

Opinion

Animal-Friendly Affinity Reagents: Replacing the Needless in the Haystack

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The multibillion-dollar global antibody industry produces an indispensable resource but that is generated using millions of animals. Despite the irrefutable maturation and availability of animal-friendly affinity reagents (AFAs) employing naïve B lymphocyte or synthetic recombinant technologies expressed by phage display, animal immunisation is still authorised for antibody production. Remarkably, replacement opportunities have been overlooked, despite the enormous potential reduction in animal use. Directive 2010/63/EU requires that animals are not used where alternatives exist. To ensure its implementation, we have engaged in discussions with the EU Reference Laboratory for alternatives to animal testing (EURL ECVAM) and the Directorate General for Environment to carve out an EU-led replacement strategy. Measures must be imposed to avoid outsourcing, regulate commercial production, and ensure that antibody producers are fully supported.

The Hidden Use of Animals in Consumer Society

A young woman visiting her local supermarket to do her weekly shopping knows that, in this conscientious-consumer-driven society, she will be overwhelmed with choices that allow her to make informed decisions about her own environmental footprint, maintain a healthy lifestyle, and patronise non-exploitative industries. The information that helps her do so is clearly visible on the packaging of the shampoo especially formulated for sensitive skin and the nutrient-supplemented, low-sugar, fair-trade cereal that she chooses as she browses the aisles. However, on this occasion the main reason for her visit is to pick up a pregnancy test that will help her plan her future. She knows that the three items she has just selected will all contribute to protecting her family and the environment they live in, but does she know that, despite the reassuring information on the packaging, she has just picked up three items that still use animals? How is that possible? The shampoo conforms to EU cosmetics regulation 1223/2009 by not being tested on animals, the cereal is animal-product free, and the pregnancy test is a simple over-the-counter diagnostic test designed for home use.

What the information on the packaging does not tell her about is the hidden use of animals in an affluent, US\$80-billion industry that creates millions of animal-derived **monoclonal** and **polyclonal antibodies** (see [Glossary](#)) to detect a vast range of molecules indicative of state of health, safety, or the environment. Antibodies are used, for example, as an indispensable link in the health-care chain, contributing to enhanced patient care and reduced public health costs. Antibody-based tests are used to diagnose and monitor infectious or chronic diseases, to manage oncology treatment, or, in rapid-test format, often available over the counter, to monitor

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Millions of animals are used for the routine production of antibodies for research, monitoring, diagnostics, and therapeutics, although the true magnitude of use is unknown.

The EU Directive on the protection of animals used for scientific purposes (2010/63/EU) requires that animals are not be used for scientific purposes where a non-animal alternative exists.

Animal-friendly affinity reagents (AFAs) from non-immunised sources and produced by phage display are mature and available to replace animal immunisation methods for antibody production.

In line with Directive 2010/63/EU, an EU-wide replacement programme must now take precedence. In this Opinion article, we outline recommended actions that must be prioritised.

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fertility, ovulation and pregnancy, sexually transmitted diseases, substances of abuse, and performance-enhancing drugs.

Also, as a vital tool to safeguard the environment and consumer industry, antibodies may be used to detect or extract the alarming number of potential chemical contaminants, allergens, microbiologicals, natural toxins, food constituents, pharmaceutical and veterinary drugs, hormones, metals, agricultural contaminants, and pesticides that can exist in our food, beverages, and water. They can predict the safe limit for a potentially harmful ingredient in a cosmetic or household product or control the quality of biological agents such as vaccines or botulinum toxin.

The tests that are employed in these examples appear to be far removed from animal experimentation since no animals were directly tested on. However, the molecule to be detected is repeatedly injected into the animal, initiating a hyperimmune response. Months later, antibodies are extracted and incorporated into an *in vitro*, animal-free test for the detection of the molecule. So in reality we are not replacing animals but simply substituting methods in which the animal use is direct for other methods where the animal use is buried several layers deep in the production process, and our ultimate aim, to replace needless animal use, is not achieved.

