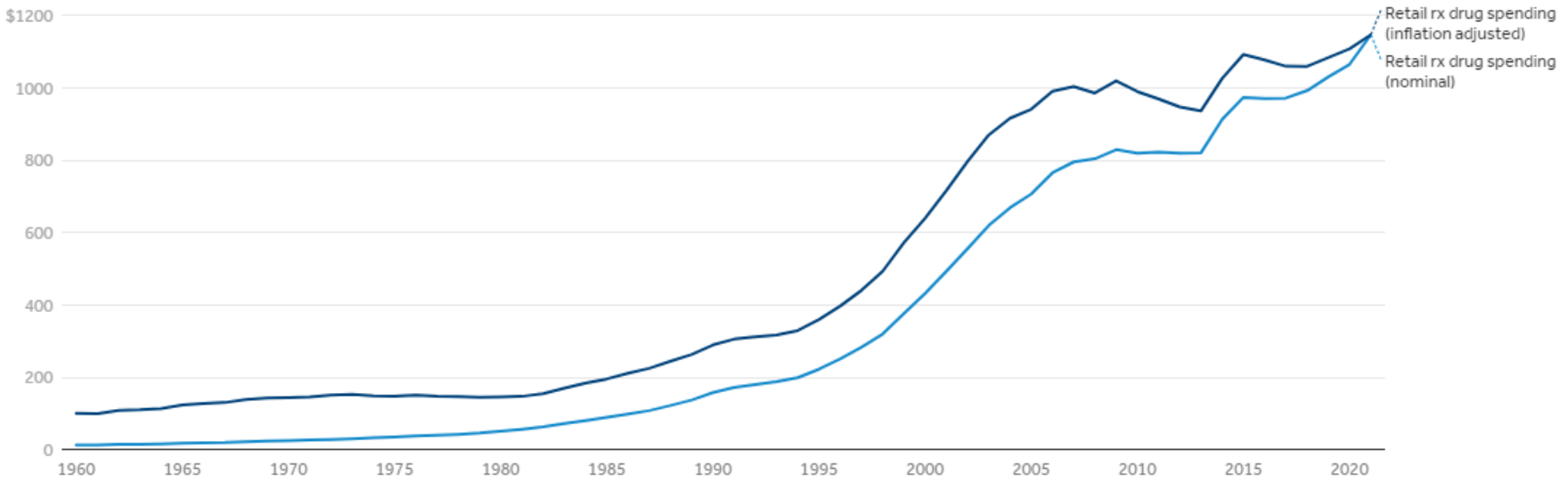


In 2022, approximately **1.48 trillion U.S. dollars** had been spent on medicines

That number is expected to increase to over **1.9 trillion** by the year 2027.

Spending on medicines has increased everywhere globally.

Nominal and inflation-adjusted per capita spending on retail prescription drugs, 1960-2021

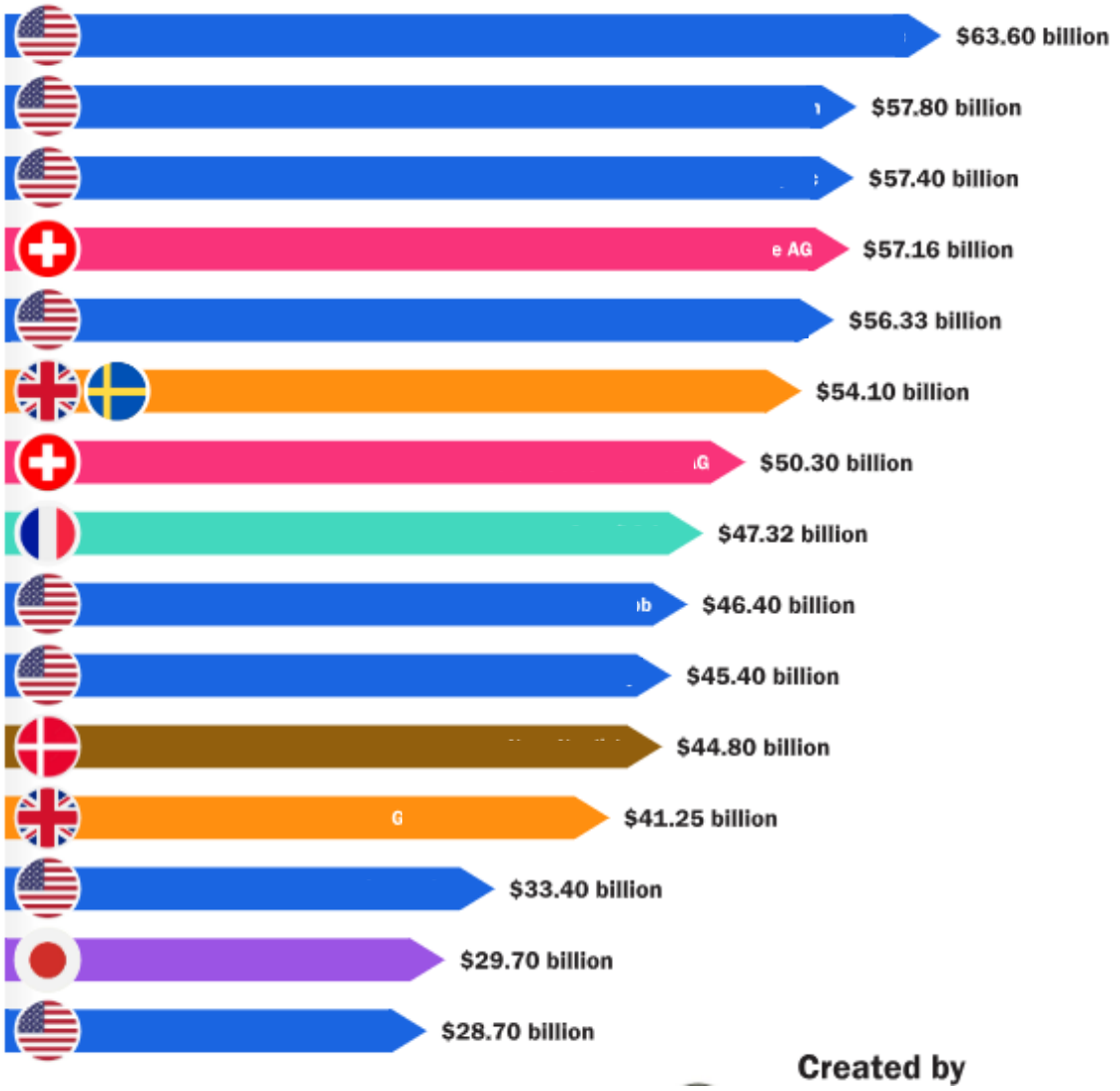


Source: KFF analysis of National Health Expenditures Accounts (NHEA) • Get the data • PNG

Peterson-KFF
Health System Tracker

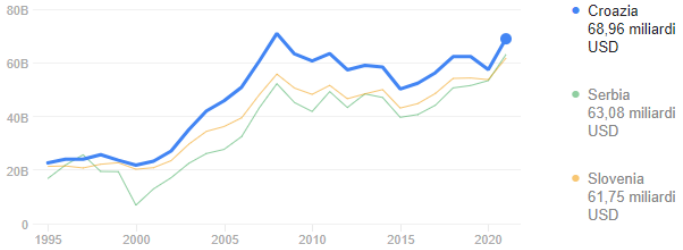
Top 15 Largest Pharmaceutical Companies in the World for 2025

Ranked by revenue from pharmaceutical drug and vaccine sales



Croazia / Prodotto interno lordo

68,96 miliardi USD (2021)



Ulteriori informazioni →

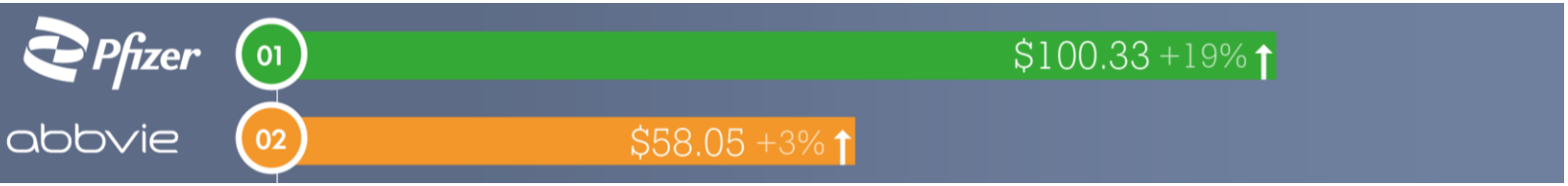
Le fonti comprendono: Banca Mondiale

Feedback

Croazia

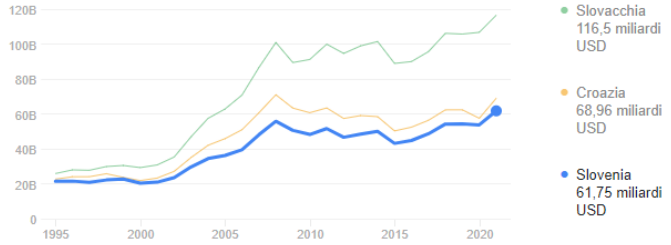
Paese nella penisola balcanica

La Croazia è un Paese dell'Europa orientale con una lunga fascia costiera sul mare Adriatico. Comprende più di mille isole ed è attraversata dalle Alpi Dinariche. La capitale, Zagabria, si trova nell'entroterra e si contraddistingue per il centro storico medievale chiamato Gornji Grad (Città Alta) e per la presenza di diversi musei. Dubrovnik è la principale città lungo la costa, caratterizzata da grandi mura del XVI secolo che circondano il centro storico con edifici gotici e rinascimentali. — Google



Slovenia / Prodotto interno lordo

61,75 miliardi USD (2021)



Ulteriori informazioni →

Le fonti comprendono: Banca Mondiale

Feedback

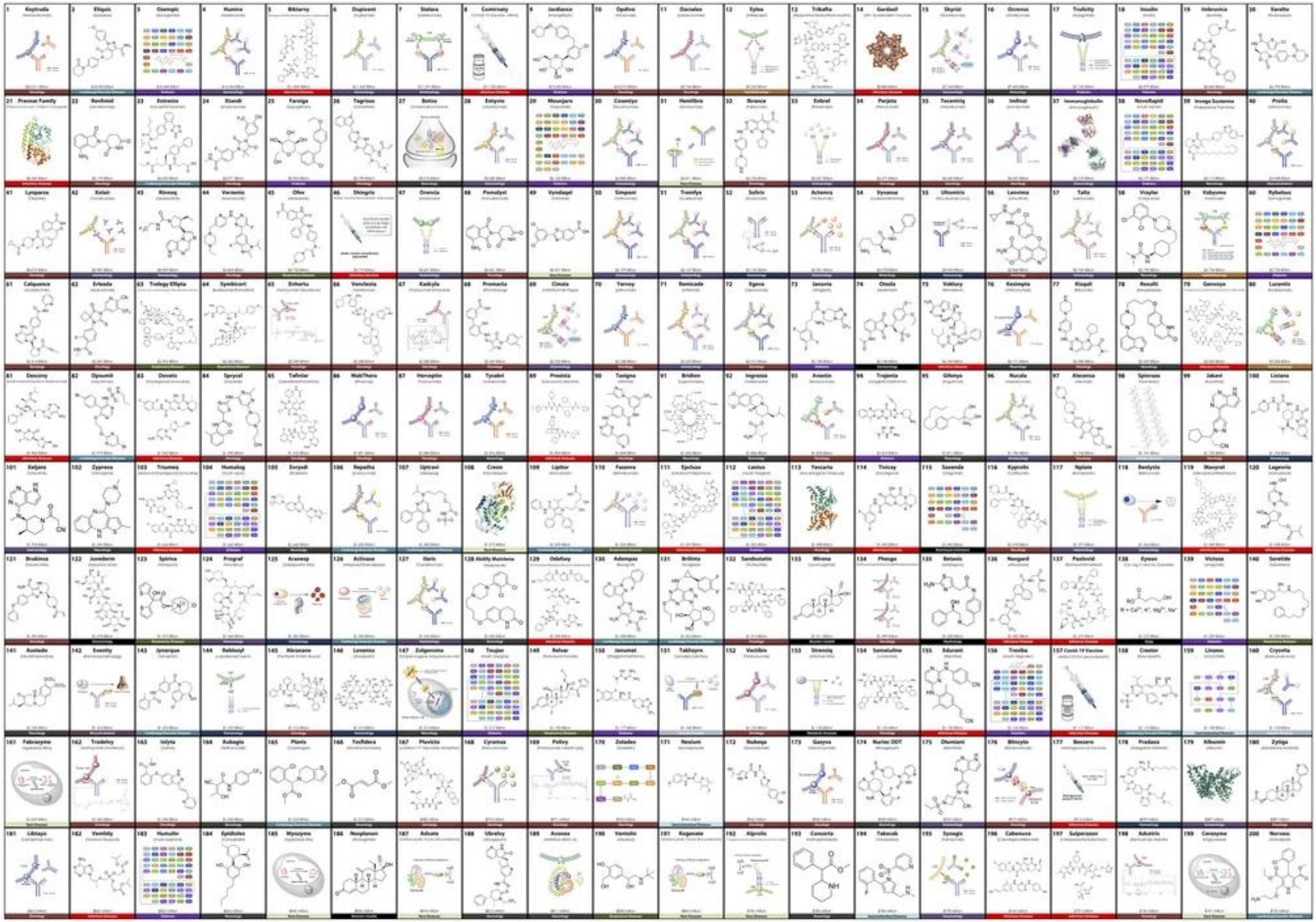
Slovenia

Paese nella penisola balcanica

La Slovenia è un Paese dell'Europa Centrale noto per le sue montagne, le stazioni sciistiche e i laghi. Sul Lago di Bled, un lago glaciale alimentato da sorgenti di acqua calda, la città di Bled custodisce un isolotto sormontato da una chiesetta e un castello medievale arroccato sulla scogliera. A Ljubljana, capitale della Slovenia, facciate barocche si mescolano con l'architettura contemporanea disegnata dal nativo Jože Plečnik, il cui iconico Tromostovje (Ponte Triplo) attraversa la stretta curva del fiume

Top 200 Drugs by Retail Sales in 2023

Compiled and Produced by Ryan E. Williams and Hayden M. Leatherwood from the Njarðarson Group (The University of Arizona)



