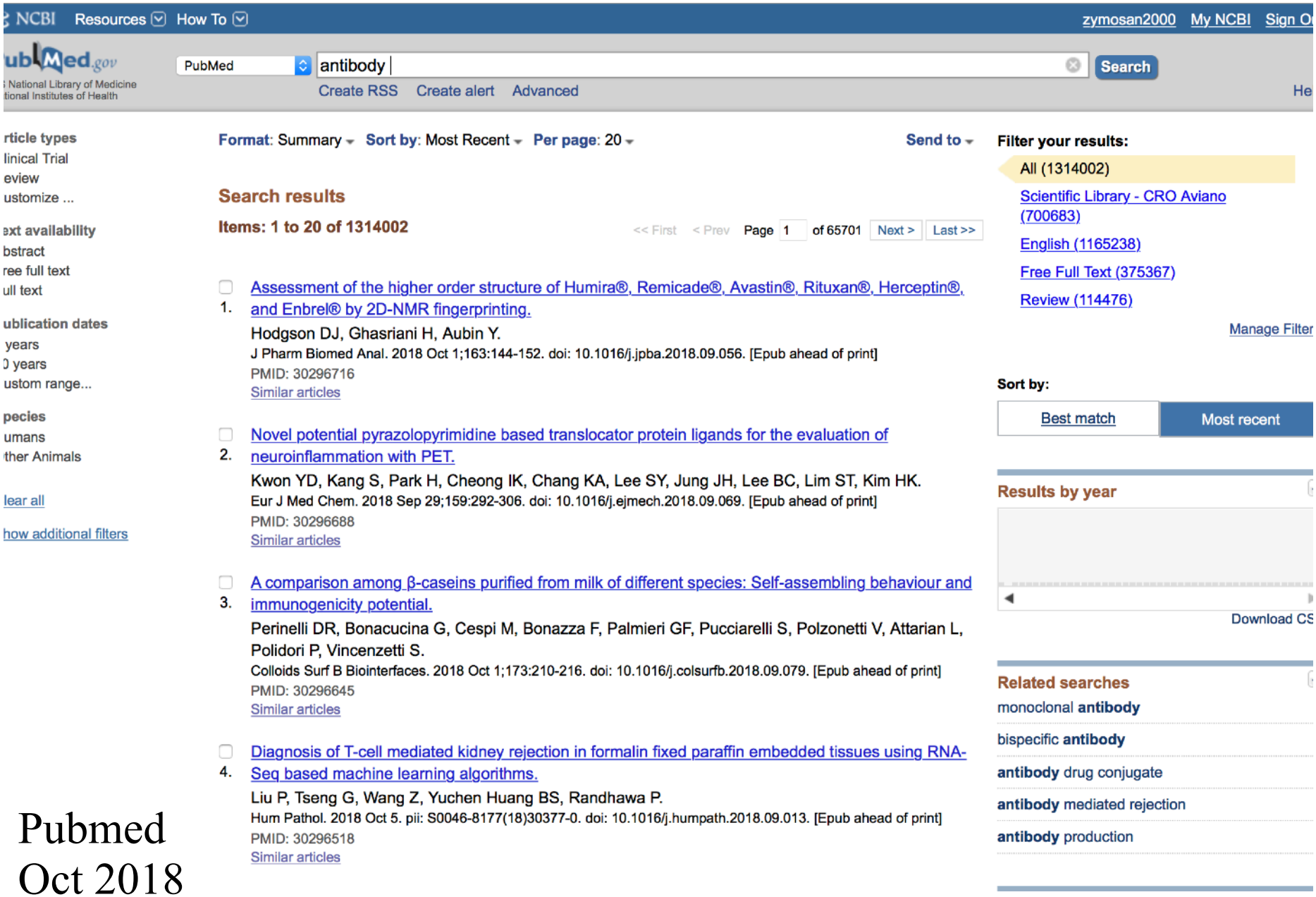
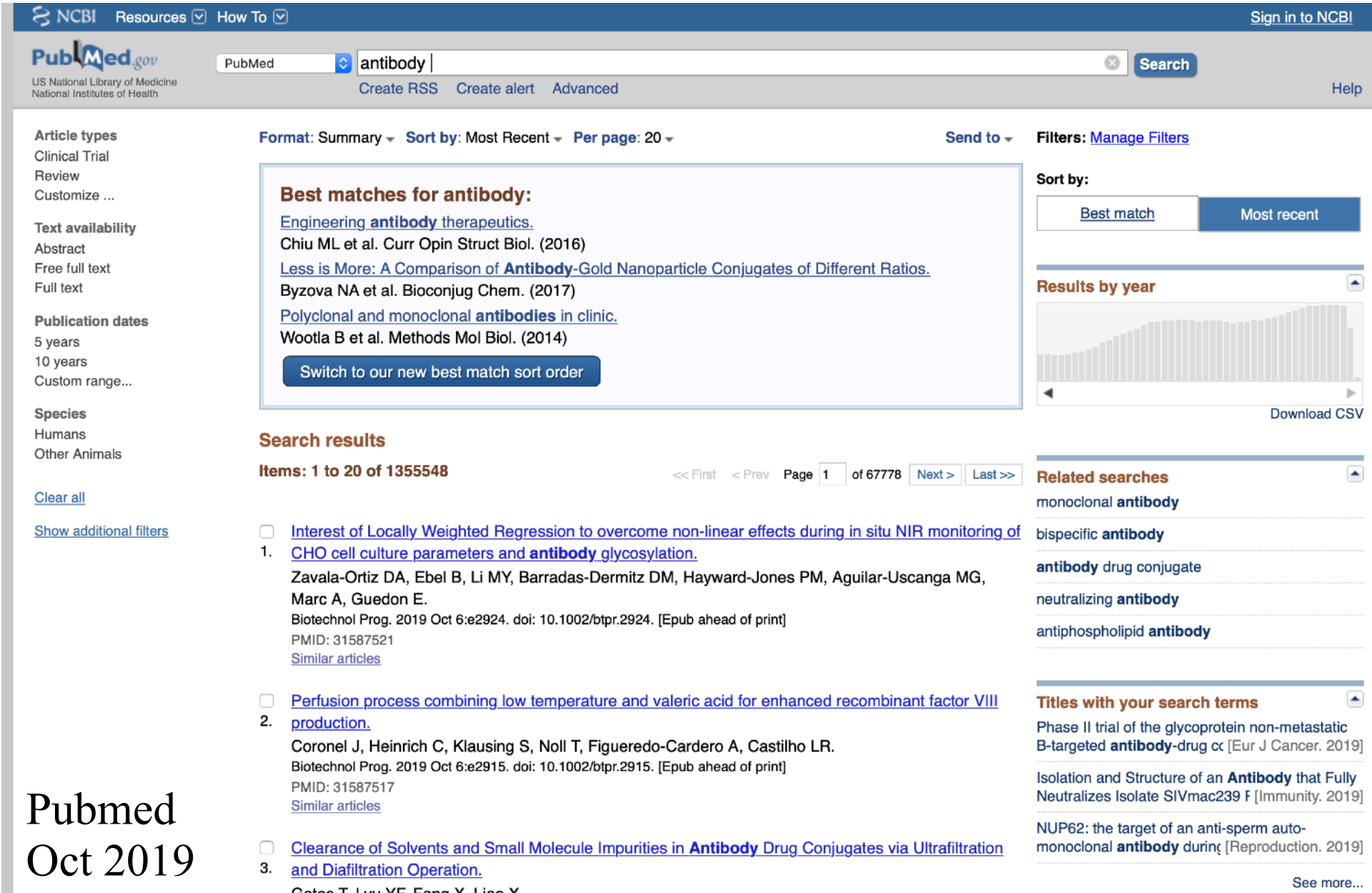
Production of polyclonal and monoclonal antibodies