Lack of Clarity about the Impact of Antibody Production on Animal Welfare

Also overlooked are the sheer numbers of animals that are sacrificed to produce antibodies and the associated and unaccounted-for animal-welfare issues [1,2].

Measures to improve animal-welfare standards by reducing or refining animal-derived antibody production methods were initiated in 1998 following the publication of a statement by the European Centre for the Validation of Alternative Methods (ECVAM) scientific advisory committee. This statement recommended that, following the immunisation protocol, the propagation of monoclonal antibodies in the ascites of the abdominal cavity was no longer scientifically necessary. Accordingly, competent authorities in European Member States responsible for the implementation of EU legislation regarding the welfare of animals used for scientific purposes should now no longer authorise project license applications proposing antibody propagation by the **ascites method**. Recommendations for refining methods for producing polyclonal antibodies were also published.

Remarkably, workshop reports that were distributed to all EU Member State competent authorities highlighted advances in novel recombinant DNA-based technologies and direct cloning into plasmids. These reports envisaged that, 'in the near future', **phage display**, an advanced technology capable of producing binders 'without prior immunisation of B cell donors (would) avoid the need to use living animals' [3–5]. So looking back, this discrepancy with reality is not easy to understand. Eighteen years later, immunised animals are still heavily relied on for **hybridoma** (monoclonal—with subsequent amplification *in vitro*), polyclonal, and even some **recombinant antibody** production methods. There are ~123 EU companies offering ~714 000 catalogue antibodies produced either in-house or through international collaborations (more than 2.5 billion worldwide). Furthermore, undetermined numbers of antibodies are generated through custom-made production by companies and research institutes. Demand is set to increase because the number of antibodies that could be generated in the future, including variants, in all different immunoassays, appears to be limitless. The human genome has 20 000–25 000 protein-encoding genes. Due to alternative mRNA splicing and post-translational modifications, the number of proteins and the potential for the generation of antibodies exceed this number tenfold. It is not uncommon to see hundreds of antibodies generated for the same target (e.g., more than 900 for P53 [6,7]). Larger numbers of animals are required to increase the chances of success if the desired antibody is expected to distinguish

Glossary

Affinity reagents: antibodies, peptides, nucleic acids, and other small molecules that bind specifically to a molecule of interest to detect, capture, or modify its mode of action.

Animal-friendly affinity reagents (AFAs): binders that are not derived from animal immunisation and do not require the use of animals at any stage of production.

Ascites method: hybridoma cells are injected into the peritoneal cavity surrounding the gut, initiating the development of tumours and the secretion of an antibody-rich fluid called ascites fluid.

B lymphocytes: cells of the adaptive immune system that bind to a foreign body or antigen and secrete highly specific antibodies to destroy or block activity. Adaptive immunity creates immunological memory after an initial encounter and leads to an enhanced response to subsequent encounters.

Combinatorial diversification [V(D)J recombination]: the unique mechanism of genetic recombination that occurs only in developing lymphocytes during the early stages of T and B lymphocyte maturation.

Hybridoma: B lymphocyte cells that bind specifically to the antigen are harvested from the spleen of immunised animals. These isolated B lymphocytes are then fused with immortal B cell cancer cells.

Human antimouse antibody (HAMA): immune response to mouse antibodies.

Monoclonal antibodies: secreted by one B cell lineage or clone. These identical, monospecific antibodies are secreted from the selected hybridoma cell.

Panning: an affinity selection technique that selects peptides or proteins binding to a specific target.

Phage display: a gene encoding a protein of interest (in this case, an antibody fragment) is ligated to the gene encoding either the minor or major coat protein of the (bacterio) phage, causing it to be 'displayed' on the outside, fused to the coat protein, where it is accessible for subsequent selection procedures, while retaining the gene for the protein on the inside.

Polyclonal antibodies: secreted by different B cell lineages. For research purposes, these antibodies are collected from the serum of immunised animals.

closely related isoforms within or across species or other subtle changes (e.g., phosphorylated versus non-phosphorylated targets).

