

THE USE OF ANIMAL-DERIVED INGREDIENTS

In Grant Applications Made to the Lord Dowding Fund for Humane Research



1. Core Policy and Mission Statement

The Lord Dowding Fund is a department of the National Anti-Vivisection Society. We believe that animal-based research is totally inapplicable to medical progress, and has often retarded it. The Fund supports research aimed at replacing the use of laboratory animals. Financial support is given for a wide range of projects including, for example, pure and applied research in the fields of biology, human and veterinary medicine, toxicology, and teaching. A wide range of techniques is supported, including cell/tissue/organ culture, computer simulation, mathematical modelling, quantum pharmacology, chemical separation/analysis, epidemiology, and genetic engineering.

The objectives of the Lord Dowding Fund for Humane Research are to support and fund better methods of scientific and medical research for testing products and curing disease which replace the use of animals; to fund areas of fundamental research which lead to the adoption of non-animal research methodology; and to fund, promote and assist medical, surgical, and scientific research, learning, educational training, and processes for the purpose of replacing animals in education and training. Therefore, our key objective is to promote and assist any research for the purpose of showing that animal research is harmful or unnecessary to humanity.

Any grant applications made must be able to show how the proposed research would help towards reducing the use of animals in research laboratories, and so a key component of any grant application to the Fund should be an estimate of the relevance of the project to our primary aim, which is replacing the use of animals in biomedical research. This should include referenced details of the animal experiments that the project is designed to replace, and estimated numbers and species of animals involved.

With this in mind, applications are more likely to succeed if the proposed project does not involve the use of animal-derived materials, including animal tissues. Such materials include monoclonal antibodies produced using the ascites method and foetal calf serum (FCS); both of which have the potential to cause pain, suffering and distress to the animals used to produce them.

This document details the remit of the Fund on the use of animal products, and provides alternative options to the use of animal-derived ingredients in research. It includes:

- Details of the Lord Dowding Fund's conditions and restrictions on the use of animal-derived ingredients, and why such a position is held
- A list of suppliers of monoclonal antibodies produced by methods which are suitable for use in projects awarded by the Fund
- A source list of serum-free media suppliers whose products can be used as alternatives to foetal calf serum for use in projects supported by the Fund

2. LDF Standpoint on the Use of Animal-Derived Products

Overall, applications are more likely to succeed if the proposed project does not involve the use of animal-derived materials, including animal tissues. This is because the Lord Dowding supports research aimed at replacing the use of laboratory animals, since it believes their use to be unnecessary. The projects that are selected for funding are those that can offer the most applicable alternative to current animal experiments and potentially have the most significant impact on reducing animal use in research.

If a proposal includes the use of animal-derived products (such as foetal calf serum or *in vivo* produced monoclonal antibodies), LDF is obliged to discuss the use of non-animal derived alternatives with the applicant. LDF is also willing to add more money to a project if there is the likelihood that by doing so, precedence could be set for the use of non-animal derived alternatives. In this document, applicants are provided with a list of approved serum-free media, and suppliers of *in vitro* produced monoclonal antibodies. If an alternative cannot be used, a full statement must be obtained from the applicant detailing the rationale for using animal derived products, including why an alternative cannot be used.

At present, the use of animals to produce monoclonal antibodies in the UK is restricted, and no animals were used for this purpose in 2003 according to the last Home Office Statistics. Of course in some countries outside of the UK, animals are still being used for this purpose. Under no circumstances can a LDF-supported project use *in vivo* produced monoclonal antibodies, because *in vitro* monoclonal antibody supply is now a well established technique.

The use of animal sera, such as foetal calf serum, is acceptable only if alternatives (human serum, serum replacements or serum-free media) are unsuitable, and reasons can be given to justify this.

It is unlikely that a grant will be approved for the use of animal tissues in culture. Preference will always be given to research using human tissues, except in cases of veterinary research where tissues from a naturally sick animal, whose individual disease is being studied as part of a course of treatment, are more appropriate.

Under no circumstances will research be sponsored where embryonated eggs (human or animal) are to be used, nor where a disease has been artificially induced into an animal during the course of veterinary research.

3. Monoclonal Antibodies

3.1. Definition

A monoclonal antibody (MAb) is an antibody that is mass produced in the laboratory from a single clone and that recognises only one antigen. Monoclonal antibodies are typically made by fusing a normally short-lived, antibody-producing B cell (type of white blood cell) to a fast-growing cell, such as a cancer cell (sometimes referred to as an “immortal” cell). The resulting hybrid cell (or hybridoma) multiplies rapidly, creating a clone that produces large quantities of the antibody, which is of exceptional purity and specificity.

