



# The UK Stem Cell Bank: Its role as a public research resource centre providing access to well-characterised seed stocks of human stem cell lines<sup>☆</sup>

Lyn Healy<sup>\*</sup>, Charles Hunt, Lesley Young, Glyn Stacey

*The UK Stem Cell Bank, Division of Cell Biology and Imaging, National Institute for Biological Standards and Control, Blanche Lane, South Mimms, EN6 3QG, UK<sup>1</sup>*

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## Abstract

The rapidly expanding field of stem cell research offers the potential to develop therapeutic agents to treat diseases such as Parkinson's, diabetes and heart disease. It is important that stem cell lines derived from quality-controlled and well-characterised cell banks should be made available to both the scientific and clinical communities to promote high-quality research and development. The requirement in the United Kingdom (UK) for rigorous regulation of the procurement and use of embryonic stem (ES) cell lines led the UK government to fund the establishment of a national bank for stem cell lines. The UK Stem Cell Bank (UKSCB) hosted at the National Institute for Biological Standards and Control (NIBSC) is committed to working closely with the clinical and research communities to provide qualified stocks of human stem cell lines of adult, foetal and embryonic origin for both research use and for use in emerging human therapy.

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<sup>\*</sup> Corresponding author. Tel.: +44 1707641502; fax: +44 1707646730.

*E-mail address:* [lhealy@nibsc.ac.uk](mailto:lhealy@nibsc.ac.uk) (L. Healy).

<sup>1</sup> *URLs:* <http://www.ukstemcellbank.org.uk>

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## 1. Introduction

Stem cells are theoretically capable of generating all tissues of the body. A growing number of *in vitro* and *in vivo* studies have indicated that these cells offer great promise for the development of clinical therapeutics. Stem cells could be used to treat a broad range of disease states by replacing and regenerating diseased or damaged tissue or treating injuries, such as paralysis. They may also prove to be of great value in gene therapy.

It is evident that in order to fulfil their potential, supply for both research and clinical therapies needs to be from a reliable source. Cell banks derived from cell lines that have been ethically sourced, from selected donors, need to be established and stored under quality conditions. In addition, they need to undergo an extensive programme to both characterise the cells and ensure their safety and efficacy.

With these requirements in mind, the UKSCB funded by the Medical Research Council (MRC) and Biotechnology and Biological Sciences Research Council (BBSRC) was launched in September 2002 to meet the needs of the scientific and clinical communities. The Bank is located at the National Institute for Biological Standards and Control (NIBSC), which is an internationally recognised UK Government laboratory experienced in ensuring the quality and safety of biological medicines (<http://www.nibsc.ac.uk>).

Probably the greatest challenge for stem cell banks is the requirement that they conserve various characteristics of the cells for which some of the key biological processes are not yet fully understood. This places a particularly heavy onus on the Bank to establish robust procedures to demonstrate that the stem cell lines remain stable in culture, that key biological indicators remain intact, and that the cell line is safe to use clinically. This paper describes the

approaches adopted by the UK Stem Cell Bank to deal with these and other practical issues.

## 2. Aims and scope of the bank

At present, the primary aim of the Bank is to enable researchers to access stem cell lines derived from adult, foetal and embryonic sources for the study of stem cell biology and related research and development. Such stem cell lines will have been subjected to rigorous characterisation and quality assurance procedures in order to guarantee their authenticity, purity and performance. Whilst the most pressing need is for access to such cell lines the Bank is also remitted to provide those working on the development of therapeutic applications with stem cell lines from clinical grade cell banks. These will be produced within a facility designed to European Union current Good Manufacturing Practice (EU cGMP) under a quality system which provides quality assurance and safety testing commensurate with the clinical use of stem cell lines.