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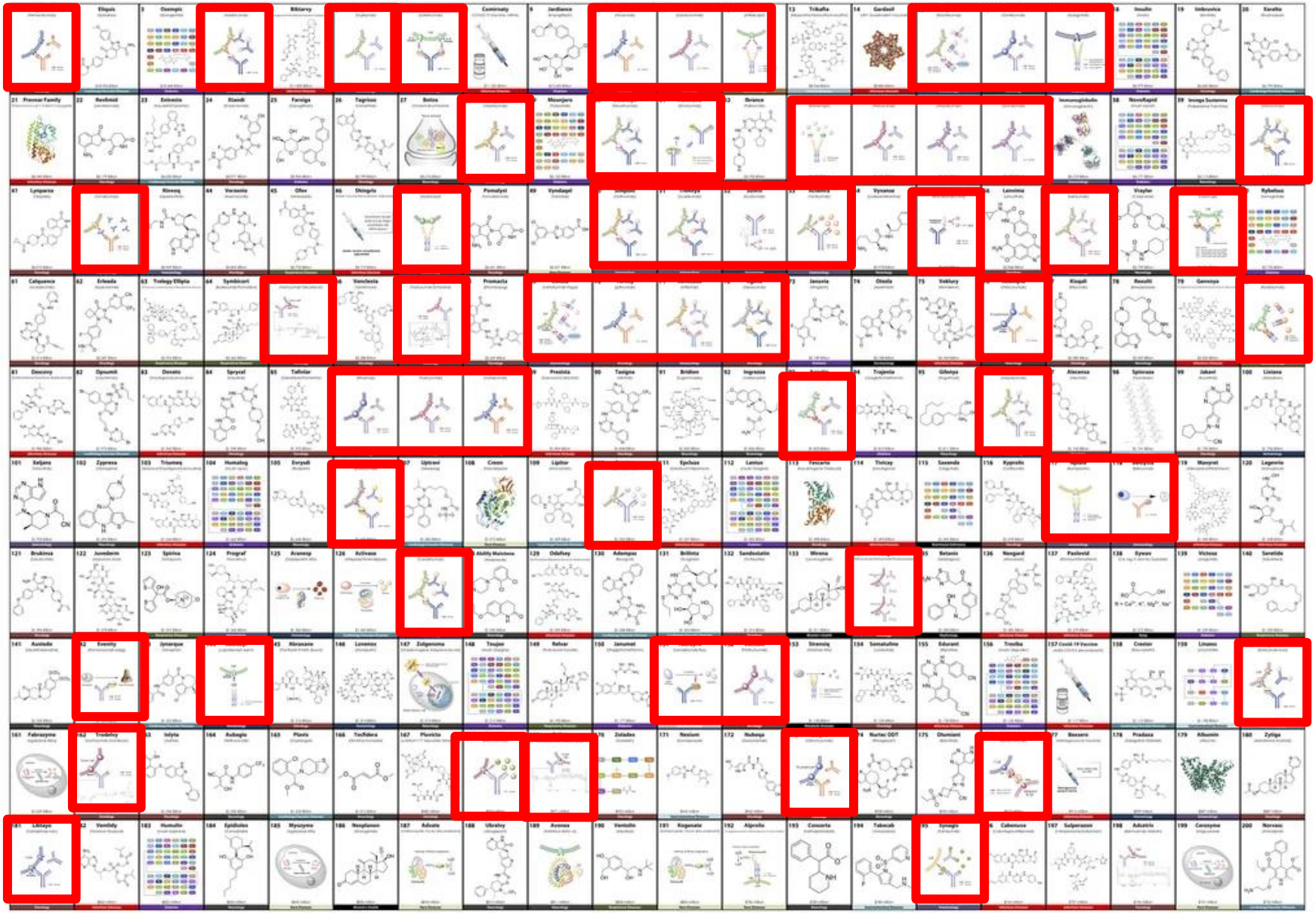
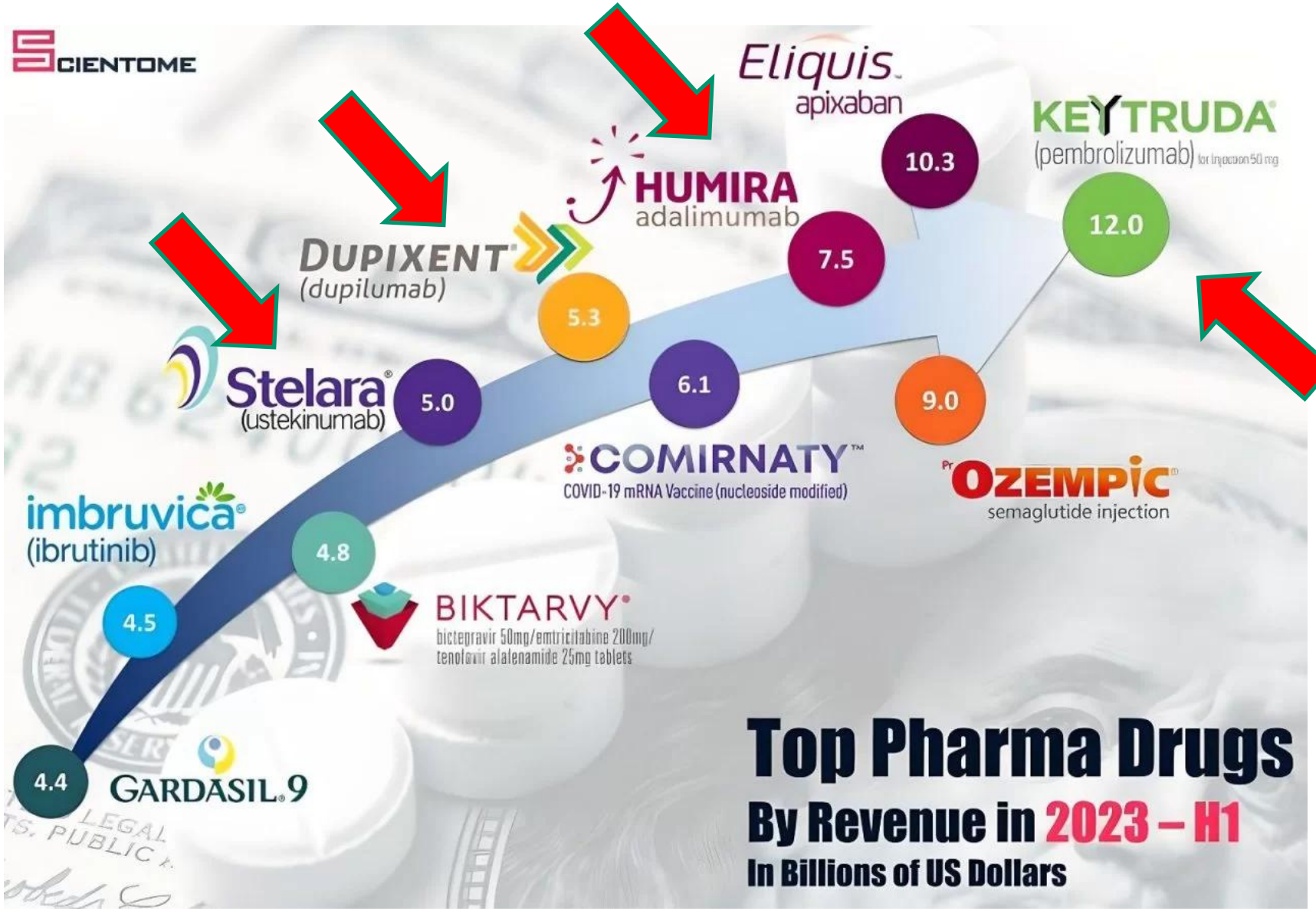
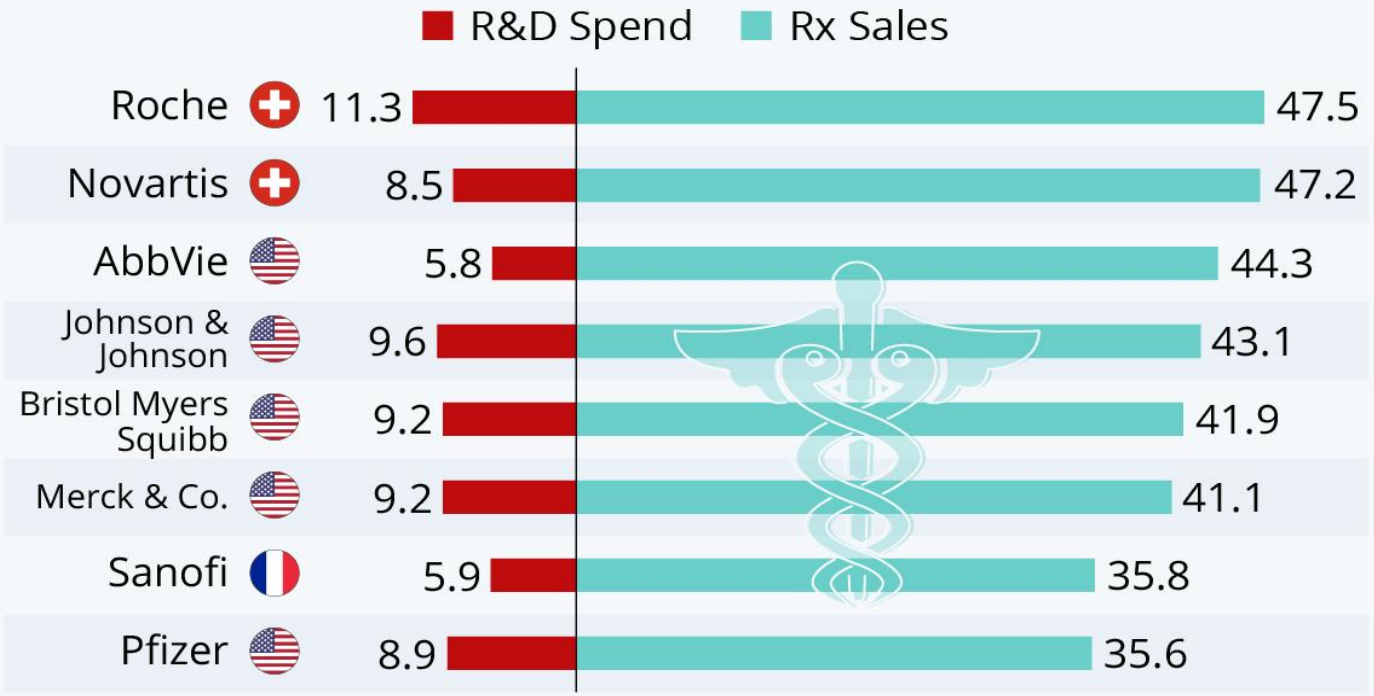



THE TOP SELLING MEDICINES OF PHARMA GIANTS Q2 2025
H1 2025 Revenue
$21.68B
sanofi
DUPIXENT
Quadracel
(polio vaccines, example)
LANTUS
$24.24B
novo nordisk
OZEMPIC
wegovy
RYBELSUS
$23.74B
Bristol Myers Squibb
Eliquis
OPDIVO
ORENCIA
$26.62B
Roche
OCREVUS
Hemlibra
(emicizumab-kxwh)
Injection
VABYSMO
$27.29B
NOVARTIS
Entresto
Cosentyx
KISQALI
$27.69B
MERCK
KEYTRUDA
GARDASIL 9
ProQuad
$28.05B
AstraZeneca
farxiga
TAGRISSO
IMFINZI
$28.29B
Lilly
mounjaro
zepbound
Verzenio
$28.37B
Pfizer
Eliquis
Prevnar 20
Vyndamax
$28.77B
abbvie
Skyrizi
RINVOQ
Humira
$29.08B
Johnson&Johnson
DARZALEX
Stelara
Tremfya
joanna-sadowska-phd
@jmsadowska


SCIENTOME
Eliquis
apixaban
10.3
KEYTRUDA
(pembrolizumab) for injection 50 mg
12.0
HUMIRA
adalimumab
7.5
DUPIXENT
(dupilumab)
5.3
Stelara
(ustekinumab)
5.0
6.1
9.0
COMIRNATY
COVID-19 mRNA Vaccine (nucleoside modified)
Pr OZEMPIC
semaglutide injection
imbruvica
(ibrutinib)
4.8
4.5
BIKTARVY
bictegravir 50mg/emtricitabine 200mg/
tenofovir alafenamide 25mg tablets
4.4
GARDASIL 9
Top Pharma Drugs
By Revenue in 2023 – H1
In Billions of US Dollars

The World's Biggest Players in Pharma

World's biggest biopharma companies ranked by Rx sales and R&D spend in 2020* (in billion U.S. dollars)



* Rx sales = prescription drug sales, R&D = research and development

Source: Pharmaceutical Executive

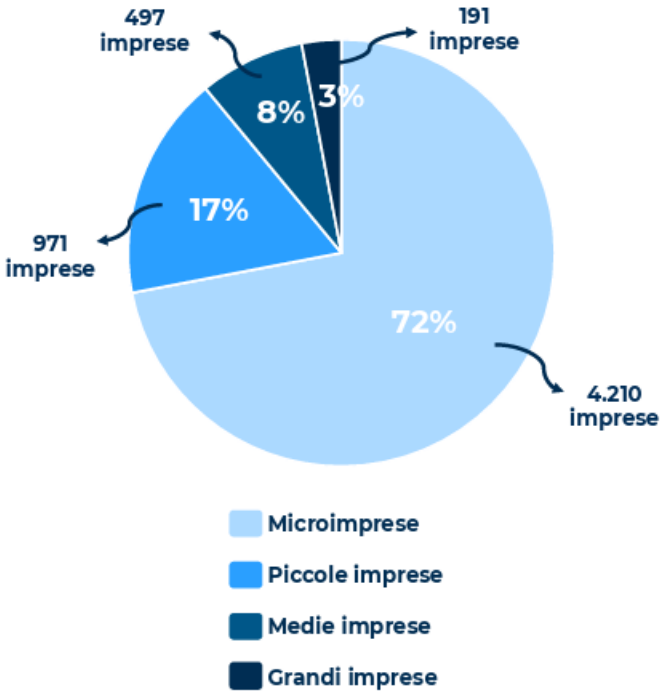
FEDERCHIMICA
ASSOBIOTEC
Associazione nazionale per lo sviluppo delle biotecnologie

IL BIOTECH IN ITALIA
Numeri, storie e trend

REPORT
2025

Figura 1 – La distribuzione delle imprese Biotech italiane per categoria dimensionale

Distribuzione delle 5.869 imprese Biotech italiane per categoria dimensionale



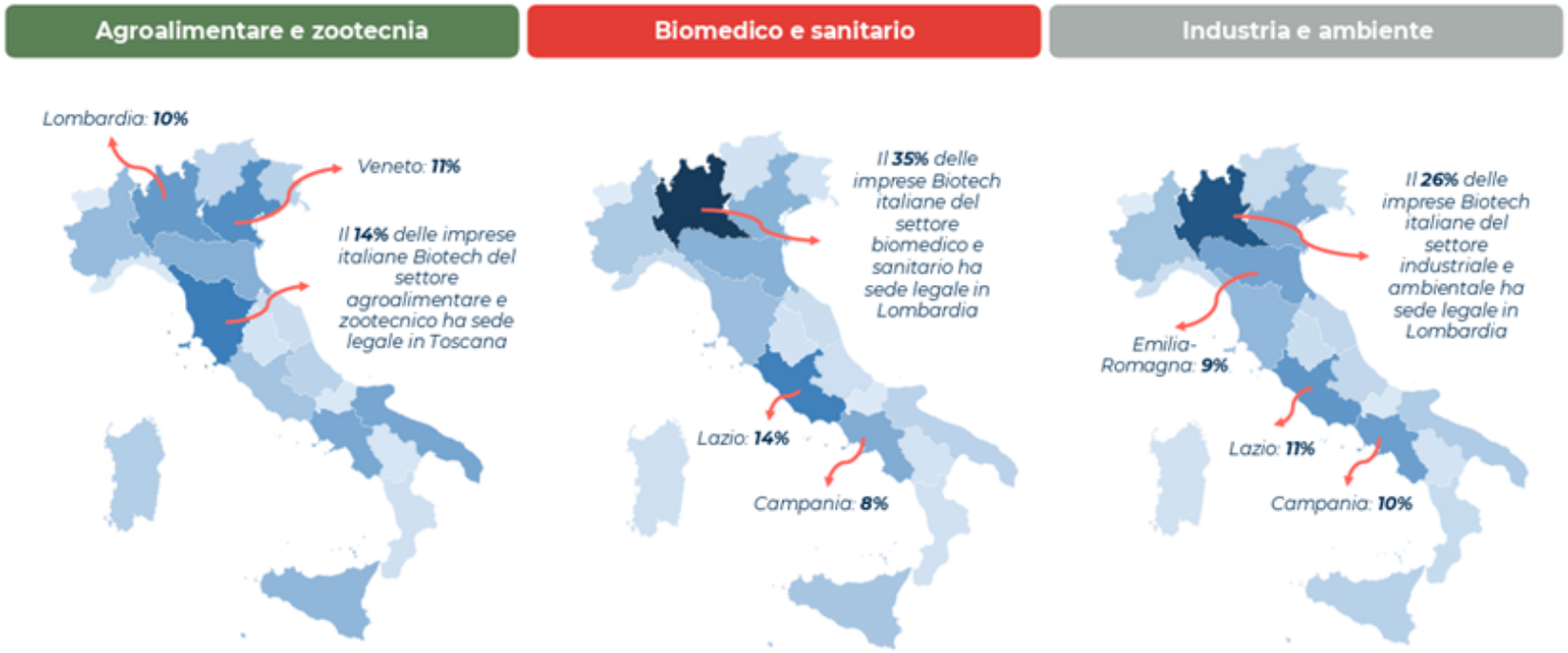
*Criteri di classificazione delle imprese **		
	Fatturato annuo	**Numero addetti**
Microimpresa	≤2 milioni di EUR	<10
Piccola impresa	≤10 milioni di EUR	<50
Media impresa	≤50 milioni di EUR	<250
Grande impresa	>50 milioni di EUR	≥250

** Per le imprese che non rispecchiano entrambi i requisiti di fatturato e addetti, la categoria di appartenenza è stata determinata dal **fatturato***

Tabella 1 – La distribuzione delle imprese Biotech italiane per categoria dimensionale nelle macroaree e macrocategorie biotecnologiche

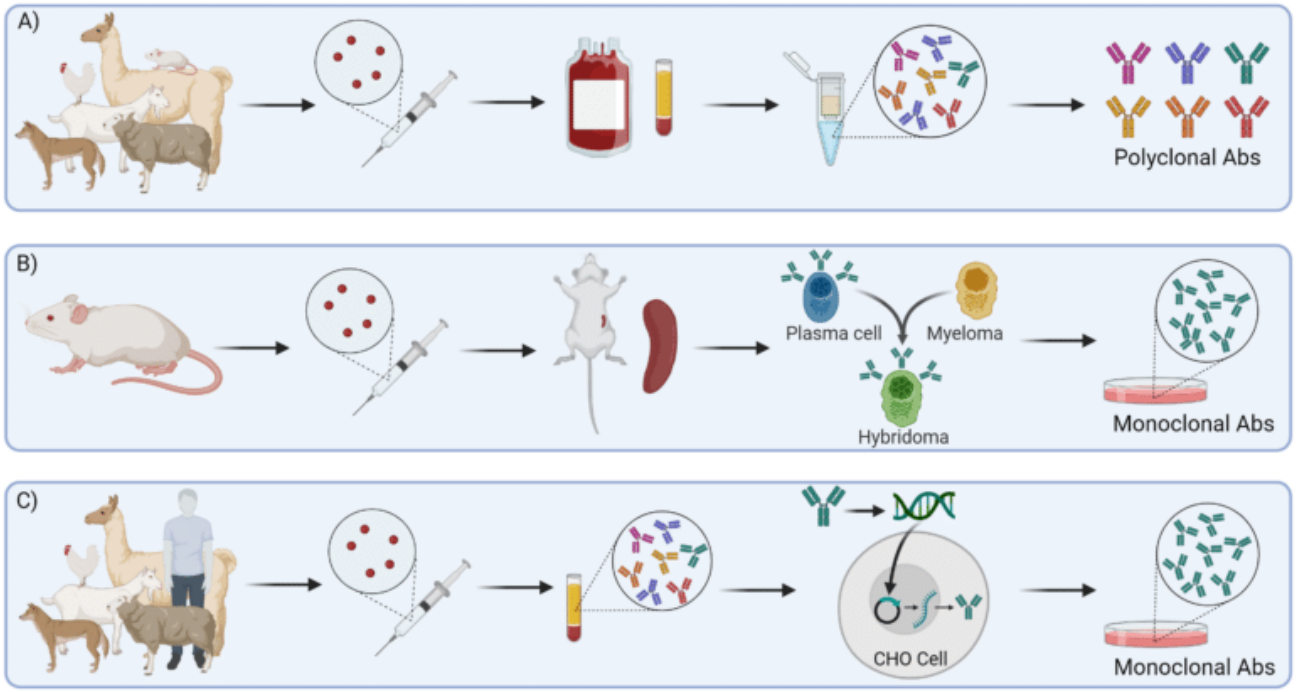
	Microimprese	Piccole imprese	Medie imprese	Grandi imprese	Totale	Microimprese	Piccole imprese	Medie imprese	Grandi imprese
Agroalimentare e zootecnia	**2687**	**699**	**342**	**106**	**3834**	**70%**	**18%**	**9%**	**3%**
Produzione di sementi o alimenti	348	229	141	46	764	46%	30%	18%	6%
Prodotti fermentati	2339	470	201	60	3070	76%	15%	7%	2%
Biomedico e sanitario	**192**	**93**	**83**	**69**	**436**	**44%**	**21%**	**19%**	**16%**
Diagnostica Biotech	145	62	33	19	259	56%	24%	13%	7%
Fabbricazione di prodotti o preparati farmaceutici	47	31	50	50	178	26%	17%	28%	28%
Industria e ambiente	**1331**	**179**	**72**	**17**	**1599**	**83%**	**11%**	**5%**	**1%**
Depurazione. trattamento acque	142	52	17	1	212	67%	25%	8%	1%
Fabbricazione di prodotti chimici	42	35	26	10	112	37%	31%	23%	9%
Produzione di bio-energia	81	28	12	1	123	66%	23%	9%	1%
Ricerca e sviluppo sperimentale	1066	63	18	4	1152	93%	5%	2%	0%
Totale	**4210**	**971**	**497**	**191**	**5869**	**72%**	**17%**	**8%**	**3%**

Figura 5 – La distribuzione delle imprese Biotech sul territorio per macroarea biotecnologica

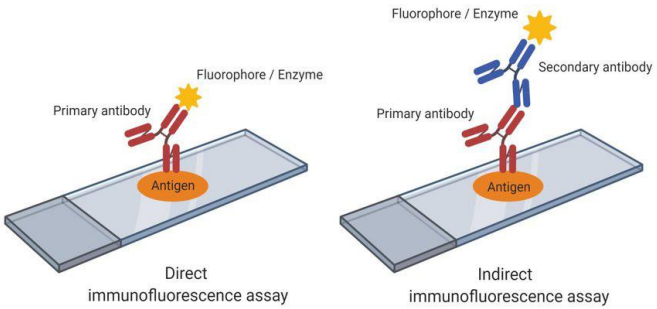


Monoclonal and polyclonal Antibodies:

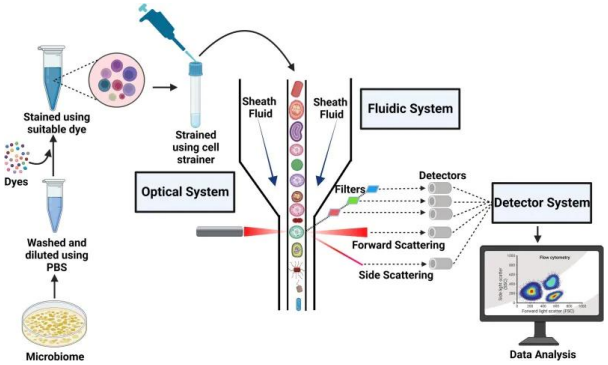
- What They Are
- How They Are Made
- Where They Are Used
- How Much They Cost



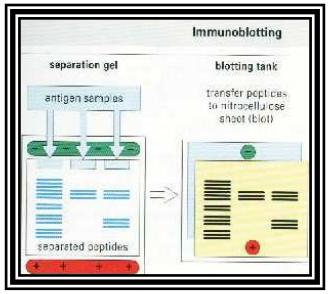
How are they used in research?