3.2. Uses

Monoclonal antibodies are currently utilised in many diagnostic procedures, including:

- measuring protein and drug levels in serum
- typing tissue and blood
- identifying infectious agents
- identifying clusters of differentiation for the classification and follow-up therapy of leukaemias and lymphomas
- identifying tumour antigens and auto-antibodies
- identifying the specific cells involved in the immune response
- identifying and quantifying hormones

Monoclonal antibodies engendered much excitement in the medical world and in the financial world in the 1980s, especially as potential cures for cancer. They have been used in laboratory research and in medical tests since the mid-1970s, but their effectiveness in disease treatment has been limited. By the mid-1990s, however, some of the technical problems had been overcome. Experimental cancer therapies have used drugs, radioactive materials, or immune killer cells attached to monoclonal antibodies that, when injected into patients, home in on antigens that grow only on the surface of cancer cells. Commercial uses of monoclonal antibodies include pregnancy tests, food safety tests, and glucose monitoring tests for diabetics.

3.3. Monoclonal Antibody Production

The production of monoclonal antibodies is a multi-step procedure:

1) Isolation of the cells that produce the antibody of interest:

- This step, at present, always involves the use of animals.

The isolation of cells that produce the antibody of interest is done by first immunizing a mouse or another animal with a specific antigen. Once the antibodies are produced, the B-cells (a type of

white blood cell that is responsible for antibody production) are collected from the spleen after the animal has been killed. The specific white blood cells from the spleen that produce the antibody of interest are isolated *in vitro*.

2) **Fusion of antibody producing cells with cancerous cells:**

Each B-cell is then fused *in vitro* with an immortal myeloma tumour cell (a type of cancerous cell that is capable of dividing indefinitely but does not produce antibodies) to form hybridoma cells. The hybridoma cells produced by fusing the B-cells with the myelomas share the properties of both types of cells and are therefore capable of repeated divisions and of secreting antibodies.

3) **Growth of hybridoma cells:**

The new fused cell line of hybridomas is grown briefly in culture and then, for the production of large amounts of monoclonal antibodies, these hybridomas can be further grown by both *in vitro* and *in vivo* methods:

- a) **In vitro methods:** Tissue culture technology is used to further grow the hybridoma cells and these cells will secrete monoclonal antibodies into the surrounding culture media.
- b) **In vivo methods:** Hybridoma cells are injected into the peritoneal cavity of a mouse where they will multiply. As a result, fluid is produced and collects inside the abdomen of the mouse. The more the hybridoma cells multiply, the more fluid they produce. As the volume of fluid (known as ascites fluid) accumulates, it distends the abdomen of the mice and causes discomfort and pain to the animals. The fluid is collected from the abdomen of the mice by holding the animal in one hand and tapping the abdomen with a 20- to 22-gauge needle, allowing the fluid to flow freely into a collection tube. Signs of distress in mice seen in mice subjected to the ascites method can include any of the following: rapid breathing rate; slow, shallow or laboured breathing; rapid weight loss; ruffled fur or rough hair coat; hunched posture; difficulty moving; hypothermia or hyperthermia; inappetence, diarrhoea or constipation. The ascites fluid collected contains large volumes of the desired monoclonal antibodies.

3.4. Factors Affecting Monoclonal Antibody Production

The purity of the antigen, the mouse strain which is used, the age and sex of the mouse, the tolerance of the individual mouse, and many other factors may affect the outcome of *in vivo* MAb-producing procedures. Because so many variables are present, one general way to maximize the animal's immune response is to use adjuvants. Adjuvants hold the antigen at the site of immunisation and release it over a long period of time. Freund's Complete Adjuvant, which is more widely used than any other adjuvant, includes killed mycobacteria which serve to increase inflammation upon injection, and thereby enhance the mouse's immune response.

Freund's Complete Adjuvant (FCA) is seen by many researchers as the most effective adjuvant for antibody production in mice, but it creates painful inflammatory lesions at the inoculation site. The resulting discomfort to mice from the use of FCA is a subject of widespread concern, and a practice which LDF is wholly against. When FCA is used, specific guidelines commonly require using smaller doses at each injection site and avoiding foot pad or intradermal injections. However, due to the pain that FCA causes, large-scale FCA use has been banned in the Netherlands and the United Kingdom.

Alternative adjuvants are effective in many situations, and *in vitro* technologies are far more preferable in any case.