Yet the actual number of animals used to generate these antibodies is not known. According to the latest EU statistics on the number of animals used for scientific purposes [8], animal **procedures** (see [Glossary](#)) for antibody production are immersed into 3 different categories, that includes all animal procedures for 'production and quality control of products and devices for human/veterinary medicine and dentistry' (categories 2.4 and 2.5) and 'other' (category 2.9). While the combined total number of animals used in these three categories is 2.7 million, or 23.18% of total animal use, it is not possible to determine what proportion of this animal use is attributed to the production of antibodies alone. Only two of the EU Member State countries publish this information in their own national statistics. In 2013, the UK reported the use of 9522 animals to produce antibodies (1433 monoclonal and 8089 polyclonal) [9]—0.25% of the combined total use of animals in categories 2.4, 2.5, and 2.9 (triple the number used in European-wide cosmetics testing, before a ban on animal use was imposed). In the same year, The Netherlands used more than double the number of animals to produce antibodies, with 25 697 animals [10] or 34% of the combined total use of animals in these three categories. These statistics give no insight into the full impact on animals because antibodies are also outsourced internationally, in countries where animal welfare is less well regulated, and imported into the EU.

Protecting the Welfare of Animals Used for Scientific Purposes, Including Antibody Production, as Defined by Directive 2010/63/EU

Directive 2010/63/EU legislates on the protection of animals used for scientific purposes, supporting **replacement, reduction, and refinement (the 3Rs)** in strict hierarchy [11]. The 3Rs are the guiding principles underpinning the humane use of animals in scientific research and are embedded in national and international legislation regulating the use of animals in scientific procedures. The Directive seeks to facilitate and promote the advancement of non-animal alternative approaches; namely, techniques that could provide the same or higher levels of information as obtained from animal procedures. Regarding the 3Rs principle of replacement (article 4) and choice of method (article 13), the Directive asserts that 'wherever possible, a scientifically satisfactory method or testing strategy, not entailing the use of live animals, shall be used instead of a procedure' and that an animal procedure should not be 'carried out if another method or testing strategy for obtaining the result sought, not entailing the use of a live animal, is recognised under the legislation of the Union'. It requires that competent authorities, appointed in each Member State, evaluate and authorise projects, including generic projects, when conducted using established methods for testing, diagnostics, or production, such as the large-scale commercial production of antibodies. The Directive also stipulates that 'The Commission should also conduct periodic thematic reviews concerning the replacement, reduction and refinement of the use of animals in procedures'.

Launched as part of the Commission's commitment to alternative approaches to the use of animals, the duties of the EURL ECVAM are defined in annex VII of the Directive and include 'coordinating and promoting the development and use of alternatives to procedures including in the areas of basic and applied research and regulatory testing; coordinating the validation of alternative approaches at Union level; acting as a focal point for the exchange of information on the development of alternative approaches; setting up, maintaining and managing public databases and information systems on alternative approaches and their state of development; and promoting dialogue between legislators, regulators, and all relevant stakeholders, in particular, industry, biomedical scientists, consumer organisations and animal-welfare groups, with a view to the development, validation, regulatory acceptance, international recognition, and application of alternative approaches'.

Procedure: 'any use, invasive or non-invasive, of an animal for experimental or other scientific purposes, with known or unknown outcome, or educational purposes, which may cause the animal a level of pain, suffering, distress or lasting harm equivalent to, or higher than, that caused by the introduction of a needle in accordance with good veterinary practice'.

Recombinant antibodies: generated *in vitro* using synthetic gene manipulation techniques.

Replacement, reduction, and refinement (3Rs): replacement—alternative methods that avoid or replace the use of animals; reduction—experimental design approaches that minimise the number of animals used per experiment; refinement—focuses on the breeding, accommodation, care, and use of animals in procedures and minimising any pain, suffering, distress, or lasting harm.

Somatic hypermutation: a mechanism that diversifies B lymphocyte receptors used to recognise foreign elements (antigens) during affinity maturation and allows the immune system to adapt its response to new threats.

