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Animal Cell Culture Collections and the Supply of Authenticated Cultures and Services for Industry and Research

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Introduction

The need for a supply of reliable authenticated cultures is not unique to animal cell technology. Culture collections for other micro-organisms have existed prior to the development of this particular branch of science but the relatively recent and rapid developments in animal cell technology have resulted in the establishment of several new Resource Centres. The purpose of this review is to give the most up-to-date information on existing collections and to provide all potential users with enough background on their activities to obtain the full benefit of the services offered.

The development of animal cell technology

The technology of cryopreservation is of fundamental importance to animal cell culture and advances have come about because of the ability to preserve most cells indefinitely in liquid nitrogen at −196°C. Maintaining a large range of cell lines as living cultures is impractical and carries the dual risk of microbial

Abbreviations: ACDP, Advisory Committee on Dangerous Pathogens; ATCC, American Type Culture Collection; CERDIC, Centre Européen de Recherches Documentaires sur les Immunoclones; CODATA, Committee on Data for Science and Technology; DKFZ, Deutsches Krebsforschungzentrum; DMSO, dimethyl sulphoxide; EBV, Epstein–Barr virus; ECACC, European Collection of Animal Cell Cultures; ECCO, European Culture Collections Organisation; EHCB, European Human Cell Bank; EPO, European Patent Office; HDB, Hybridoma Data Bank; ICSU, International Council of Scientific Unions, IDA, International Depository Authority; IUIS, International Union of Immunological Societies; JCRB, Japanese Cancer Resources Bank; MINE, Microbial Information Network Europe; MSDN, Microbial Strain Data Network; NBCS, new-born calf serum; NIA, National Institute of Aging; NIGMS, National Institute of General Medical Sciences; RFLP, restriction fragment length polymorphism; WDCM, World Data Centre on Microorganisms, WFCC, World Federation for Culture Collections; WIPO, World Intellectual Property Organisation.

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contamination and genetic drift (leading to a loss of unique characteristics) in successive cell generations. The technique on which long-term storage depends has developed from fundamental observations made in the late 1940s that the addition of glycerol to fowl semen improved cell survival during freezing and subsequent storage for prolonged periods at low temperatures (Polge, Smith and Parkes, 1949). Cryopreservation in the presence of glycerol was then applied successfully to other cell types; another important discovery was the ability of dimethyl sulphoxide (DMSO) to cryoprotect (Lovelock and Bishop, 1959). The need for well-characterized authenticated reference material is the general principle behind the establishment of all service culture collections. However, it is still evident that all too often poorly characterized and inadequately quality controlled cell stocks are exchanged between laboratories. Often such poor material can be the basis of major research programmes. The increasing use of cell lines for the generation of unique protein products intended for therapeutic use emphasizes the need for rigid adherence to quality control measures. This is essential for compliance with regulatory requirements mandatory for substances used in medicine. The particular quality control problems associated with animal cell cultures will developed later but they can be summarized into two specific problem areas. First, the presence of mycoplasma (a prokaryotic organism that does not possess a cell wall) as a frequent contaminant in cell cultures (Barile, Happs and Grabowski, 1978) and, secondly, the high incidence of inter- and intraspecies cross-contamination (Nelson-Rees and Flandermeyer, 1977; Nelson-Rees, Daniels and Flandermeyer, 1981). To remove the risk of using contaminated material, access to the well-documented and well-characterized stocks of a culture collection must be a prerequisite of any research and development programme in animal cell technology. The requirement for animal cell lines developed in the early 1950s as a demand from the vaccine industry, with movement away from *in vivo* methods of production (Rowlands, 1987). Vaccine cell substrates of well-defined and well-characterized material, e.g. the WI38 human diploid cell strain (Hayflick and Moorhead, 1961), were established in tissue culture laboratories in government institutes and academic departments, and were released to industry and into public collections. However, this later became a contentious issue with regard to intellectual property rights (Hayflick, 1984). The development of animal cells in biotechnology, although slower to emerge than exploitation of micro-organisms, has formed the basis of a major new industry and continues to develop. This stemmed from the use of hybridoma technology to produce cell lines giving specific antibodies and also differentiated cell types producing interleukins and growth factors. Technology has now advanced so that genes may be incorporated for the production of a rapidly increasing range of valuable protein products that are of major importance in both diagnosis and therapy. New advances in Baculovirus vector systems has resulted in the promotion of insect cell lines for protein production with increased yields—currently one of the major drawbacks of any animal cell production process (Ratafia, 1987).

Information centres

New Resource Centres to maintain and distribute cultures are being established in many countries (*Table 1*). Up-to-date information is collated by the World Federation for Culture Collections' (WFCC) World Data Centre on Microorganisms (WDCM) at the Riken Institute in Japan. It is important to use