Monoclonal Antibodies



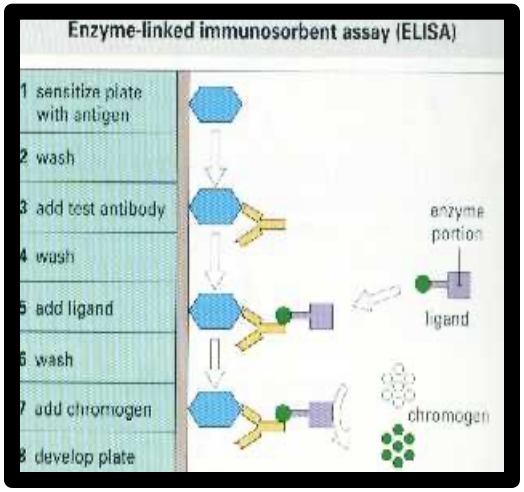
Pubmed
Oct 2018

Monoclonal Antibodies

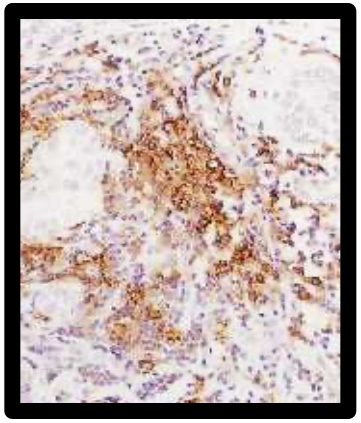


Pubmed
Oct 2019

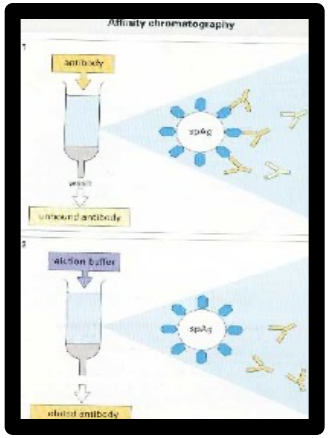
Use of monoclonal antibodies



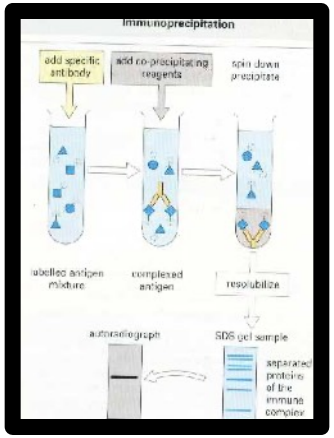
ELISA



Immuno-histochemistry

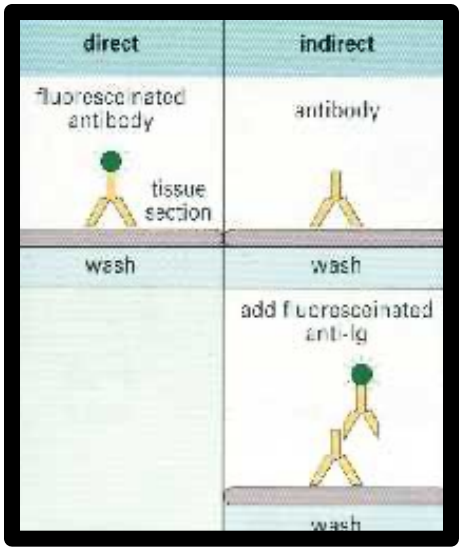


Purification

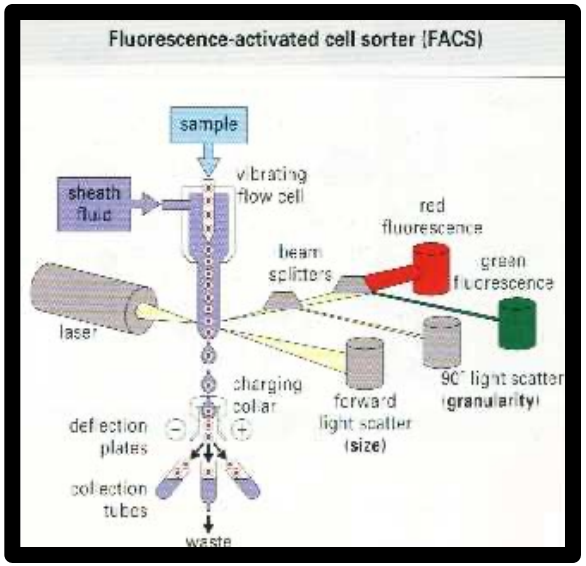


Immuno-precipitation

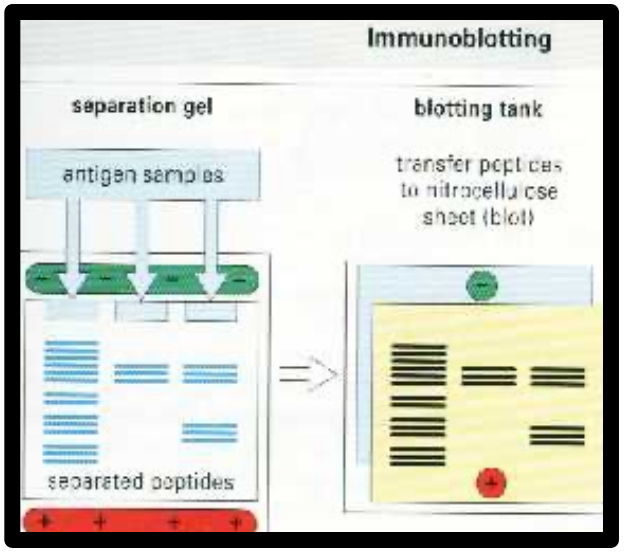
Use of monoclonal antibodies



Immuno-fluorescence



Flow cytometry



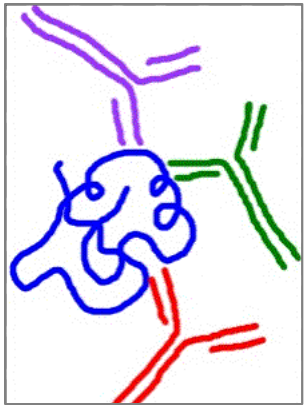
Immunoblotting

Antibody Production

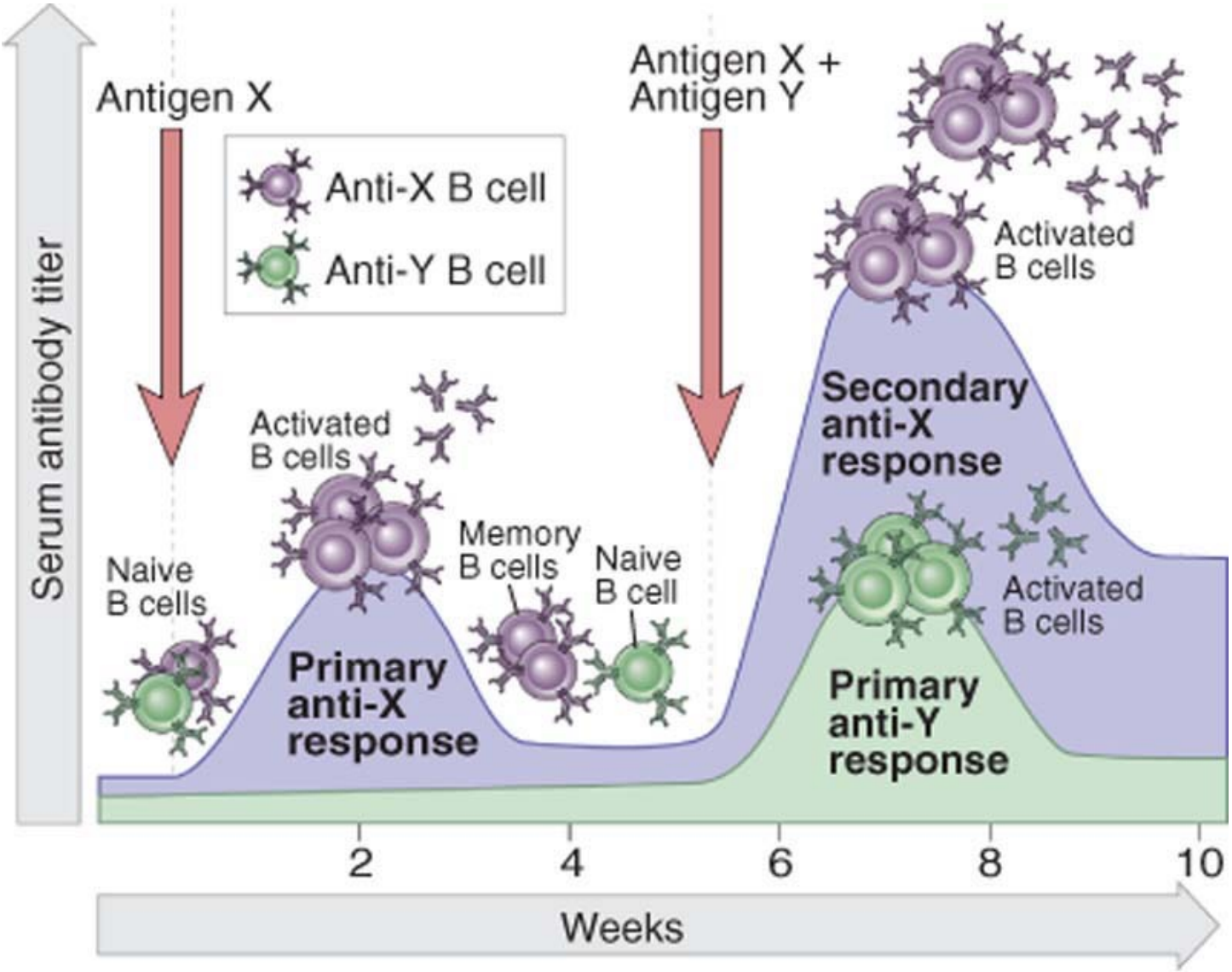
Polyclonal:
Antibodies are collected from sera of exposed animal,
- or -
a combination of monoclonal colonies is combined.

Can be any animal: **Rabbit**, **Goat**, Horse, Rat, Sheep, etc…

Suite of antibodies recognizing multiple antigenic sites of injected biochemical.



Timing for Ab production



Disadvantages of Polyclonal Antisera

- Antiserum is composed of a mixture of **high** and **low** affinity antibody populations
- Antiserum is composed of a **mixture of antibodies** with different specificities - not all recognize the target of interest
- If the animal has had an infection, antibodies against the infecting organism will be present - can lead to "**non-specific" binding**