The handling of stem cell lines, whether for clinical or research purposes, requires considerable care since, when manipulated and passaged *in vitro*, they can be prone to subtle changes which may not only damage their ability to replicate as stem cells but also cause a loss of their original capacity to differentiate into different cell types. Whilst it is likely to prove an extremely hard or even an impossible task to standardise the cells themselves, the framework of procedures and conditions under which they are cultured, preserved and characterised can be carefully controlled and documented so that these may be reproduced in the recipient's laboratory. This process requires the Bank to work closely with depositors of cell lines in order to carefully capture their procedures and practices and translate these into standardised protocols which may be

passed onto the recipient of the cell line. Thus, the ability of the recipient to best utilise the UKSCB is ensured through a combination of:

- Reliable and qualified stocks of cryopreserved cells;
- Accurate and user-friendly protocols which include best practice adopted by the depositor;
- Support for training and education that may also involve, or be driven by, the depositors themselves.

Thus, the remit of the Bank encompasses a spectrum of varied activities, including:

- Establishment of well-characterised and reliable banks of stem cell lines available to researchers in the UK and elsewhere;
- Provision of cell banks as starting materials appropriate for clinical use;
- Ensuring appropriate safety testing regimes tailored separately for research and clinical grade cells;
- Performing appropriate studies in collaboration with the depositor to demonstrate the characteristics of the material to be released by the Bank;
- Assessing the performance of the cell lines at different passage levels;
- Ensuring appropriate agreements are in place to enable unhampered research whilst protecting the intellectual property of the depositors;
- Supporting opportunities for training in the culture, preservation and characterisation of stem cells in line with the requirements of the stem cell community.

In the following sections, we will describe how the UK Stem Cell Bank is approaching these various elements of its remit and the perceived challenges for stem cell banking centres.

### 3. Regulation, management and facilities

The complex legal and ethical issues involved in the use of embryos to generate stem cell lines led to the setting up by the House of Lords of a high-level Steering Committee to provide direction in these areas in the UK. The committee, chaired by Lord Patel, includes senior representatives of various regulatory bodies (e.g., Medicines and Healthcare Products

Regulatory Agency (MHRA), the Human Fertilisation and Embryology Authority (HEFA)), the National Blood Service, key research groups, ethicists and legal advisory groups. The Steering Committee, in its capacity as overseer of the Bank, has developed a Code of Practice for the UK Stem Cell Bank ([www.mrc.ac.uk](http://www.mrc.ac.uk)) with which the Bank has to comply. In addition, as a future participant in the generation of cells for clinical therapies, the Bank must also comply with the current Department of Health guidelines for UK tissue banks [1]. The Bank has been inspected under the Code by the UK regulatory authority (the MHRA) and was accredited in June 2004.

The ethos of the Bank is to work in a transparent and accountable manner. It is also required to remain independent and free of any conflicts of interest. To this end, it has been required to refrain from any involvement in commercial product development or basic research into stem cell biology. However, it is vital that the Bank engages not only with stem cell researchers, in grant-funded projects (to provide support on cell banking issues) but also to provide companies developing new products with advice on quality and safety issues as NIBSC already does for a broad range of biological medicines.

High-quality cell lines for research and clinical therapy require facilities which can produce banks of cells under appropriate conditions. Research grade lines are currently produced in the recently renovated clean-rooms built for the Division of Cell Biology and Imaging which provide a clean air environment within which to process the cell lines. Clinical-grade cells (those that will in the future provide material for clinical trials and therapies) require a more stringent environment similar to that employed within the pharmaceutical industry. To this end, the Bank has established a suite of laboratories to EU cGMP [2]. The GMP facility comprises three self-contained Grade B laboratories, each with a Class II microbiological safety cabinet providing the Grade A air environment required for open processing of cell materials (Fig. 1) (for a comparison of the different international classifications for airgrade, see [3]).

The laboratories are self-contained and independent of each other in order to minimise the potential for cross-contamination (Fig. 2A). Each laboratory is capable of operating independently in fumigation mode. This will allow cell banking to be carried out

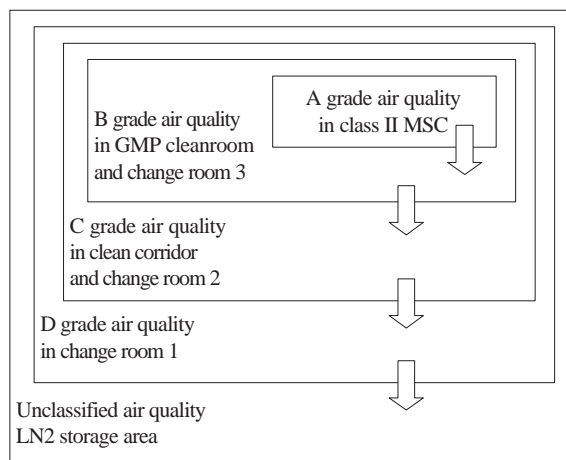


Fig. 1. Flow of air grade quality in the EU cGMP facility of the UKSCB.