Immuno- fluorescence

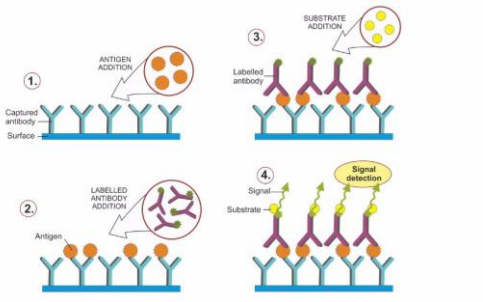


Flow cytometry

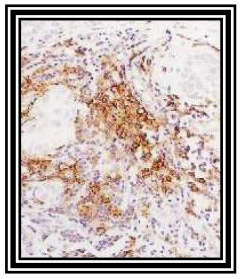


Immunoblotting

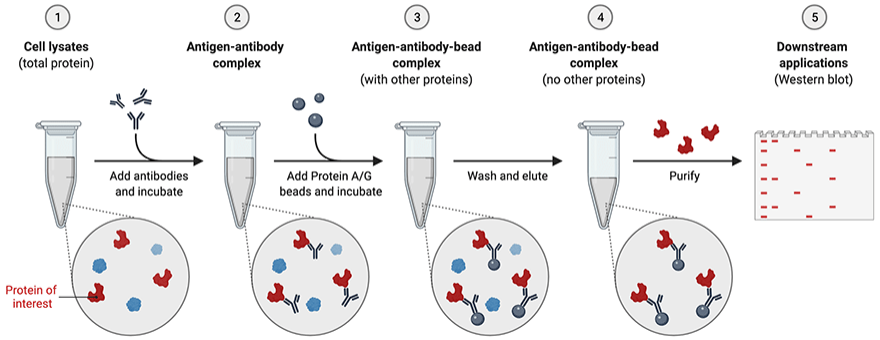
How are they used in research?



ELISA



Immuno-histochemistry



Immuno-precipitation

Glossary

Antibody
(anti-foreign body) is a protein produced by a white cell (B lymphocyte).

Antigen
(antibody generating substance) is any agent, such as a chemical or microorganism that is recognized by the antibody. Not all antigens are immunogens (e.g hapten).

Immunogen:
Any substance to which an animal responds by making antibodies. All immunogens are antigens.

Epitope : the part of a target to which an antibody binds, also known as an antigenic determinant

Antigen binding site - relatively small region of an antibody that binds to the antigen.

Immunogenicity

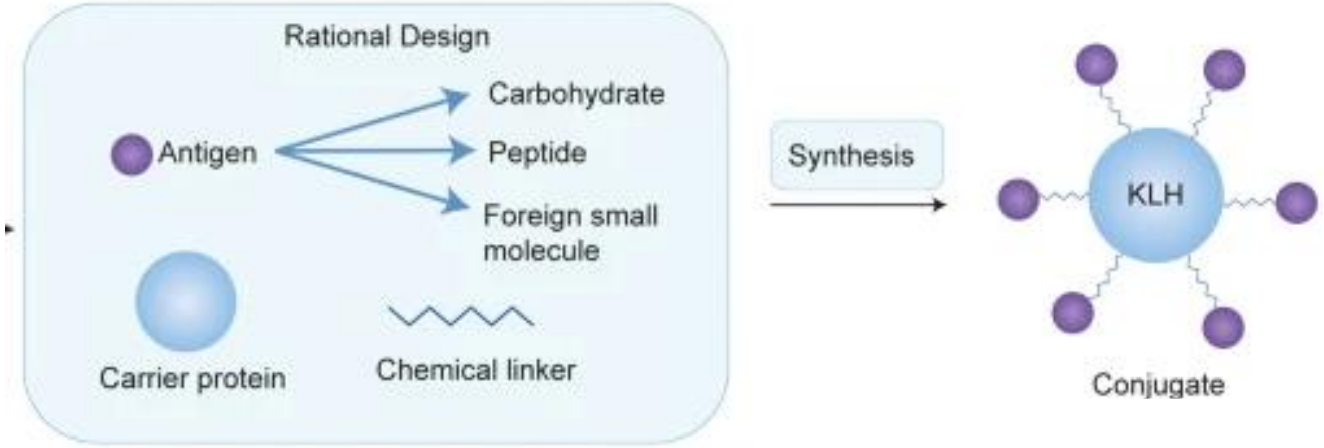
Ability of a molecule to induce an immune response

Proteins, peptides, carbohydrates, nucleic acids, lipids

Must be **larger than 3000-5000** daltons -

Antigen

(hapten)



Carrier protein

e.g. BSA Thyroglobulin, KHL

Carbodiimide

Glutaraldehyde

MBS–Heterobifunctional reagents

Glossary

Antibody (**anti**-foreign **body**) is a protein produced by a white cell (B lymphocyte).

Antigen (**anti**body **gen**erating substance) is any agent, such as a chemical or microorganism that is recognized by the antibody. Not all antigens are immunogens (e.g hapten).

Immunogen : Any substance to which an animal responds by making antibodies. All immunogens are antigens.

Epitope :

the part of a target to which an antibody binds, also known as an antigenic determinant

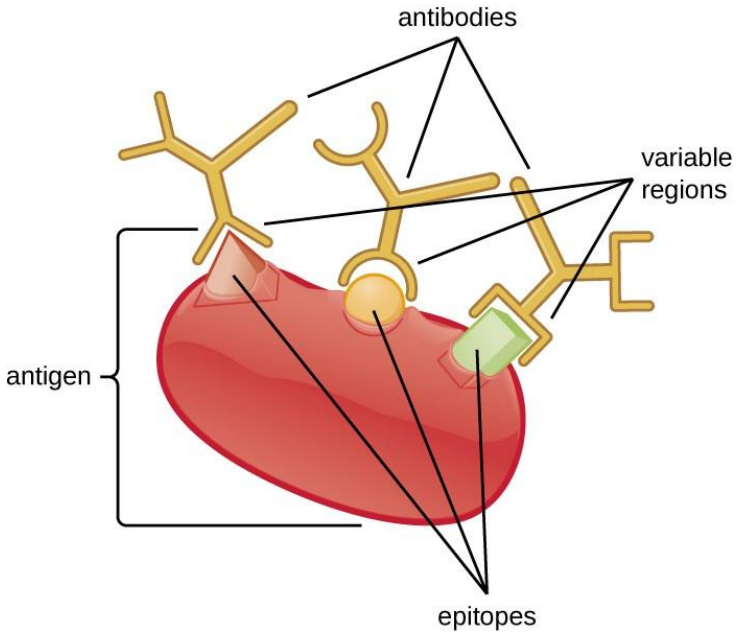
Antigen binding site

relatively small region of an antibody that binds to the antigen.

What are **epitopes**?

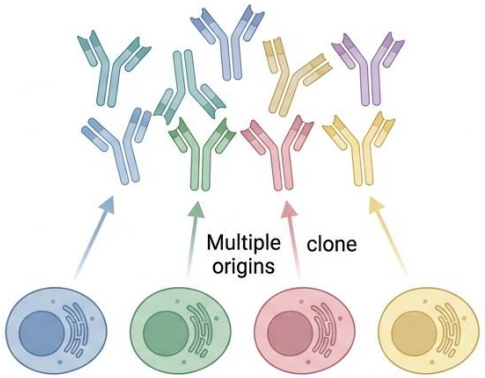
Different portions of a target that can be recognized by different antibodies

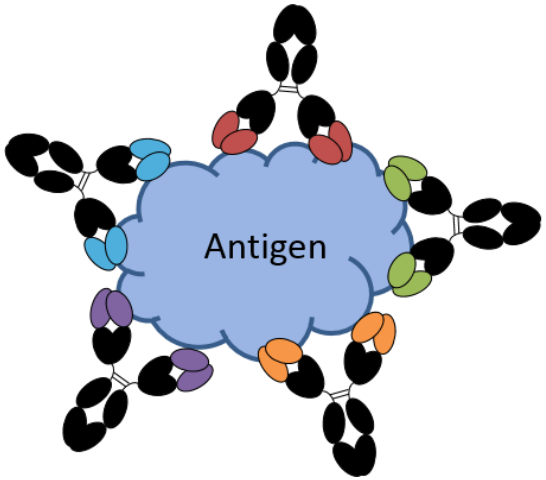
Each **individual antibody** only recognizes a **single epitope**



Antigen binding site - relatively small region of an antibody that binds to the antigen.

Polyclonal Antibodies

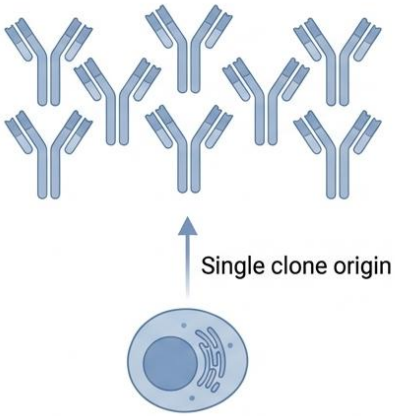


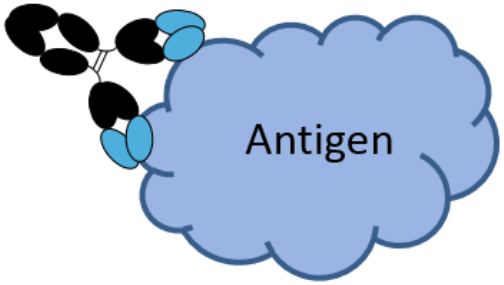


Polyclonal antibodies

React with various epitopes on a given antigen

Monoclonal Antibodies

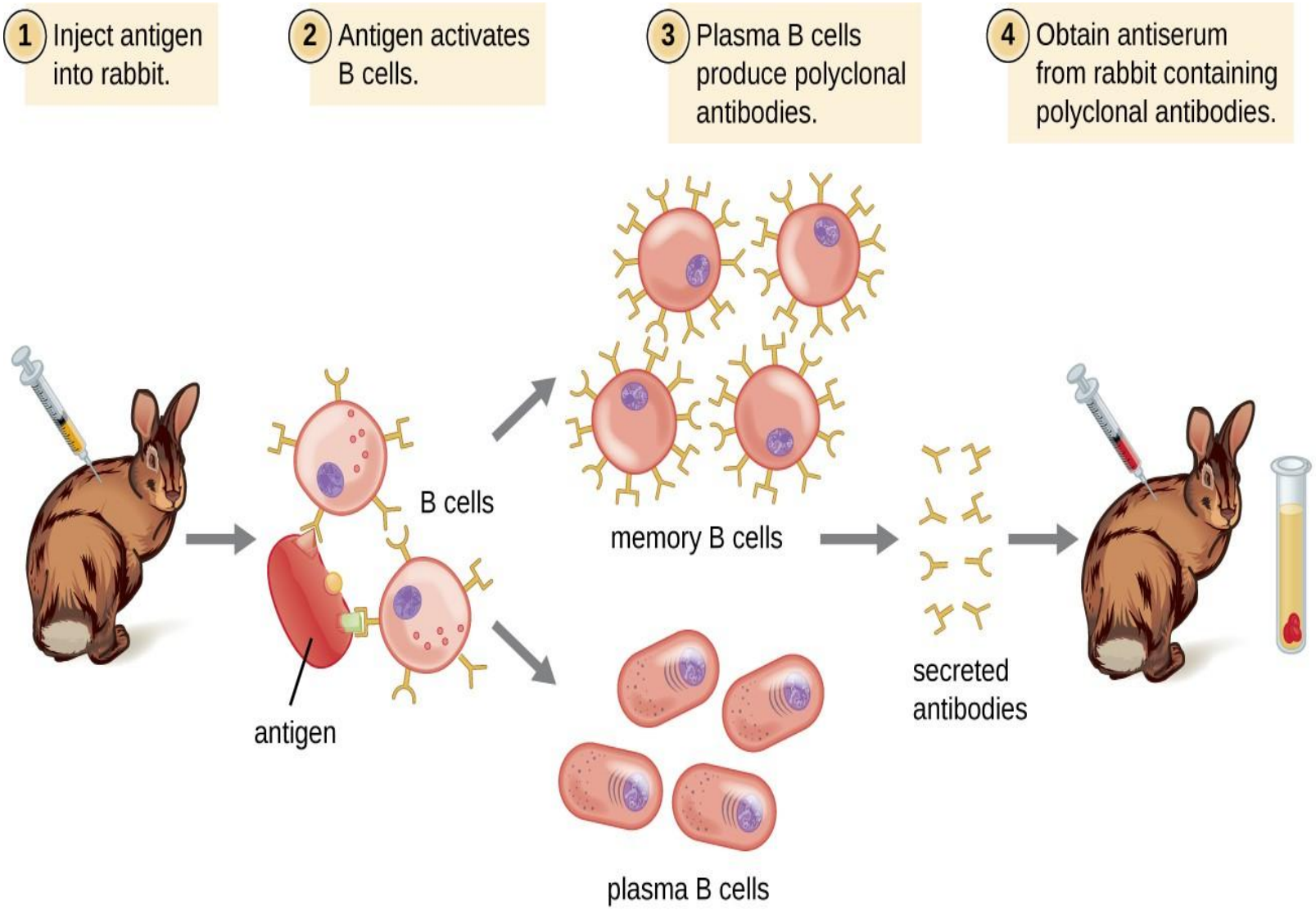




Monoclonal antibodies

React with a specific epitope on a given antigen

Polyclonal antibodies



Polyclonal antibodies

Animals immmunized

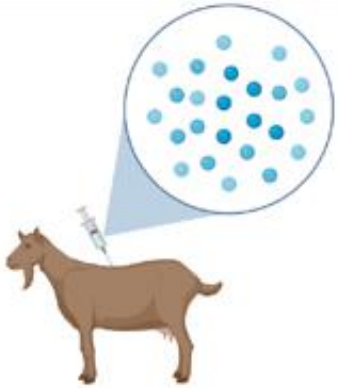
Mouse/ rat

Goat/Sheep

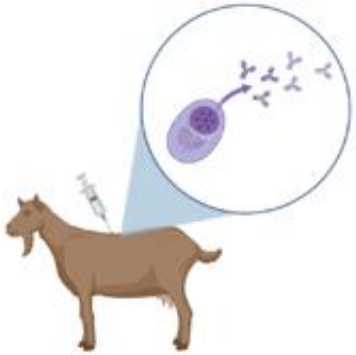
Horse/donkey

Rabbit

Llama

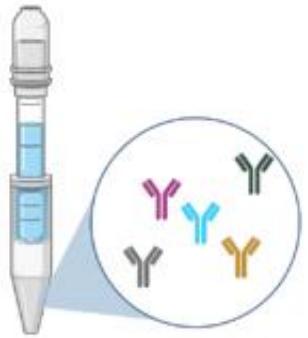


1. An animal, such as a goat or a rabbit, is injected with an immunogen plus adjuvant.



2. Booster injections are given to the animal every 2-3 weeks until the proper titer of antibody is reached.

3. Blood is harvested from the animal and centrifuged to isolate the serum, which contains the antibodies.



4. The serum is further processed to purify the polyclonal antibody population.

Table 5 Maximum blood volumes to be collected from animals during experimentation [a]

Animal	Maximum blood volume (mL)
Rabbits	15 [b]
Mice	0.3
Rats	2
Guinea pigs	5
Hamsters	0.3
Sheeps	200-600 [b]
Goats	150-400 [b]
Horses	500-7000 [b]

Freund's adjuvant

is a solution of **antigen** emulsified in **mineral oil** and used as an **immunopotentiator (booster).**

- non-metabolizable oils (paraffin oil and mannide monooleate).

- The **complete** form, **Freund's Complete Adjuvant**,(**CFA** or FCA) is composed of inactivated and dried mycobacteria (usually M. tuberculosis);

- the **incomplete form (IFA or FIA)** lacks the mycobacterial components (hence just the water in oil emulsion).

