Antibody Production and Engineering

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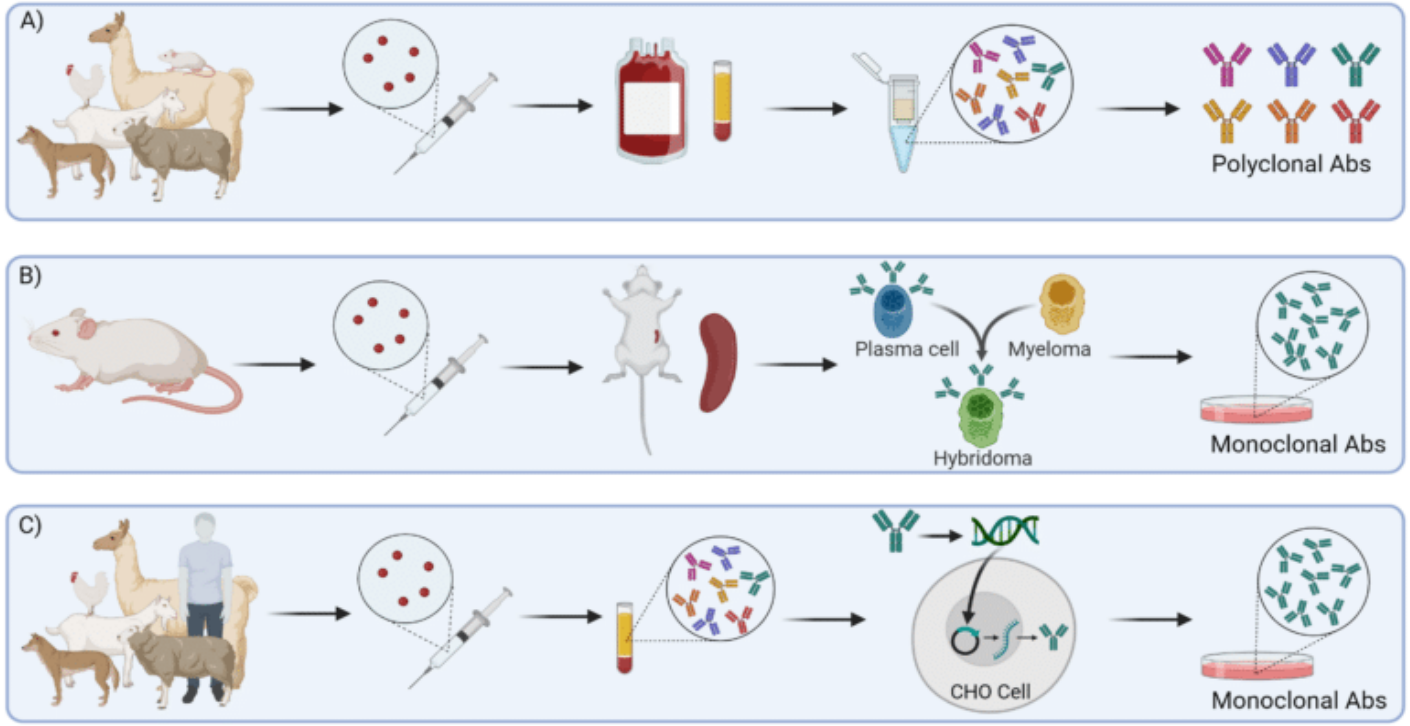
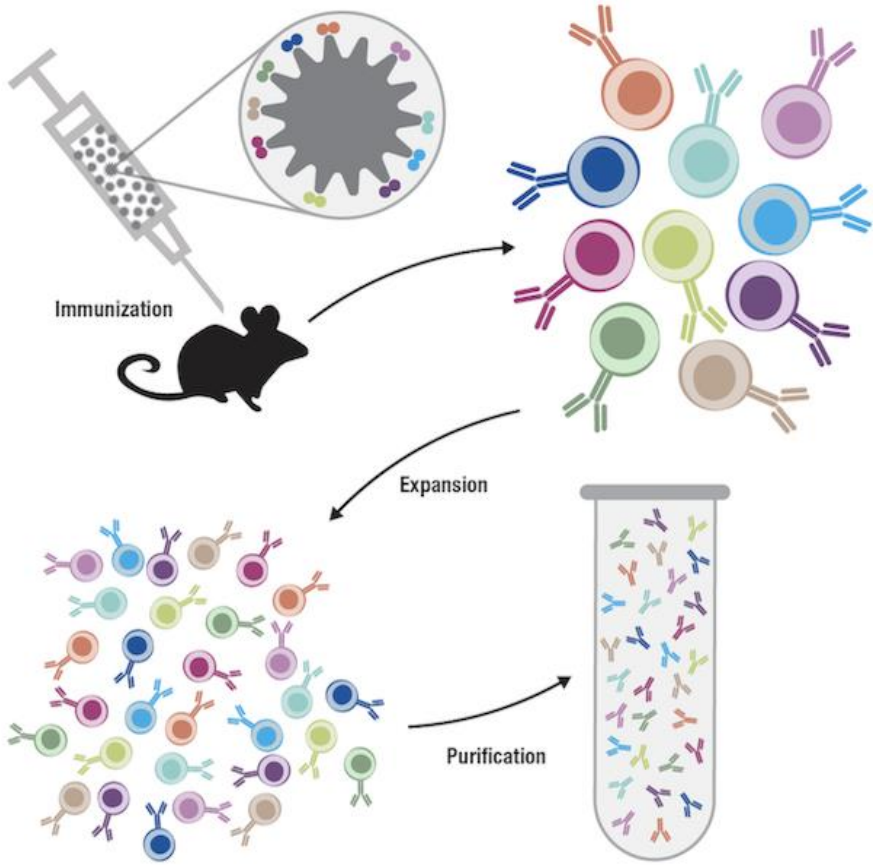
Overview of Antibody Production Strategies

- Antibodies used in research, diagnostics, and medicine are typically generated by immunising an animal with a target antigen.
- The immune response stimulates proliferation of antigen-specific B-lymphocytes and production of antibodies directed against the target.
- Depending on the intended application, antibodies may be collected directly from serum as polyclonal antibodies or isolated from individual B-cell clones to generate monoclonal antibodies.
- Each approach offers distinct advantages and limitations in terms of specificity, reproducibility, cost, and production time.



Production of Polyclonal Antibodies

- Polyclonal antibodies are produced by immunising an animal such as a rabbit, goat, sheep, or mouse with the target antigen.
- Multiple B-cell clones respond to different epitopes on the antigen, resulting in the production of a diverse antibody population.
- Following immunisation, blood is collected and the serum harvested.
- Antibodies specific for the antigen can then be enriched using affinity purification methods.