- **Quantity** of antiserum is **limited** by amount of serum and **life of immunized animal**.
- **Antigen must be pure**.

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, functional assay)

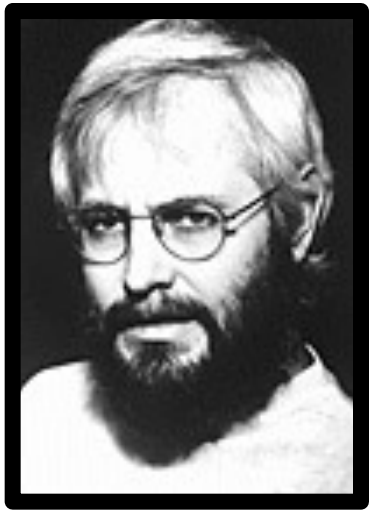
- Can be affinity purified to eliminate the non-specific binding antibodies

Production of monoclonal antibodies

One of the most important example of induction and selection of stable cell mutant and cell fusion is the production of **hybridoma**

Hybridomas are hybrid of cells obtained fusing lymphocytes and a tumor cell line obtained from multiple myeloma.

The aim is to obtain a stable clone of lymphocytes in order to obtain in vitro, for long time and in high amount, monoclonal antibodies that usually lymphocytes produce after their differentiation to plasma cells.



&

Georges Köhler

(1946-1995)

Cesar Milstein

(1927-2002)

Nature Vol. 256 August 7 1975

Continuous cultures of fused cells secreting antibody of predefined specificity

Method to produce monoclonal antibodies -1975

Nobel Prize - 1984

How to make a monoclonal antibody

1. Immunize mice
2. Test the serum
3. Purify lymphocytes from the spleen
4. Perform a fusion
5. Screen the fusion for the right cells
6. Grow the hybridomas
7. Harvest the antibody
8. Concentrate and purify the product