on a campaign basis followed by post-campaign fumigation of the laboratory and all equipment. The laboratories are accessed via an airlock leading onto a central access corridor (itself a Grade C area). Access to this central corridor is via a two-stage airlock, which controls movement into and out of the cleanroom environment. These facilities, built over the course of 2003, have undergone a full programme of validation prior to inspection and accreditation by the MHRA in 2004.

#### 4. Cell banking, safety testing and quality assurance

An important means of establishing appropriate quality systems for the Bank was the preparation of 'process maps' for all activities undertaken in the Bank. An example of such a map is shown in Fig. 3. Detailed process mapping has enabled the Bank to identify clearly all the steps in critical procedures and ensure that the entire process from deposition of cells to provision to recipient is properly controlled and documented, thereby ensuring a consistent approach and assure as far as is possible a reproducible product.

Cell banking begins with the receipt of cell material into the Bank (see Fig. 3). On arrival at the Bank, the cells are placed in frozen storage in quarantine until pre-banking safety criteria are met.

Once met, the cells are released for the production of the Pre-master Cell Bank. This is a limited bank of cells, most of which is used for the initial safety and other quality assurance tests necessary to permit the Master Cell Bank to be produced. From the Master Cell Bank are produced over time the Distribution Banks. This tiered banking system is important to ensure that cells can be provided over decades at the same passage level.

In order to proceed from the Pre-master Cell Bank to the Master and thence to the Distribution Bank, a number of quality assurance and safety tests must be performed and produce results within acceptable limits. These are particularly stringent in the case of clinical grade material and NIBSC is already well versed in the requirements for such cell lines as it produces a range of cell lines for diagnostics, virus isolation and the manufacture of vaccines. During the banking process segregation of fully tested cell

A



B



Fig. 2. Equipment in the UKSCB: (A) one of the three clean-room laboratories. (B) Liquid nitrogen storage vessels for the banking of the stem cell lines.