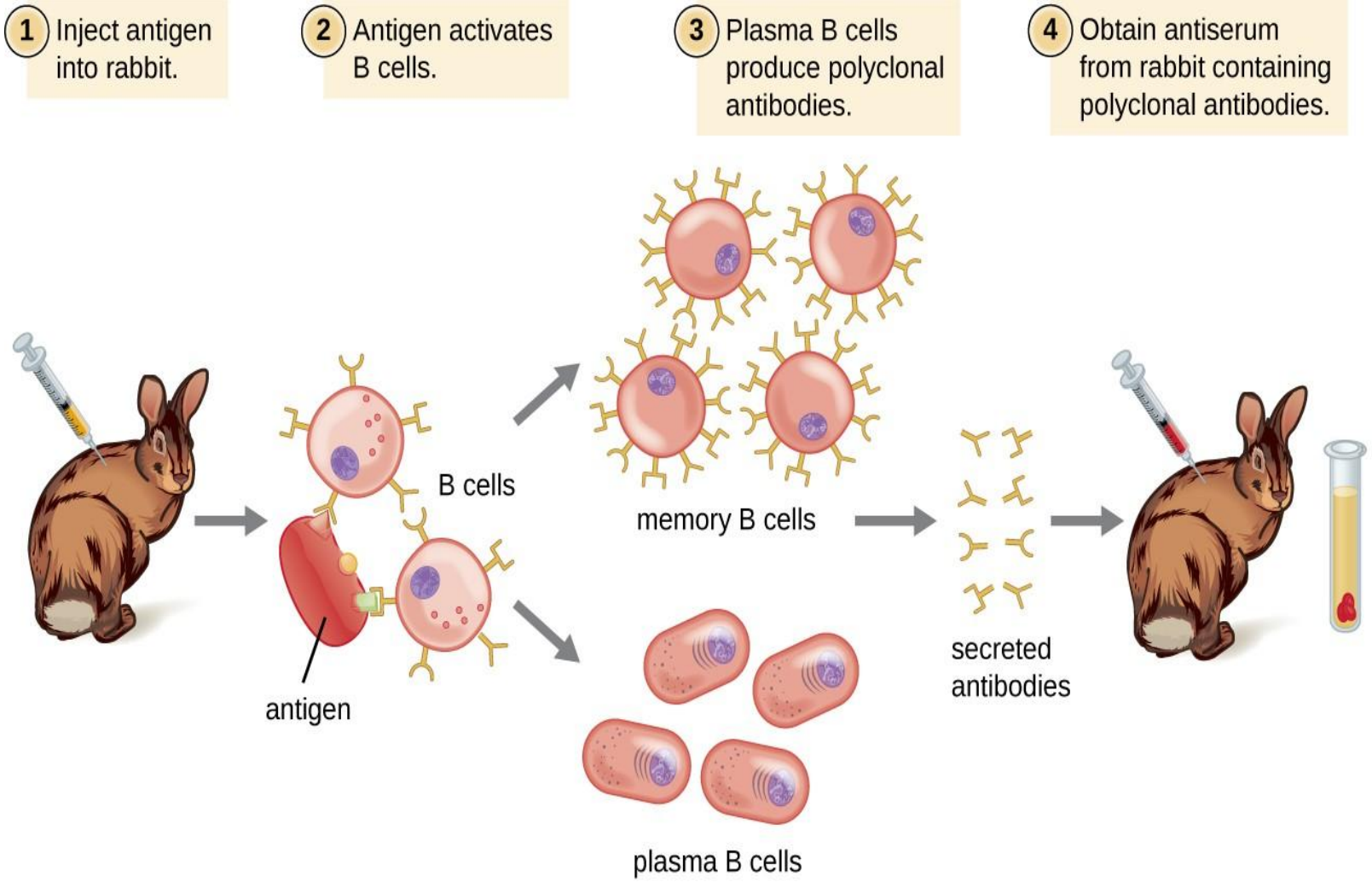
- It is named after Jules T. Freund.

Freund's Adjuvant is designed to

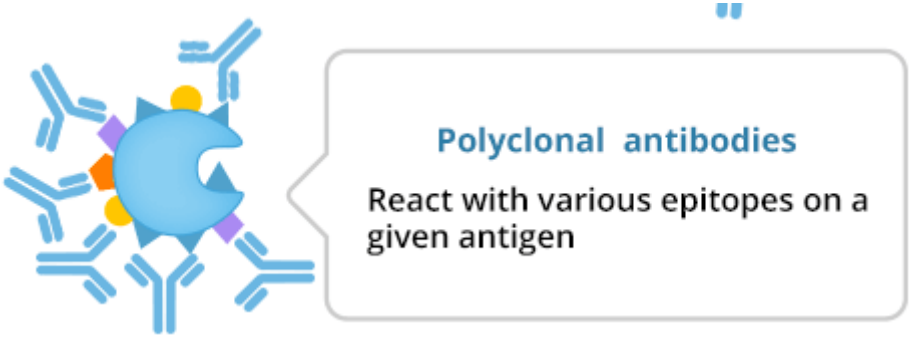
1- provide continuous release of antigens necessary for stimulating a strong, persistent immune response.

2- The mycobacteria in Complete Freund's attracts macrophages and other cells to the injection site which enhances the immune response.

For this reason, **the Complete Freund's Adjuvant is used for the initial injections**.


1 Inject antigen into rabbit.
2 Antigen activates B cells.
3 Plasma B cells produce polyclonal antibodies.
4 Obtain antiserum from rabbit containing polyclonal antibodies.
B cells
antigen
memory B cells
plasma B cells
secreted antibodies

Advantages of Polyclonal Antisera



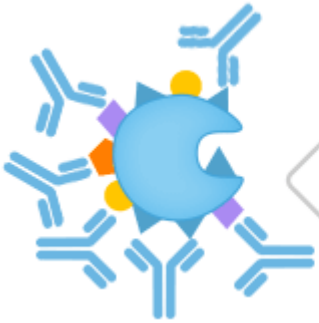
- Antiserum recognizes **many different epitopes** on the target

- Can usually be used for **many different research procedures**

  Immunohistochemistry, immunofluorescence, immunoprecipitation, ELISA, precipitation assays

- Can be **affinity purified** to eliminate the non-specific binding antibodies

Disadvantages of Polyclonal Antisera



Polyclonal antibodies

React with various epitopes on a given antigen

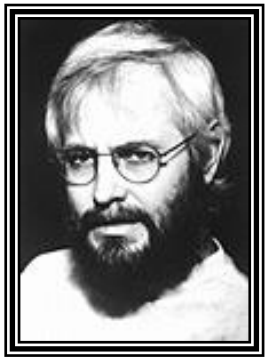
- Antiserum is composed of a **mixture of antibodies** with different specificities - **not all recognize the target of interest**
- Antiserum is composed of a mixture of **high** and **low** affinity antibody populations
- **Quantity** of antiserum is **limited** by amount of serum and **life of immunised animal**.

A Perspective of Monoclonal Antibodies

1. What are monoclonal antibodies?

2. What are the principles involved in their production?

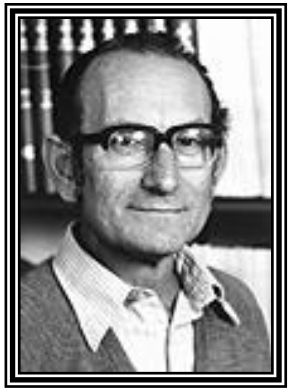
3. How do they differ from conventional antisera?



Georges Köhler

(1946-1995)

&



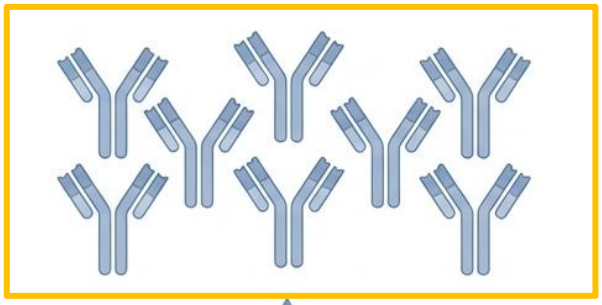
Cesar Milstein

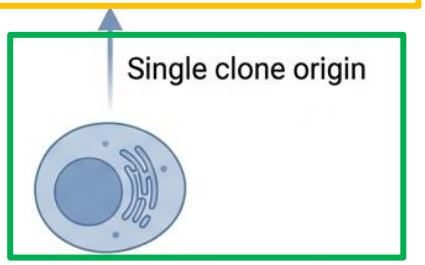
(1927-2002)

Nature Vol. 256 August 7 1975

Continuous cultures of fused cells secreting antibody of predefined specificity

Monoclonal Antibodies





The Nobel Prize in Physiology or Medicine 1984

Photo from the Nobel Foundation archive.

Niels K. Jerne

Prize share: 1/3

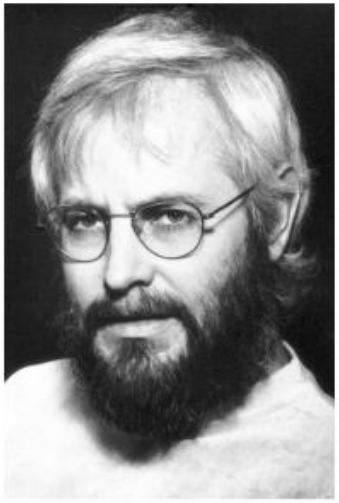


Photo from the Nobel Foundation archive.

Georges J.F. Köhler

Prize share: 1/3

Photo from the Nobel Foundation archive.

César Milstein

Prize share: 1/3

The Nobel Prize in Physiology or Medicine 1984 was awarded jointly to Niels K. Jerne, Georges J.F. Köhler and César Milstein "for theories concerning the specificity in development and control of the immune system and the discovery of the principle for production of monoclonal antibodies."

7 Oct 1976

NRDC

National Research Development Corporation
PO box 236 Kingsgate House 66/74 Victoria Street London SW1 E 6SL
Telephone 01-828 3400 Telegrams Nardec London SW1 Telex 23580

Your ref

Our ref EJT/AED

7th October 1976.

Mr. L.D. Hamlyn,
Medical Research Council,
20 Park Crescent,
London, W1N 4AL.

Dear Jimmy,

Continuous Cultures of Fused Cells

We have now had an opportunity to study the paper by Kohler and Milstein to which you referred in your letter of 24th September addressed to Ron Homer.

Although the authors suggest that the cultures which they have developed, or rather similar cultures, could be valuable for medical and industrial use, I think this statement should be taken as a matter of long term potential rather than immediate application. It is certainly difficult for us to identify any immediate practical applications which could be pursued as a commercial venture, even assuming that publication had not already occurred. I would add that the general field of genetic engineering is a particularly difficult area from the patent point of view and it is not immediately obvious what patentable features are at present disclosed in the Nature paper.

In summary, therefore, unless further work indicates a diagnostic application or industrial end product which we can protect, despite the disclosure in the Nature paper, we would not suggest taking any further action ourselves.

Kind regards,

Yours sincerely,

Eric

E.J. Tridgell
Biosciences Group

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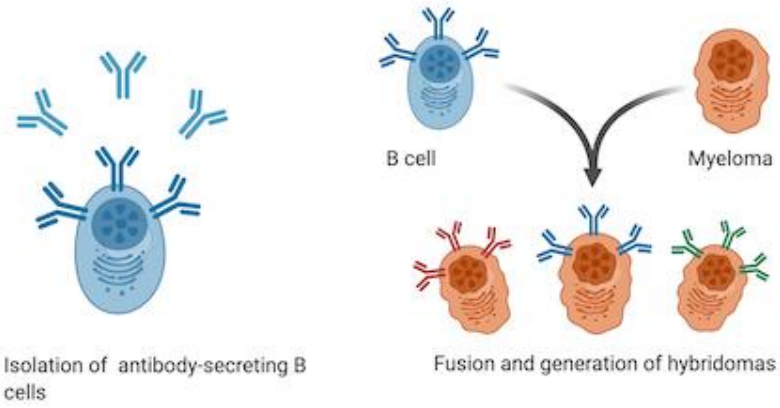
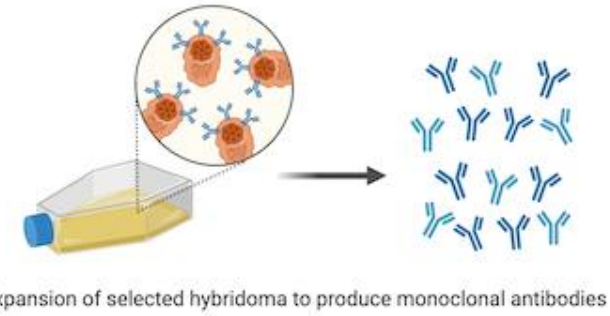
Definition of A Monoclonal Antibody

1. Mabs comprise **identical antibody molecules**.

2. They only recognise **a single antigenic determinant**.

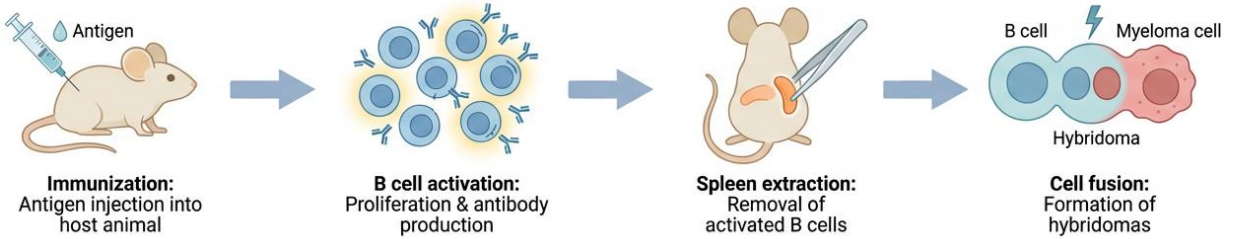
3. They are secreted by a **clone of hybrid cells** in culture.



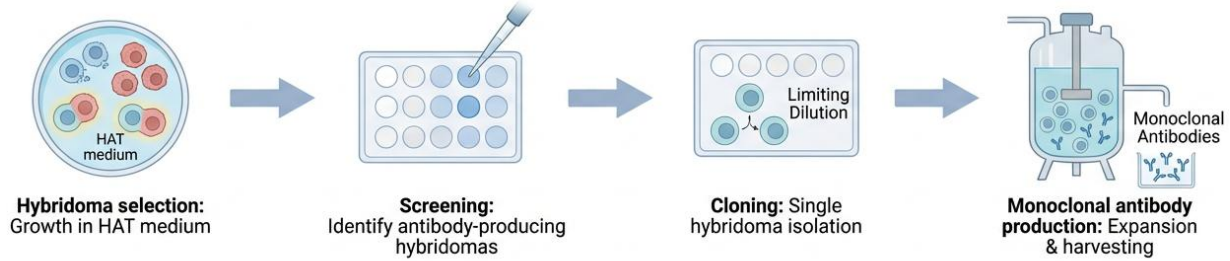


How to make a monoclonal antibody

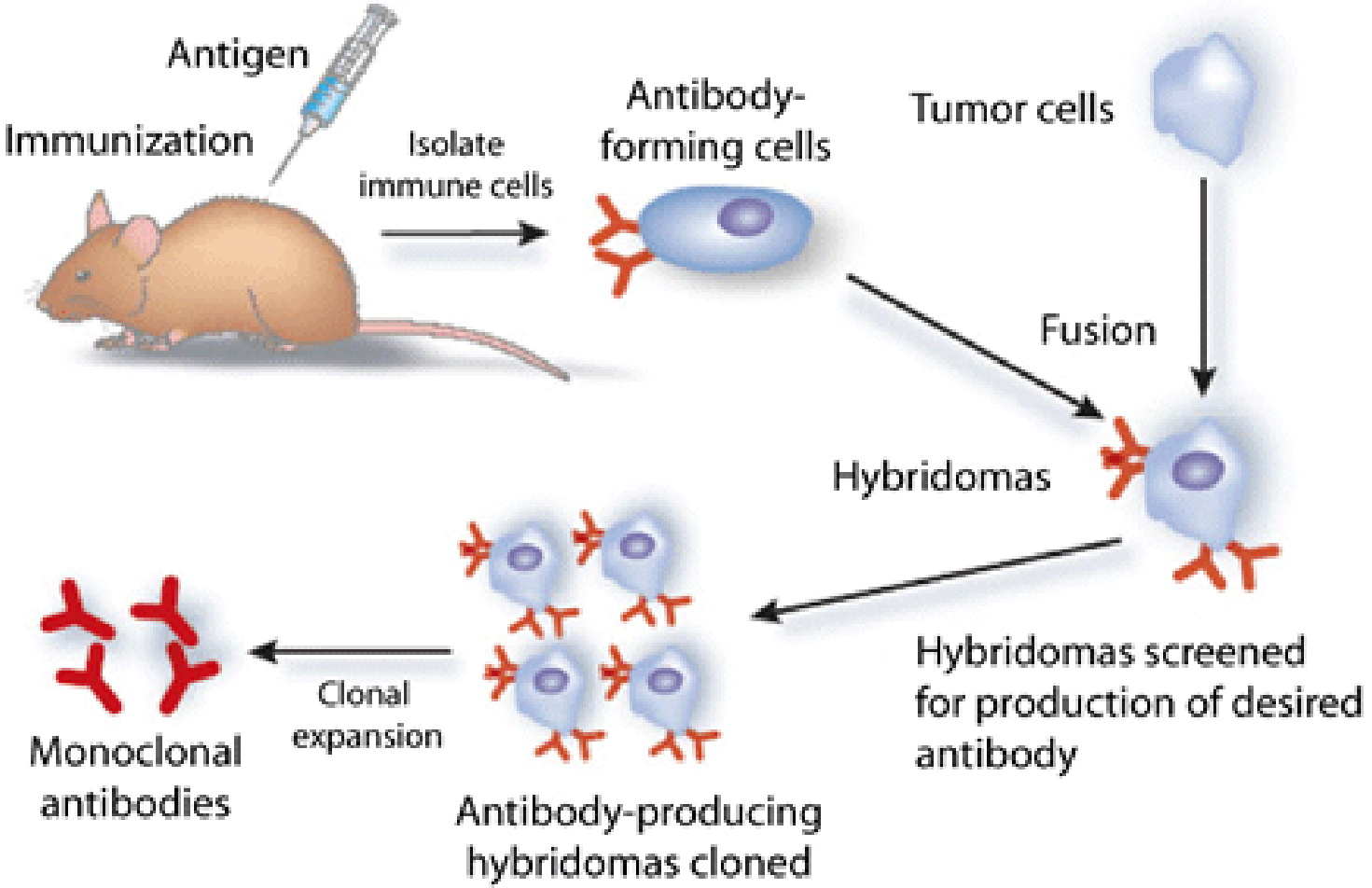
- Immunize mice
- Test the serum
- Perform a fusion