Production of Monoclonal Antibodies

Immunisation with antigen

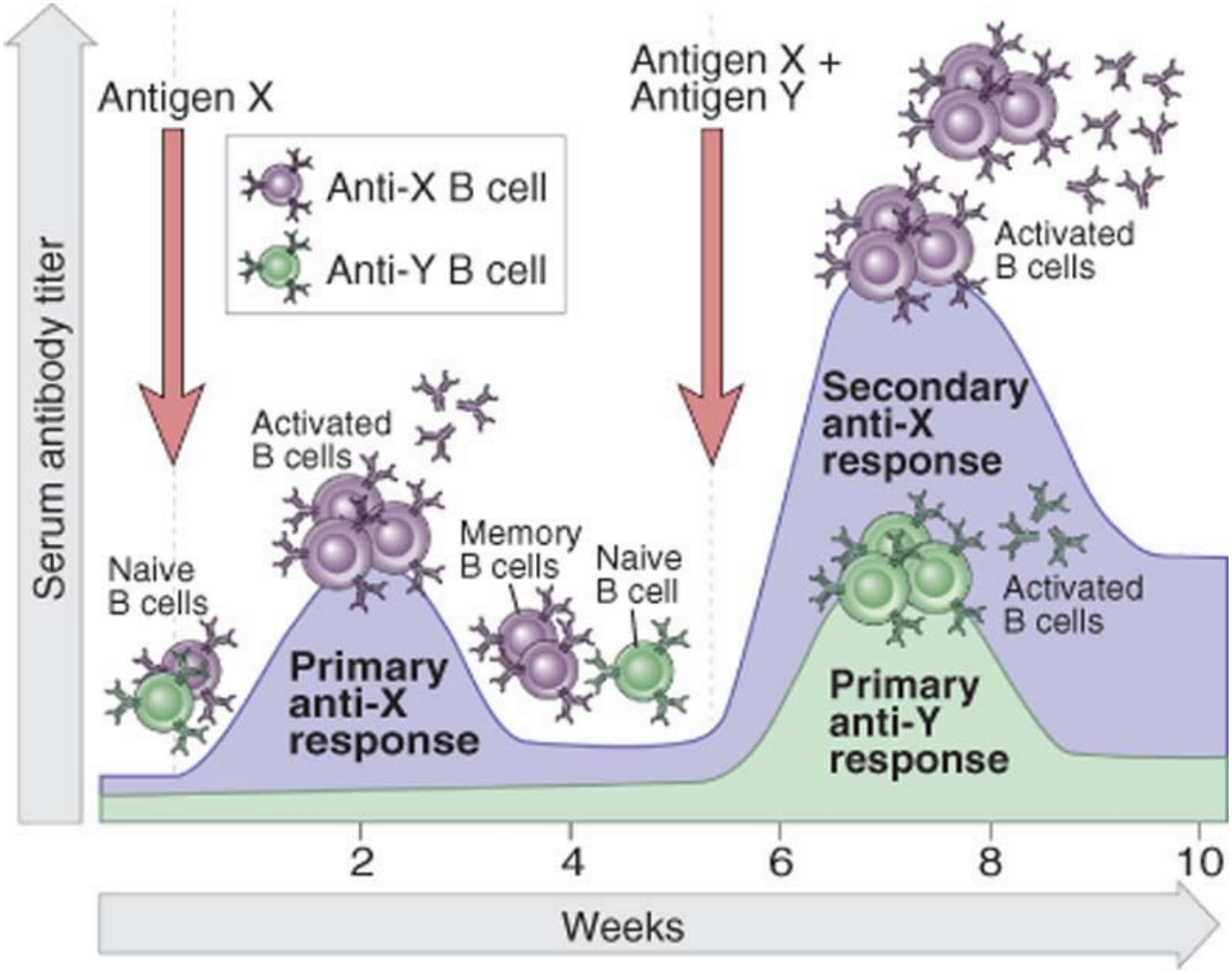
Ab titre from serum

Ab_1 Ab_2 ... Ab_n

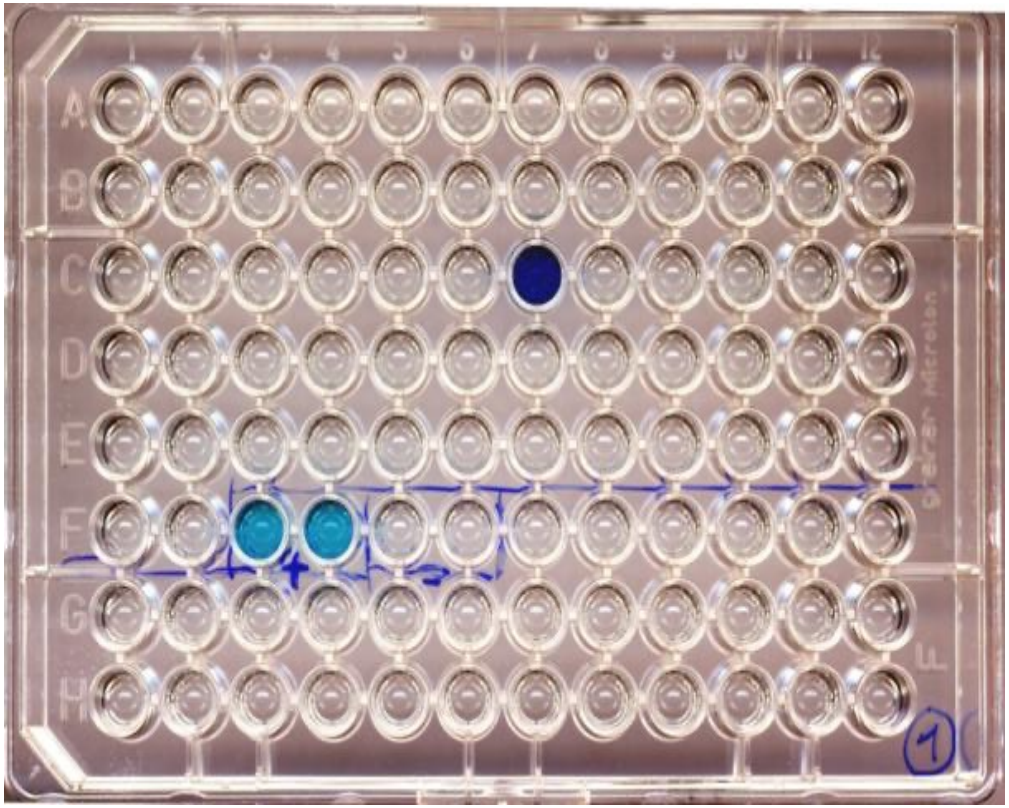
Immunize the mice - Inoculation

- The mice are aseptically inoculated with the antigen combined with an adjuvant.
- Inoculations are done either **sub-cutaneously** or **intra-peritoneally.**
- Normal dose per mouse is between **20 and 100 micrograms** of protein.
- Inoculations are performed every 14 to 21 days.

Timing for Ab production



Test the serum – In the lab



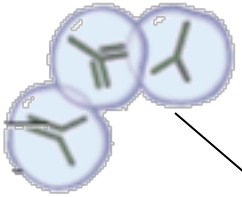
Test the serum - Decision time

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

- Sometimes additional testing is done (Western blots, immunoflurorescence) to determine whether the serum response is specific for the selected antigen.
- The mouse with the strongest, most specific response is chosen for the fusion.

Production of Monoclonal Antibodies

Immunisation with antigen



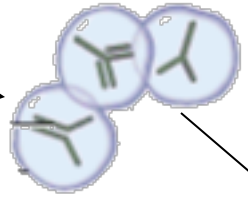
Spleen suspension

Ab titre from serum

Ab_1 Ab_2 ... Ab_n

Production of Monoclonal Antibodies

Immunisation with antigen



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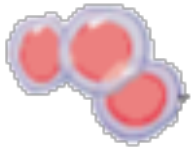