Finding the Needle in the Haystack *in vitro* by Adopting the Same *in vivo* Mechanism

Technology has evolved over the past 20 years and antibody production by animal-friendly methods has grown to a level of scientific sophistication that outweighs obsolescent animal immunisation protocols.

Increasing numbers of different animal friendly affinity reagents (AFAs) have been developed or are commercialised. They are generated independently of any living immune system and are available to replace animal immunisation techniques for a wide range of applications. AFAs are selected *in vitro* by phage, ribosome, or yeast display. These are typically antibodies, but also include non-antibody **affinity reagents** such as DARPs, affibodies, monobodies, anticalins, and others [6,12]. In this Opinion article, we particularly focus on recombinant antibodies produced by phage display because they are the most technologically mature and compatible with the usual scientific applications.

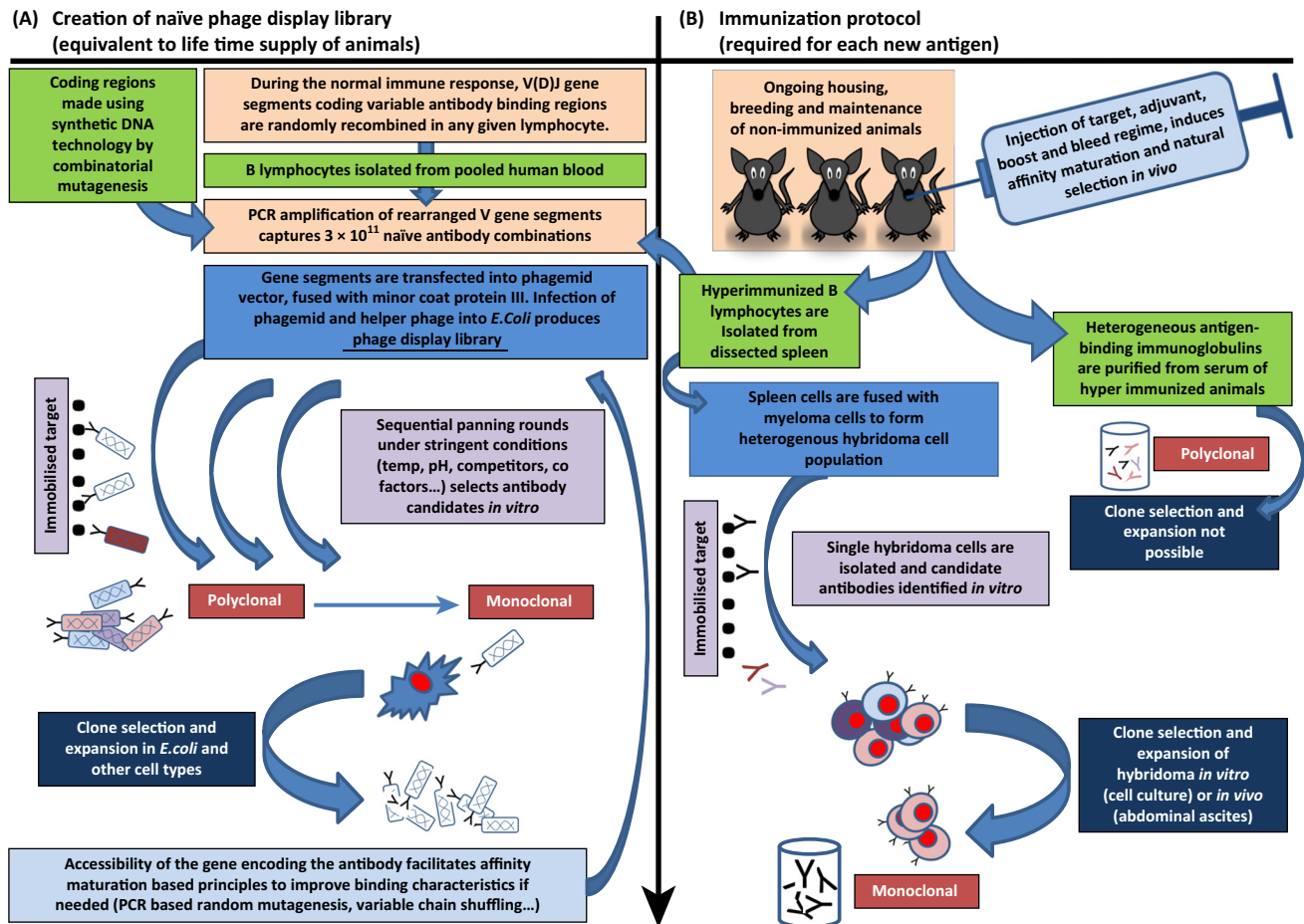
Rather than being manufactured to 'look' or 'act' like antibodies, antibodies produced by phage display are the genuine article, adopting the same biological mechanisms that are employed by the *in vivo* adaptive immune system and that are also commercially exploited to produce animal-derived antibodies (Figure 1). The resulting phage display antibodies are thus developmentally, functionally, and structurally indistinguishable from those produced *in vivo*. This supports the requirement within Directive 2010/63/EU for a non-animal alternative to provide the same or higher level of information as the animal procedure, justifying its replacement.