Table 1. Information centres

Address	Contact
World Federation for Culture Collections' World Data Centre, Riken, 2–1 Hirosawa, Wako, Saitama 351–0, Japan	Fax: 0484 64 5651 Electronic Mail: Dialcom 42: CDT 0007
ECCO Information Centre, DSM, Mascheroder weg 1, 3300 Braunschweig, FRG	Tel: 0531 618715 Fax: 0531 618718 Electronic Mail: BT Gold 10075: DBI 0178
Hybridoma Data Bank, CERDIC, 2° C.A.I. -Solarex, Avenue des Maurettes, 06720 Villeneuve-Loubet, France	Tel: 33 93 20 01 80 Fax: 33 93 20 01 81 Electronic Mail: BT Gold 10075: DBI 0006
Hybridoma Data Bank, Bioinformatics Department, ATCC, 12301, Parklawn Drive, Rockville, MD 20852, USA	Tel: 301 231 5585 Telex: 898 055 ATCC NORTH Fax: 301 231 5826 Electronic Mail: Dialcom 42: CDT 0004
Microbial Strain Data Network, Institute of Biotechnology, Cambridge University, 307 Huntingdon Road, Cambridge CB3 OJX, UK	Tel: 0223 276622 Fax: 0223 277605 Electronic Mail: BT Gold 10075: DBI 0001

these information centres as the interphase between the user community and the Resource Centres (*Table 1*). They can be accessed by electronic mail systems, which are becoming of increasing importance for the rapid transfer of information and documents. In Europe an information centre for all culture collection supplies and services has been established at the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH at Braunschweig, West Germany. This centre has the backing of the European Culture Collections Organisation (ECCO) and is financially supported by the Commission of the European Communities. It is an independent body with an international board of control to oversee its activities. The aim of the centre is to improve interaction between users and all collections in Europe. It provides a focal point for obtaining information on all aspects of culture collection

activities, services, contract research and development and training, as well as the primary role of culture supply.

Data bases

Other information can be sought via international data bases. These include MINE (Microbial Information Network Europe), sponsored by the Commission of the European Communities, and HDB (the Hybridoma Data Bank), sponsored by the Committee on Data for Science and Technology (CODATA). Keeping ahead with new developments is often difficult because of the cross-disciplinary nature of animal cell technology, which involves veterinary, biotechnological, agricultural and medical interests. These problems are now being met by the increasing and effective use of rapid electronic systems for data recovery.

CODATA/IUIS HYBRIDOMA DATA BANK

The Committee on Data for Science and Technology (CODATA) of the International Council of Scientific Unions (ICSU) and the International Union of Immunological Societies (IUIS) jointly sponsor the HDB. Contributing nodes of the HDB exist at the American Type Culture Collection (ATCC); the Riken Institute, Japan; Centre Européen de Recherches Documentaires sur les Immunoclones (CERDIC), France; The National Facility for Animal Tissue and Cell Culture, Poona, India; and The European Collection of Animal Cell Cultures (ECACC), UK. The role of the HDB network is to collect and disseminate data on hybridomas and other cloned cell lines of immunological interest, termed 'immunoclones' by Alain Bussard (Bussard, 1989). The types of information kept by the HDB are:

1. Developers of specific immunoreactive cell lines and products;
2. Immunocyte donors;
3. Immunizing agents and procedure;
4. Methods of conferring immortality;
5. Immortal fusion partners;
6. Antibody reactivities;
7. Uses and applications;
8. Availability and distribution.

The co-ordination centre for these activities is at ATCC in the USA. The number of searchable records is now over 15 000. This information is obtained directly from research workers or from the academic literature or commercial catalogues. Full addresses for the HDB nodes are given in *Table 1*. Any of these nodes will conduct specific searches on request. The CERDIC node also publishes *Immunoclones* a subscription journal of information summarizing new important journal articles and specific reviews of interest.

THE MICROBIAL STRAIN DATA NETWORK (MSDN)

There are several data bases available on the MSDN system–HDB, MSDN central directory to microbial and cultured cells with specific features, culture collection catalogues (ATCC, ECACC) and ATCC human gene probe and chromosome libraries. There is also international access to other data bases, through storage on the MSDN computer or via an electronic gateway to the host computer (for example, Information Centre for European Culture Collections, World Data Centre on Collections of Microorganisms).

The network uses the telecommunications services of the Dialcom family of service providers (Dialcom, Telecom Gold), thus allowing usage by scientists in over 100 countries throughout the world, whether academic or non-academic, organizations or individuals. The requirements to join the system are minimal. Access is by use of a PC and modem or through a mainframe computer. No special skills or equipment are needed. Registration incurs a single payment (currently US$15)—to purchase the user's handbook. Subsequent costs are for connect time and associated costs. The charges are related to usage alone and are therefore self-regulated.

Resource Centres

There are several ways in which Resources Centres have developed, either from a comprehensive culture collection such as the ATCC or the development from collections arising from specific research interests within an institute. Resource Centres receive financial support from government agencies usually in the form of grants or contracts, but in most cases the fees charged for cultures and services are also important sources of revenue. The major Resource Centres are listed in *Table 2*.

COLLECTION ACTIVITIES

Most core functions of the major Resource Centres are essentially similar:

Accession procedures for culture deposits

There are standard accession procedures for cell culture deposits covering the precise method for acquisition, characterization, preservation, cataloguing and distribution of deposits (Hay, 1984).

Acquisition

Depositors see the advantage to themselves of making material available through recognized repositories, having to fulfil requests from other laboratories can take up a lot of time and carries additional expense. However,

