Cell line provenance

R. Ian Freshney

Cancer Research UK Department of Medical Oncology, Glasgow University, Garscube Estate, Bearsden, Glasgow, U.K.

(E-mail: I.Freshney@beatson.gla.ac.uk)

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Abstract

Cultured cell lines have become an extremely valuable resource, both in academic research and in industrial biotechnology. However, their value is frequently compromised by misidentification and undetected microbial contamination. As detailed elsewhere in this volume, the technology, both simple and sophisticated, is available to remedy the problems of misidentification and contamination, given the will to apply it. Combined with proper records of the origin and history of the cell line, assays for authentication and contamination contribute to the *provenance* of the cell line. Detailed records should start from the initiation or receipt of the cell line, and should incorporate data on the donor as well as the tissue from which the cell line was derived, should continue with details of maintenance, and include any accidental as well as deliberate deviations from normal maintenance. Records should also contain details of authentication and regular checks for contamination. With this information, preferably stored in a database, and suitable backed up, the provenance of the cell line so created makes the cell line a much more valuable resource, fit for validation in industrial applications and more likely to provide reproducible experimental results when disseminated for research in other laboratories.

Introduction

The word 'provenance', literally defined, means the source or origin of a particular item. It has been used extensively in the world of antiques to mean the collective history of an object, including details of its origin, ownership, special characteristics, modifications, and any information confirming the identity and antiquity of the object. There are significant parallels with the origin and evolution of cell lines, and the word has been used in the field of cell line validation to describe the origin and culture history of a cell line, including its transfers among laboratories and repositories, its manipulation (physicochemical or genetic), tests for and elimination of microbial contamination, genotypic and phenotypic characteristics, and authentication of its identity. This information is vital to the successful use of a cell line, particularly if it used extensively as a research tool by many different labor-

atories, or is adopted commercially for the production of a biopharmaceutical.

In general, most pharmaceutical companies will have strict procedures in place for specific operations (Specific Operating Procedures, SOPs) and will keep proper records stored on a computer database with input at terminals throughout the work area, in compliance with Good Manufacturing Practice (GMP), and, where research and development are taking place, it will be in compliance with Good Laboratory Practice (GLP) (DHSS, 1986; FDA, 1992). Although much of what will be presented here may be of use to industrial concerns, most will be, or should be, already incorporated into the appropriate SOPs. The main difficulties lie with independent research laboratories and academic institutions where, on the one hand, procedures tend to change more rapidly and SOPs are often not defined because they become redundant too quickly and need updating frequently, and, on the other hand, academic research, by its very nature, demands innov-

Figure 1. Diagrammatic representation of derivation of user stock of a cell line.

ation and experimentation and attracts those who are less willing to be restricted by the confines of strict SOPs and meticulous record keeping. Not that records are not kept, but each worker will define the needs of his or her own records, and these are not always translatable among individuals, far less among laboratories. Frequently these records do not contain aspects of information that may be important to another user if they are not of prime importance to the initiator. So this article is primarily directed at such research laboratories in the hope that a modest degree of standardisation in handling procedures and record keeping may be possible, making cell lines more useful to a wider community and more readily exploited by the biopharmaceutical industry.

Tracking the origin of a cell line

It is important to know not only the specifications of the species, age, sex, tissue and pathology of the donor, but also the way that the tissue, and cells derived from it, were handled at isolation and subsequently. An outline of this is shown in Figure 1. It should include a record of everything that has been done to the cell line, accidentally as well as deliberately, and should be constantly updated when fresh seed stocks are frozen. All too often this information is required retrospectively, when a particular cell line becomes important, and, regrettably, gaps are then found in the record. This problem need not arise if proper records are kept, but frequently, particularly where large numbers of primary cultures are being prepared, or cell lines handled, records become minimal and important details are found to be missing when a ret-

rospective analysis of a cell line is carried out. It is, therefore, critical to keep full records from the outset.

Keeping records

The main reasons for failing to keep complete records are:

- The records are too detailed and complex.
- The operator is too busy and postpones completion of the record until spare time is available.
- The manipulation is not different from that carried out previously, so why bother?

Records need to be detailed to be of any value in later consultation, but all details need not be completed each time the cells are handled if a strict protocol is adhered to. In this case it is only when the operator deviates from the protocol, deliberately or accidentally, that a note must be inserted in the record. It is also important, if a deviation becomes routine, to redefine the protocol to save future repetition.

Permanence and accessibility are best guaranteed by maintaining records in a computer database with provision for back-up. Many laboratories may find it impractical to have a terminal for keyboard entry of data at the point where sterile work is being carried out and in this case, standard format record sheets should be used, and the data keyed in later. This has the additional advantage that records are then kept in hard copy and well as electronic, a useful precaution in case of computer and back-up failure.