Cell line –myeloma

P3– NSI/I – Ag4 – 1

X63 – Ag8 - 653

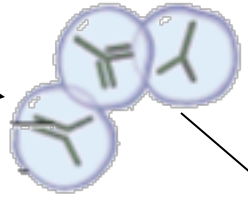
Ab titre from serum

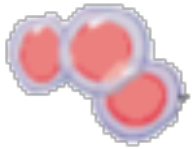
Ab_1 Ab_2 ... Ab_n

Production of Monoclonal Antibodies

Immunisation with antigen

Spleen suspension





Cell line –myeloma

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Ab titre from serum

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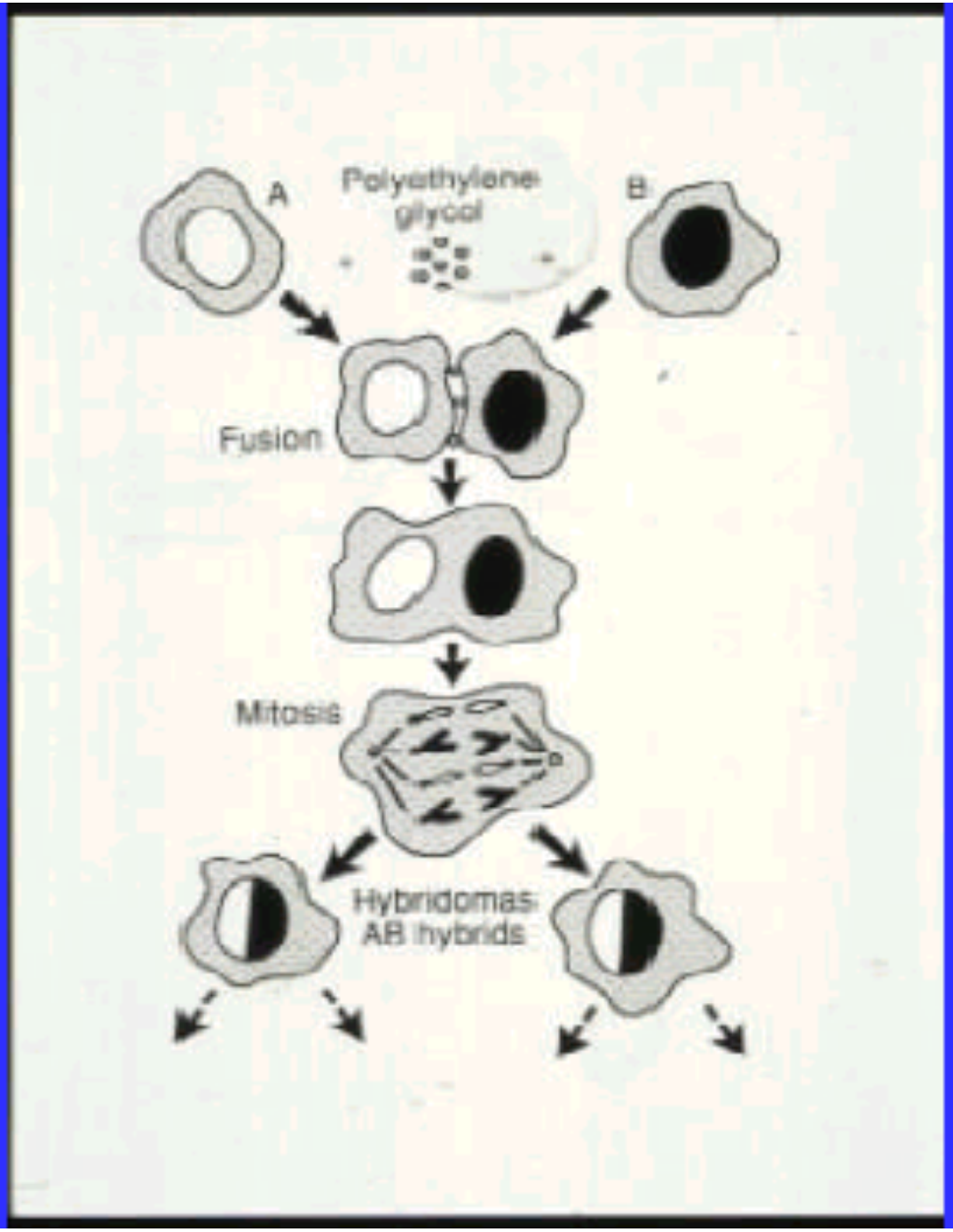
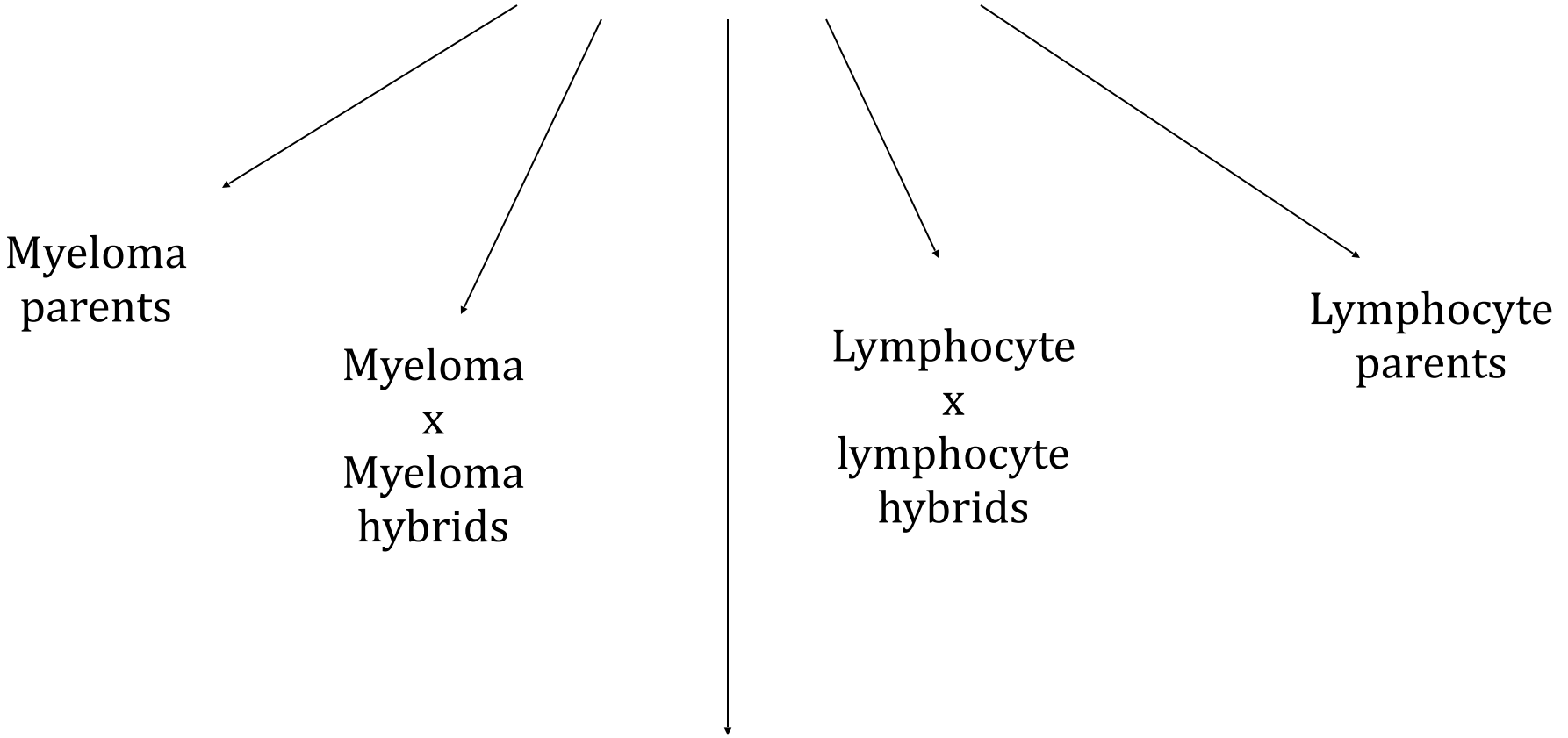
Fusion with polyethylene glycol (PEG)


A
Polyethylene glycol
B
Fusion
Mitosis
Hybridomas
AB hybrids

Cell types present after fusion

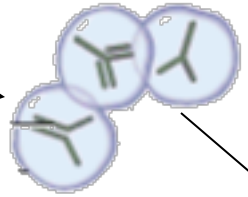


What does HAT do?