A New Mindset for Project Application, Evaluation, and Authorisation

Misconceptions regarding practical measures such as cost, intellectual property stipulations, implementation, relevance, scientific applicability, and being limited to pharmaceutical applications obviously still broadly exist, along with the assumption that these misconceptions apply to all antibodies and other AFAs produced by phage display rather than being rare and exceptional cases. Consequently, project applications for antibody production in animals, from smaller academic to large-scale (5-year duration) commercial projects, are still authorised by Member State competent authorities. These misconceptions are addressed in Table 1.

This notion should now be overturned and project applications should be authorised only in rare and exceptional cases of single antibody production, to be determined only on a case-by-case basis. Practical competence may be acquired by gaining access to the abundance of publications, including full methodology breakdowns, that exists to assist researchers and technical staff wishing to establish the new technology in their facility. In the hands of competent personnel, the design of a good phage display library and routine **panning** and selection rounds will be adequate to produce antibodies of affinity at least equivalent to those produced using immunised animals for the vast majority of antigens. Otherwise, there are many *in vitro* techniques available to improve antibody binding affinity, specificity, and other characteristics in cases where routine panning and selection does not produce the desired result and further intervention is required, without resorting to animals. Acquisition of genetic material from the **B lymphocytes** of immunised donors (human or animal depending on the intended target) enriches the antibody population for certain antigens. However, this produces a non-renewable library unsuitable for the selection of multiple antibodies to unrelated targets and should be proposed only in exceptional cases where *in vitro* affinity maturation techniques have not produced a suitable candidate.

Interestingly, several large-scale comparisons have been completed or are ongoing in academic programmes aiming to generate binders to many antigens; for example, the Structural Genomics Consortium SH2 Pilot, the EU programme Affinity Proteome and AFFINOMICS, and the



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Figure 1. Schematic Showing How the Biological Mechanisms Employed by the *in vivo* Adaptive Immune System Are Adopted to Produce Phage Display or Animal-Derived Antibodies. Same-colour boxes highlight areas of similarity [7,12,18–26]. Colour-matched boxes depict similar processing stages in (A) and (B). The three immunoglobulin loci (IgG heavy and light kappa and lambda chains) contain multiple copies of three V(D)J gene segments encoding the variable (binding) regions of the antibody (pink). In a process called **combinatorial diversification**, evolved by nature and unique to antibody genes, one copy of each type of gene segment is randomly recombined in any given lymphocyte (green). This allows a small number of genes to produce huge molecular diversity (roughly 3×10^{11} combinations) at the antibody-binding site [18]. This combinatorial mechanism is exploited in antibody production. Whereas animal immunisation and myeloma cell fusion are required to produce an antibody-expressing immortal hybridoma for each new target, the recombined antibody-encoding gene segments from human B lymphocytes can simply be copied and transferred into a non-immunised phage display library [12,19] to achieve vast molecular diversity *in vitro* (blue). In a highly efficient selection process analogous to finding a needle in a haystack and resembling that which takes place in nature, a phage antibody is selected during consecutive ‘panning’ rounds (purple) and expanded (dark blue) [7,19,20]. During the course of a host’s immune response, **somatic hypermutation** in the variable, antigen-binding coding sequences followed by competitive clonal selection ensures that the antibody affinity continues to improve. Similar affinity maturation based improvements are easily adopted post selection, if required, because the gene encoding the antibody is accessible inside the phage. This generates diversity beyond that achievable by the natural immune response, in consequence of accessibility to a much larger combinatorial space and facilitating raising antibodies against basically any structure (light blue) [21–25].