Table 2. Major culture collections

Collection	Activities	Holdings	Catalogue(s)
General			
European Collection of Animal Cell Cultures PHLS CAMR, Salisbury, UK (ECACC) Tel: 0980 610391 Fax: 0980 611315	Safe deposit Patent deposits Cell culture Cataloguing and distribution Microbial quality control Species verification Training	1500, including hybridomas and human genetic variant cell lines	Catalogue of cell lines and hybridomas (3rd Edn, 1988) HLA-defined cell lines European Human Cell Bank cell lines and DNA probes On-line through MSDN
American Type Culture Collection, Rockville, USA (ATCC) Tel: 0301 881 2600 Fax: 0301 231 5826	As above	2650 cell lines and hybridomas	Cell line and hybridoma catalogue On-line through MSDN
Specialist			
Japanese Cancer Resources Bank, Riken Gene Bank, Tsukuba, Tokyo, Japan (JRCB) Tel: 029075 43611 Fax: 029075 42616	Cell line storage and distribution	300 animal cell lines in eight constituent laboratories	Cell line catalogue
National Institute of General Medical Sciences (NIGMS); National Institute of Aging, Cell Culture Repository, Camden, USA (NIA) Tel: 0609 966 7377	Cell line storage and distribution	3700 cell lines for human genetic studies	Cell line
Tumorbank, Deutsches Krebsforschungzentrum, Heidelberg, FRG (DKFZ) Tel: 06221 4841	In collaborating laboratories	500+	Tumor cell bank catalogue

increasingly there are the problems of intellectual property rights and the potential commercial exploitation of material deposited with public collections and thus made freely available (Morris and Doyle, 1988). The Patent Depository procedure may be one route to retaining rights over the material deposited (Bousfield, 1989). However, it is often difficult to persuade potential depositors to release their material to public collections, although in many cases this may be resolved by offering to delay release for a pre-determined period once the material is received [e.g. 2 years with European Human Cell Bank (EHCB) deposits at ECACC].

Deposit procedures

Following discussion with the collection staff, deposits are shipped to the collection in the form of frozen ampoules and are accompanied by a deposit form (*Figure 1*) which gives full details on the species, strain, tissue of origin and their product/function of interest. At the initial culture stage basic quality control will be carried out and an initial 'token freeze' stock is made. The tests performed are for microbial contamination, including mycoplasma, together with isoenzyme analysis for species verification. Once these tests are completed a master cell bank ('seed' stock) and working cell bank ('distribution' stock) are prepared. This procedure is completed in the minimum number of cell passages in order to prevent the problem of genetic drift or to avoid the onset of senescence in cell lines with a finite lifespan. The master cell stock remains untouched until a replacement working cell bank is required on depletion of distribution stocks.

Basic quality control procedures are repeated on the working cell bank. Information on the deposits is given in the latest edition of the culture collection catalogue. Increasingly, on-line information services of culture collection deposits are available.

It is usual for the culture collection to return a sample of the distribution stock or, alternatively, with hybridomas or lines producing growth factors a sample of cell culture supernatant is returned for assay by the original depositor. In some cases further extensive characterization work is carried out to give an important cell line certified deposit status. This can include karyotyping, HLA typing, reverse transcriptase assay for retrovirus detection and tumorigenicity testing.

Services

Each collection listed in *Table 2* has a number of core functions in common, most have cataloguing and distribution services, provision of cultures and safe deposit facilities. In addition, some have Patent Depository status. Patent Deposit procedures are regulated internationally by the Budapest Treaty, 1977. As part of a patent application in addition to written disclosure it is necessary to place an example of the organism in a recognized public collection. Eventually each deposit is made available to the public. The importance of the Budapest Treaty deposit system is that it is necessary to make only one deposit

ACCESSION FORM FOR CELL CULTURE DEPOSITS

ECACC use only

Date Received .... Viability test ....

Depositor No. .... Contamination test ....

Lab. Ref. No. .... Distribution status ....

DEPOSITOR INFORMATION

Name of depositor ....

Institute .... Hybridoma ....

Department .... Genetic Recombinant ....

Address .... Gene Marker ....

.... Other ....

....

ORIGIN OF CELL CULTURE

Identification Code ....

Name in full ....

Species and strain ....

Organ/Tissue ....

IF HYBRIDOMA

Immunised animal ....

species ....

strain ....

Myeloma designation ....

CULTURE CONDITIONS

Medium (give full details on additional sheet if special formulation)

....

Sodium bicarbonate (mg/l) .... %/serum and type ....

pH .... Temperature .... Gaseous phase ....

Any additional supplements (including antibiotics) ....

....

give concentrations ....

Optimal split ratio .... Limited lifespan *in vitro* Yes .... No ....

Technique for routine sub-culture ....

Sterility checks already performed:

Bacteria Yes .... No .... Fungi Yes .... No ....

Mycoplasma Yes .... No .... Viruses Yes .... No ....

Known contaminants ....

CELL STORAGE CONDITIONS

Composition of medium .... Cell concentration ....

Method ....

(cont'd)

ADDITIONAL INFORMATION

Cell products/characteristics ....

Morphology .... Growth in suspension .... Ploidy (Modal No.) ....

If Hybridoma — state antibody specificity/subclass ....

....

Secretion stable Yes .... No ....

Principal literature reference (if any) ....

....

Culture deposited elsewhere Yes .... No ....

If YES state where and reference no. ....

....

Address, telephone and telex number where additional technical enquiries can be made

....

....

....

Tel. No.: .... Telex No.: ....

Signature .... Date ....

EUROPEAN COLLECTION OF ANIMAL CELL CULTURES
PHLS Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wilts, SP4 0JG, UK

Figure 1. ECACC accession form for cell culture deposits.

with a recognized International Depository Authority (IDA) and this is then recognized by many national patent offices (including those in the USA, UK, West Germany, France and Japan) and, indeed, the European Patent Office (EPO). IDA status is regulated by application to the World Intellectual Property Organisation (WIPO) based in Geneva. Each collection must meet specific requirements to make a valid patent deposit, and detailed information is available elsewhere (Bousfield, 1989). In addition, there are other activities which are offered as contract services: information/data banks, training courses, quality control testing, bulk culture and cell evaluation studies.