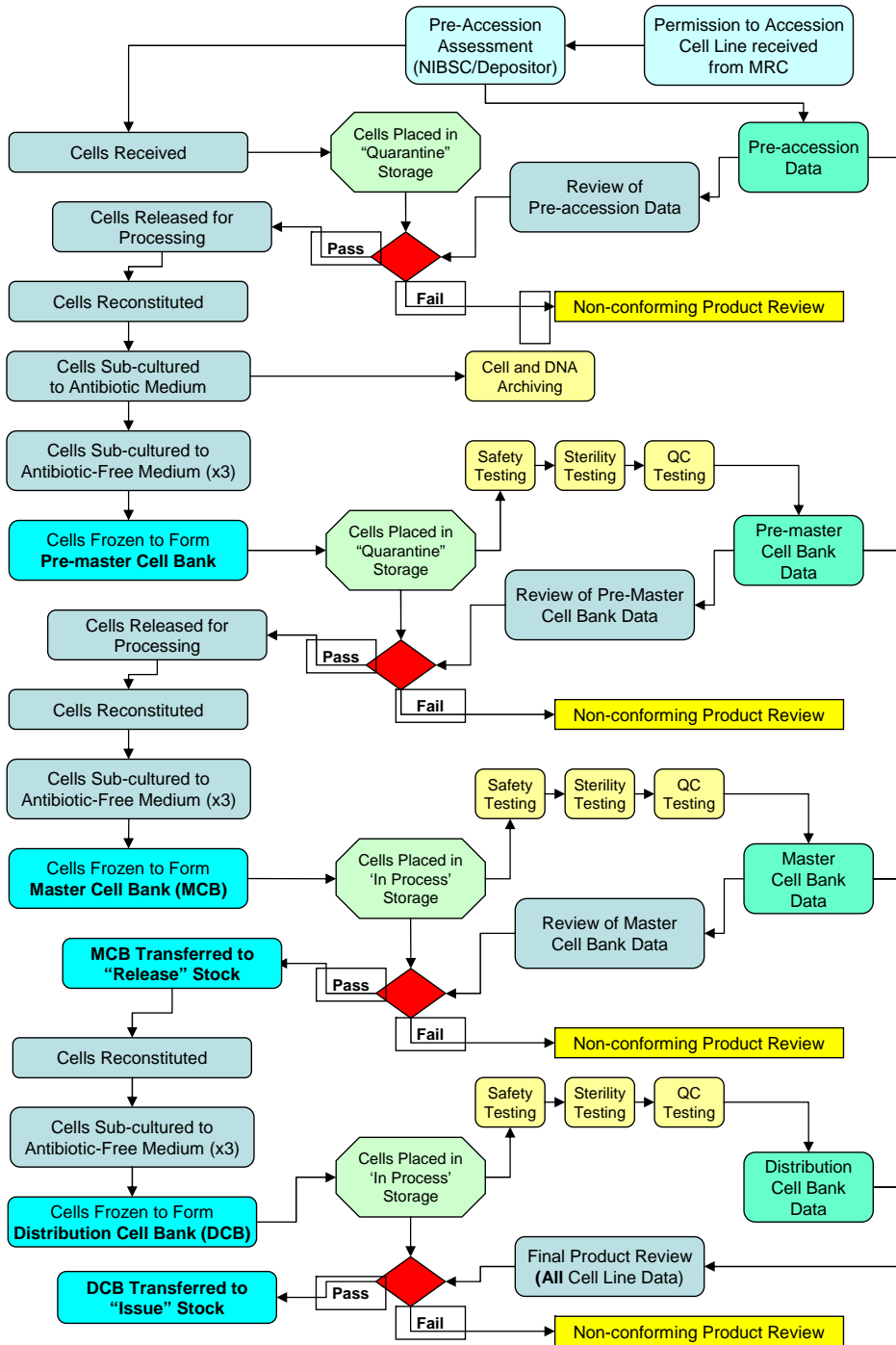


Fig. 3. Overview of the receipt, quarantine, processing, storage and issue of stem cell lines.

banks from partially tested and untested banks is ensured by physical separation of cell banks in one of three liquid nitrogen storage refrigerators for “Quarantined”, “In-process” and “Released for use” material (Fig. 2B).

Both safety testing and many quality control criteria are based on a detailed risk assessment undertaken in conjunction with the depositor and UKSCB advisors. Many of these tests will be common to all cell lines but others will be specific to particular cell lines. While many tests can be performed “in-house” under the scope of the quality system ISO 17025, some will be outsourced to specialist, accredited laboratories. Core safety tests will include:

- Tests for viruses including HIV 1 and 2, HBV, HCV, and HTLV 1 and 2;
- Tests for mycoplasma by PCR and culture;
- Tests for sterility based on the European Pharmacopoeial guidelines;
- Tests for adventitious agents including electron microscopy and cell line inoculation.

For clinical grade cells, a much more extensive set of tests for adventitious agents will be applied based on the risk assessment. These will include tests for agents that may be introduced in cell culture reagents of animal origin or from xenogeneic feeder layers currently used in the growth of many embryonic stem cell lines.

In addition to the safety testing regime, a number of quality assurance tests will need to be performed. Viability testing will be the cornerstone of Quality Assurance (QA) and tests will need to be developed to supplement the routine methods of assessment currently used in most cell banks. Each line must also be authenticated by DNA fingerprinting in order for depositors and users to be confident that the lines are bona fide and are not cross-contaminated with pre-existing cell lines. Cross-contamination has been an ongoing problem for many other types of immortalised cell lines [4–8].

Epigenetic changes may also need to be investigated, since these may effect subtle changes in the characteristics of the stem cells. Phenotyping of cells will be undertaken using a panel of well-defined antibodies, selected by experts in the field, and tailored to each line. Though such a panel is not yet

available, the Bank is involved in a new international project to characterise approximately 80 ES cell lines worldwide (see below). The Bank is providing a broad range of potentially useful antibodies which may facilitate the development of a standard panel for characterisation of ES cells. All cell lines will undergo both molecular and phenotypic characterisation. As a matter of routine, the stability of stem cell lines in culture also will be addressed by establishing cell banks at high passage for comparison with the Master Bank. This process is especially important for stem cell lines in view of recent research which indicates that the karyotype of ES stem cells is not stable [9].