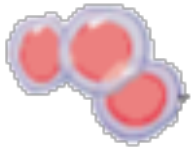
- Myeloma cell fusion partners were selected for **the loss of the ability to synthesize hypoxanthine-guanine phosphoribosyl transferase (HGPRT)**
- HGPRT enables cells to synthesize purines using an extracellular source of hypoxanthine as a precursor.
- Normally, the absence of HGPRT is not a problem 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 contains:
  - Hypoxanthine
  - Aminopterin
  - Thymidine
- 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.

Production of Monoclonal Antibodies

Immunisation with antigen



Spleen suspension



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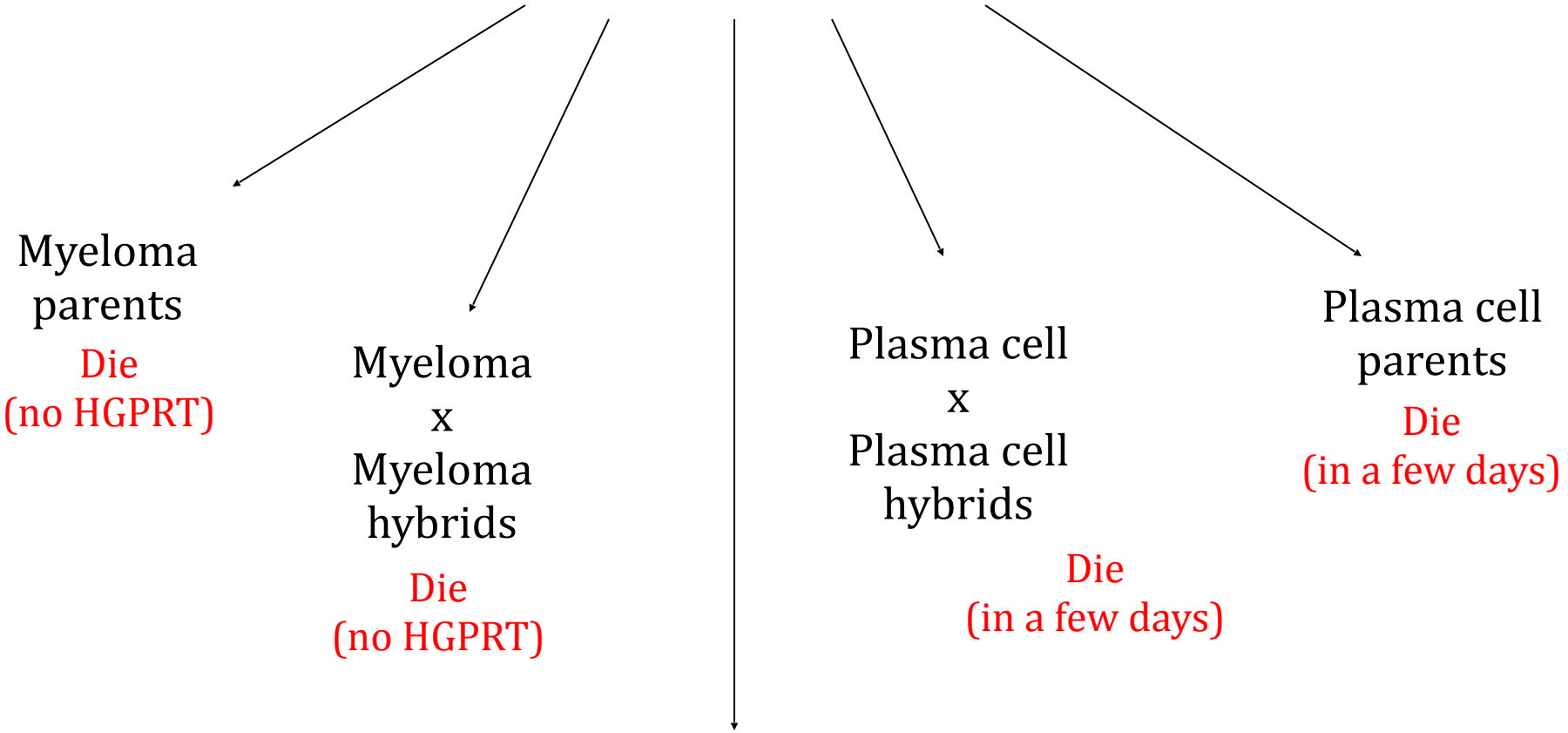
Ab titre from serum

Ab_1 Ab_2 ... Ab_n

Fusion with polyethylene glycol (PEG)

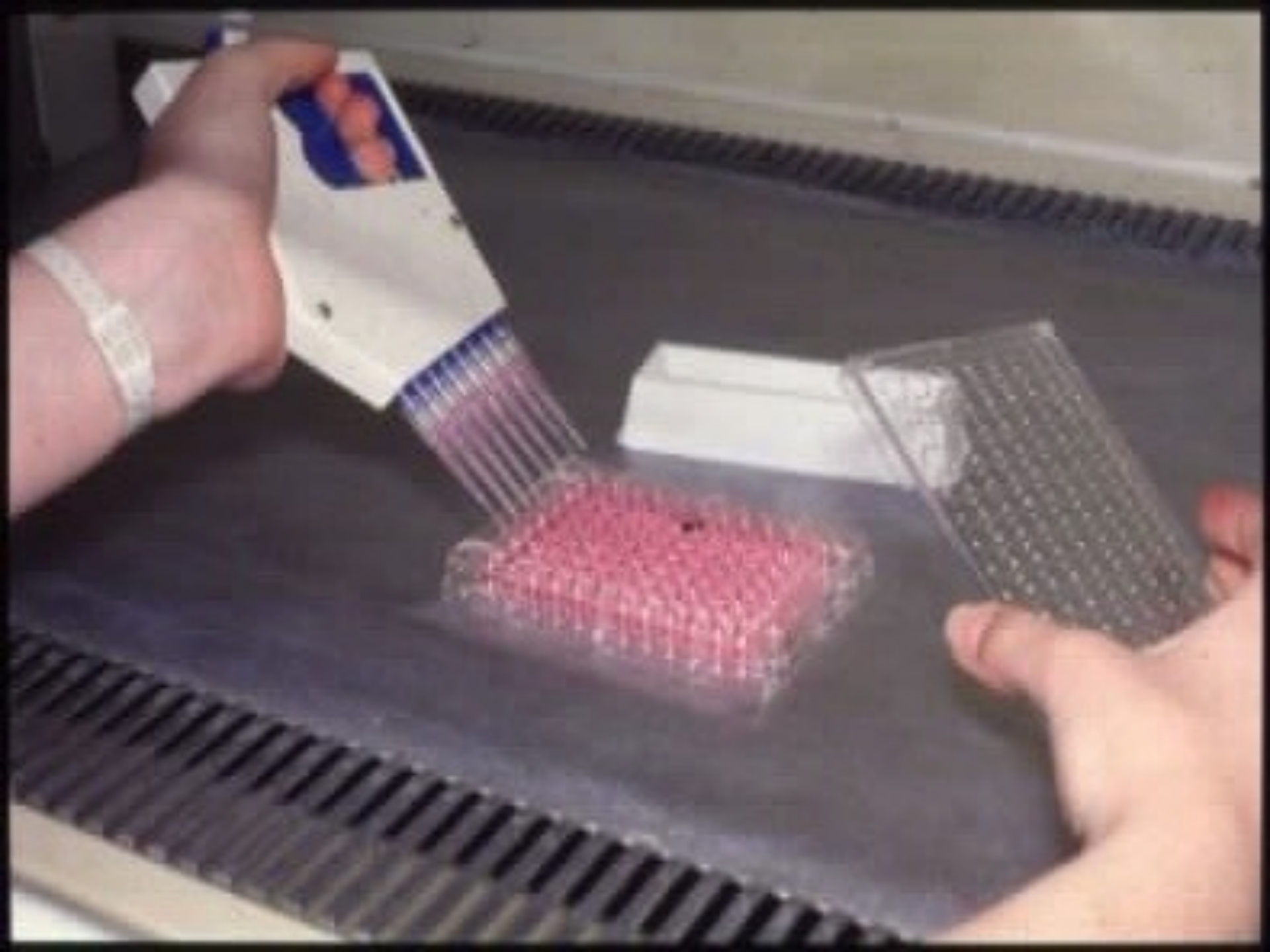
Selection with H.A.T.