Distribution

All cell stocks are held as frozen deposits in liquid nitrogen storage. Distribution can be either of frozen stock, on dry ice, or as growing cultures. Shipment can be via postal services (e.g. in Europe, Datapost) or by international air freight. Ideally, samples should not be kept at dry ice temperatures for more than 72 hours. Growing cultures are normally distributed with the culture flask completely filled with growth medium or maintenance medium. Under these conditions cells despatched subconfluent can survive for up to 5 days if ambient temperatures are maintained.

Order procedure

Collections accept orders by mail, telephone, telex, fax or electronic mail. In some cases it is necessary to complete a disclaimer before the release of certain cultures together with a statement of intended use. With ECACC this is

AGREEMENT BETWEEN THE EUROPEAN COLLECTION OF ANIMAL CELL CULTURES AND RECIPIENT OF CELL LINE(S)

The cell line(s) listed below will be made available subject to the following conditions.

1. The cultures are to be used either by
   a) Scientists affiliated to a non-profit Research Institute in pursuance of an academic research programme.

   or

   b) if by commercial or similar interests, they will not be offered for sale or be utilised in commercial processes involved in the preparation for sale of any biological material or in any other type of commercial activity without having secured written agreement from the ECACC on behalf of the original depositor. ECACC will refer back to depositors all such requests.
2. The cell line(s) will not be distributed to third parties.
3. If the cell line(s) are referred to in any publication, then correct reference will be made to the work of the original depositor and no alteration will be made to its ECACC title or acronym.
4. It is understood that neither ECACC or its depositors accept any liability whatsoever in connection with the handling or use of the cell line(s).

Signed for and on behalf of ....

By the recipient ....

Name: .... Date ....

Position: ....

Address: ....

....

Cell line(s) ....

....

EUROPEAN COLLECTION OF ANIMAL CELL CULTURES

PHLS Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wilts. SP4 0JG, UK

Figure 2. ECACC disclaimer/agreement form for the release of cultures.

GOVERNMENT INSTITUTIONS
FORM TO BE COMPLETED BEFORE ATCC CAN SHIP CERTAIN CELLS

Cells Provided From The Human Tumor Cell Bank and/or The Hybridoma Bank (under NIH Contracts) and some of the TIB lines to Investigators at Government Institutions

I hereby agree that the cell lines provided are for research purposes only. Cell lines and their products shall not be sold or used for commercial purposes. Nor will cells be distributed further to third parties for purposes of sale, or producing for sale, cells or their products. Secondary distribution shall only be made under the terms outlined in this form. The cells are provided as a service to the research community. They are provided without warranty or merchantability of fitness for a particular purpose or any other warranty, express or implied.

Accepted:

Typed or Printed Name ______ Agency ______

Signature ______ Date ______ Division or Department ______

City/State ______

Cell Lines of Human Origin

I understand that, although human cells distributed by the American Type Culture Collection have been subjected to stringent tests and observations which indicate the absence of extraneous agents and deleterious properties, the ATCC accepts no responsibility for any injury (including injury resulting in death), damage or loss that may arise from the use of the cells, either directly (including use for diagnostic purposes) or in the preparation of a product. I assume all risks and responsibility in connection with their receipt, handling, storage and use.

Typed or Printed Name ______ Agency ______

Signature ______ Date ______ Division or Department ______

City/State ______

Upon receipt of this signed understanding, the ATCC will be able to meet this request for these cells and any further requests you may make in the future.

Human Tumor Cell Bank, brief statement of intended use:

______

______

______

American Type Culture Collection
12301 Parklawn Drive
Rockville, MD 20852 USA

telex: 908768 ATCCROVE
FAX: 301-231-5826

Figure 3. ATCC disclaimer form for the release of cultures (government institutions).

necessary with Research Council and certain other deposits (*Figure 2*), for ATCC separate agreements are needed for scientists in government (*Figure 3*) and non-government (*Figure 4*) institutions for Hybridoma Bank (HB) and Tumour Immunology Bank (TIB) lines, and a disclaimer is necessary if cell lines are of human origin. For the National Institute of Aging (NIA) and National Institute of General Medical Sciences (NIGMS) Bank a disclaimer noting that material is derived from patients is used.

Handling of cell lines on receipt

It is recommended that cell lines shipped frozen on dry ice are immediately resuscitated on arrival. However, if this presents difficulties for the receiving

NON-GOVERNMENT INSTITUTIONS
FORM TO BE COMPLETED BEFORE ATCC CAN SHIP CERTAIN CELLS

Cells Provided From The Human Tumor Cell Bank and/or The Hybridoma Bank (under NIH Contracts) and some of the TIB lines to Investigators at Non-Government Institutions

I hereby agree that the cell lines provided are for research purposes only. Cell lines and their products shall not be sold or used for commercial purposes. Nor will cells be distributed further to third parties for purposes of sale, or producing for sale, cells or their products. Secondary distribution shall only be made under the terms outlined in this form.

The cells are provided as a service to the research community. They are provided without warranty or merchantability of fitness for a particular purpose or any other warranty, express or implied. In addition, the recipients of the cell lines agree to indemnify and hold harmless the United States from any claims, costs, damages, or expenses resulting from any injury (including death), damage, or loss that may arise from the use of the cell lines.

Accepted:

Typed or Printed Name — Institution

Signature — Date — Department

City/State

Cell Lines of Human Origin

I understand that, although human cells distributed by the American Type Culture Collection have been subjected to stringent tests and observations which indicate the absence of extraneous agents and deleterious properties, the ATCC accepts no responsibility for any injury (including injury resulting in death), damage or loss that may arise from the use of the cells, either directly (including use for diagnostic purposes) or in the preparation of a product. I assume all risks and responsibility in connection with their receipt, handling, storage and use.