- Screen the fusion for the right cells
- Grow the hybridomas
- Harvest the antibody
- Concentrate and purify the product

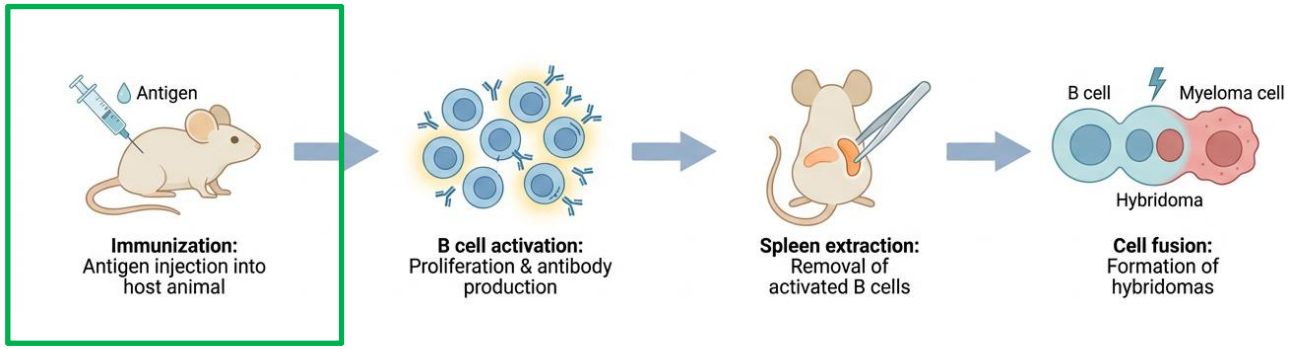


Outline Of Monoclonal Antibody Production



How to make a monoclonal antibody

- **Immunize mice**
- Test the serum
- Perform a fusion



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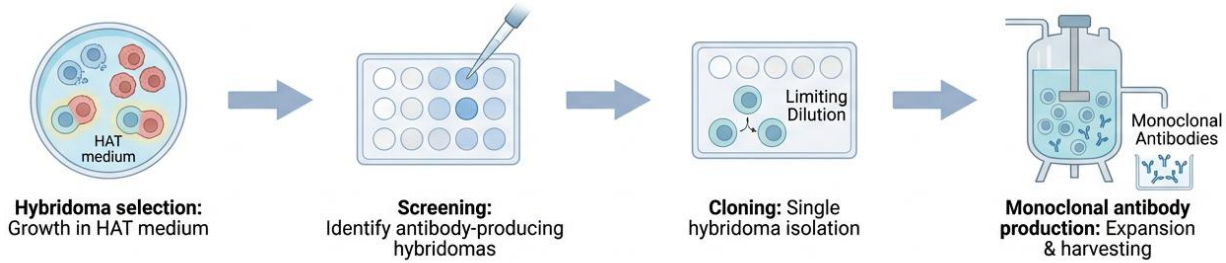
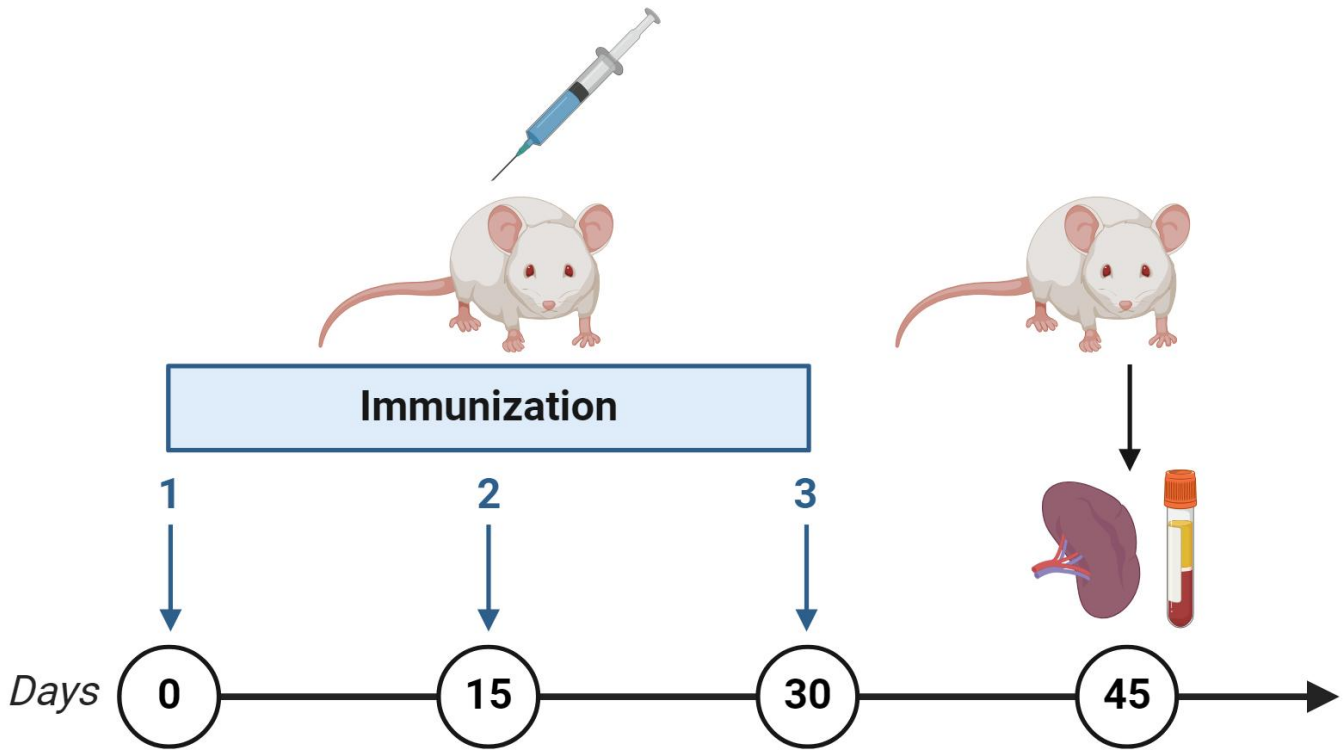


Immunize the mice

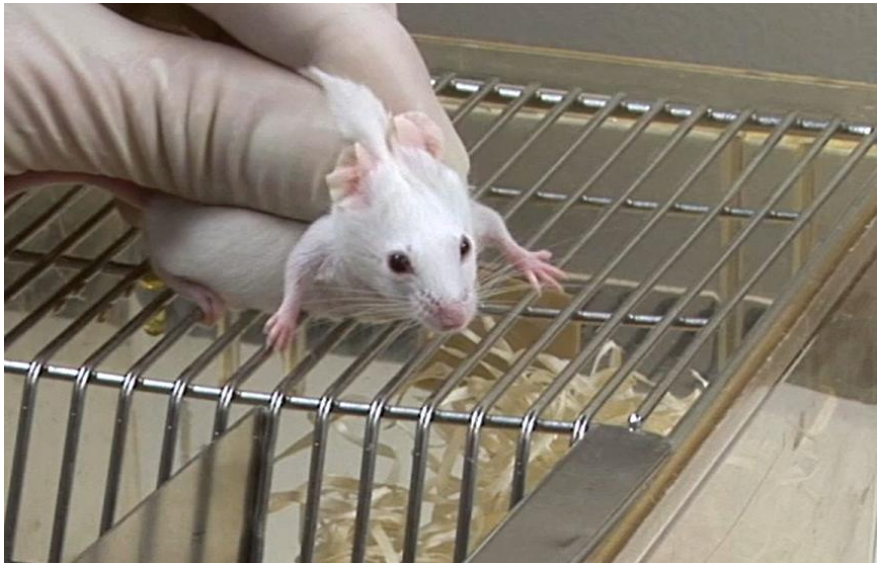
Common Administration Routes in Laboratory Mice



Immunize the mice - Inoculation

- The mice are aseptically inoculated with the antigen combined with an adjuvant.
- Normal dose per mouse is between **50 and 100 micrograms** of protein.
- Inoculations are performed every 21 to 28 days.

sub-cutaneously

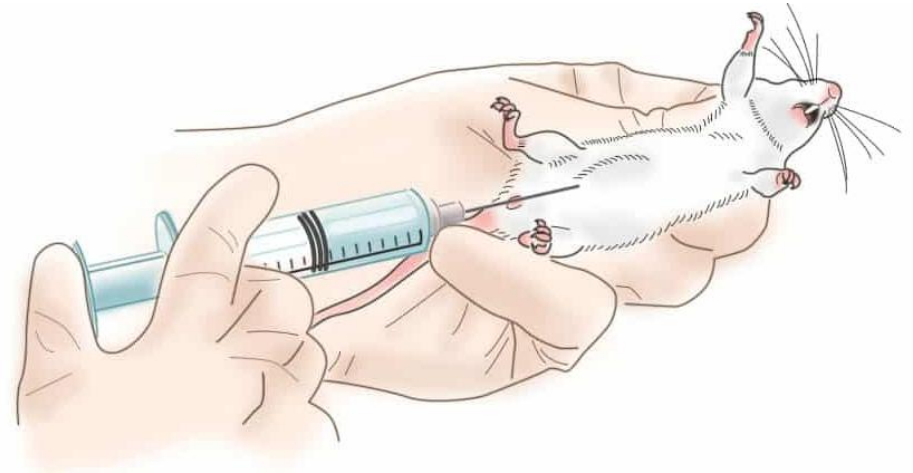


https://researchanimaltraining.com/articles/subcutaneous-injection-in-the-mouse/

Immunize the mice - Inoculation

Intraperitoneal (IP) Injection
Definition: Delivery of a substance directly into the peritoneal cavity —

Peritoneal cavity:
Potential space in abdomen **between visceral and parietal peritoneum**, usually containing only small amount of peritoneal fluid (for lubrication)



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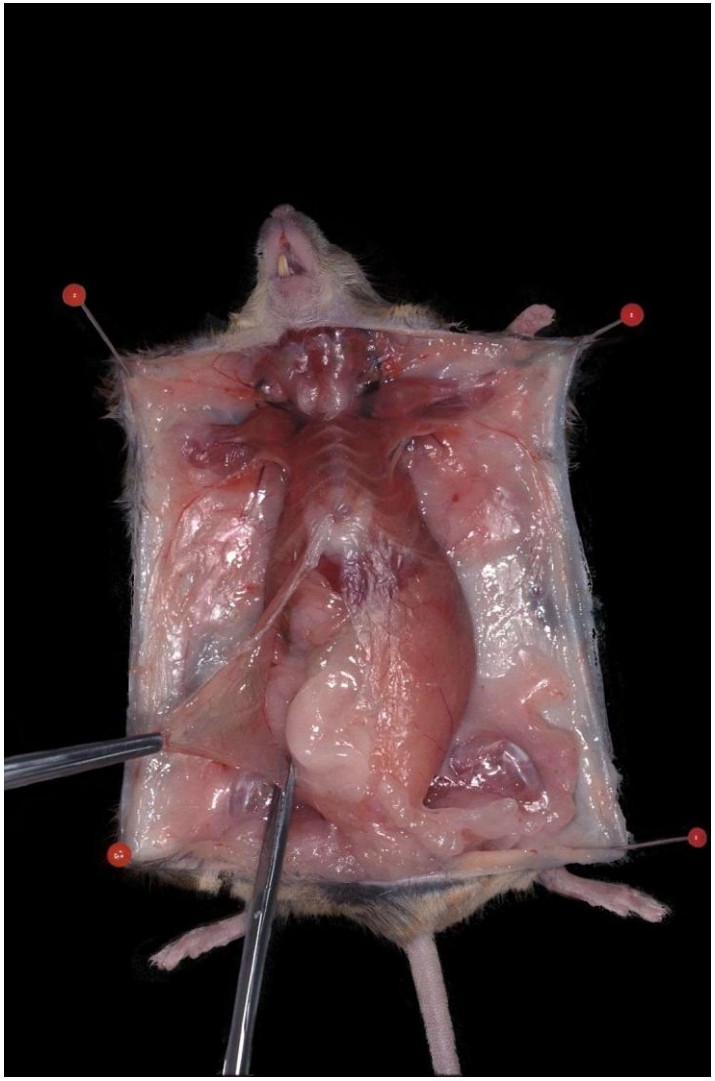
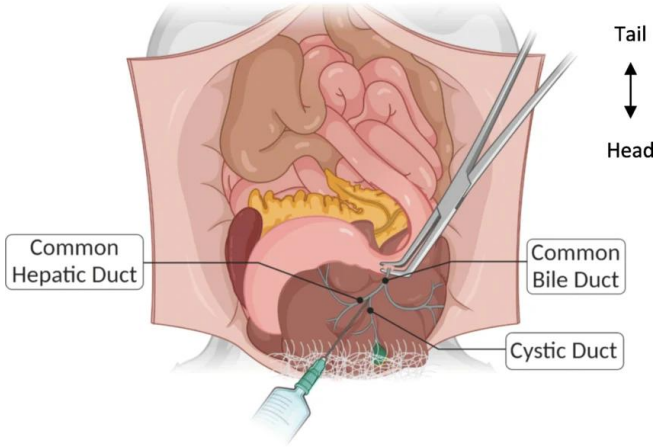
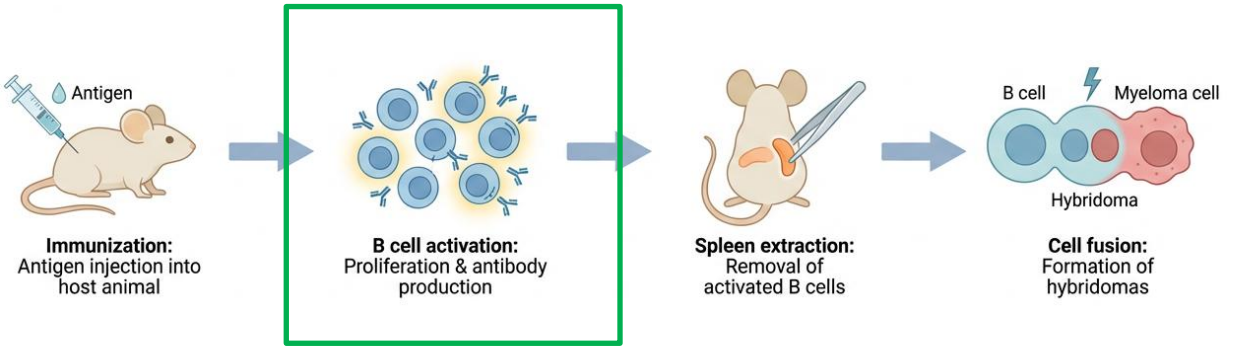




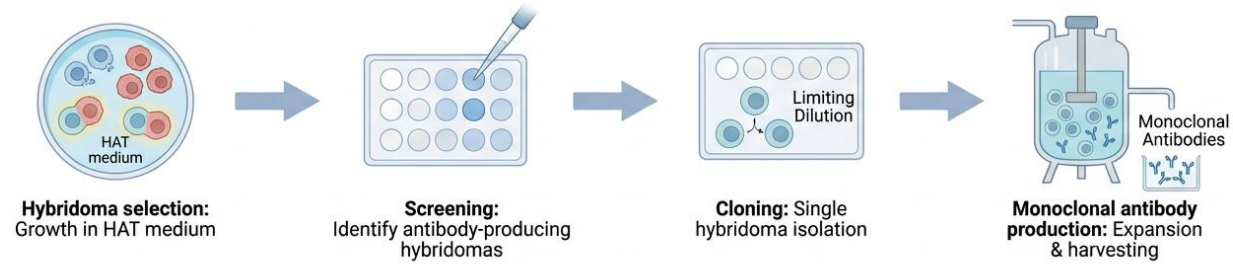
IMMUNIZE THE MICE

How to make a monoclonal antibody

- Immunize mice
- **Test the serum**
- Perform a fusion



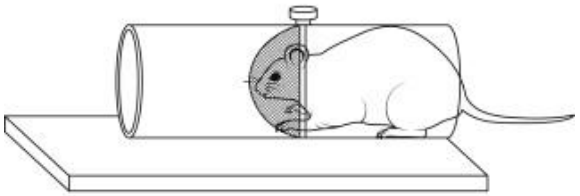
- Screen the fusion for the right cells
- Grow the hybridomas
- Harvest the antibody
- Concentrate and purify the product



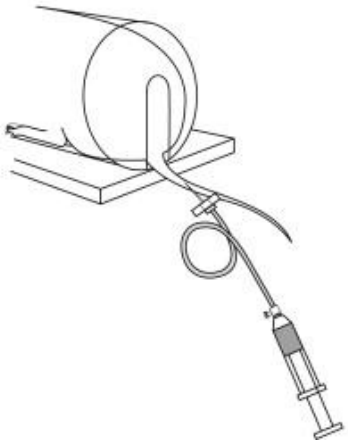
Test the serum - Bleeding the mice

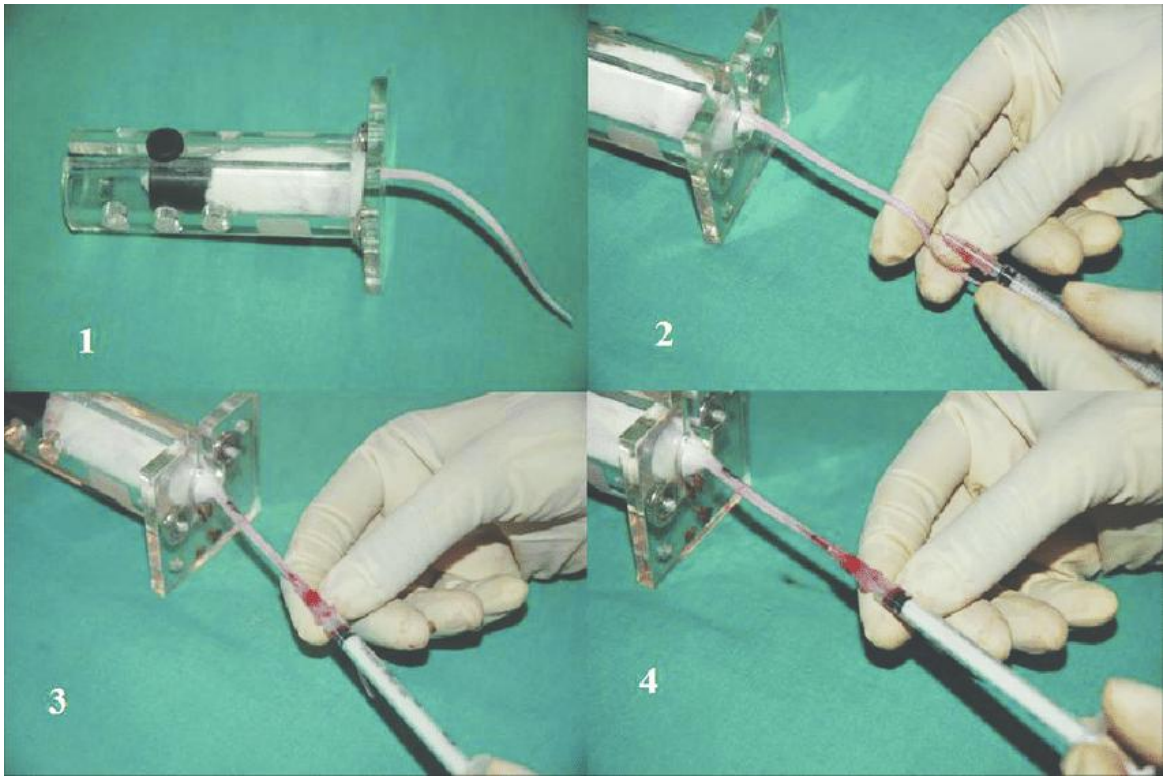
- A capillary tube is applied to **nicked vein**
- Blood is drawn into the tube.

