National Centre
for the Replacement
Refinement & Reduction
of Animals in Research

Impact report 2026

Accelerating the use of non-animal reagents for *in vitro* research

Pioneering Better Science

Headline impacts

Inputs

£905k
invested across ten awards.

40
core team members supported.

9
research organisations funded in Coventry, Hertfordshire, Leeds, Leicester, London, Nottingham and Strathclyde.

Outputs

200+
non-animal derived reagents have been characterised, validated or optimised – including non-animal derived antibodies, synthetic matrices and culture media supplements.

18
project partners across academia and industry provided expertise and training, and/or access to facilities, reagents and equipment.

£390k+
direct and in-kind contributions provided by host institutions and project partners.

£970k+
follow-on funding secured to support further optimisation, validation and adoption of animal-free products and reagents.

Thousands of animals
will be replaced annually.

Introduction

Many areas of biomedical research are dependent on the use of products such as antibodies and cell culture components that are derived from animals. In this report we describe the outputs and impacts of a funding call that we ran to support a shift away from the use of these products to animal-free alternatives.

In 2024, the NC3Rs invested £905k to accelerate the adoption of animal-free products and reagents[1] in *in vitro* research, as part of a package of £5M funding from the Department for Science, Innovation and Technology (DSIT). The investment focused on generating robust comparative data to support the systematic characterisation and assessment of non-animal derived products and reagents. Ten awards were funded for projects that included the evaluation of recombinant antibodies, chemically-defined media and synthetic matrices for routine cell culture studies.

Animal-derived products and reagents such as foetal bovine serum (FBS), extracellular matrices and antibodies are widely used in *in vitro* research. Their generation can involve significant suffering for the animals involved – plus undefined composition, batch-to-batch variability and limited standardisation or quality control for many products and reagents can compromise the reproducibility of the experiments they are used in. Non-animal alternatives are available, with significant scientific and ethical advantages, however, their use has been limited by a lack of comparative performance data in routine research applications.

> "Moving away from animal-derived products requires researchers to have confidence that alternatives will work reliably in their own experimental systems. These awards show how targeted investment can generate the evidence needed to drive wider adoption, with early impacts demonstrating their potential to meaningfully replace animals."
>
> Katie Bates, Head of Research Funding

[1]The terms 'animal-free' and 'non-animal derived' are used interchangeably throughout this report to describe the use of reagents or products developed without the use of animals.

Building the evidence base for non-animal derived affinity reagents

Antibodies are one of the most commonly used reagents in biomedical research. Due to a lack of reporting, it is not possible to determine the number of animals used globally in their production but it is reasonable to assume that the number is very high and likely to be in the hundreds of thousands. Non-animal derived affinity reagents such as recombinant antibodies, aptamers and Adhirons are available. These have improved specificity and reproducibility compared to traditional animal-derived products, however there is a lack of confidence in their performance for routine applications. To help address this, we funded three projects focusing on characterising and benchmarking these alternatives reagents for western blotting and in diagnostic lateral flow tests.

Large-scale characterisation of recombinant antibodies to replace animal-derived reagents

Dr Harvinder Virk's team at the University of Leicester used their award to accelerate the replacement of animal-derived antibodies with recombinant alternatives. The team characterised 150 conventionally-produced (i.e. by animal immunisation) primary and secondary antibodies for 15 protein targets relevant to cardiovascular, respiratory and oncology research. Using standardised industry-endorsed protocols, antibody performance was assessed in cells expressing the protein-of-interest and corresponding knockout controls. This identified high-performing recombinant antibodies (e.g. in terms of specificity) suitable for replacing animal-derived monoclonal and polyclonal reagents for various applications including western blotting, immunoprecipitation, immunofluorescence and flow cytometry.

Delivered in partnership with the YCharOS[2] international consortium, the project attracted additional industry engagement with antibody manufacturers Sigma Aldrich and Miltenyi contributing in-kind reagents. All datasets are publicly available through the YCharOS Gateway, with raw data deposited on the Zenodo data sharing website. Nine research groups from the UK, Canada and the USA, are already using the recombinant antibodies tested for cardiorespiratory and neurodegenerative disease research.