Typed or Printed Name — Institution

Signature — Date — Department

City/State

Upon receipt of this signed understanding, the ATCC will be able to meet this request for these cells and any further requests you may make in the future.

Human Tumor Cell Bank, brief statement of intended use:

American Type Culture Collection
12301 Parklawn Drive
Rockville, MD 20852 USA

telex: 908768 ATCCROVE
FAX: 301-231-5826

FORM-100-NG

Figure 4. ATCC disclaimer form for the release of cultures (non-government institutions).

laboratory the material can be returned to liquid nitrogen, but this could result in viability problems. If there are specific hazards associated with the handling of the shipped material these are indicated. It is the responsibility of each collection to ensure that the receiving laboratory has suitable facilities for the safe use of pathogenic material. This has been emphasized by recent legislation in the UK (Control of Substances Hazardous to Health Act, 1988), e.g. the expression of hepatitis B surface antigen (HBsAg) by the PLC/PRF 5 cell line means that suitable containment conditions must be used in handling. The collection must be assured that correct handling facilities exist prior to despatch. Also, if cell lines are derived by Epstein–Barr virus (EBV)

transformation of peripheral blood lymphocytes, or derived from other patient material (including biopsy), there should be minimum guidelines for the handling of cultures, which may be largely uncharacterized and not examined fully for the presence of potentially hazardous contaminants. However, this does not infer that all cell lines are potentially hazardous, but there should always be a cautious approach where laboratory safety is concerned.

An example of such guidelines, published in the ECACC European Human Cell Bank catalogue, are given below:

Safety considerations on the handling and use of Epstein–Barr virus transformed human B-lymphoblastoid cell lines. Epstein–Barr virus (EBV) is an ACDP (Advisory Committee on Dangerous Pathogens) category 2 pathogen (ACDP, 1984). EBV is not considered to be a significant hazard to health—up to 90% of individuals are already infected with the virus.

Laboratory facilities

1. There should be adequate space (24 m^3) in the laboratory for each worker.
2. Work may be conducted on the open bench except where there is the likelihood of aerosal generation, in which case an appropriate safety cabinet is recommended.
3. If the laboratory is ventilated mechanically, there should be a negative airflow hepafiltered direct to atmosphere from the laboratory. If an independent plenum and extract system is not available, this can be achieved by the use of a suitable safety cabinet. All facilities should be properly maintained to ensure their efficiency.
4. The laboratory should contain a wash-basin located near the laboratory exit to allow hand washing on completion of work.

Laboratory practices

1. Laboratory coats or gowns should be worn at all times and should be removed before leaving the laboratory.
2. Eating, drinking, smoking and the application of cosmetics must not be permitted in the laboratory.
3. Mouth pipetting must not be allowed. A number of automatic or semi-automatic pipetting devices are commercially available.
4. The swabbing of work surfaces with effective disinfectants should be a routine part of laboratory procedure.
5. Discarded materials should be stored safely prior to their decontamination and disposal. Transportation of any potentially infectious material should be in robust and secure containers so that accidental spillage is impossible.

Supervisory considerations

1. All staff should be adequately trained in aspects of cell culture and the

handling of potential pathogens. They should be aware of safety policy and any national or local codes of practice. They should also be capable of dealing with emergencies if they arise.

2. Medical surveillance is important and should include the taking of baseline serum samples from staff on initial deployment to the laboratory. There should also be screening procedures for monitoring the serum antibody titres of staff exposed to EBV from B-lymphoblastoid cell lines over a long period.
3. All accidents should be recorded and reported to the appropriate authorities.

Preservation techniques and long-term storage of animal cell lines

As already discussed, the preservation of animal cells at −196°C in liquid nitrogen is the basis of animal cell technology (Doyle, Morris and Armitage, 1988). However, there are several principles to be followed in order to ensure maximum viability of cryopreserved cell stocks. In summary, the technique can be described as 'slow freeze' and 'fast thaw' in the presence of high protein concentrations and a cryoprotectant (e.g. DMSO). Cultures must be in the log phase of growth (ensured by the addition of fresh growth medium 24 h prior to freezing) and only in exceptional circumstances should cells with less than 80% viability be frozen, because the resulting debris formed on thawing may inhibit cell recovery. If cells are adherent and are removed from the monolayer by using a proteolytic enzyme (e.g. trypsin), it is essential to neutralize the enzyme by the addition of serum or by washing the cells prior to cryopreservation.

The freezing medium is an important consideration. At ECACC, 91% NBCS (new-born calf serum) and 9% DMSO is used. The high protein concentration is protective and the use of whole serum avoids the possibility of a pH change in phosphate-buffered media during the cryopreservation step. Storage is carried out in either plastic (Nunc A/S, Roskilde, Denmark) or glass (Wheaton Scientific, Millville, NJ, USA) 1 ml ampoules. Despite the long-term advantages of the latter, there can be problems with ensuring that ampoules are correctly sealed. They should be quality controlled after sealing (placed in methylene blue solution at 4°C for 30 min) to avoid entry of nitrogen into improperly sealed ampoules and subsequent explosions.

Liquid nitrogen storage facilities can vary in size and capacity from small (3000 ampoules) to large (40 000 ampoules, *Figure 5*). It is important to ensure that an alarm system is installed to monitor the level of nitrogen in the refrigerator to prevent accidental loss from leaks or evaporation.