(a)

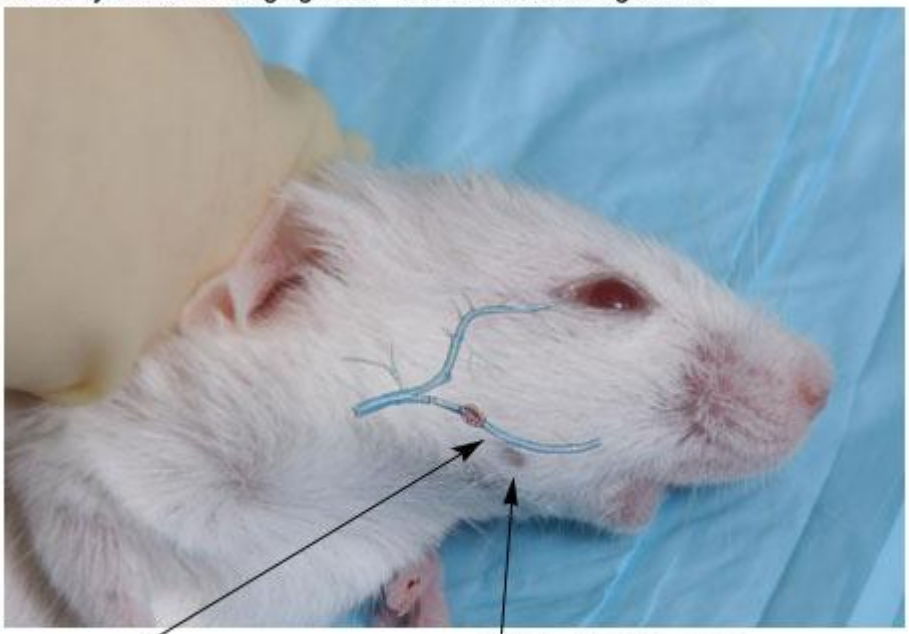


(b)





Note: Eyes are not bulging here. Risk of not obtaining blood.

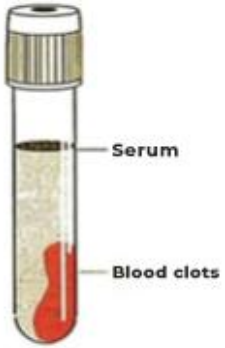


Freckle. Easily seen on
white mice, also visible on dark mice.

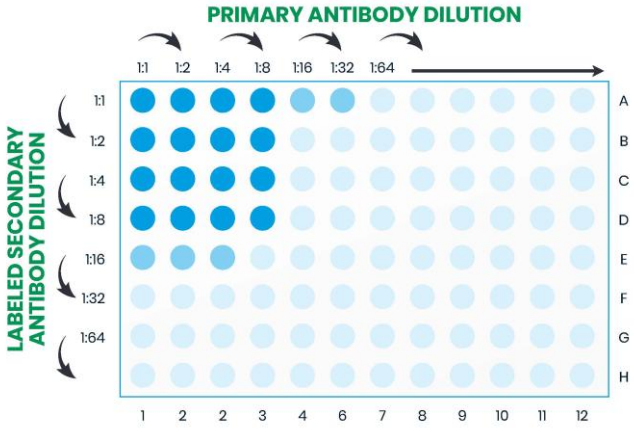
Facial Vein. Your target blood vessel, running
just along the bottom of the mandible (jaw).

Test the serum - In the Lab

- Whole blood from the immunized mice is transported to the lab.

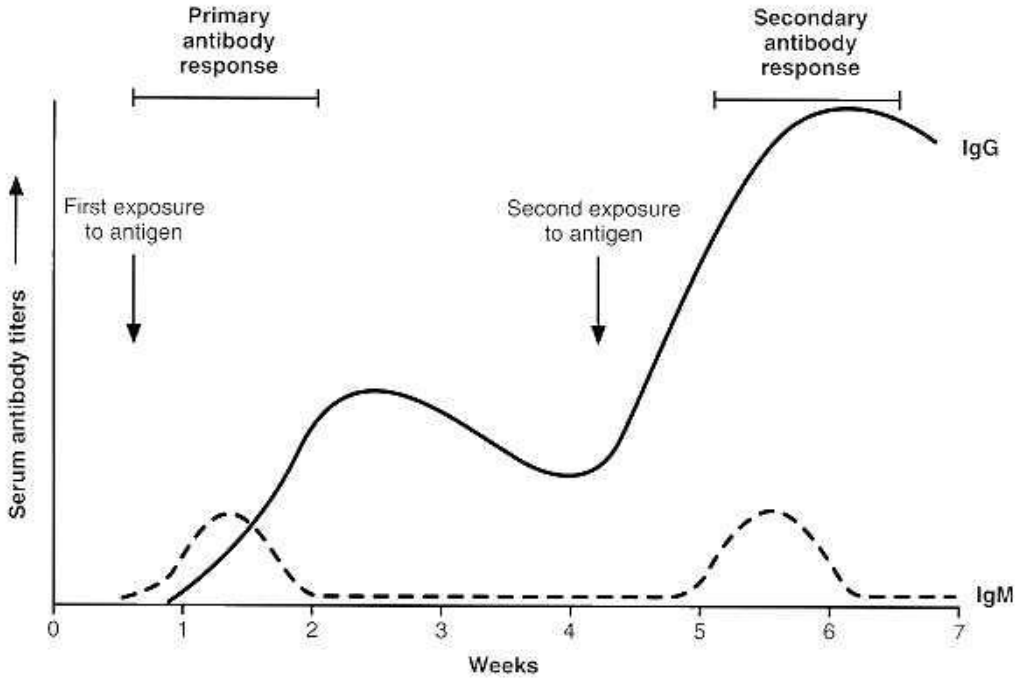


- The whole blood is spun down in a centrifuge to separate **the serum.**



- The serum is diluted in a series from **1:300 to 1:300,000**. and tested by **ELISA**

Test the serum - Decision time



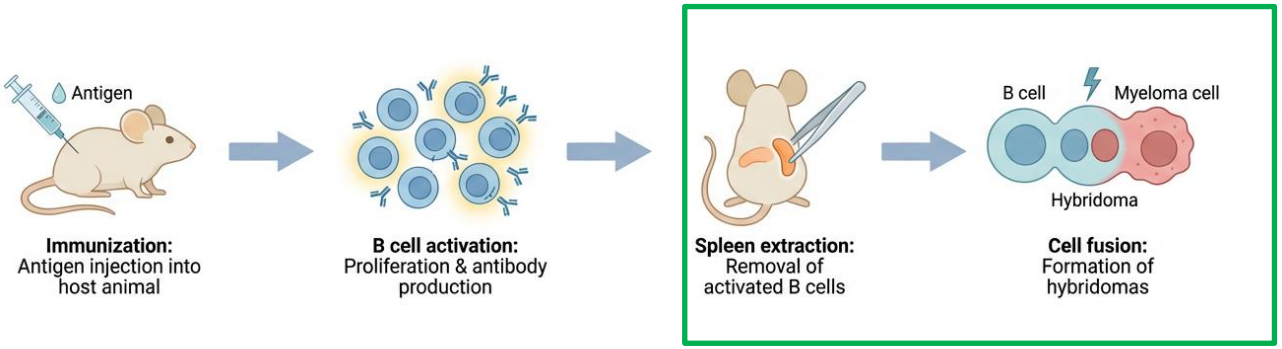
A *fusion* is done when
- the IgG level is high
- and the IgM level is low.

When the serum titer of the mice has **reached a plateau**, an additional ELISA test is performed to determine the predominant *isotype* present.
The two *isotypes* that are most common in mouse serum are IgG and IgM.

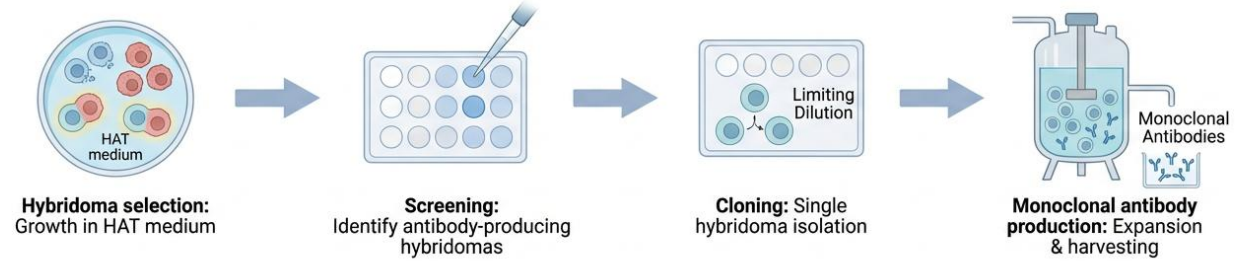
The mouse with the **strongest, most specific response is chosen for the fusion.**

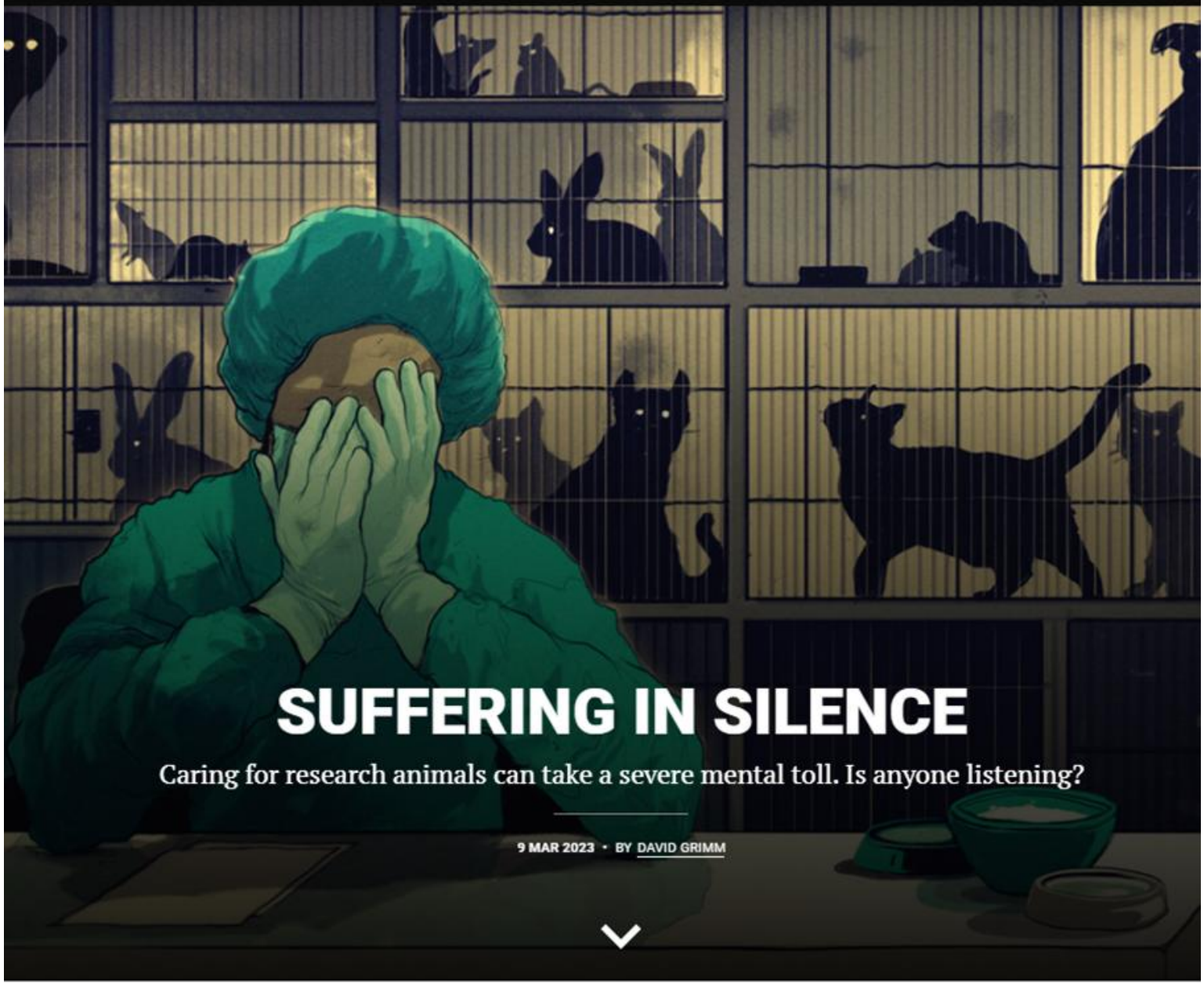
How to make a monoclonal antibody

- Immunize mice
- Test the serum
- **Perform a fusion**



- Screen the fusion for the right cells
- Grow the hybridomas
- Harvest the antibody
- Concentrate and purify the product

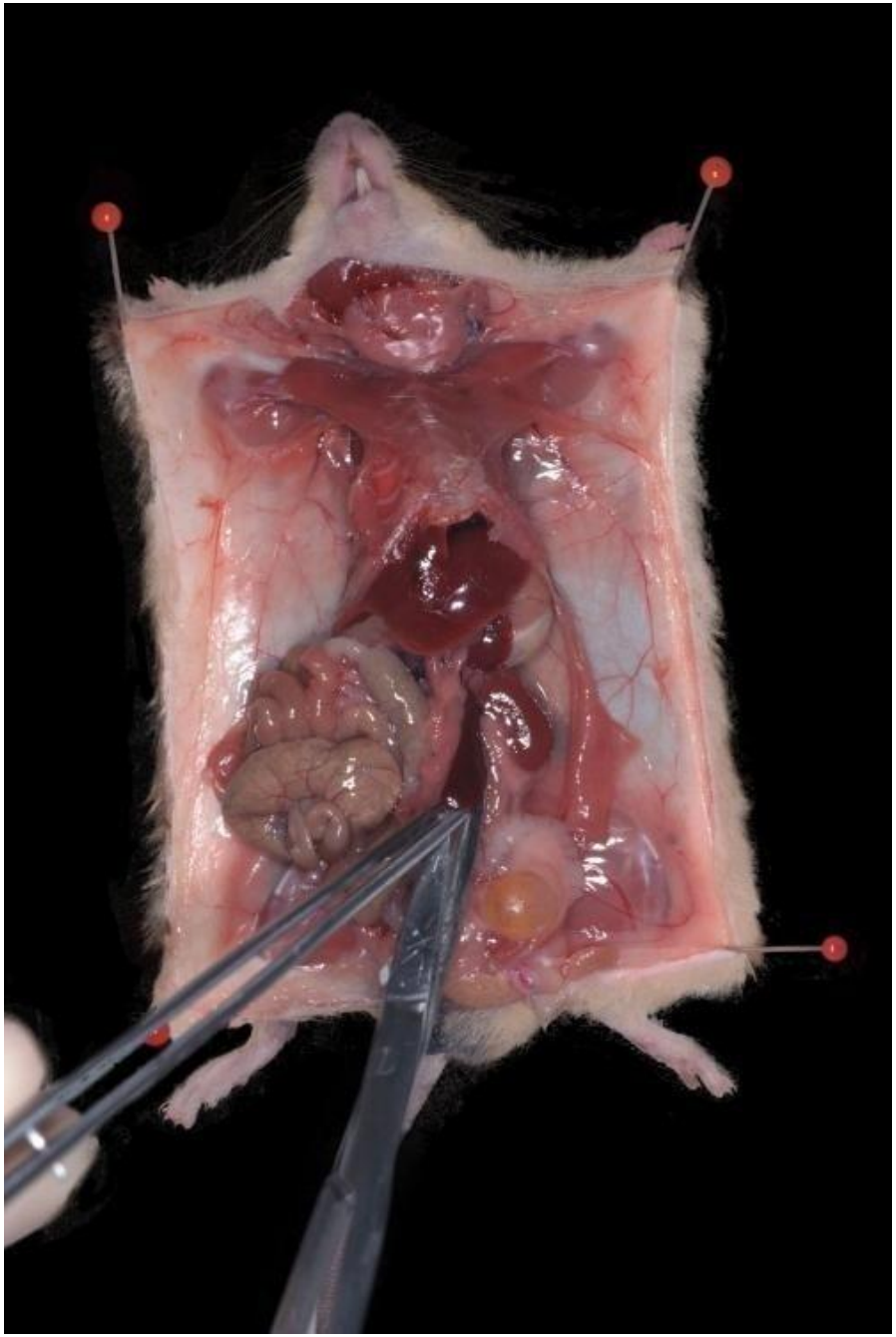




SUFFERING IN SILENCE

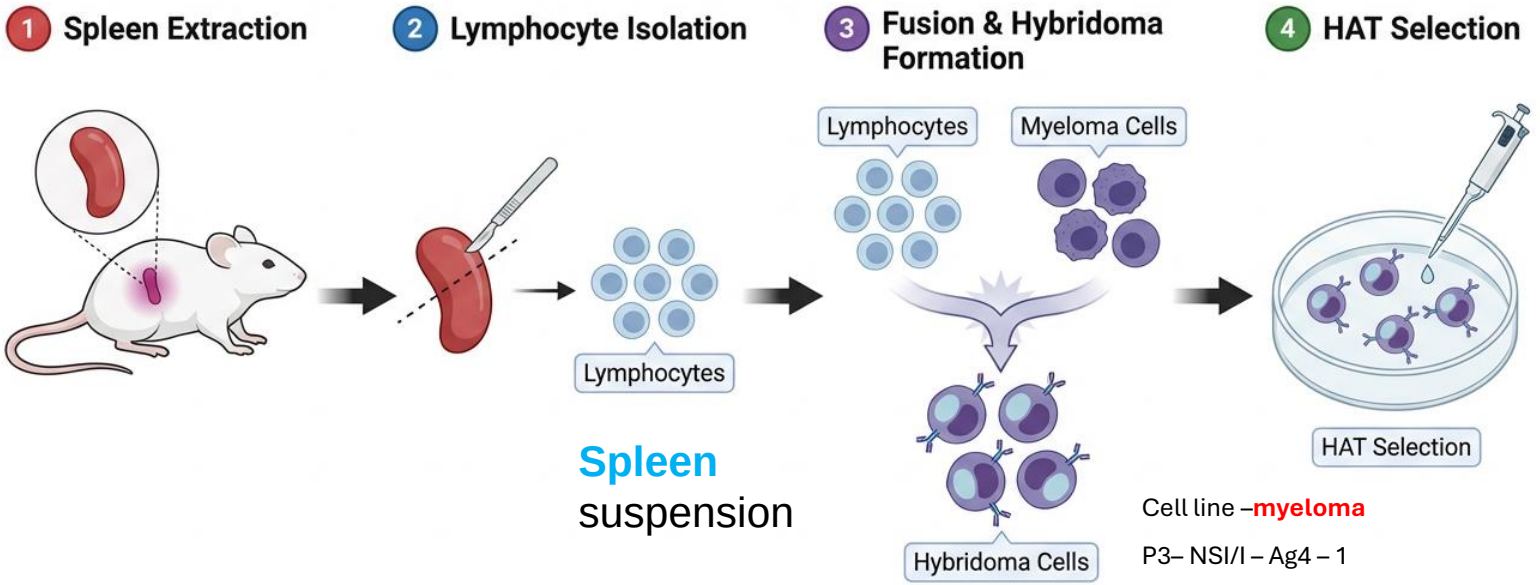
Caring for research animals can take a severe mental toll. Is anyone listening?