3.5. LDF Conditions and Restrictions on the Use of Monoclonal Antibodies

Overall, monoclonal antibodies may be produced from hybridomas either *in vitro* or from animals. To produce a desired monoclonal antibody using the animal method involves the production of an ascites tumour, which yields fluid containing the monoclonal antibody of interest. In addition, *in vivo* monoclonal antibody production uses Freund's Complete Adjuvant in order to maximise the immune response. Both the ascites development and the use of adjuvant are methods that have the potential to cause considerable levels of pain, suffering and distress to the animals used, and are therefore unacceptable to the Lord Dowding Fund. *In vitro* technologies possess a number of advantages in addition to being more humane; in the majority of instances they can now replace the use of ascitic animals, and should be used whenever possible.

The UK Government has restricted the use of animals in monoclonal antibody production, although *in vivo*-produced monoclonal antibodies imported from abroad are still widely available. Applicants should therefore be aware that they may be inadvertently supplied ascitic-derived monoclonal antibodies from an otherwise *in vitro* supplier; companies that produce or supply antibodies do not always make clear whether their monoclonal antibodies have been raised in animals or cultured *in vitro*.

The Lord Dowding Fund is against the use of *in vivo*-produced monoclonal antibodies where production involves the ascites method or the use of FCA

Hybridomas are available commercially and can be obtained 'off the shelf'. Many suppliers often carry hundreds of hybridomas, produced many years ago in some instances. A researcher seeking specific monoclonal antibodies can choose to have a supply produced either *in vitro* in cell culture or one which was produced using the ascites method. If an applicant was seeking 'off-the-shelf' hybridomas, we would request that they choose those produced using the *in vitro* method.

New (custom-made) hybridomas to produce novel monoclonal antibodies will involve the killing of further animals to complete step 1 of the MAb production procedure, and if such antibodies were to be used in a research project, then LDF would be unable to support their use. The use of 'off-the-shelf' hybridomas, if developed using the *in vitro* method, would however be acceptable.

The Lord Dowding Fund does not provide funds for the production of ascitic-derived monoclonal antibodies, nor to establish new hybridomas

It is the responsibility of grant holders to ensure that:

- Only *in vitro* sources of monoclonal antibodies are used
- The use of any monoclonal antibody is derived from previously established hybridoma cell lines (either in-house or commercial).

3.6. Human Monoclonal Antibodies

The production of MAbs has opened many doors in the area of immunotherapy. Human antibodies, however, are much more useful in this capacity than are those of mice, which may be rejected by the human immune system. Also, in some cases, MAbs from mice may elicit different responses than those from humans.

Human MAb production has proved to be quite complex. One current strategy involves transforming cells with Epstein-Barr virus and then stabilising them by extensive cloning. Another popular technique is the "humanisation" of mouse MAbs. In this procedure, regions of the human myeloma protein are joined to the variable region of a mouse antibody. If this technique for producing human MAbs was involved in a research project being put forward for LDF funding, then such a project could not be supported by LDF. The production of true human antibodies is possible with the use of the Polymerase Chain Reaction, but limitations of this procedure are still in need of attention.

3.7. *In Vitro* Production

The expansion of hybridomas in animals is becoming less acceptable due to humane and economic concerns. Several European countries have incorporated legislation limiting antibody production in mice. MAbs are extensively produced *in vitro* in Switzerland and Germany. Although *in vivo* production is relatively inexpensive in comparison to *in vitro* production, ascites fluid of mice may yield commercially unsuitable antibody. Two popular alternatives are bulk tissue culture in encapsulated or hollow fibre systems, and the expression of cloned antibody genes in high producing eukaryotes. The latter is accomplished through recombinant DNA technology.

3.8. Suppliers of *In Vitro* Monoclonal Antibodies for Research Use

The following companies are all able to supply *in vitro* produced monoclonal antibodies to a high specificity and purity. This list has been drawn up to help researchers make an informed choice about the source of the monoclonal antibodies that they may utilise in the study for which Lord Dowding Fund support is sought. The list has been compiled on the basis of information available from the companies involved, and should avoid the possibility that researchers may inadvertently be supplied ascitic-derived monoclonal antibodies from an otherwise *in vitro* supplier;

These companies have been approved by the Lord Dowding Fund. The use of antibodies obtained from the companies listed here would be acceptable to the Fund if they were involved in a grant application to be considered for funding (provided that the antibodies obtained are 'off-the-shelf' hybridomas and not custom-made).

The Antibody Resource Centre (previously Sheffield Hybridomas)

Firth Court, Western Bank, Sheffield S10 2TN, UK.