There are very few cell lines that cannot be stored successfully—at ECACC a particular problem has been encountered in the long-term storage of human T-cell lines. However, the substitution of glycerol instead of DMSO, together with the inclusion of certain growth factors (IL-2) in the storage medium, produce better results (Morris and Doyle, 1988).

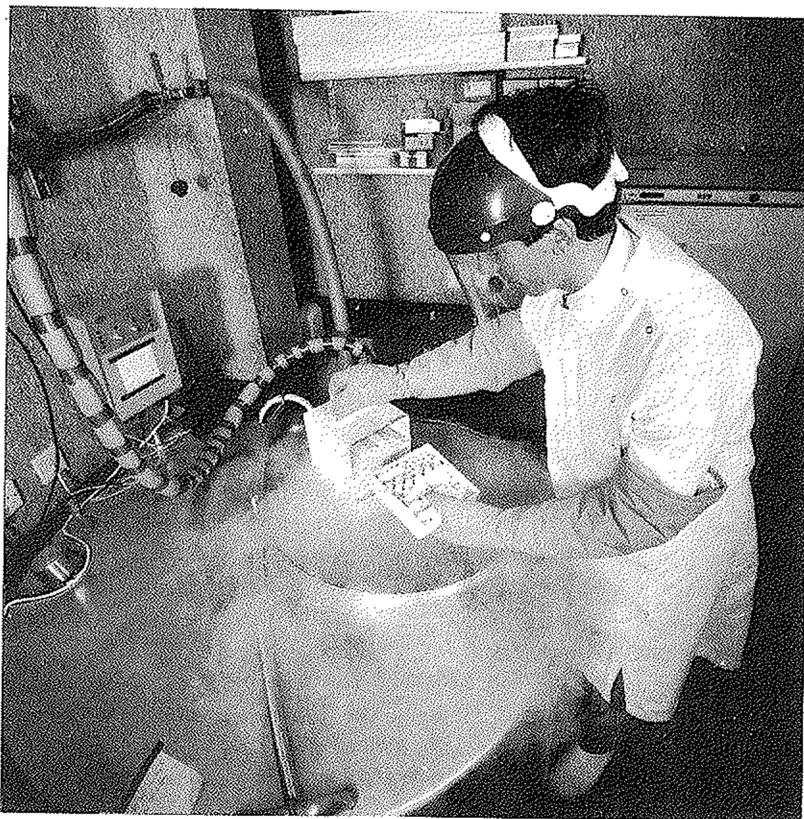

Figure 5. Liquid nitrogen storage tank fitted with automatic level controller and alarm system. The ampoules are kept in a storage tank inventory system.

Quality control techniques and cell characterization studies and research and development within culture collections

Culture collections routinely perform cell characterization techniques and are therefore in a position to offer services on a commercial or contract basis.

ISOENZYME ANALYSIS

Traditionally cell identification has been based on cytogenetic analysis but this is a time-consuming technique and may produce unusual results with tumour cell lines that have been in culture for some considerable time. Increasingly, isoenzyme analysis has been an important tool and systems such as the 'Authentikit' of Innovative Chemistry Inc. (Marshfield, Mass., USA) has enabled the production of rapid and reliable fingerprinting results in a day.

Isoenzymes from different animal species [e.g. glucose 6-phosphate dehydrogenase (EC 1.1.1.49) and mannose phosphate isomerase (EC 5.3.1.8)] have unique electrophoretic mobilities. By selecting several enzymes an overall pattern is produced for each species. By comparing these to a standard (mouse) and control (human) a comparison with known values for known species can be made. Thus the species may be easily and quickly verified. The technique can detect contaminating species down to a level of 10%, but in our experience it is not sensitive enough to differentiate between strains of the same species.

DNA FINGERPRINTING BY RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP) METHODS

A new development is the use of probes to produce characteristic DNA fragment patterns (RFLP) for individual cell lines. Minisatellite probes were first described for this purpose by Jeffreys, Wilson and Thein (1985) and are marketed by ICI Cellmark Diagnostics, Abingdon, UK. The method involves digestion of cellular DNA with specific restriction enzymes, fractionation by agarose gel electrophoresis, followed by transfer of the DNA digest to a membrane by Southern blotting. In the method developed by Jeffreys, Wilson and Thein (1985) the membranes are exposed to radioactive minisatellite probes that react with complementary DNA fragments, the positions of which can be identified on autoradiography of the processed membranes. An example of this is given in *Figure 6*. Evidence suggested that JM cells were derived from the JURKAT line isolated from an acute lymphocytic leukaemia patient. Although they are thought to be identical there are certain morphological distinctions between these T-cell lines. Comparison between the two DNA fingerprints shows that with the multilocus probe 33.15 they are identical but the single locus probe mix does show some differences. These results show clearly that these lines are not identical, but are very closely related and almost certainly originated from the same person.

A further use of this technique has been in the detection of somatic changes in human cancer DNA (Thein *et al.*, 1987). The changes in most cases consisted of increased or decreased intensity of bands but in two cases of rectal carcinoma and one of gastro-oesphageal carcinoma additional bands were identified. The use of these probes is not restricted to human cell lines but can be applied to other animal species such as the Chinese hamster (Thacker, Webb and Debenham, 1988) and mouse hybridomas (ECACC and Cellmark Diagnostics, unpublished results). DNA fingerprinting by RFLP may be carried out using a variety of restriction enzymes and probes other than the original minisatellite probes developed by Jeffreys, Wilson and Thein (1985). Ryskov *et al.* (1988) reported the use of a 563-nucleotide sequence from phage M13 as a probe in RFLP methods to generate 'fingerprints' from the DNA of a variety of organisms, including mammalian cells, invertebrates, plants, yeasts and bacteria. There is also the possibility that means other than autoradiography, e.g. epifluorescence or enzyme-linked visualization, may be employed for fragment identification.