9 MAR 2023 • BY DAVID GRIMM



Down to size: Mouse spleens illustrate the reducing effects of a promising drug for Cooley's anemia patients.

Schematic of Mouse Hybridoma Generation for Monoclonal Antibody Production



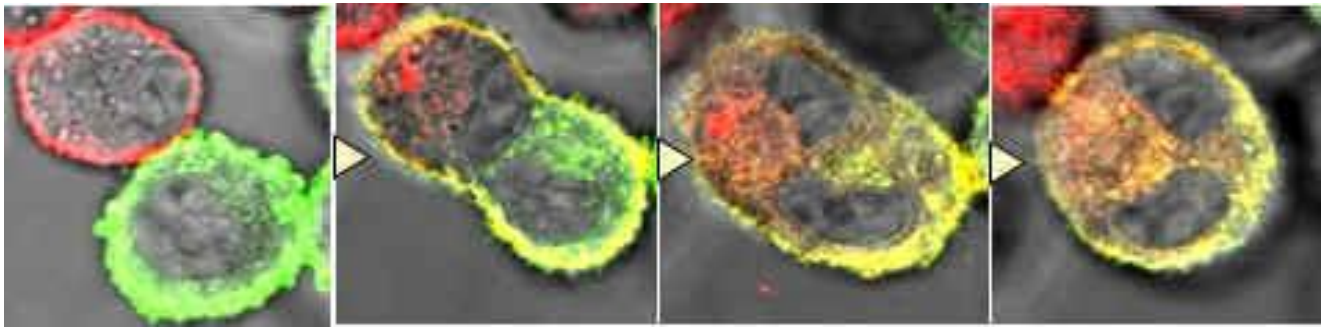
Spleen suspension

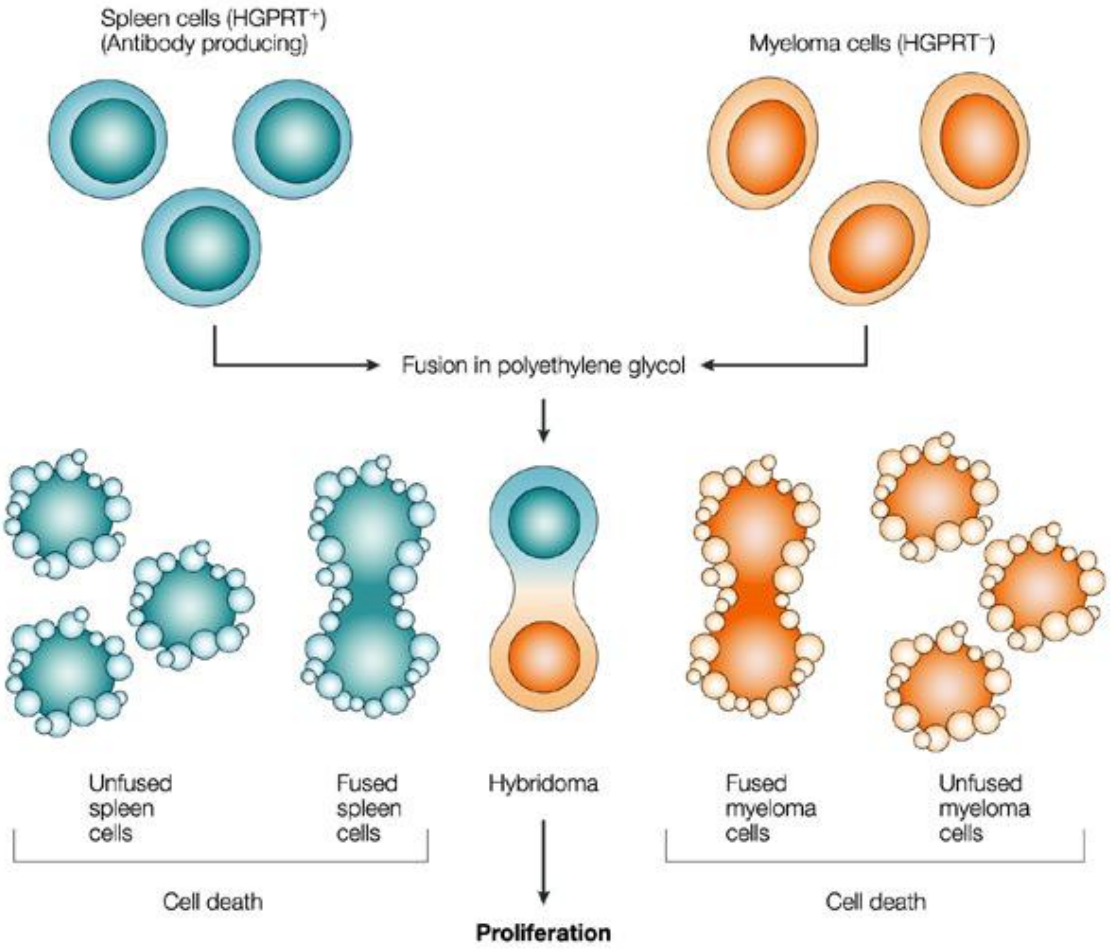
Cell line –**myeloma**

P3– NSI/I – Ag4 – 1

X63 – Ag8 - 653

Fusion with polyethylene glycol (PEG)





Die in HAT because they have a limited life span

Survive in HAT because they have HGPRT and contain the unlimited potential of cancer cells

Die in HAT because they lack HGPRT

What does HAT do?

- **HAT** contains:
  - **H**ypoxanthine
  - **A**minopterin
  - **T**hymidine

- **Myeloma** cell fusion partners were selected for **the loss of the ability to synthesize hypoxanthine-guanine phosphoribosyl transferase (HGPRT)**

- HGPRT enables cells to synthesize purines using an extracellular source of hypoxanthine as a precursor.

- Normally, the absence of HGPRT is not a problem because cells have an alternate biochemical pathway (termed the rescue pathway) they use to synthesize purines. The rescue pathway allows myeloma cells to divide normally.

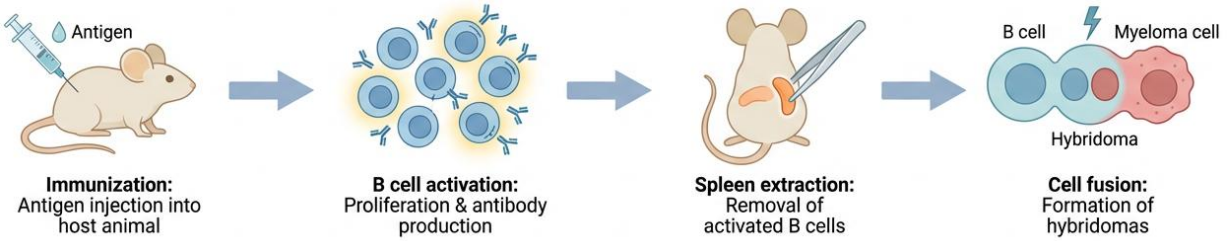
- **The rescue pathway is inhibited by aminopterin**. In the presence of aminopterin, HGPRT is essential for survival.

- HAT is selective for fused, (hydridoma) cells because:
  - Unfused myeloma cells cannot grow because they lack HGPRT
  - Unfused normal spleen cells cannot grow indefinitely because of their limited life span.

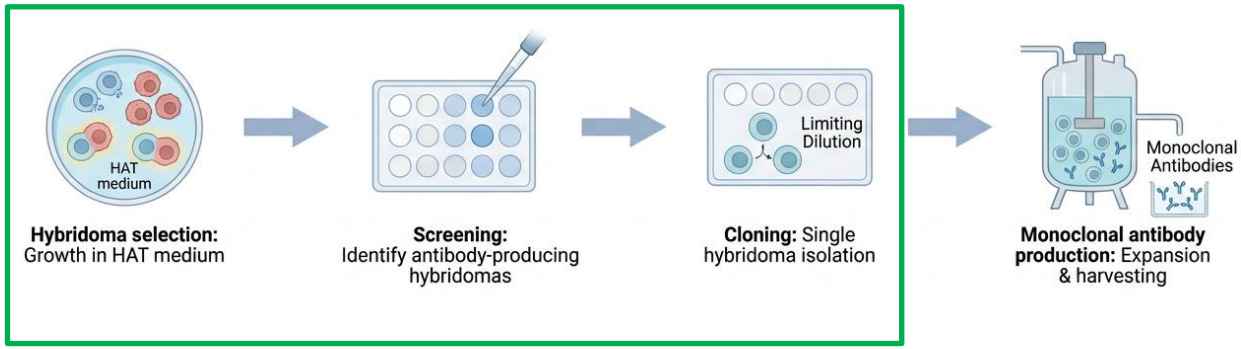
- In hybridomas the spleen cell partner supplies HGPRT and the myeloma partner is immortal because it is a cancer cell, overcoming the growth block of spleen cells.

How to make a monoclonal antibody

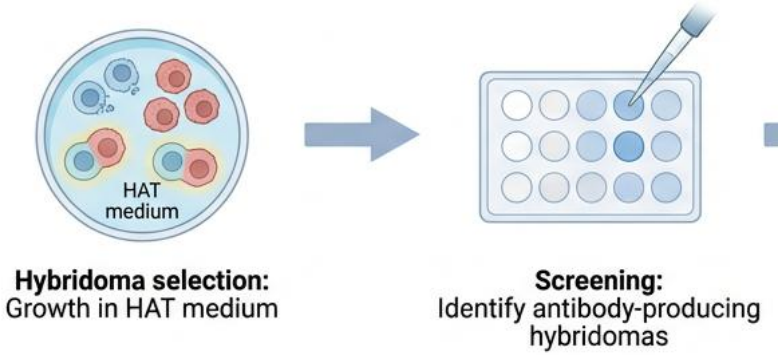
- Immunize mice
- Test the serum
- Perform a fusion

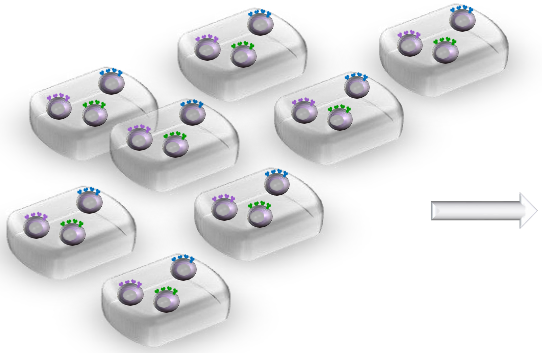


- **Screen the fusion for the right cells**
- Grow the hybridomas
- Harvest the antibody
- Concentrate and purify the product



Hybridomas selection





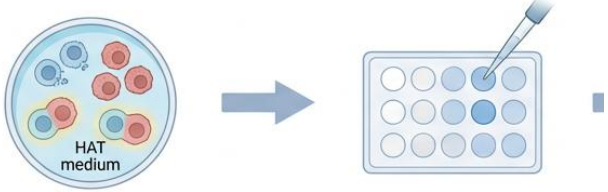
More than **24.000** hybridomas

10 days in the incubator

Perform a fusion - Growing the cells (hybridomas)

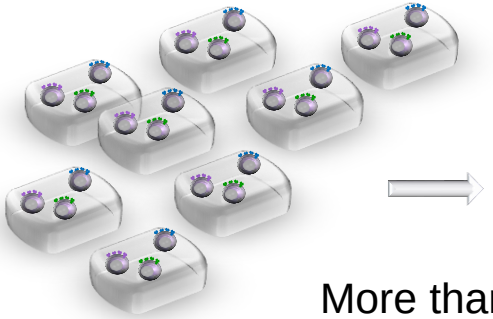
- Cells are grown in a 37° C incubator.
- Cells are kept in an atmosphere of about 6% CO_2.
- The cells are fed after 7 days of incubation.
- The cells are checked for growth after **10 days of incubation.**

Hybridomas selection



Hybridoma selection:
Growth in HAT medium

Screening:
Identify antibody-producing hybridomas



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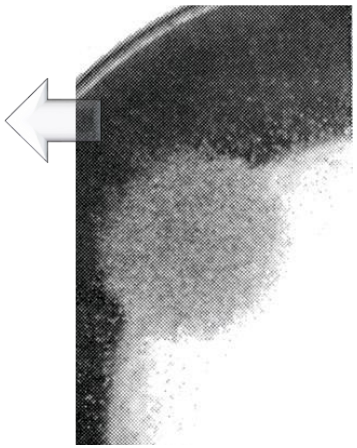
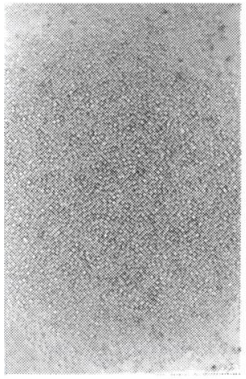
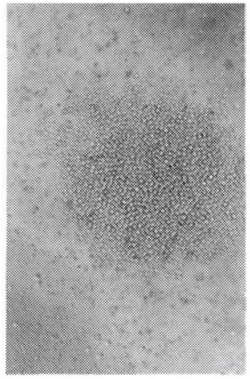
S C R E E N I N G