large, ongoing NIH Common Fund Protein Capture Initiatives. All of these large programmes explicitly support animal-free binder generation methods and the results clearly demonstrate that phage display-derived affinity reagents of various types not only are equivalent to hybridoma antibodies, but have bypassed hybridoma technology [12,13].

These programmes broadly demonstrate that this method has been standardised to an extent that yields consistently high-quality products and facilitates ease of understanding of the technology and the adoption of the method by the wider scientific community. In addition, there are many other academic and commercial groups that have published step-by-step

Table 1. A Comparative Overview of the Technical Considerations in Phage Display and Animal Immunisation-Derived Antibody Production^b

Technical Factor	Recombinant Antibody Expression From Naïve Phage Display Libraries	Monoclonal Antibody Production	Polyclonal Antibody Production	Refs
<i>Reliance on Animals</i>	None. Not reliant on <i>in vivo</i> immune response. One-off development of library, renewable resource, equivalent to lifetime supply of animals.	Derived from animal immunisation: new animals required for each new target.	Derived from animal immunisation; new animals required for each new target, higher volumes, new batches.	[7,12,25–27]
<i>Facilities Required</i>	Cell (mammalian and bacterial) culture facilities, standard equipment for molecular biology and protein analysis.	Animal housing facilities upkeep, immunisation and animal care, cell (mammalian) culture facilities, standard equipment for protein analysis.		[12]
<i>Time Required</i>	Six months or less for one-off development of library. Weeks for selection and clonal expansion of antibody candidates, from existing libraries.	Typically 6–8 months (protein antigen) or 12–15 months (smaller, less immunogenic molecules) for development of immune response, fusion, and characterisation.	Typically 2–4 months for development of immune response, harvest, and purification.	^a
<i>Reliability of Candidate Selection</i>	High. Acquisition of rare specificities to targets away from the influence of the natural immune response (e.g., against conserved epitopes, toxins, pathogens, non-immunogenics, closely related epitopes, cross-reactivities).	Varied. Natural immunity may limit response to certain targets including toxins, pathogens, non-immunogenic molecules, and closely related epitopes. May require conjugation to carrier protein and careful identification strategy. Antibodies vulnerable to negative selection.		[12,25,27]
<i>Antibody Specificity</i>	Excellent. Achieved by exposure of antibody candidates to multiple solution targets of interest for reduced cross-reactivity or DNA directly and readily accessible for post-selection affinity maturation-based techniques.	Varied. Dependent on immunisation response and efficiency of clonal selection procedure.	Varied. Dependent on immunisation response.	[12,17,25,27,28]
<i>Affinity</i>	nM to μ M: optimised by design of biological milieu and can be improved to pM or fM level by affinity maturation-based techniques.	nM to μ M: limited by immune response and affinity ceiling effect of the B cell response.	Undefined: due to multiple epitope recognition and limited by immune response.	[12,25,27]
<i>Validation Issues</i>	Easy access to sequencing data for high-level scientific validation. Immortal expression of antibody to target of interest	Sequencing data rarely acquired. Hybridoma produces indefinite supply of selected antibody but random mutation/genetic drift	Nonspecific binders and batch-to-batch variation (even in the same animal) meaning that they may not recognise the same target and	[7,17,29,30]