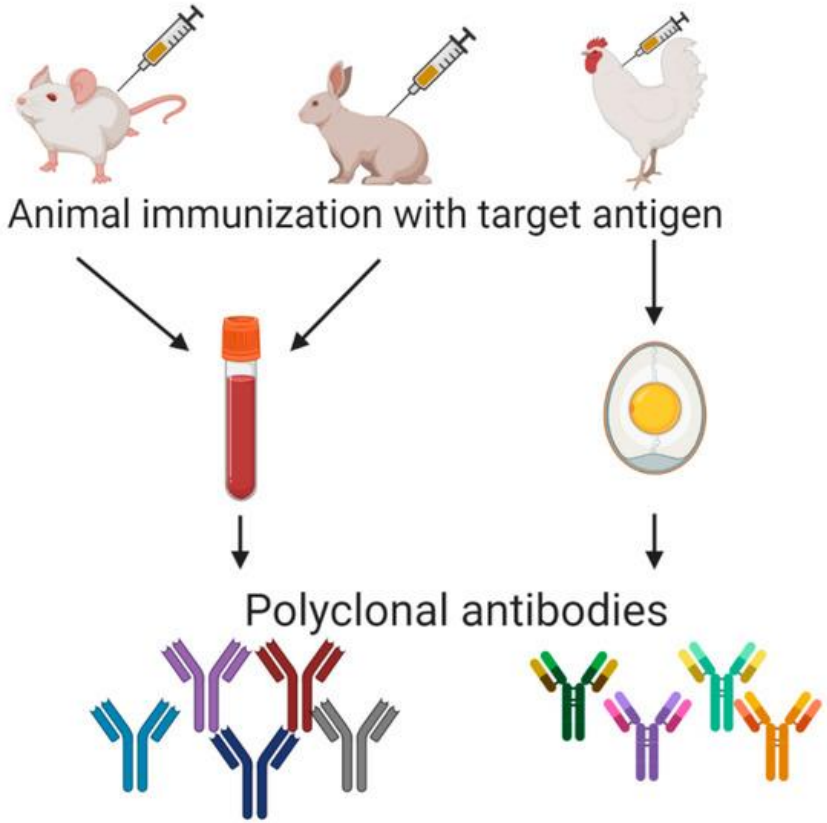


Timeline of Polyclonal Antibody Production

- Following immunisation, antigen-specific antibodies gradually accumulate within the circulation.
- Booster immunisations are often administered 2 - 3 weeks after the initial injection to increase both antibody titre and affinity.
- Blood collection is typically performed several weeks after immunisation once an adequate immune response has developed.
- Rabbits often generate useful antibody titres within 4 - 6 weeks, whereas larger animals may require longer immunisation schedules.
- Depending on the antigen and host species, useful polyclonal antisera can often be generated within 4 - 8 weeks.

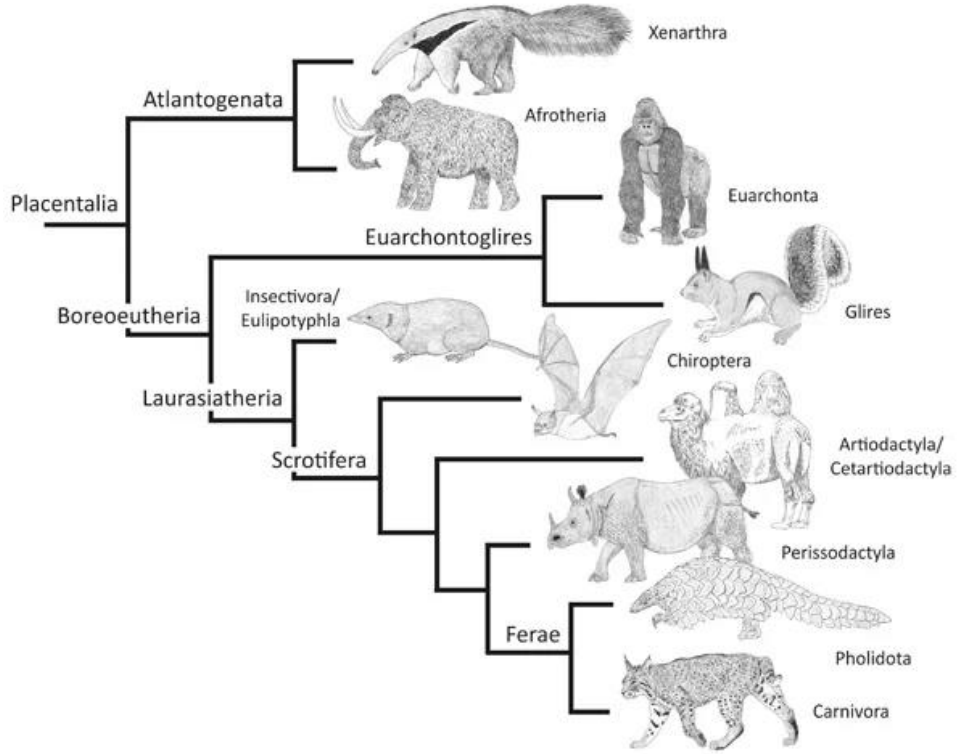
Host Species for Polyclonal Antibody Production

- The choice of animal influences both antibody yield and production cost.
- Rabbits are commonly used because they are inexpensive, easy to handle, and generate robust immune responses.
- Larger animals such as goats and sheep provide substantially greater serum volumes when larger quantities of antibody are required.
- Horses may be used when very large-scale antibody production is needed.



Additional Considerations When Selecting a Host Species

- Successful antibody generation depends not only on antibody yield but also on antigen recognition.
- Animals generally produce stronger responses against proteins that are evolutionarily distinct from their own.
- For example, generating high-affinity antibodies against a mouse protein in another mouse is often difficult because immune tolerance suppresses the response.
- The phylogenetic relationship between host species and antigen source should therefore be considered during experimental design.