The robustness of stem cell culture procedures will also need to be explored to facilitate standardization, since the complex biology of stem cells is reflected in their *in vitro* growth characteristics. Each line may be expected to have its own set of unique procedures for growth and preservation. ES cell lines require a mouse fibroblast feeder layer to promote their proliferation in an undifferentiated state and the Bank will need to provide and ensure the quality of the feeder layers required for the growth of ES cell lines. It will also be engaged in optimising cultures and preservation protocols as well as developing alternative cell-free methods. For cells intended for use in humans, as part of the regulations of the MHRA accreditation (see Section 4) all media components used within the GMP facility will be fully traceable, fulfilling a requirement important to clinical users.

## 5. Scientific collaboration

The UKSCB is committed as part of its remit to establishing a programme of research that improves the banking of stem cells including cryopreservation and cell characterisation. During 2004, the Bank became involved in an international stem cell project initiated by the MRC. This project, known as the International Stem Cell Initiative, will explore the characteristics of embryonic stem cells at both the cellular and molecular level. The UKSCB and NIBSC are acting as the ‘technical hub’ laboratory sending out reagents and receiving material for processing. The project is intended to

characterise a majority of human ES cell lines currently available worldwide by identifying the salient features of ES cell lines, thus determining the degree of diversity between lines. More than 15 stem cell research groups are now engaged in the project from the UK, USA, Canada, Japan, Australia, Israel, Czech Republic, Sweden and Finland. Under the coordination of Professor Peter Andrews (University of Sheffield, UK), these centres will be characterising over 80 different ES cell lines most of which have been established since 2000. Through the use of standardised methodologies, cell culture reagents and reference reagents such as reference antibodies, samples of each cell line will be provided by the participants, encoded at the UK Stem Cell Bank and forwarded to expert laboratories for investigation. Each cell line will undergo:

- Phenotypic profiling, using a panel of 18 antibodies to a range of markers known to be expressed in undifferentiated and differentiated ES cell lines;
- Gene expression profiling, using a panel of 48 genes known to be expressed in stem cells;
- Genetic identity tests to exclude cases of cross-contamination with early established ES cell lines;
- Microbiology tests for sterility, mycoplasma and adventitious agents.

In addition, histopathology of sections of tumours produced in animals will be compared centrally by an expert histopathologist and karyological data produced by each centre on their cell lines will also be collated. Further information on this project is available on the MRC web site ([www.mrc.ac.uk](http://www.mrc.ac.uk)).

## 6. Communication, training, and education

The UKSCB has established a close liaison with many in the scientific community both in the UK and abroad. This has been important in establishing the role of the Bank and in the longer term will enable the Bank to respond to both the needs and views of users and depositors. The Bank has begun to forge links with expert centres around the world as well as with national and international stem cell research networks. In addition, members of the Bank are actively

engaged in a programme of presentations to communicate its aims and purposes.

Involvement in training is also seen as a valuable role for the Bank. Over the last 2 years, the Bank has participated in the “Practical Course on Working with Stem Cell Lines” developed by the Centre for Stem Cell Biology at the University of Sheffield. It has hosted a number of 1-day symposium and workshops for stem cell researchers and IVF clinics and is actively seeking opportunities to develop this important aspect of its work. The Bank has a website which can be found at <http://www.ukstemcellbank.org.uk>.

## 7. Concluding remarks

In conclusion, the UK Stem Cell Bank has been established to work with the scientific and clinical community to assure the quality of human stem cell lines used in research and therapy. By providing a centralised resource not only for stem cell lines but also for expertise in cell banking, cryopreservation, quality systems and regulatory frameworks, it will seek to ensure that developing research technology can be rapidly translated into the stringent quality systems and standards required for the development of stem cell therapies. It is hoped that the quality assurance and safety procedures employed in the UKSCB may provide useful standards for other stem cell banks.

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