In this way, service culture collections can not only set high standards for the

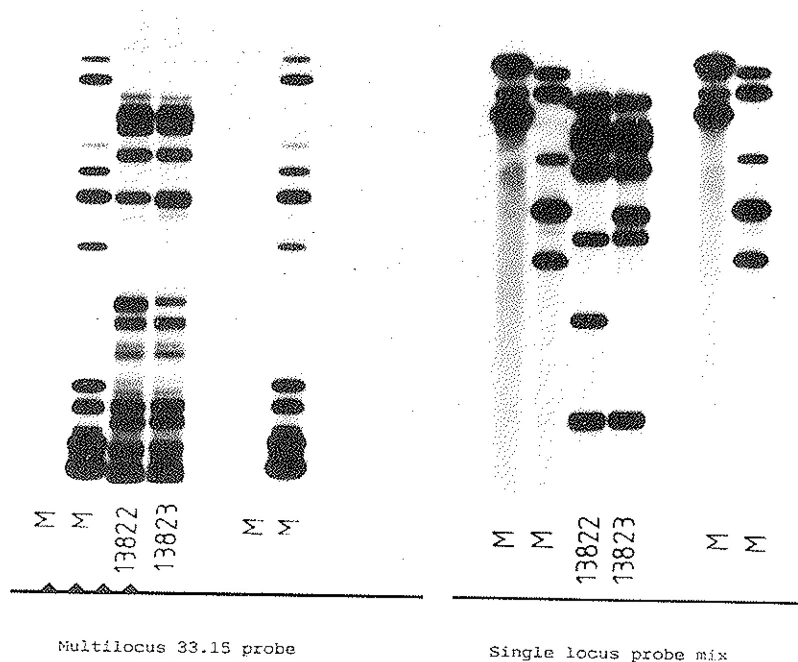

Figure 6. DNA fingerprinting analysis of human T-cell lines JURKAT (13822) and JM (13823). Lanes M are from standard reference proteins; lanes 13822 and 13823 are derived from JURKAT and JM cells, respectively. The multilocus (33.15) probe shows virtual identity whereas small genotypic differences are revealed by the single locus probe mix.

performance of quality control techniques, thereby generating data of the standard required for presentation to the regulatory authorities (e.g. the US Food and Drug Administration), but can also be at the forefront in the development of new techniques.

MYCOPLASMA DETECTION

At ECACC approximately 15% of cell lines received are contaminated with mycoplasma, although this is probably an artifically low figure compared with the actual incidence in most laboratories. Because of this particular problem cell lines are routinely held in a 'quarantine' laboratory on receipt so that preliminary studies can be completed before allowing the cell line to be handled elsewhere in the facility. Several techniques are available for the detection of mycoplasma. The most reliable methodology involves a combination of techniques, including Hoechst stain (*Figures 7 and 8*) and culture methods. *Table 3* lists all the techniques available with the relevant advantages and disadvantages.

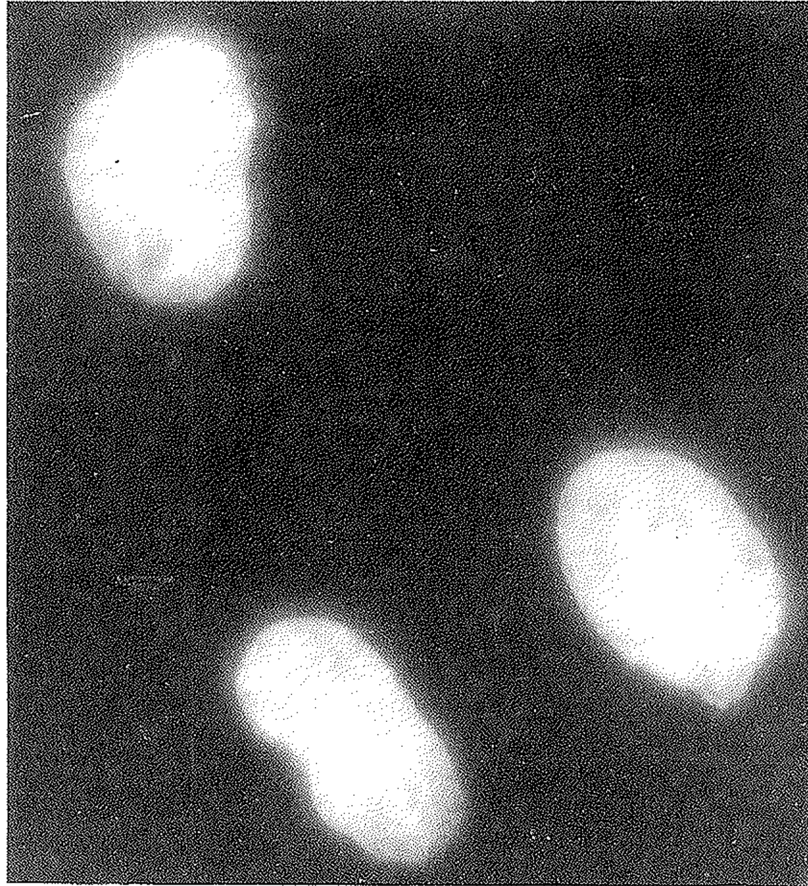

Figure 7. Hybridoma cells stained with Hoechst 33258. Only nuclear fluorescence is visible.