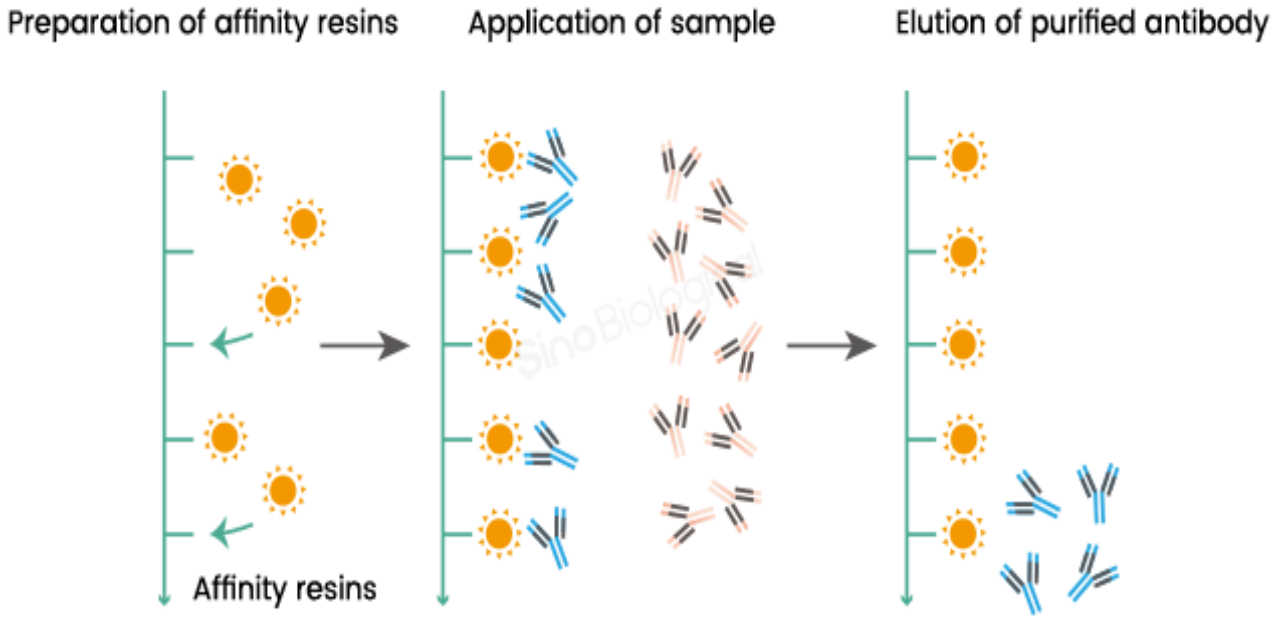


Selection of Antigen and Adjuvant

- The quality and purity of the antigen are critical for successful antibody production.
- Even trace quantities of contaminating proteins may stimulate unwanted antibody responses.
- Adjuvants are administered alongside antigen to enhance immune activation and increase antibody production.
- Freund's Complete Adjuvant is commonly used for primary immunisation, while Freund's Incomplete Adjuvant is often used for booster injections.
- Aluminium-based adjuvants are also widely used because of their established safety profile.

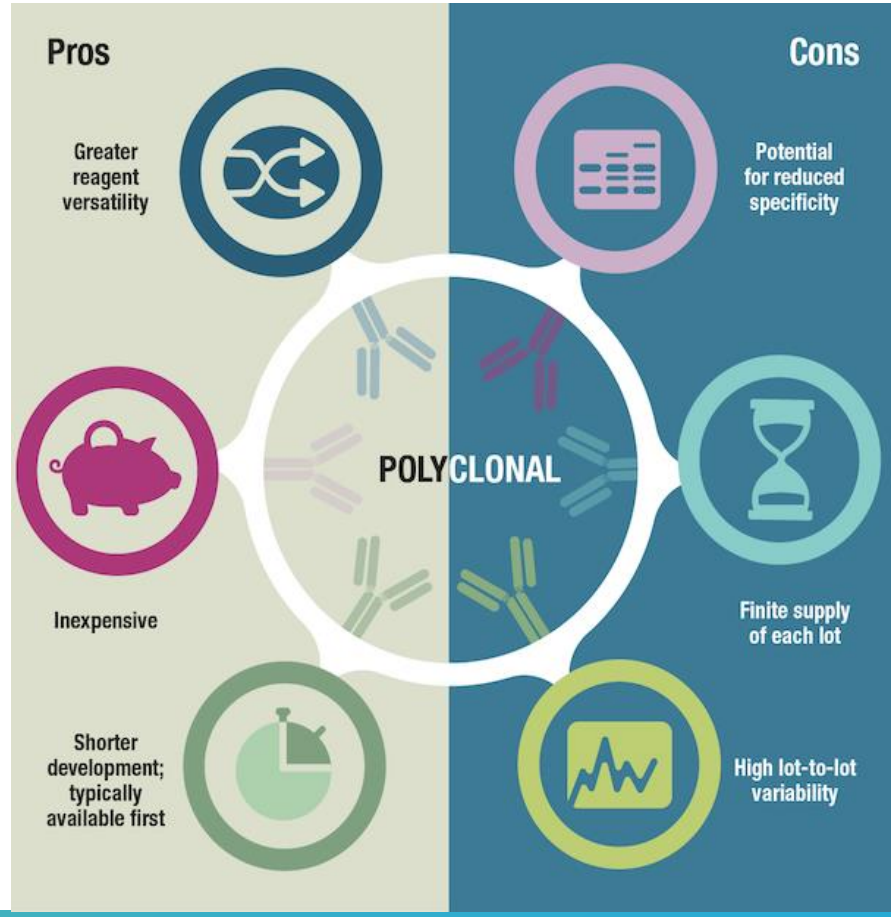
Affinity Purification of Polyclonal Antibodies

- Serum collected from immunised animals contains thousands of different antibody molecules.
- To improve specificity, the serum can be passed through a column containing immobilised target antigen.
- Antigen-specific antibodies bind to the column while unrelated antibodies are washed away.
- Altering buffer conditions subsequently releases the bound antibodies for collection.
- This process substantially reduces background binding in downstream assays.



