Antibody production for diagnostic applications and therapeutic development: is it possible to reduce the use of animals?

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INTRODUCTION/OBJECTIVE

Traditionally, antibodies have been produced using the hybridoma technique, which involves immunization and the sacrifice of animals. Although this has revolutionized the practice of "tradicional" immunohistochemistry, it currently raises a number of ethical questions regarding animal suffering. Therefore, it's important to develop methods that are viable to obtain quality antibodies for human diagnosis and therapy, while also having a less significant impact on animal welfare. There are several promising methods, such as the use of mammalian cells, the expression of antibodies in phage display and even in yeasts. This review aims to explore alternative methods for antibody production, highlighting their advantages and limitations.

METHODOLOGY

Systematic review of scientific articles from the PubMed database, applying key terms combined with Boolean operators (AND/OR). Following the PRISMA methodology, 1004 articles were initially identified and underwent two rounds of screening based on predefined inclusion and exclusion criteria. Ultimately, the following were excluded: 12 articles due to restricted access, 1 duplicate, and 2 studies focusing on regions outside the European Union (China and India). Additional sources were consulted, as referenced in [5] [10] [11] [14] [15] [17] [18]. The selection process was independently reviewed by two researchers, resulting in the inclusion of 18 studies in this review.

Keywords: ("*antibody production*" *OR* "*antibodies*") *AND* ("*phage display*" *OR* "*yeast display*" *OR* "*mammalian display*") *AND* ("*in vitro*" *OR* "*non-animal*" *OR* "*animal-free*").

1004 articles → **31 articles after the 1st screening** → **11 articles after the 2nd screening** + **7 additional sources** → **18 articles included**

Inclusion criteria:
- **Publication date:** Less than 10 years *
- **Language:** English *
- **Type of study:** Reviews/systematic reviews *
- **Population:** Humans *
- **Antibody production in the European Union**

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Exclusion criteria:
- **Articles without free access** *
- **Studies conducted in countries outside the European Union**
- **Studies in exclusively all-animal models**
- **Duplicates**

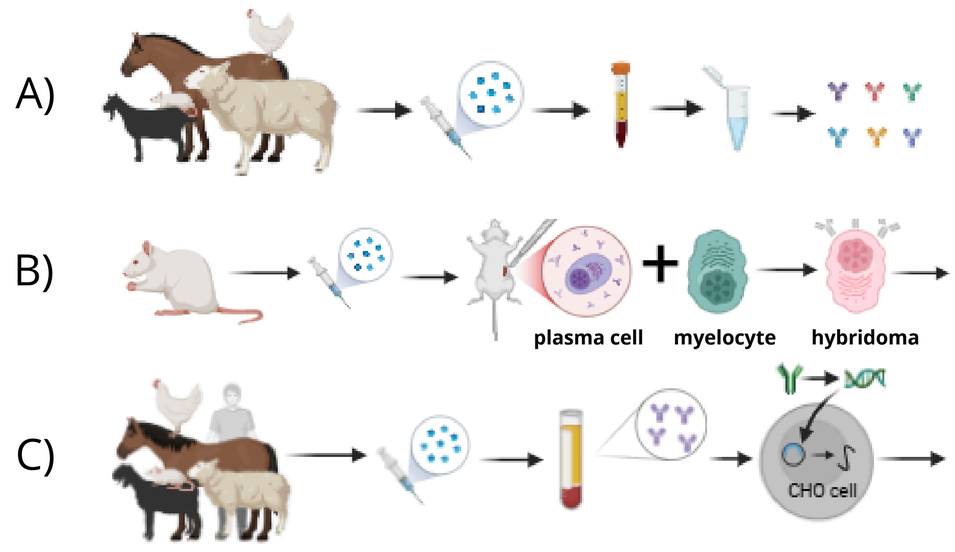

Figure 2 - Overview of antibody production methods
A) Immunization in animals, B) Hybridoma technology,[1] and C) Recombinant expression system.
Gene cloning involves inserting the gene that codes for the antibody into a plasmid, a circular DNA molecule. Promoters are then added. In addition, other genes, such as selective markers, can be inserted to identify cells that have taken up the plasmid. Created at https://BioRender.com

The study by Gray, et al. [11] determined that the main reasons for still using animals in antibody production are economic barriers and the preference for traditional methods. However, these methods offer common advantages, such as improved reproducibility, a renewable source of antibodies and more versatile reagents.[11] The different methods also offer specific advantages for the purpose of producing the antibodies (Table 1), showing themselves to be promising candidates for eliminating the use of animals in this area.

RESULTS/DISCUSSION

One of the methods reported by Sheehan and Marasco[2] is **Phage display (PD)**, a method of presenting polypeptides on the surface of filamentous phages,[3] such as the M13 phage, which only infects strains of Escherichia coli (E. coli), allowing the selection of antibodies with any specificity and format.[4] This enables the recombinant production of reagents with various health applications.

Another method is **Yeast display (YD)**, which allows several copies of the target protein to be displayed on the surface of yeasts, such as *Saccharomyces cerevisiae*, using plasmid vectors.[5] In this method, the gene of interest is fused to an anchor protein, which attaches the target to the cell wall. It is used to create libraries of protein variants [5] and allows screening using techniques such as flow cytometry cell sorting (FACS)[6] (Figure 1).