At ECACC the Hoechst and culture methods give a greater than 95% correlation and the techniques have the advantage of recognition by the US Food and Drug Administration in their *Points to consider in the manufacture of biologicals*, November 1987 (Esber, 1987).

Future trends

Two major problems face all culture collections. The first is the need to maintain a wide and representative range of cultures of required genetic diversity. The second is the problem of acquisition of organisms to meet potential demand despite the reluctance of some workers to deposit. This is particularly true in the animal cell culture field where commercial exploitation of cell line products can be a major consideration (Morris and Doyle, 1988a).

It has been calculated that only a fraction of available material in the natural environment is currently stored in culture collections. Limited manpower and storage resources inevitably impose a restriction on collections, often forcing

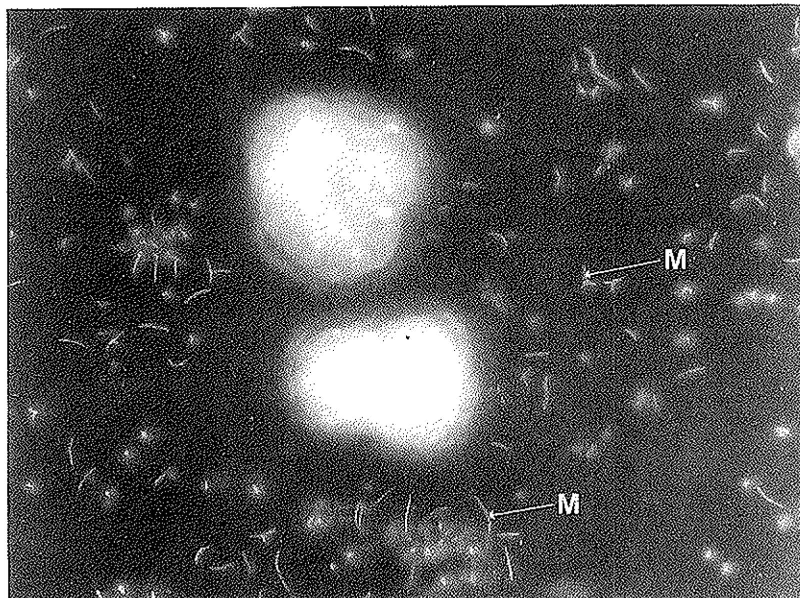

Figure 8. Mycoplasma-contaminated hybridoma cells stained with Hoechst 33258. The mycoplasma are present in the form of short filaments, marked M.

them to select those organisms thought to have the greatest scientific or commercial value.

Resolving the problem of covering genetic diversity and encouraging deposition essentially involves a change in attitude from both research-funding bodies and the scientific community. An appreciation of the need to conserve natural resources has clearly been seen in many countries over the past decade. If this work is to continue and even expand, the interaction between governments and organizations such as the WFCC must increase. An educational programme co-ordinated by the WFCC to increase awareness of the resources available now includes a range of training courses and bursaries.

The immediate greater problem is one of deposition, especially in the case of animal cell culture collections. The first obstacle encountered is a lack of awareness that such collections exist and their *modus operandi*. Originators are often reluctant to deposit their cell lines either because they fear commercial exploitation, or more commonly because they believe they will not be acknowledged in future publications and would often wish to carry out further work on the cell lines prior to general release.

In an endeavour to overcome this last problem, ECCO is approaching editors of scientific journals to ensure that all submitted articles clearly state the source of organisms. Another approach would be to adopt the system used in the USA, whereby many cultures derived from government-funded work are

Table 3. Techniques for mycoplasma detection

Technique	Sensitivity	Advantage	Disadvantage
DNA stain (e.g. Hoechst 33258)	10^4 cfu ml^{-1}	cheap, fast	interpretation of slide
Culture	1 cfu per test sample	speciation sensitive	time (28 days), fails to detect non-cultivable *M. hyorhinis* stains
6MPDR 6-methylpurine deoxyriboside) 'Mycotect'	no independent information available	no specialist equipment needed	time taken (4–5 days)
DNA probe 'Gen-probe-II'	10^7 cfu ml^{-1}	fast (1 day)	expensive, need microcentrifuge and scintillation counter

cfu, Colony-forming unit.
Suppliers: Sigma Chemical Co. Ltd, Fancy Road, Poole, Dorset, BH17 7NH, UK;
Gibco Ltd, Unit 4, Cowley Mill Trading Estate, Longbridge Way, Uxbridge, Middx, UB8 2YG, UK;
Laboratory Impex Ltd, 111–113, Waldegrave Road, Teddington, Middx, TW11 8LL, UK.

deposited at the ATCC under contract. Currently, the ECACC receives a grant from the UK research councils, for instance, which allows UK grant-funded workers to deposit their cell lines with the ECACC, and have them banked and distributed free of charge. As a minimum precaution against the loss of in-house frozen stocks, all scientists should consider placing a safe deposit with collections as an insurance measure.

Finally, a scientific problem facing all animal cell culture collections is that of standardizing *in vitro* environmental culture conditions. There is increasing evidence that the characteristics of cell lines can change with prolonged culture. The majority of cell lines require the addition of bovine serum to media formulations, and as some variation in the quality of bovine serum is unavoidable this can seriously modify cultural behaviour. Secreted proteins, surface markers, cytoskeletal components and growth rate are all affected by a multitude of stimulatory and inhibitory factors (Spier, 1988). In an attempt to control this variation the bovine serum level is often either reduced or replaced with defined components. Understanding the roles these components play in phenotypic drift has now become a major goal of the animal cell culture collections.

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