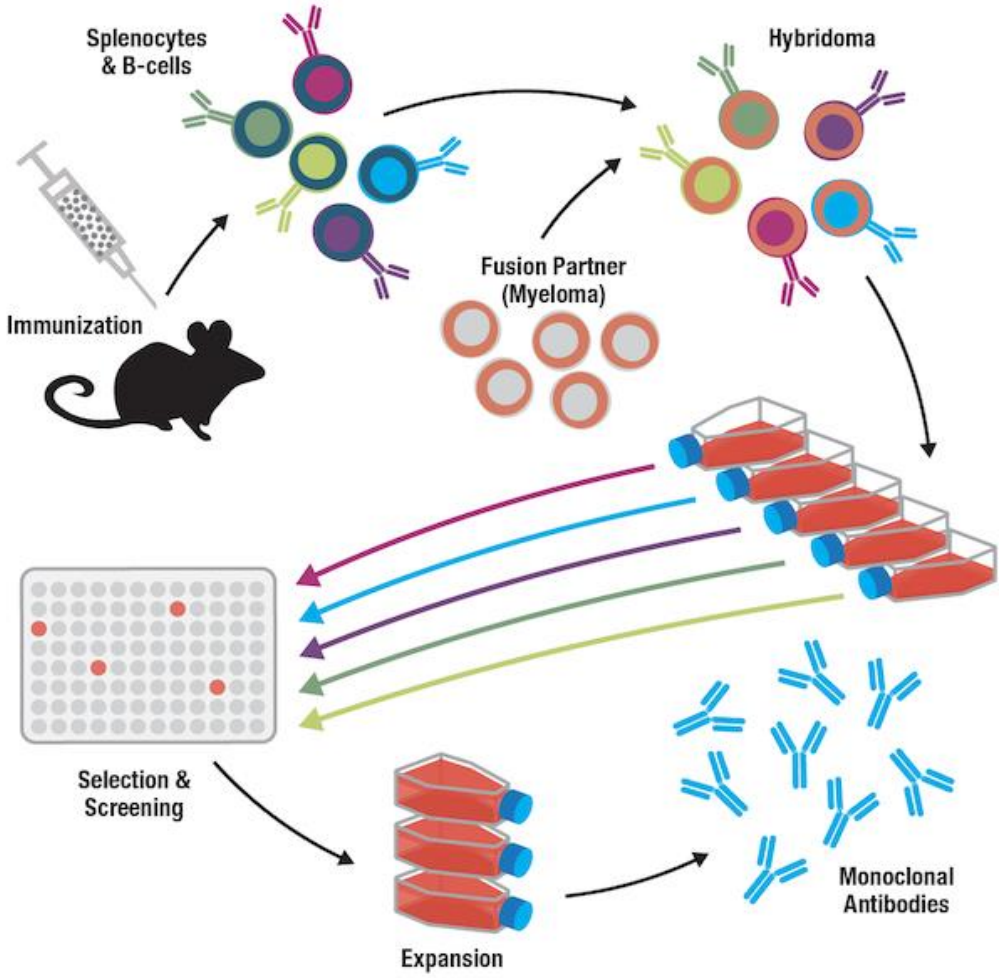
Advantages and Limitations of Polyclonal Antibodies

- Polyclonal antibodies can usually be produced rapidly and at relatively low cost.
- Because multiple antibody clones recognise the antigen, strong signal generation is often achieved.
- However, antibody composition varies between animals and between immunisations.
- Off-target binding is generally more common than with monoclonal antibodies.
- Polyclonal antibodies are unsuitable for therapeutic use because animal immunoglobulins are highly immunogenic in humans.



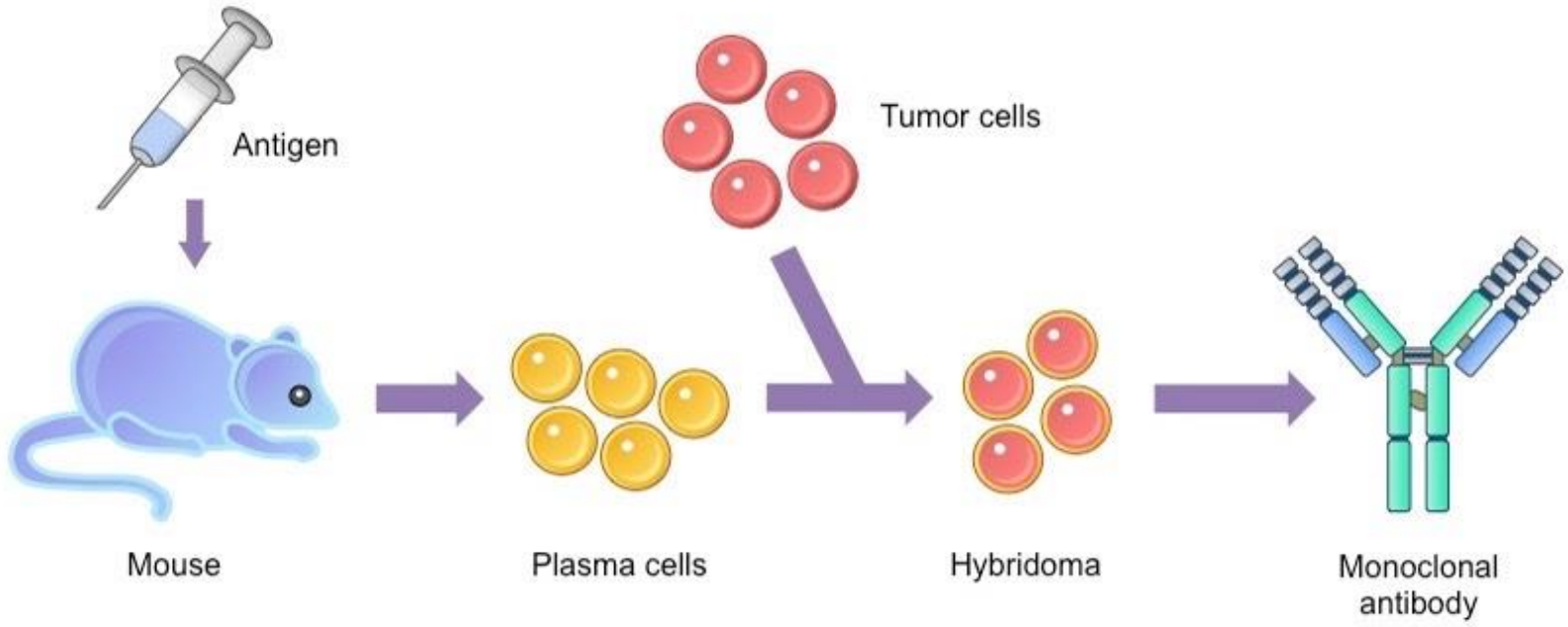
Production of Monoclonal Antibodies

- Monoclonal antibody production also begins with immunisation of an animal using the target antigen.
- Rather than collecting serum, antibody-producing B-cells are isolated from the spleen.
- Individual antibody-producing clones are subsequently identified and expanded.
- The resulting antibody preparation contains only a single antibody specificity.