Table 1. (continued)

Technical Factor	Recombinant Antibody Expression From Naïve Phage Display Libraries	Monoclonal Antibody Production	Polyclonal Antibody Production	Refs
	achievable by expression in cell line. Resulting excellent lot-to-lot consistency means revalidation not required.	can occur. May exhibit poor binding characteristics or specificity.	each batch must be revalidated. May exhibit poor binding characteristics or specificity.	
<i>Formats</i>	ScFv or Fab fragment compatible with bacterial expression systems. Post-selection engineering produces various formats including whole-antibody monoclonal isotypes (IgG, IgA, IgE), ScFv-Fc, and polyclonal compatible with all standard laboratory applications.	Various formats available reliant on the output generated by the immune response and success of the purification process.	No control over format.	[7,12,31]
<i>Quantity of Antigen Required</i>	1–100 µg (selection by ELISA).	Typically 4 mg for five mice (immunisation and selection by ELISA).	Typically 1–2.5 mg for two rabbits or 4 mg per goat (immunisation and selection by ELISA)	[12] ^a
<i>Antibody Generation Pipeline</i>	Amenable to automation and high throughput, multiple target selection, and miniaturisation.	High throughput possible for selection of candidates only after lengthy immunisation process.	Not amenable to high throughput.	[7,12,25,27]
<i>Therapeutic Applications</i>	100% humanisation since derived from human B lymphocytes or synthetic. Immunogenicity lower, making these more likely candidates for therapeutic applications.	Up to 95% humanisation possible. High immunogenicity against therapeutic candidates [human antimouse antibody (HAMA)] requiring resolution by humanisation or deimmunisation strategies.	Not applicable.	[12,26,28]
<i>Expertise</i>	Requires knowledge of molecular biology techniques (PCR, cloning, vector design, sequencing, immunodetection) and competence in a range of mammalian/bacterial cell culture techniques.	Requires competence/training in animal care and handling, extensive knowledge of immunisation strategies and immunodetection, and competence in a range of cell culture techniques.		[12]

^a<http://www.thermofisher.com/uk/en/home/life-science/antibodies/custom-antibodies/custom-antibody-production.html>

^bAbbreviations: ScFv, single-chain fragment variable; Fab, - fragment antigen- binding; Fc, fragment crystallisable.

methodologies, available at online bookstores and libraries (for example, [7,14–16]). Commercial entities like AbD, and Yumab and the Recombinant Antibody Network universities consortium have picked up the technology to make animal-free antibodies commercially available to everybody. Therefore, individuals not wanting to produce antibodies themselves can rest

assured that phage display-produced antibodies and custom services are commercially available, intended for use in the pharmaceutical, *in vitro* diagnostics (IVD), and life science sectors.

Call for the Replacement of Animal Immunisation Techniques

The existence of AFAs has reduced the reliance on animal use to a very minor extent. However, these mature methods currently coexist alongside animal-derived antibody production techniques and the technology remains unadopted by the wider scientific community. It is thus time to break down the barriers built on foundations of misconception. In light of the emergence and maturation of AFAs and the implementation of the 3Rs through a strict hierarchy imposed by Directive 2010/63/EU, it is indisputable that an EU-wide replacement programme must now take precedence.

To facilitate this replacement, in recognition of the EURL ECVAM's responsibilities as outlined in Directive 2010/63/EU to promote the scientific and regulatory acceptance of alternative methods that are of importance to basic and applied research and regulatory testing, we have engaged in discussions with the EURL ECVAM and Directorate General for Environment with a view to carving out a strategy for the replacement of animal-derived antibody production methods. We have focussed on the phage display antibody production methodology as a scientifically applicable and practically available alternative that provides a replacement method as accessible as the validated and accepted alternative methods featuring in Organisation for Economic Co-operation and Development (OECD) regulatory testing guidelines. However, in order that the full range of options can be reviewed, we encourage other AFA competent experts to engage with us.

We recommend that the following actions are prioritised.