Cell types present after fusion



Perform a fusion - Growing the cells (hybridomas)

- Cells are grown in a 37^{o} C incubator.
- Cells are kept in an atmosphere of about 5% CO_2.
- The cells are fed after 7 days of incubation.
- The cells are checked for growth after 10 days of incubation.



Production of Monoclonal Antibodies

Immunisation with antigen

Ab titre from serum

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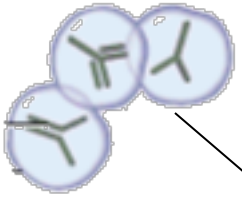

Spleen suspension

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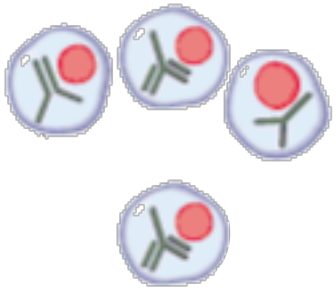


Selection with H.A.T.

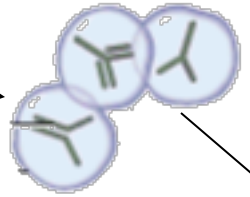
Screen for Ab – secreting hybrids

Production of Monoclonal Antibodies

Immunisation with antigen

Ab titre from serum

Ab_1 Ab_2 ... Ab_n



Spleen suspension

Cell line –myeloma

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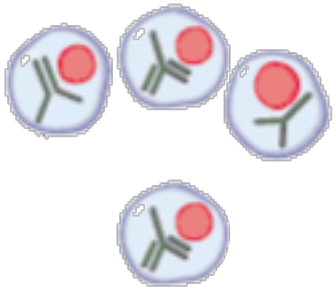
Fusion with polyethylene glycol (PEG)



Selection with H.A.T.

Screen for Ab – secreting hybrids

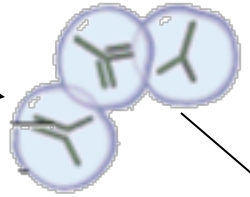
Clone Ab secreting hybrids

Production of Monoclonal Antibodies

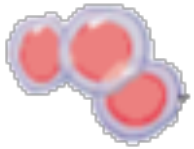
Immunisation with antigen

Ab titre from serum

Ab_1 Ab_2 ... Ab_n



Spleen suspension



Cell line –myeloma

P3– NSI/I – Ag4 – 1

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Fusion with polyethylene glycol (PEG)

Selection with H.A.T.