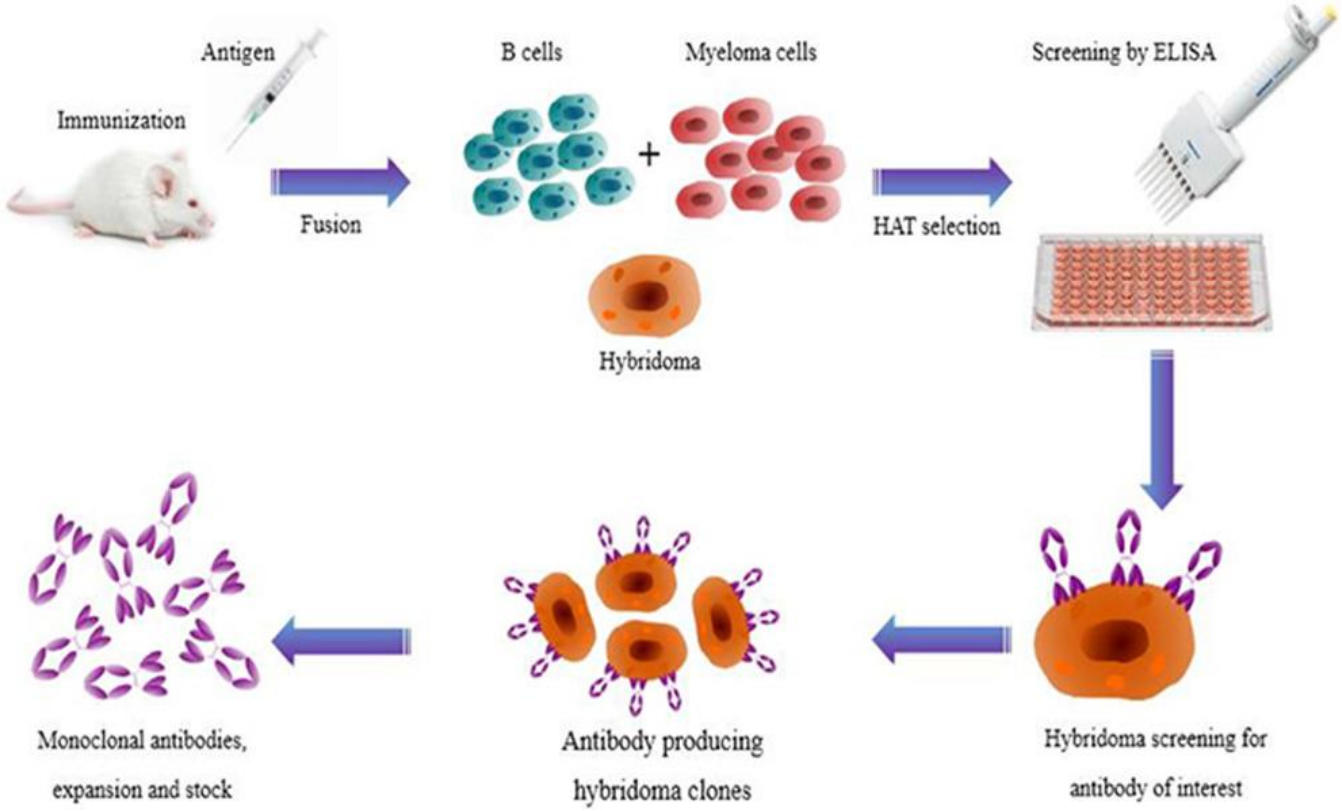
Generation of Hybridoma Cells

- Antibody-producing B-cells have limited lifespan outside the animal.
- To overcome this limitation, splenic B-cells are fused with immortal myeloma cells.
- The resulting hybridomas combine continuous growth with antibody production.
- Each hybridoma clone secretes only one antibody specificity.
- Hybridoma technology remains one of the most important developments in modern antibody production.



Screening Hybridoma Clones

- Hybridoma fusion generates thousands of independent clones.
- Cells are diluted and distributed into 96-well plates so that individual wells contain a single hybridoma cell.
- As colonies grow, culture supernatants are screened for antigen binding using assays such as ELISA.
- Positive clones are identified and selected for further expansion.
- Several rounds of screening may be required to identify the most suitable clone.

