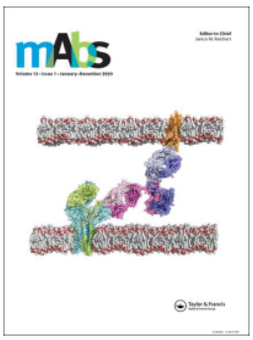

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PERSPECTIVES

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Animal- versus *in vitro*-derived antibodies: avoiding the extremes

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ABSTRACT

Recent recommendations from the European Union Reference Laboratory regarding the generation of antibodies using animals have stimulated significant debate. Here, four of the scientists who served on the Scientific Advisory Committee provide clarification of their views regarding the use of animals and *in vitro* platforms in antibody generation.

Abbreviations: EURL ECVAM, European Union Reference Laboratory for alternatives to animal testing. ESAC, EURL ECVAM Scientific Advisory Committee

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Antibody; phage display; immunization; antibody discovery; antibody therapeutics

The European Union Reference Laboratory for alternatives to animal testing (EURL ECVAM) has the mandate of advancing the Replacement, Reduction, and Refinement (the 3Rs) of animal procedures, in that order. In this context, it assembled a Scientific Advisory Committee (ESAC) *ad hoc* working group, of which we were members. We write as independent scientists to clarify our position regarding recent communications.[1–8] These communications uniformly disagree with the EURL ECVAM recommendations, particularly in the generation of therapeutic antibodies, but also in the fields of diagnostics and research, indicating that restricting the use of animal-derived antibodies would severely impact the competitiveness of EU research, the health of the European pharmaceutical industry, access of EU patients to the best medicines, and even society at large. As members of ESAC, we were tasked to "review the scientific validity of non-animal methods for the development and production of antibodies," specifically excluding therapeutic antibody discovery and development. We were also instructed not to consider economic or other nonscientific factors. We concluded that well-characterized recombinant non-animal-derived antibodies are mature reagents generated by a proven technology, offering significant additional scientific benefits, including improved reproducibility. However, we also acknowledged the general lack of availability of non-animal-derived antibodies as a key impediment to their widespread adoption.

The EURL ECVAM Recommendation on Non-Animal-Derived Antibodies[9] reiterated previously stated positions:[10–12] the widespread adoption of high-quality recombinant antibodies is essential for improving biological research quality and reproducibility. Unlike almost all other reagents, animal-derived antibodies are for the most part not molecularly defined, and sold for what they purportedly do, rather than what they are, a problem recombinant antibodies can overcome. Once sequence-defined, antibodies become immortal: gene synthesis allows the production of essentially identical reagents *ad infinitum*, as well as variants, where antibody genes are fused to functional moieties or altered, for additional applications. As antibody genes from hybridomas can now be easily sequenced, the same advantages potentially extend to preexisting monoclonals. Thus, independently of the 3Rs, there are strong scientific arguments for sequence-defined recombinant antibodies, which of course must be of high quality and well characterized.[3,11]

As referenced in the recommendation, *in vitro* selection from large, well-designed antibody libraries can now yield antibodies as good as animal-derived antibodies. This was not always the case, reflecting extensive advances in the technology over 30 years.

In general, the needs for effective research or diagnostic antibodies are stringent, requiring the detection of antibody binding with high affinity and specificity within a particular assay (e.g., immunofluorescence). For therapeutics, the demands are higher. Binding alone is usually insufficient: biological activity often requires interaction with a particular epitope at a particular geometry with a particular affinity and defined cross-reactivity. Sometimes the optimal molecules are selected from *in vitro* display systems, sometimes from mice, which may be inbred or transgenic. Different *in vitro* display systems, different antibody libraries, and different animals each perform differently, making it currently impossible to predict in advance which platform will be most successful for any particular therapeutic target. Consequently, many drug discovery programs today use parallel approaches to generate antibody therapeutics, increasing the chances leads will be found and reducing the time required.

We believe it would be unacceptable for future patient benefit to restrict such searches or require prolonged serial searches, exploring different *in vitro* platforms and libraries, before moving to animal immunization. While several approved therapeutic antibodies have been derived from non-animal-derived antibody libraries, many more have come from

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animal immunization. This likely reflects the longer history of immunization, as well as patents (now expired) that once restricted the use of display technologies. Potential developability advantages of animal-derived antibodies over those from older *in vitro* library technologies[13] have been overcome by more recent advanced platforms. It is important to keep both options in the future. Whatever the ultimate origin or discovery technology used, the final therapeutic antibody format is always recombinant, sequence defined, and of high quality.

The way forward

We are strong proponents[10–12,14] of the use of *in vitro* methods to generate antibodies to improve research quality and reproducibility. However, to encourage this switch to antibodies from *in vitro* technologies, the platforms must become more widely available. They should compete with animal-derived antibodies and become adopted organically for their quality and flexibility.

The requirements to effectively establish *in vitro* selection platforms are too demanding for many individual laboratories. Likewise, most individual laboratories do not have access to their own animal houses and so outsource immunization. However, whereas many companies provide immunization services, far fewer provide custom non-animal antibody generation. In order to encourage the transition to sequence-defined recombinant antibodies, we would suggest that public funding may be required to subsidize the initial implementation of commercial and institutional antibody selection services at reasonable cost.

All existing hybridomas should be cloned and supplied recombinantly to improve reproducibility, an approach some manufacturers have started to implement. Notwithstanding the recognized economic challenges for suppliers to provide antibody sequences of their products,[15] solutions to provide them should still be pursued, and at least the use of uniform clone reference codes should be encouraged, allowing researchers to ensure reagent continuity.

For therapeutics, we agree with Prabakaran et al.[16] that, due to the extended time necessary for drug development, the best approach would be to apply parallel methods, using as many simultaneous and different technologies as possible, including immunization, *in vitro* platforms, single-cell techniques, and even machine learning-based methods once they are validated. We believe this should continue for the foreseeable future.

While we expect the intrinsic advantages of *in vitro*-derived antibodies to become generally recognized as they become more broadly available, a restriction on the use of immunization today, without substantial efforts to improve general access to non-animal-derived antibodies, would indeed significantly hamper research.

Disclosure statement

A.R.M.B.: CSO, cofounder and shareholder of Specifica Inc., involved in the supply of antibody library and discovery services for the therapeutic market. S.D.: co-founder and shareholder of companies that employ animal-free antibody generation—namely, mAb-factory GmbH, Yumab GmbH, and Abcalis GmbH. A.P.: cofounder and shareholder of companies (Morphosys AG and Molecular Partners AG) that employ animal-free antibody and binding protein generation for therapeutics. A.K.: shareholder of companies (Morphosys AG, Molecular Partners AG, and Bio-Rad Laboratories) that employ animal-free antibody and binding protein generation. All authors are independent advisors on the EURL ECVAM scientific advisory committee (ESAC) for the scientific validity of alternative methods for antibody production. ESAC is composed of external scientists who are appointed on the basis of their scientific expertise; they act independently, in the public interest, and do not represent their company's, the EU's, or external interests of any kind.

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