- (i) The replacement of animal immunisation methods for antibody production (including the generation of polyclonal antibodies in animal serum, the production of hybridomas from animal spleen cells fused to myeloma cells, and the subsequent amplification of monoclonal antibodies by the ascites or tissue culture supernatant method, and the use of hyper-immunised animals for the production of recombinant antibodies) within EU Member States or through international cooperation, including the import of antibodies and antibody-containing products, unless it can be demonstrated on a case-by-case basis that AFAs cannot be applied.
- (ii) An expert working group should be established to set up a roadmap for moving away from animal immunisation-based techniques for antibody production, in light of the scientific feasibility and commercial availability of AFAs.
- (iii) Implementation programmes should be set up to facilitate the transfer of establishments to the new technology. These should include centres of excellence for training in AFA-based technologies to ensure that antibody producers are fully supported.
- (iv) Measures should be taken to ensure that animal-derived antibodies manufactured outside the EU adhere to European standards to avoid ethics dumping in regions where animal welfare is less well regulated.
- (v) The EURL ECVAM should extend its field of activities with its international collaborative partners to include the production of AFAs and their subsequent use.
- (vi) EU and national agencies who are committed to the 3Rs and who execute EU regulations at an operational level for the commercial production of cosmetics, medicines, household products, and food or to safeguard our health or the environment should reinforce this action and no longer permit the import or use of animal-derived antibodies and antibody-containing products aimed to monitor, detect, diagnose, or extract targets of interest.

(vii) Subsequent reports from the Commission to the Council and the European Parliament on the statistics on the number of animals used for experimental and other scientific purposes should include data on the use of animals for antibody production as an independent category.

Concluding Remarks

Antibody production by phage display adopts the same mechanistic principles as those employed by the *in vivo* adaptive immune system and exploited for scientific research and commercial purposes. Consequently, the antibodies produced by these methods are functionally indistinguishable to the extent that recombinant antibodies provide the 'same or higher level of information as the animal procedure', as stipulated by Directive 2010/63/EU.

Despite the emergence of better-quality *in vitro* technologies (see Outstanding Questions) to tackle a problem that continues to be overlooked [17], obsolete animal-based antibody production methods persist. We have at our disposal a mature and widely used technology that is set to have an enormous impact on animal use owing to the fact that the reliance on antibodies by biomedical scientists, health-care professionals, and consumers impacts all areas of research, development, and safety testing. For that reason, it is bewildering to consider that, despite the readiness of new molecular methodologies and the wealth of literature to support implementation, and despite the growing availability of companies offering AFAs, antibodies are continuing to be produced using animal immunisation techniques. It is even more perplexing that we are not already deeply committed to a programme of replacement of animal-derived antibody production techniques. To encourage technical discussions that will address any arising issues and feed into an EU-led replacement programme, to ensure that a consolidated expert opinion is reached and that a level playing field exists for all antibody producers, we have made available a [LinkedIn.com](#) group forum: AFAs–Animal Friendly Affinity-reagents. To stimulate discussion, please see Outstanding Questions.

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Outstanding Questions

Not including socioeconomic reasons such as time constraints, cost, convenience, or lack of expertise, in the hands of competent experts what exceptional circumstances might lead to project authorisation for animal-derived antibody production against a target antigen?

What barriers exist to the adoption of recombinant methods of antibody development/production and use by research institutes, industry, and end users? How can these barriers be overcome?

What role could be played by regulatory authorities, ethics committees, publishers, industry, funders, and end users to encourage the routine production and commercialisation of AFAs or to restrict animal-derived antibody production, including their import?

What are the advantages of recombinant antibody production methods over monoclonal and polyclonal antibody production methods?

How can phage display antibody production be adapted to produce the characteristic desired in a polyclonal—namely, multiple epitope recognition? What advantage can AFA polyclonals offer over animal-derived polyclonals?

If the initial selection procedure does not produce an antibody candidate with the desired specificity and affinity, what methods can be used to improve these characteristics without resorting to animal immunisation methods?

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