Telephone: 0114 222 7480

Fax: 0114 222 7483

Email: arc@sheffield.ac.uk

Website: <http://www.shef.ac.uk/arc/index.html>

- A university service unit providing *in vitro* monoclonal antibodies for industry and academia. They will carry out contract commercial work, and can also provide serum/protein-free media. They offer a monoclonal service using *in vitro* bioreactors, whereby totally precluding the need for making ascites fluid. Furthermore, they have a two-year grant from the NC3R's to reduce the numbers of animals used in making polyclonal antibodies; one rabbit is used for a number of different immunogens (up to three) and then each individual antibody is affinity purified from the serum. So although the method still uses an animal to make polyclonal antibodies, the number is heavily reduced.

Biosynergy (Europe) Ltd. (previously **QBS Ltd.**)

12 Pembroke Avenue, Denny Industrial Centre, Waterbeach, Cambridgeshire CB5 9BP, UK.

Telephone: 01223 579345

Fax: 01223 579349

Email: rmason@biosynergieurope.com

Website: <http://www.biosynergy.co.uk/index.htm>

- A contract provider of monoclonal antibodies produced *in vitro* using hollow-fibre bioreactors.

NB: This company provides custom and standard ('off-the-shelf') *in vitro* monoclonal antibodies, as required. The custom MAbs produced by this company would not be acceptable to LDF, but their standard MAbs are all produced using *in vitro* methods, and so would be acceptable.

Diatec.com AS

Gaustadalléen 21, N-0349 Oslo, Norway

Telephone: (+47) 22 95 86 25

Fax: (+47) 22 95 86 49

Email: diatec@diatec.com

Website: <http://www.diatec.com>

- This company provides monoclonal antibodies for worldwide distribution; all are produced *in vitro*, yielding antibodies with a higher and more consistent quality than is routinely produced by ascites production in mice.

European Collection of Cell Cultures

CAMR, Salisbury, Wiltshire SP4 OJG, UK.

Telephone: 01980 612512

Fax: 01980 611315

Email: ecacc@camr.org.uk

Website: <http://www.ecacc.org.uk>

- This company is a provider of cell cultures and associated services to industry and the academic research community. It has an extensive collection of suitable monoclonal-secreting hybridomas (available 'off-the-shelf').

International Blood Group Reference Laboratory

Southmead Road, Bristol BS10 5ND, UK.

Telephone: 0117 991 2100

Fax: 0117 959 1660

Website: <http://www.bloodnet.nbs.nhs.uk/ibgrl/default.htm>

- This laboratory provides a range of monoclonal antibodies for immunohaematological research. All antibodies are grown using *in vitro* culture, and ascitic *in vivo* methods are not used.

Lonza Biologics Plc. (previously **Celltech**)

228 Bath Road, Slough, Berkshire SL1 4DX, UK.

Telephone: 01753 777000

Fax: 01753 777001

Email: contact.slough@lonza.com

Website: <http://www.lonzabiologics.com>

- This company claims to be the world's leading provider of monoclonal antibodies and recombinant proteins.

Serologicals Limited (previously **Bioscot Ltd.**)

Fleming Road, Kirkton Campus for Science & Technology, Livingston EH54 7BN, Scotland

Comprises 3 Business Units: Celliance Corporation, *Chemicon International Inc.* and Upstate Group

Telephone: 0150 640 4000

Fax: 0150 640 4001

Website: <http://www.chemicon.com/Product/ProductIndex.htm>

- Chemicon International Inc. offers a complete range of antibodies, assay kits, and purified proteins for the scientific research market. They are a recognized leading provider for the research fields of Neuroscience, Stem Cell Biology, Matrix Biology, and Apoptosis and Cancer, with over 6,000 products available for the research market. This company manufactures monoclonal antibodies for blood typing reagents and diagnostics. All of these are produced using *in vitro* cell culture.

4. Animal Serum

4.1. Background

Animal serum is routinely added to culture media as a source of nutrients and other ill-defined factors, despite technical disadvantages to its inclusion and its high cost. Technical disadvantages to using serum include the undefined nature of serum, batch-to-batch variability in composition, and the risk of contamination. There are increasing concerns about animal suffering inflicted during serum collection that add an ethical imperative to move away from the use of serum wherever possible.

4.2. Foetal Calf Serum (FCS)

The preferred source of serum for cell culture is foetal calf serum (FCS). FCS is prepared from blood extracted from foetuses removed from cows found pregnant at slaughter. The foetus is removed during evisceration and blood extracted via cardiac puncture without any anaesthesia.