The project demonstrated that an estimated 96 animal-derived antibodies could be replaced with recombinant alternatives, avoiding the use of around 300 mice or rabbits in their production, with further animal replacement potential as uptake increases. The team have secured over £580k in follow-on funding to expand their antibody characterisation pipeline, identifying 28 further protein targets with the potential to replace 179 animal-derived antibodies with recombinant alternatives.

[2]YCharOS is an international consortium working with global antibody manufacturers.

Replacing animal-derived antibodies in western blotting with animal-free reagents

Dr Jamie Meredith's team at the MRC London Institute of Medical Sciences used their award to evaluate animal-free affinity reagents as alternatives to animal-derived antibodies for use in flow cytometry, microscopy, proteomics and tissue culture. In western blotting studies the team showed that fluorophore-labelled His-tag aptamers produced signal intensities comparable to conventional primary and secondary antibodies derived from animals, while also reducing assay time and reagent use. The team further identified the experimental conditions under which phage display-derived antibodies perform reliably using standard western blot membranes and imaging systems.

By removing the need for animal-derived antibodies in western blotting workflows, Jamie estimates that wider adoption of these affinity reagents across the Institute could replace approximately 3,200 animals annually. Further reproducibility studies are now being planned with the team also sharing protocols across the Institute.

Evaluating Adhirons as animal-free alternatives in lateral flow diagnostics

Dr Christian Tiede's team at the University of Leeds used their award to build on the outputs of an earlier NC3Rs-funded PhD studentship (completed in 2024) to advance the use of Adhirons – engineered animal-free binding proteins previously known as Affimers – as replacements for animal-derived secondary antibodies used in diagnostic lateral flow tests. Secondary antibodies are conventionally produced by immunising host animals, commonly goats or sheep, with antibodies from another species, such as mice or rabbits. The antibodies generated by the host are then purified for use as secondary antibodies. Working in partnership with Surescreen Diagnostics, a UK-based lateral flow test developer, the team benchmarked anti-mouse and anti-rabbit Adhirons against commercial antibody-based lateral flow tests. The Adhirons demonstrated comparable sensitivity and enhanced biochemical stability, including improved tolerance to thermal, chemical and processing stresses relevant to *in vitro* point-of-care diagnostics.

In lateral flow test applications, secondary antibodies are typically required at the hundreds of milligrams scale. Substituting these animal-derived reagents with Adhirons could replace the use of hundreds of goats or sheep per application, given that typical antibody yields are only 100 to 200 mg per animal. Beyond lateral flow tests, wider adoption of Adhiron technology could replace up to 675 goats and sheep used annually in the UK for secondary antibody production.

Qualifying animal-free reagents in *in vitro* cell culture systems

The supplementation of basic cell culture media with FBS has remained a fundamental technique in cell culture since the 1950s. Derived from blood collected by cardiac puncture from un-anaesthetised foetal calves this undefined growth supplement is associated with significant animal suffering and has notable batch-to-batch variability that compromises the reproducibility of experiments. In comparison, the composition of animal-free media supplements is well defined, with high levels of consistency between batches. Despite these benefits, transitioning to animal-free cell culture conditions is often considered challenging due to the time and cost required for characterisation and validation studies. To address this, we funded four projects to generate robust comparative data to support the use of animal-free reagents in routine cell culture, regulatory assays and advanced *in vitro* models.

Evaluating animal-free serum formulations for routine cell culture

Dr Manuela Natoli and Dr Corina Kohler, together with the Cell Science, Science Technology Platform team at the Francis Crick Institute used their award to conduct a large-scale evaluation of four commercially-available animal-free serum formulations in 50 commonly used cell lines in oncology, renal and immunology research. All of the cell lines adapted to the new culture conditions, with more than 90% adapting to media containing 75% animal-free serum and 25% FBS, with comparable growth characteristics, morphology and bioenergetic profiles to those maintained in 100% FBS.