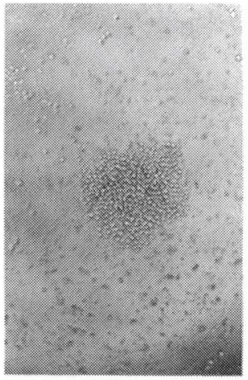
10 days



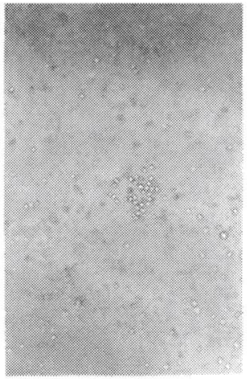
9 days



7 days



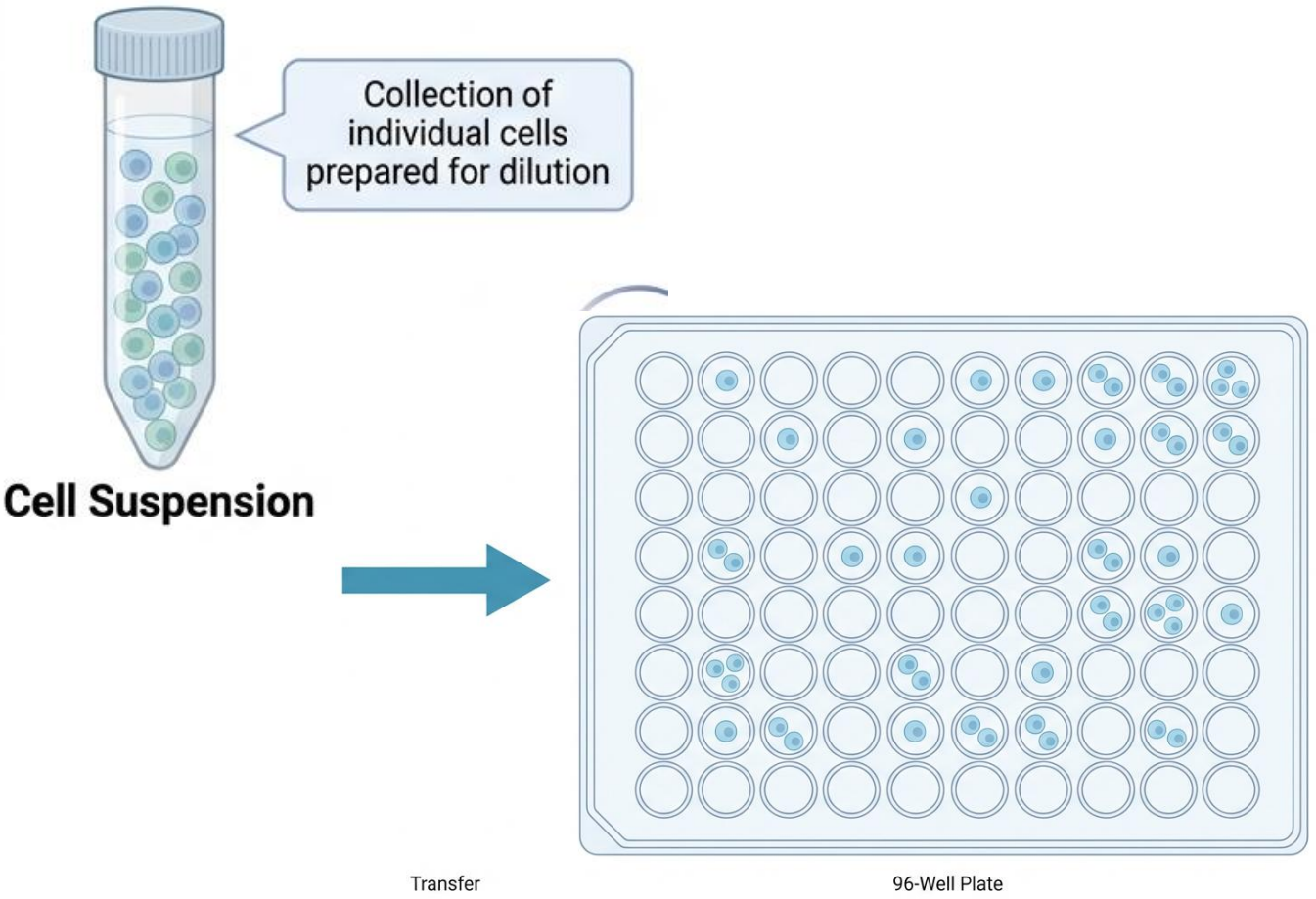
5 days



3 days

1 day

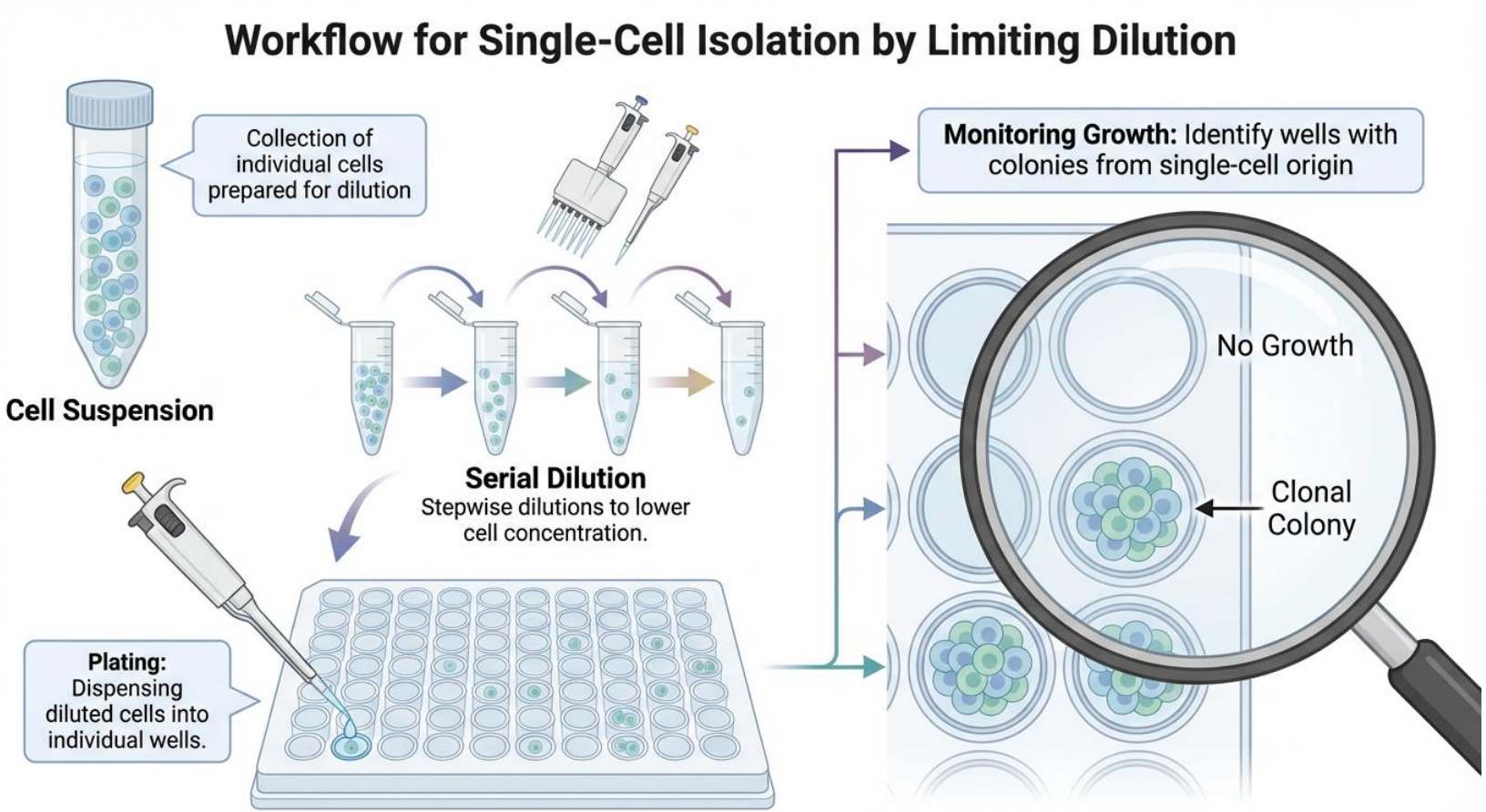
Is this really a MONO (single) clone ?

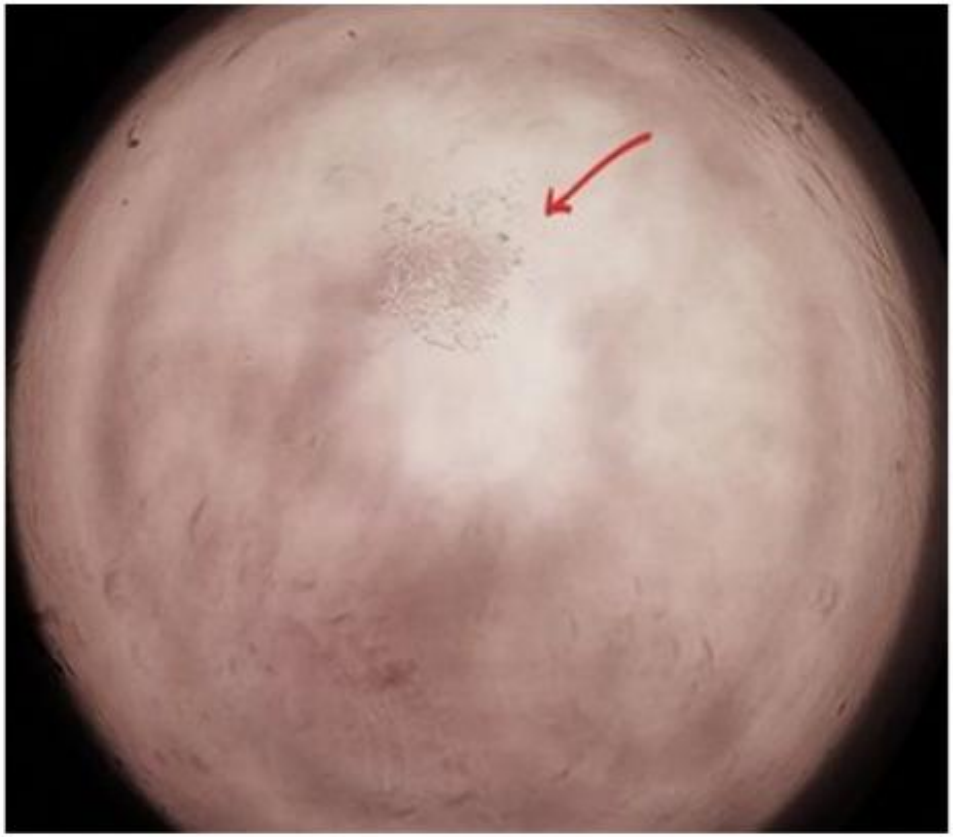


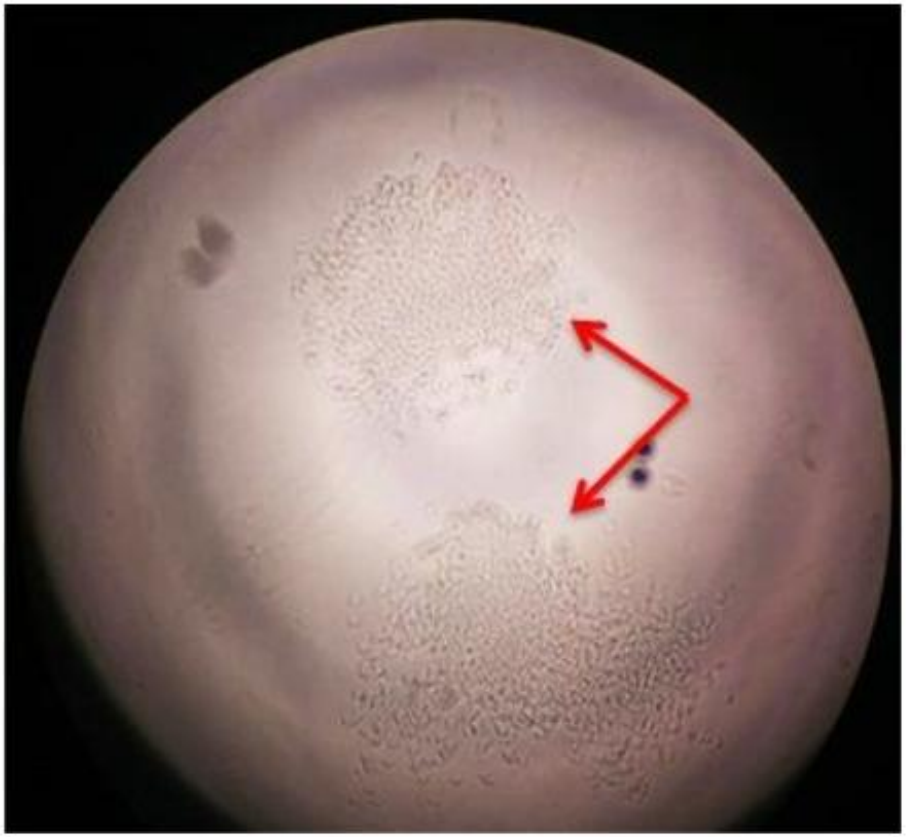
Limiting dilution

is a technique used to isolate **single cell clones** from a population, based on **statistical probability**.

It involves **diluting cells** to a concentration where, on average, **each well contains only one cell**.

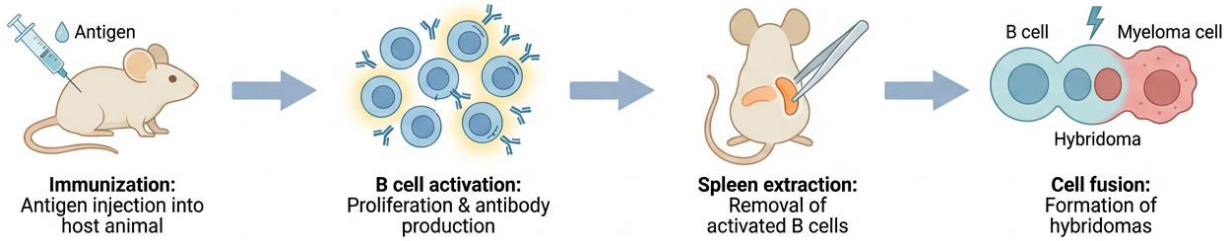






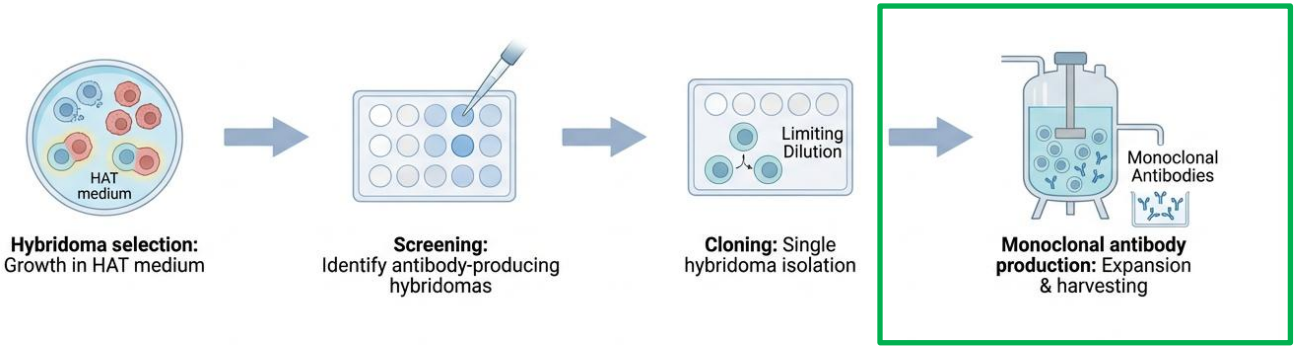
How to make a monoclonal antibody

- Immunize mice
- Test the serum
- Perform a fusion

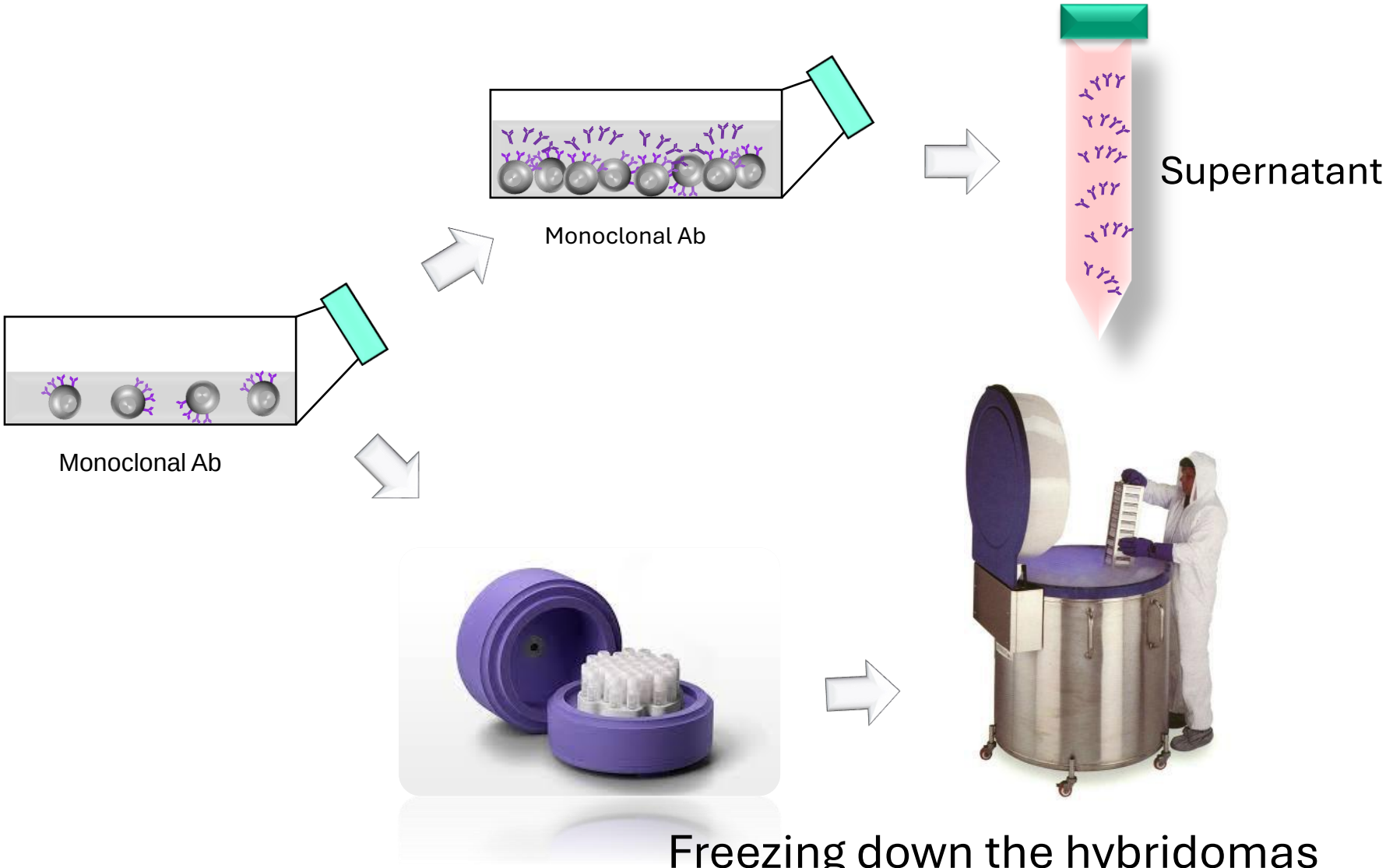


- Screen the fusion for the right cells

- **Grow the hybridomas**
- **Harvest the antibody**
- **Concentrate and purify the product**



Ab production



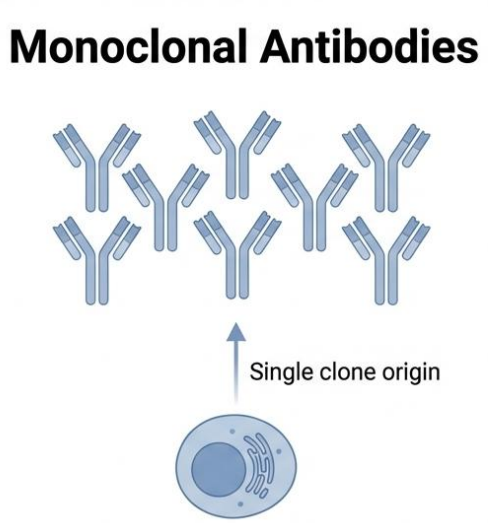
Advantages

1. Possible to select Mabs with the **required specificity.**

2. **Large quantities** of antibodies can be obtained easily.

3. **Pure antibodies** can be obtained more easily.

4. **Indefinite supply.**



Disadvantages of Monoclonal Antibodies

1. Labour intensive.
2. Costly.
3. Longer time span.