Recent years have seen an increased awareness of foetal sensitivity to pain, and growing evidence of resistance to anoxia in mammalian foetuses. Consequently calf foetuses are likely to be alive and have normal brain function during blood collection, and can be expected to experience suffering until death actually occurs. It is estimated that one to two million bovine foetuses are subjected each year to this inhumane process, yet many scientists regularly using FCS for cell culture remain unaware of the animal suffering involved in its collection.

4.3. Suppliers of Serum-Free Media for Research Use

With this in mind, the Lord Dowding Fund (as part of Focus on Alternatives, a group of British organisations working together to advance the replacement of animal experiments) produced a serum-free media table, which provides an overview of the range of commercially available serum-free media for cell culture. This document can be found online at <http://www.focusonalternatives.org.uk/>, by clicking on the 'Current Initiatives' link. It can also be found through the Lord Dowding Fund website.

The members of Focus on Alternatives (FoA) currently include the Lord Dowding Fund for Humane Research, the Dr Hadwen Trust for Humane Research, FRAME, Humane Research Trust, RSPCA, St Andrew Animal Fund, and the UK Human Tissue Bank.

The table was compiled to highlight the range of serum-free media currently available, and to encourage a move away from the use of animal serum in cell culture. The table lists companies selling serum-free media in alphabetical order, and details almost 200 serum-free media products.

Some media are designed to support a particular cell type, but others are for general purpose and will support a range of cell types.

The index at the back of the document can be used to identify the most suitable products for particular cell types. Media that are entirely free of animal ingredients are highlighted. More detailed information on particular products should be obtained by contacting the supplier directly.

4.4. Content of Serum-Free Media

Many companies offer to provide full details of the ingredients in their serum-free media directly to interested researchers, and some list the ingredients on their websites or in technical product datasheets. However, because serum-free media is a commercially competitive field, a few companies wish to keep their patented media formulations a secret.

By definition, serum-free medium lacks whole serum as an ingredient, but it may not be entirely free of serum-derived products. FoA has not researched in detail the sources of other animal-derived ingredients with regard to animal welfare, but both the Lord Dowding Fund and FoA's primary concern is the extraction of blood from living fetuses. Products derived from adult animals, or at slaughter may cause less suffering, but media containing no animal-derived ingredients are preferable.

4.5. Animal-Free Culture Media

The purest and most consistent cell culture environment is a totally chemically defined medium that is entirely free of animal-derived components. Several companies now offer such specialised animal-free media and these are highlighted in the serum-free media table.

4.6. Weaning and Performance

Most companies can provide technical advice to researchers on persuading cells to grow in serum-free medium, a process that usually involves gradual weaning. Some companies offer a limited range of cells already adapted to their own serum-free media products.

During weaning it is necessary to monitor cellular functions, as changes in culture conditions may affect aspects of cellular function of interest to the investigator. However the major benefits gained from switching to serum-free media can outweigh the effort required to overcome these initial hurdles. Many companies claim that their serum-free media can outperform comparable serum-supplemented media, and publish literature displaying results for particular cell types that show comparable or better growth of cells, at higher densities, and/or producing higher yields of end products.

Once cells have been adapted to serum-free media, researchers can benefit from improved control over culture conditions; the elimination of contaminant interference; improved reproducibility between cultures; consistency of media that avoids the need to screen batches; and avoidance of any serum cytotoxicity.

4.7. The Future for Serum-Free Media in Research

There is both a moral and legal imperative (European Directive 86/609/EEC as amended by Directive 2003/65/EC) for scientists to use alternatives to animals wherever possible. Cell cultures have already proved immensely valuable in replacing procedures on living animals. Their value to animal welfare would be further enhanced by the removal of serum, in particular FCS, from culture media.

Economic and safety reasons have already provided the impetus for industry to switch to serum-free culture conditions for biopharmaceutical production, and have paved the way for biomedical researchers to follow suit. Weaning cells off serum-supplement medium and onto serum-free conditions may cost researchers some time and effort, but this investment would be repaid in terms of consistency and quality of results.

4.8. LDF Conditions and Restrictions on the Use of Animal Serum

The use of animal sera, such as foetal calf serum, is acceptable to the Fund only if alternatives (human serum, serum replacements or serum-free media) are unsuitable, and sufficient reasons can be given to justify this. If an alternative cannot be used, a full statement must be obtained from the applicant detailing the rationale for using animal derived products, including why an alternative cannot be used.

The Lord Dowding Fund is against the use of animal serum where alternatives can be used

Therefore, the Lord Dowding Fund is more likely to support a project which uses serum-free media, rather than one which uses animal serum

Updated: August 2005