Use of the adapted cell lines by researchers across the Francis Crick Institute is estimated to replace serum derived from approximately 30 calf foetuses per researcher per year. The cell lines will be available through the Institute's cell banking and restocking services, supporting approximately 120 research groups and five commercial companies, including Cancer Research Horizons, hosted within the Institute. A user-friendly protocol for adapting cell lines to animal-free serum has been developed and will be shared publicly to support broader adoption of animal-free serums in routine cell culture.

Evaluating an animal-free product protocol for the OECD Test guideline 487 – the micronucleus assay

Dr Victoria Hutter's team at the University of Hertfordshire used their award to conduct a validation of an animal-free product version of the OECD Test Guideline 487 – the *in vitro* micronucleus assay which is widely used to assess the genotoxicity of chemicals. The work built on the outputs of a previous NC3Rs-funded **CRACK IT Challenge**, in which ImmuONE (a spin-out from the University) adapted the assay to remove animal-derived components, including replacing FBS with a chemically-defined media[3]. The protocol was benchmarked against OECD performance criteria and evaluated by ImmuONE and the University of Hertfordshire. Testing with four OECD-recommended control chemicals demonstrated reproducible dose-dependent micronucleus responses, with cytotoxicity within the acceptable limits for assay validity and dose-responses comparable to those obtained using standard serum-containing protocols.

Based on routine reagent use, local implementation of the animal-free protocol at the University of Hertfordshire and ImmuONE is estimated to replace approximately 60 calf foetuses per year. Future work will focus on further inter-laboratory reproducibility testing, protocol sharing and regulatory engagement to support wider uptake of the animal-free protocol for genotoxicity testing to support incorporation into international guidelines.

Qualifying an animal-free media formulation for mammary organoid culture

Dr Cinzia Allegrucci and her team at the University of Nottingham used their award to optimise animal-free approaches for culturing induced-pluripotent stem cell (iPSC)-derived mammary organoids for breast cancer research. The team successfully characterised a fully animal-free culture media for the differentiation and growth of mammary organoids *in vitro*, replacing animal-derived components including FBS and porcine-derived media supplements. Organoids cultured under animal-free conditions showed consistent growth and morphology, with the gene expression profiles of organoids carrying mutations closely aligned with patient tumour signatures, demonstrating the ability to replicate patient tumours sharing the same genotype.

In Cinzia's lab, use of the animal-free media is estimated to replace animal-derived materials from approximately five calf foetuses and ten pigs over the next five years. Building on this progress further work is focused on optimising synthetic matrices to replace Matrigel and collagen and validating animal-free antibodies for mammary organoid characterisation.

Optimising animal-free culture conditions for *in vitro* airway nanotoxicology

Dr Fiona Murphy and her team at the University of Strathclyde collaborated with Scinora GmbH to evaluate the company's chemically-defined serum-free media formulations and their Xplace cell culture supplement as an alternative to the use of FBS in *in vitro* airway nanotoxicology models. The study used Calu-3 bronchial epithelial cells and THP-1 monocytes to represent epithelial and immune components of the airway. Biocompatibility testing assessed effects of candidate formulations on cell growth, phenotype and epithelial barrier formation.

[3]The aim of the CRACK IT Challenge was to adapt established OECD Test Guidelines that involve *in vitro* assays to remove animal-derived products. For Test Guideline 487, this included replacing both FBS used in cell culture and rat liver S9 fraction used for metabolic activation.

Under the conditions tested, serum-free formulations supported key airway epithelial characteristics in Calu-3 cells and maintained viability and functional behaviour in THP-1 cells under test conditions.

Based on current FBS use, adoption of Scinora's animal-free media formulations in Fiona's lab is estimated to replace serum derived from approximately 14 calf foetuses per year. Future work will focus on further optimising Scinora media formulations and generating additional validation data to support wider use of animal-free media in airway nanotoxicology research.