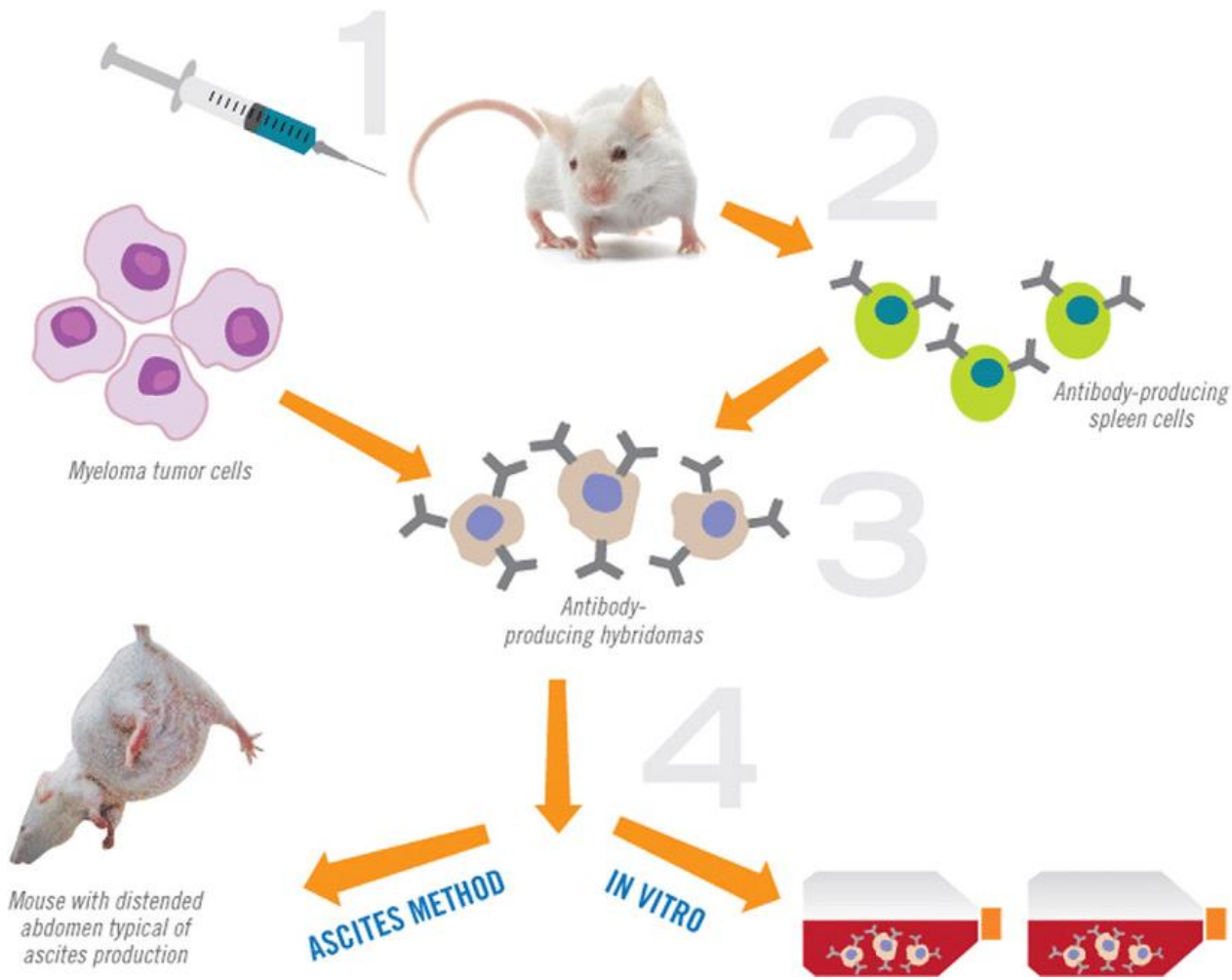


Expansion of Selected Hybridoma Cultures

- Hybridoma clones producing the desired antibody are expanded in tissue culture.
- Small-scale culture may be sufficient for many laboratory applications.
- Additional screening is often performed to confirm specificity and performance.
- Clones with the most desirable properties are selected for long-term propagation.

Ascites-Based Production of Monoclonal Antibodies

- When larger quantities of monoclonal antibody are required, hybridoma cells can be injected into the peritoneal cavity of a mouse.
- The hybridoma cells continue to proliferate and secrete antibody within the abdominal cavity.
- Antibody-rich fluid known as ascites gradually accumulates and causes swelling of the abdomen.
- The ascites fluid contains very high concentrations of monoclonal antibody and can be harvested repeatedly using a syringe.
- Historically this was a common method for large-scale monoclonal antibody production before advances in tissue culture technology.

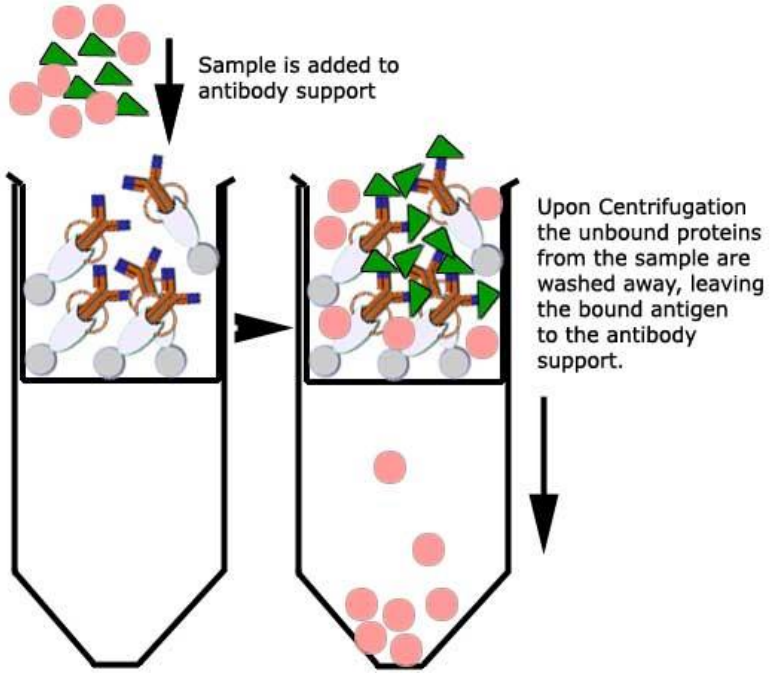


Why Ascites Production Has Declined

- Although highly effective, the ascites method raises significant animal welfare concerns.
- Mice may experience pain and distress due to abdominal tumour growth and the accumulation of large volumes of fluid.
- Repeated harvesting of ascites fluid by needle aspiration can further compromise animal welfare.
- Ascites fluid contains host-derived proteins, cytokines and other biologically active molecules that may contaminate antibody preparations.
- Production commonly requires several months and is considerably slower than polyclonal antibody production.
- For these reasons, most laboratories now favour in vitro cell culture systems and bioreactor-based antibody production.

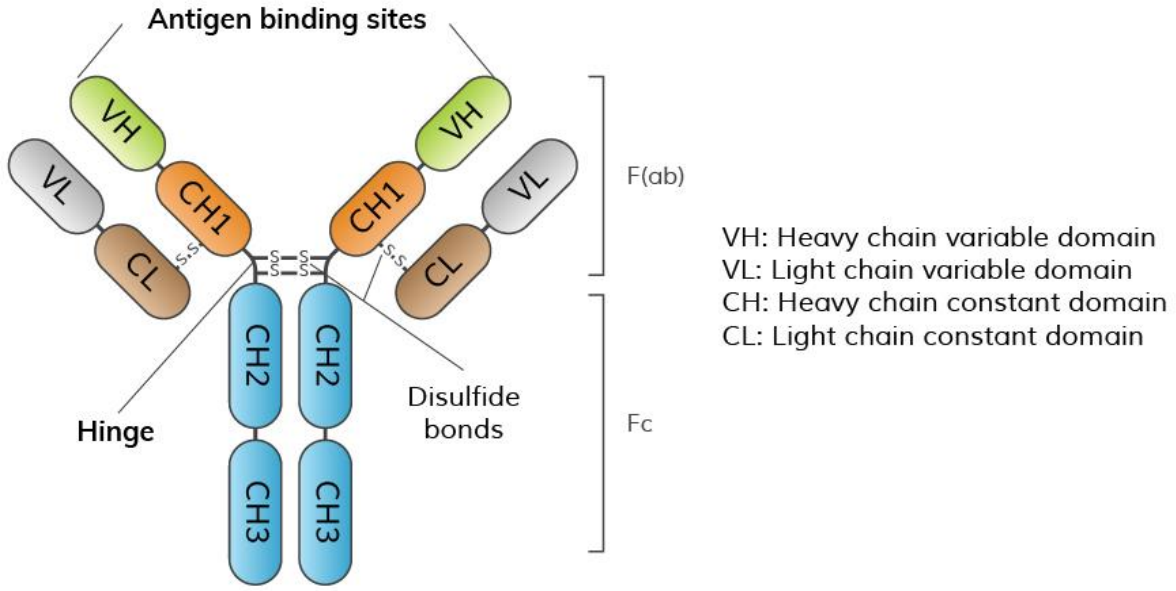
Purification of Monoclonal Antibodies

- Monoclonal antibodies can be purified from culture supernatant or ascitic fluid.
- Protein A and Protein G affinity chromatography are commonly used because they bind the Fc region of IgG molecules.
- Protein A binds strongly to many IgG subclasses and is widely used in commercial antibody purification workflows.
- Following purification, dialysis may be used to remove salts and exchange buffers.
- The resulting preparation contains highly enriched monoclonal antibody.