METHOD	ADVANTAGES	LIMITATIONS
PHAGE DISPLAY	• **Identification and fast isolation of high affinity and specificity antibodies;** • **Inexpensive process after implementation.** [2] [12]	• **Need for specialised technicians;** [13] • **Lack of glycosylation patterns in E. coli.** [7]
YEAST DISPLAY	• **Compatibility with flow cytometry technique;** [5] • **Defined glycosylation patterns.** [6]	• **Multivalent bonding;** [6] • **Library variability.** [6]
MAMMALIAN ANTIBODIES	• **Lower risk of endotoxin contamination, therefore safer for therapies;** [14] • **Generation of antibodies and antibody fragments with high specificity and functionality.** [14]	• **High cost of equipment and maintenance;** [11] • **Failure to produce specific antibodies in some cell lines.** [10] [18]

Table 1 - Advantages and limitations of the Phage Dispay, Yeast Display and Mammalian Antibody methods.
Currently, PD is more suitable for screening large antibody libraries (10^11),[6] while YD is more suitable for smaller libraries (10^7 - 10^9), but for the production of more complex and biologically active antibodies. Glycosylation in yeast plays an important role in producing antibodies with better binding affinity, biodistribution and pharmacokinetics, as it more closely resembles the human system.[7] Since mammalian antibodies are derived from animal cells, they allow antibodies to be functionally equivalent to natural ones, especially for therapeutic applications and high-precision.

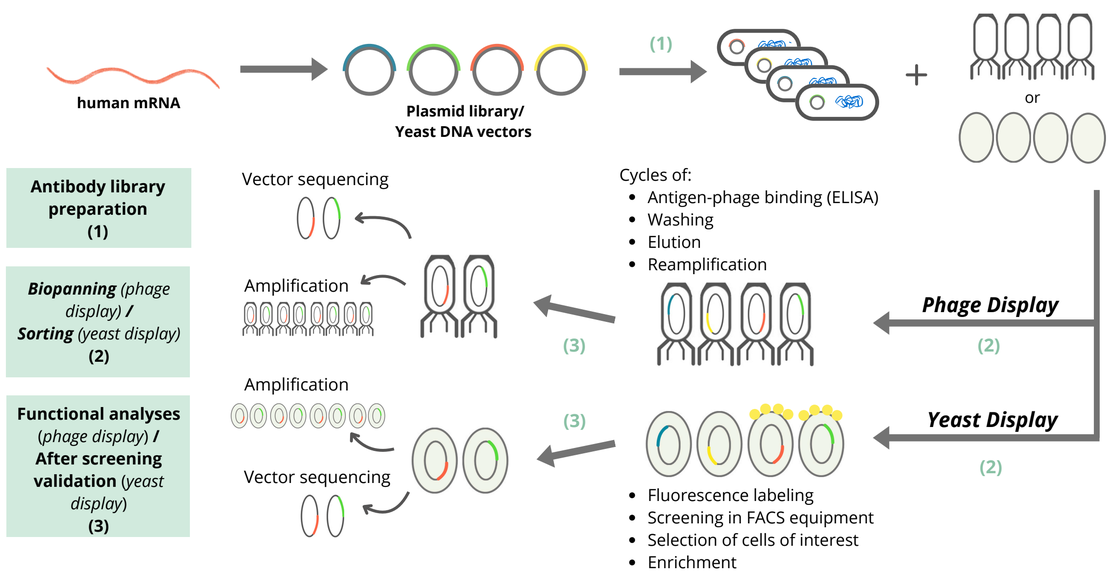

Figure 1 - Antibody production process using the Phage and Yeast Display methods.
Both methods can begin with a common sample, mRNA, which will be subjected to reverse transcription, amplification and, finally, binding to the surface of the competent phage or yeast, expressing the desired antibody **(1)**. Next, in the screening phase, we select antibodies based on their antigen-binding affinity (PD) or quantitative and qualitative characteristics (e.g. binding intensity or cell size) (YD). While Biopanning is based on antigen-antibody binding techniques. [7-9] Sorting uses a more complex technique, Flow Cytometry (FACS) (2).[6] Finally, in PD, the phages are validated by ELISA and the selected clones are amplified,[8] with their vector sequence.[7] In the YD, the screened proteins can be characterized directly in yeast by FACS (3)

Mammalian antibodies are immunoglobulins synthesized by mammalian plasma cells in response to antigens. According to Jina Ryu et al.,[10] these antibodies can also be produced in the laboratory using cultured mammalian cells, such as Chinese Hamster Ovary (CHO) cells, which closely resemble natural antibodies in both structure and function. This process involves the construction of an expression plasmid (Figure 2).

Following the initial isolation of the antibody gene or the generation of antibody libraries, the entire subsequent procedure occurs in vitro, eliminating the need for immunization or the continuous use of animals.

The methods are widely used to develop targeted therapies for autoimmune diseases and in the oncology field.[15] Phage display is currently used, for example, in the production of therapeutics for rheumatoid arthritis, such as Adalimumab (Humira).[16] Yeast display's greatest contribution is the optimisation of antibodies against biomarkers, a good example being PD-L1, a protein expressed by tumour cells. [17] In 2021, Mayrhofer et al.[18] reported that CHO cells make it possible to develop new anti-SARS-CoV-2 antibody formats.

CONCLUSION

These methods represent a significant advance in the practice of Immunohistochemistry, making it possible to obtain specific and functional antibodies and eradicating the future use of animals. These approaches offer several advantages; however, they face technical and economic challenges that limit their uptake. Continuous optimisation of these technologies is essential to promote an ethical and sustainable transition, making them viable substitutes for traditional methods.

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