Qualifying animal-free hydrogels, synthetic matrices and engineered tissue platforms for *in vitro* modelling

Advances in biomimetic matrices are enabling the development of physiologically-relevant 3D *in vitro* models. However, many models still rely on animal-derived products directly extracted from animal tissue, such as rat tail collagen or Matrigel which is derived from mouse tumours. These products are prone to biological variability and a lack of standardisation but are often regarded as the gold standard. We funded three projects to characterise and apply the use of animal-free matrices and model components across diverse applications, spanning disease modelling, microsurgical training and human tissue-based safety assessment.

Characterising a 3D human *in vitro* model of Alzheimer's disease using peptide hydrogels

Dr Mattéa Finelli at the University of Nottingham characterised a human 3D model of Alzheimer's disease using iPSC-derived neurons and astrocytes cultured in a defined peptide hydrogel, in collaboration with PeptiMatrix, StrataStem Ltd and Dr Richard Elsworthy at the University of Birmingham. The peptide hydrogel, originally developed through NC3Rs funding and now commercially available via PeptiMatrix, enabled more than a 95% reduction in animal-derived material use compared with standard Matrigel-based systems. Replacing Matrigel with the peptide hydrogel in Mattéa's lab has reduced usage by around eight bottles per year, equivalent to replacing the use of approximately 15 mice annually. When benchmarked against current gold standard models, the system showed comparable cell survival and differentiation, alongside improved reproducibility across experiments.

Building on these findings the industry partner StrataStem, providers of the iPSC cell lines, are incorporating animal-free reagents into their iPSC culture workflows. The platform is compatible with 96-well formats and automated imaging, supporting higher-throughput studies of Alzheimer's disease mechanisms and therapeutic screening. The team has secured over £70k in follow-on funding from Alzheimer's Research UK and the Alzheimer's Research UK Midlands Network to further characterise the model and extend its applications, including to study the effects of exercise *in vitro*.

Advancing animal-free microsurgical training with synthetic vessel models

Dr Deepak Kalaskar at University College London used his award to validate bioprinted vascular constructs made from VascuBiomatrix™, a fully synthetic, non-animal derived hydrogel as an alternative to rat blood vessels for microsurgical training. Using established microsurgical performance measures, the team assessed elasticity, tensile strength and mechanical integrity, demonstrating that the material can reproduce key biomechanical properties of native vascular tissue required for realistic suturing practice. Independent evaluation by surgical trainers at the Griffin Institute confirmed that the synthetic vessels provide suitable handling and suturing characteristics for early-stage training. A typical microsurgical training course can use up to 60 live rats – adoption of the validated synthetic vessels into these training courses could replace approximately 20% of this animal use. This project has generated £286k in follow-on funding, including from British Heart Foundation.

Characterising engineered human heart tissues for cardiovascular research

Professor Helen Maddock at Coventry University used her award to characterise the performance of engineered human heart tissue generated from human iPSC-derived cardiomyocytes for use in cardiovascular research and safety assessment. The work generated comparative performance data to support the use of human cardiomyocytes as an alternative to animal-derived cardiac preparations and *in vivo* studies. The team combined engineered human heart tissue developed by Professor Katja Gehmlich at the University of Birmingham with the work-loop assay, an *in vitro* system that measures cardiac contractile function under physiologically-relevant loading conditions. Benchmarking against established preclinical cardiac assays demonstrated reproducible measurements of tissue contractility and mechanical performance, supporting the suitability of human iPSC-derived heart tissue to investigate drug-induced cardiovascular effects.

The team estimate that routine application of the system in cardiovascular disease modelling and drug testing research could replace over 500 rats per year within Helen's laboratory.

NC 3Rs
Gibbs Building
215 Euston Road
London NW1 2BE
T +44 (0)20 7611 2233
enquiries@nc3rs.org.uk
www.nc3rs.org.uk