Screen for Ab – secreting hybrids

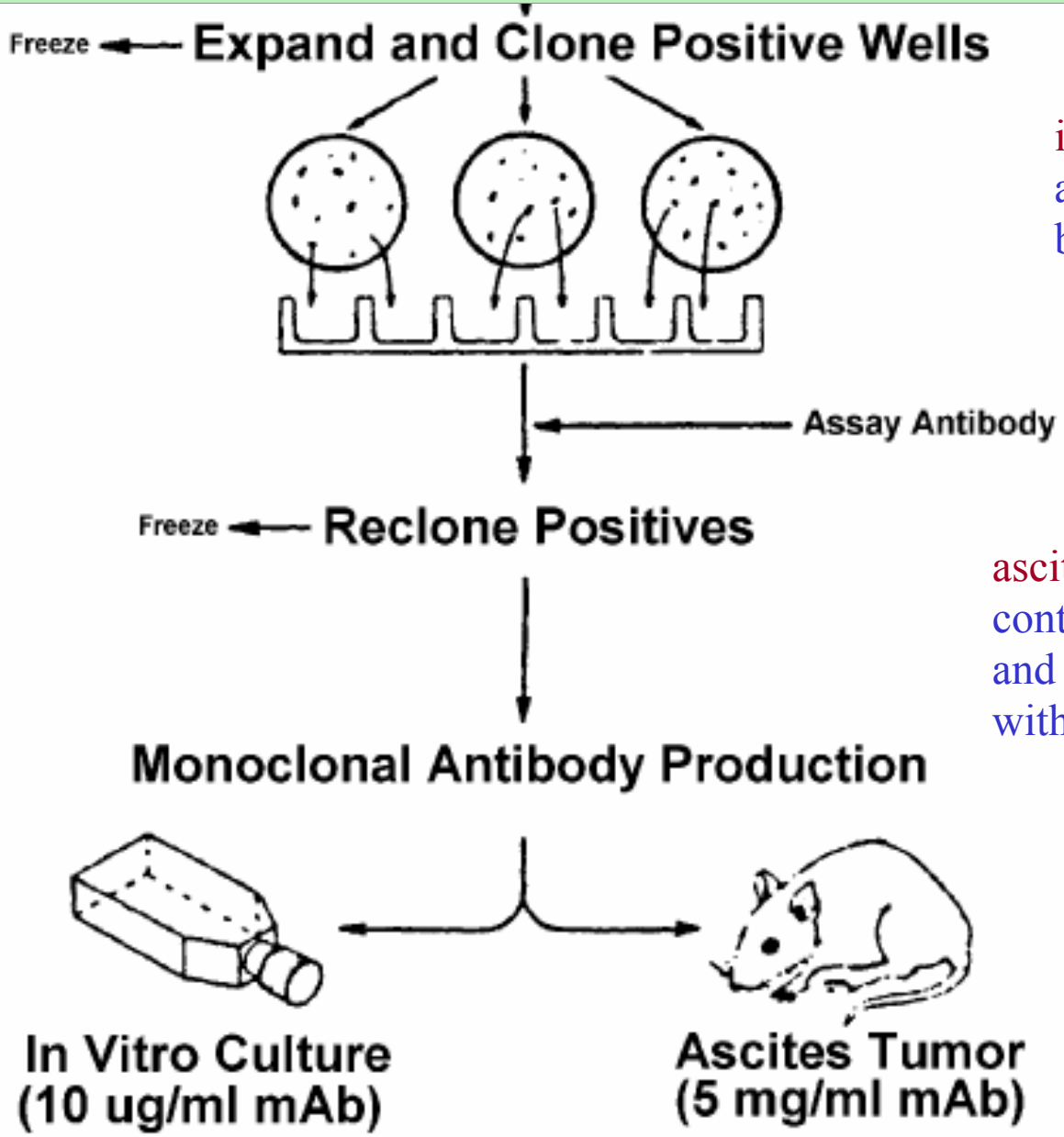
Clone Ab secreting hybrids

Clone 1 Clone 2 Clone n

Freeze cells


Fire exit
LIQUID NITROGEN

Expand in mice or expand in vitro



in vitro material is less concentrated and contains bovine serum

ascites fluid contain high [mAb] and minor contamination with mouse Ig

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.**

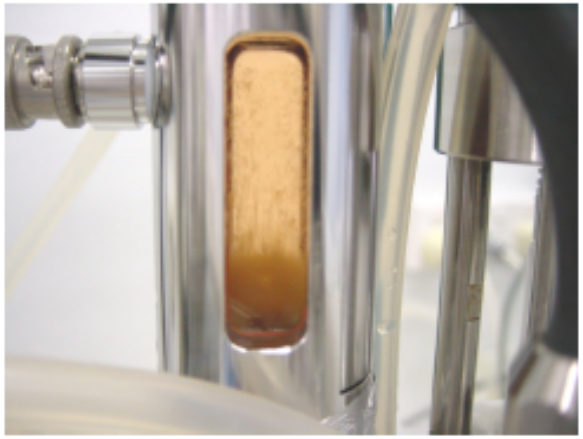
Disadvantages of Monoclonal Antibodies

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

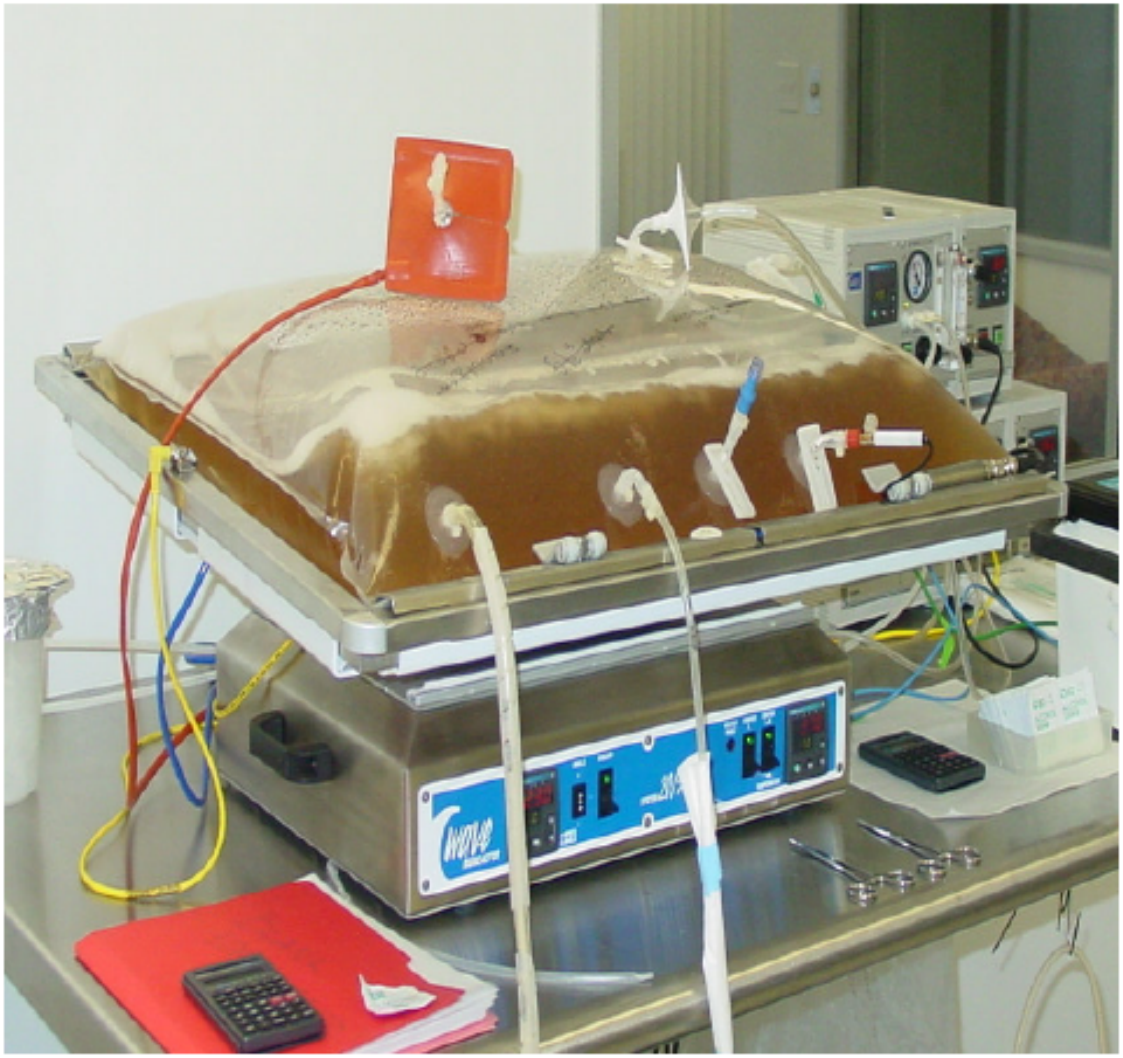
Roller bottles

- Old technology, principally for attached cell culture
- Bottle rotates at 1-3 rpm
- Effective OTR related to rotation speed and operating volume
- Scalable by replication

Bioreactors for Suspension Systems (examples)



Disposable systems



1-25 L

10-100 L

http://www.wavebiotech.com/

20,000 liter mammalian cell ferment or - Lonza Biologics - Portsmouth, NH

Cost

- Facilities cost $20-50 million to build
- Antibodies cost $500-1,000/gram
- High costs present challenges for reimbursement in chronic diseases

High level production in mammalian cells: the math

Lonza (contract manufacturer) claims = **5.5 g/L yield in 24 days**

30,000 L reactor:
30,000 L. X 5.5 g/L = **165 kg in 24 days**,
x 12 = 1,980 kg/year = **2,000,000 g/year**

One mAb dose = 500 mg = **0.5 g**
2,000,000/0.5 = **4 million doses per reactor per year.**
6 doses per patient per year?
4,000,000/6 = **600,000** patients per year per reactor.
At $10,000 per patient per year → **$6B** in sales /per 30KL reactor

Not bad!!