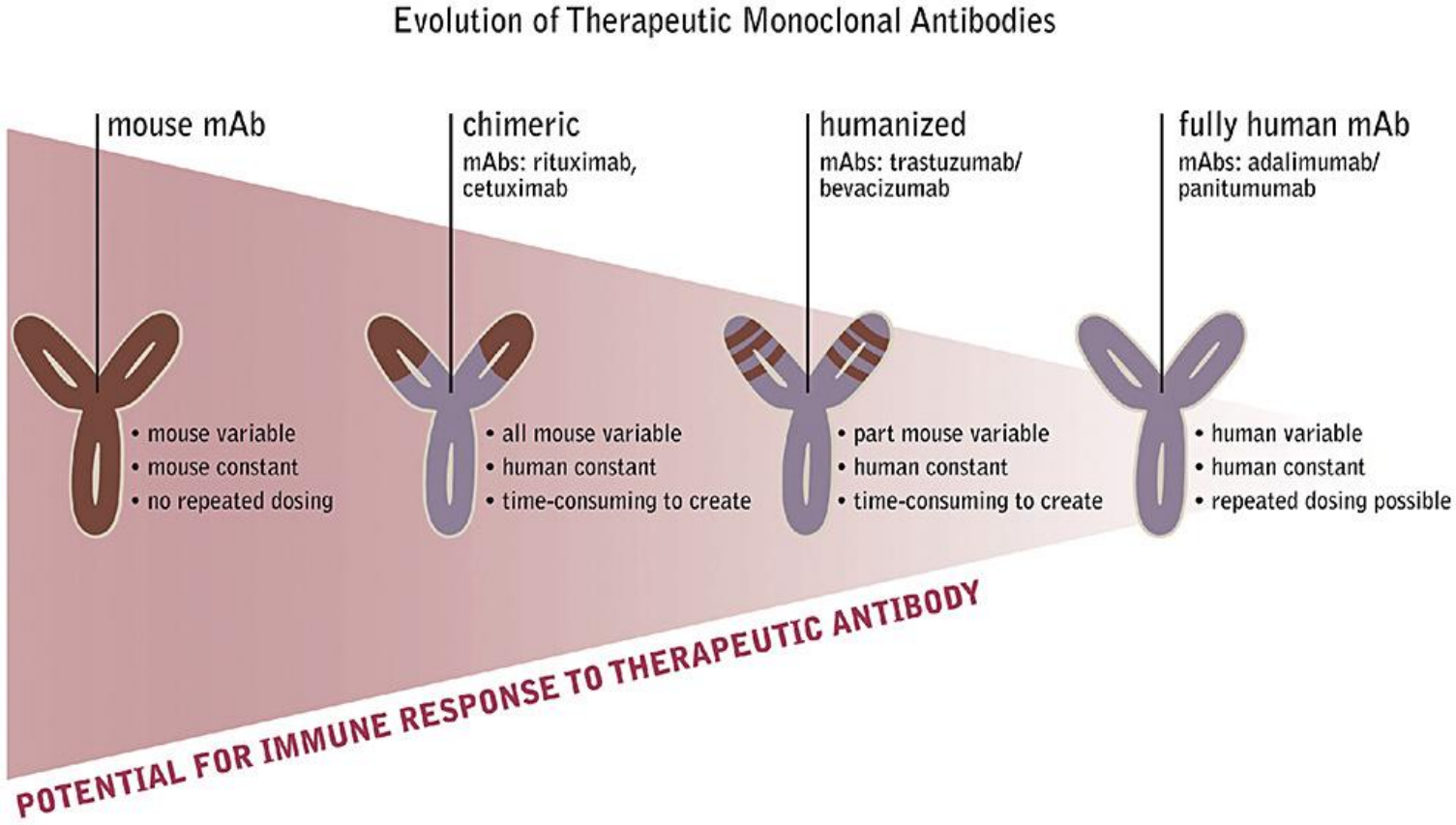
Immunogenicity of Murine Antibodies

- Early therapeutic monoclonal antibodies were produced directly from mouse hybridomas and initially showed promising clinical activity.
- However, many patients gradually developed Human Anti-Mouse Antibody (HAMA) responses against the therapeutic antibody.
- The amino acid sequence of mouse immunoglobulins differs sufficiently from human antibodies for the immune system to recognise them as foreign proteins.
- Patients therefore generated antibodies directed against the mouse antibody itself, particularly against regions outside the antigen-binding site.
- Once formed, these anti-mouse antibodies accelerated clearance of the therapeutic antibody and could neutralise its activity, causing treatment efficacy to decline.



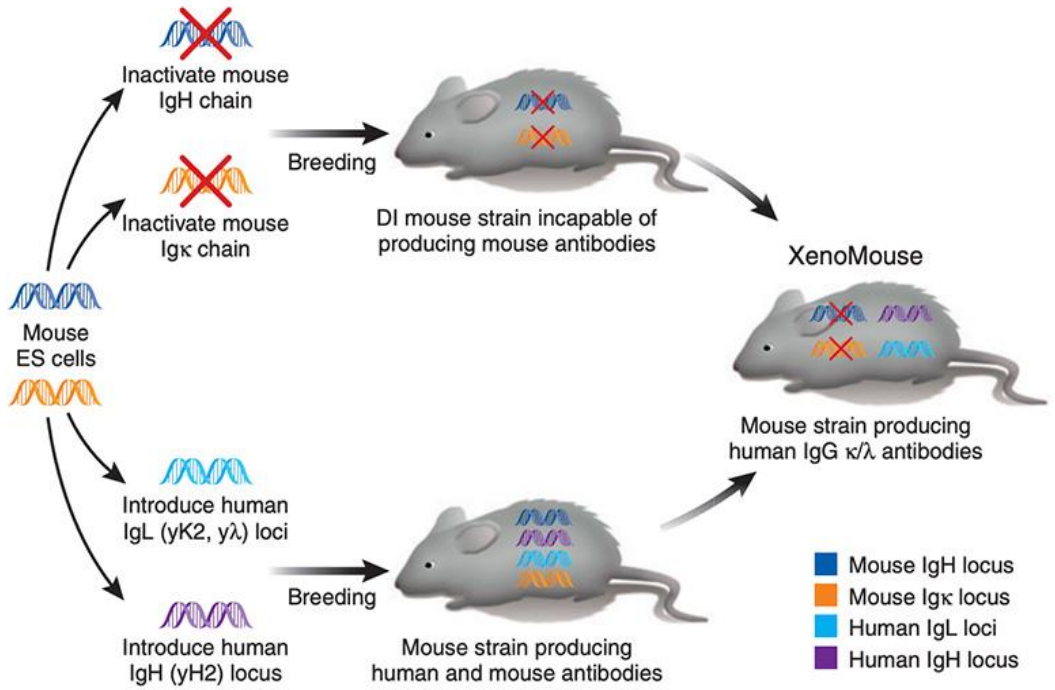
Antibody Humanisation

- Humanisation was developed to reduce the immunogenicity of therapeutic antibodies.
- Mouse framework sequences are replaced with human equivalents while retaining the antigen-binding complementarity determining regions (CDRs).
- This preserves target recognition while reducing immune rejection.
- Humanised antibodies such as trastuzumab and bevacizumab have become widely used in clinical medicine.



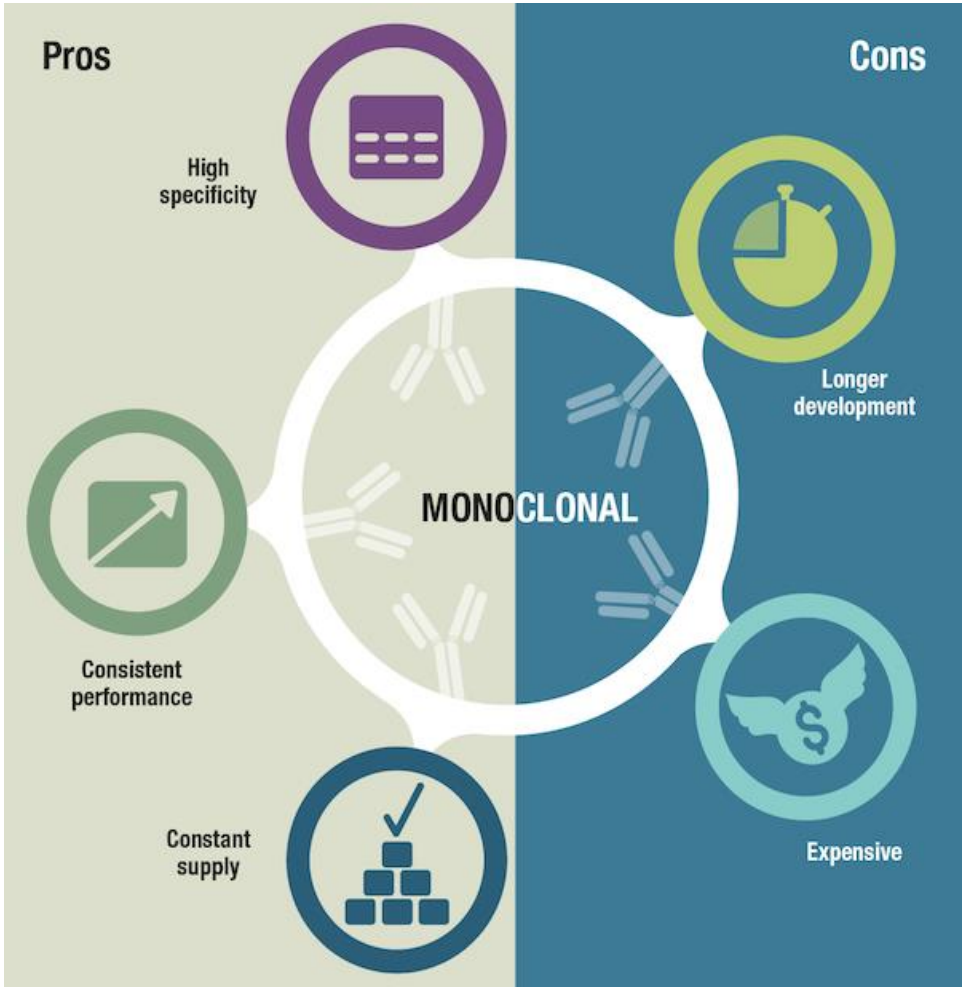
Generation of Fully Human Antibodies

- Humanisation significantly reduced immunogenicity, but traces of mouse sequence remain within the antibody.
- To overcome this limitation, transgenic mice carrying human immunoglobulin loci have been developed.
- Following immunisation, these animals produce antibodies with fully human variable and constant regions.
- Alternative approaches include phage display, where very large libraries of antibody fragments are screened in vitro against a target antigen.
- These technologies now underpin many modern therapeutic antibody discovery programmes.



Advantages and Limitations of Monoclonal Antibodies

- Monoclonal antibodies recognise a single epitope and therefore show much less off-target binding than many polyclonal preparations.
- Because every antibody molecule is identical, monoclonal reagents generally behave more consistently between experiments and between production batches.
- Antibody genes can be sequenced and expressed recombinantly, enabling large-scale manufacturing.
- However, monoclonal antibody development requires more time, expertise, and financial investment than polyclonal antibody production.
- The complete process may take several months from immunisation to final clone selection.



Summary

- Antibodies for research and clinical applications can be produced as either polyclonal antibodies or monoclonal antibodies.
- Polyclonal antibodies are generated by immunising an animal and collecting serum. They recognise multiple epitopes on the target antigen and can usually be produced within a few weeks.
- Monoclonal antibodies are generated from a single B-cell clone following hybridoma production. They recognise a single epitope and provide excellent specificity and batch-to-batch reproducibility.
- Polyclonal antibodies are generally faster and less expensive to produce, whereas monoclonal antibodies require more time, screening and cell culture but provide more consistent performance.
- Both antibody types can be purified using affinity chromatography and remain important tools in research, diagnostics and biotechnology.
- Therapeutic antibody development has largely focused on monoclonal antibodies, with humanised and fully human antibodies overcoming many of the limitations of early mouse-derived antibodies.

Quiz

- Why might a researcher choose a polyclonal antibody instead of a monoclonal antibody?
- Why are monoclonal antibodies generally preferred for therapeutic applications?
- What are the major ethical concerns associated with antibody production in animals?
- Why did early mouse-derived therapeutic antibodies often lose effectiveness in patients?
- How has antibody humanisation improved the clinical utility of monoclonal antibodies?
- What are the advantages and disadvantages of generating fully human antibodies using transgenic mice?
- If you were developing an antibody against a novel cancer target, would you choose a polyclonal or monoclonal approach?. Why?