Recording primary cultures

Table 1 suggests the type of parameters which should be recorded each time that a primary culture is attempted. If a standard protocol is adopted then this need only be detailed the first time used and there after only the deviations from this protocol, or variables, such as cell yield, need be recorded. This type of record can be used as an individual sheet (Table 1a) per operation or as a continuous record sheet (Table 1b).

This record will contain a field for the cell line designation. When applying a designation to a new cell line, it is useful to use an institute identifier, e.g., MOG, Medical Oncology Glasgow; NCI, National Cancer Institute; or IMR, Institute for Medical Research, to avoid confusion if two initiators use the same number or initials to identify a cell line. It is

convenient to follow the institute identifier by a series identifier, e.g. G, glioma; H, human; L, lung, etc., and a serial number generated from the record number to give the complete designation such as MOG-G123 or NCI-H69. Using the donor's initials should be avoided as this can lead to a breakdown in confidentiality.

When human material is used, ethical consent must be obtained from the hospital ethics committee, and informed consent obtained from the donor or a close relative (Table 2). The issue of ownership is complicated, as there may be several agencies involved, the hospital, the clinician, the scientist, his/her academic institution, the donor, the granting agency, and any commercial company if one is involved. It is simplest to get the donor to waive ownership rights and sort out any conflicting interests among sponsors before a series of cultures is initiated.

It is important to save a sample of DNA or tissue from the donor and freeze this at -70°C for future use in identification by DNA profiling should any valuable cell line be derived from this material.

Recording maintenance of cultures

Medium changing

It may be necessary to change the medium during primary culture or between subcultures of any derived cell line. Medium change or 'feeding' should also be recorded on a separate table within the same database, for example, as in Table 3.

Subculture

The culture becomes known as a cell line following the first subculture. This and all subsequent subcultures should be recorded in another table within the database (Table 4). Each subculture increases the passage number by one (Figure 2) and this should be recorded, but if a finite cell line is being cultured, the critical figure is the generation number (number of population doublings, PD). Finite cell lines will cease proliferation after a set number of generations, characteristic of the cell type and species; usually 50 ± 20 PD for normal human diploid fibroblasts (Hayflick and Moorhead, 1961; Sasaki et al., 1994). Hence the number of population doublings must be recorded. The simplest method of doing this is to ensure that dilution for re-seeding after each subculture is done by a power of two. For example, a slow growing cell line might be split 1:2, equivalent to one population doubling, in which case the passage number equals the generation number. However, if the cells grow rapidly from a low

Table 1a. Primary culture data record

Date: Time: Operator: Record no.¹

Origin of tissue	Species	
	Race or strain	
	Age	
	Sex	
	Path. # or animal tag #	
	Tissue	
	Site	
	Stored tissue / DNA location	
Pathology		
Disaggregation agent	Trypsin, collagenase, etc.	
	Concentration of agent	
	Duration of incubation	
	Temperature	
	Solvent (e.g. PBSA, PBS/EDTA)	
Cell count	Concentration after resuspension (C_i)	
	Volume (V_i)	
	Yield ($Y = C_i \times V_i$)	
	Yield per g (wet weight) tissue	
Seeding	Number & type of vessel (flask, dish, or plate wells)	
	Final concentration	
	Volume per flask, dish or well	
Medium	Type	
	Batch no.	
	Serum type and concentration	
	Batch no.	
	Other additives	
	CO ₂ concentration	
Matrix coating	e.g. fibronectin, Matrigel, collagen	
Other parameters		

¹ This number, incorporating a letter code for the institution and culture type, may, ultimately, become the cell line designation.

Table modified from Table 11.3, Culture of Animal Cells, 4th ed., Freshney (2000), and reproduced by permission of Wiley-Liss, a Division of John Wiley & Sons, New York.

Table 1b. Primary culture data record

Record no.²						
Date						
Time						
Operator						
Origin of tissue	Species					
	Race or strain					
	Age					
	Sex					
	Path. # or animal tag #					
	Tissue					
	Site					
	Stored tissue / DNA location					
Pathology						
Disaggregation agent	Trypsin, collagenase, etc.					
	Concentration of agent					
	Duration of incubation					
	Temperature					
	Solvent (e.g. PBSA, PBS/EDTA)					
Cell count	Concentration after resuspension (C_1)					
	Volume (V_1)					
	Yield ($Y = C_1 \times V_1$)					
	Yield per g (wet weight) tissue					
Seeding	Number & type of vessel (flask, dish, or plate wells)					
	Final concentration					
	Volume per flask, dish or well					
Medium	Type					
	Batch no.					
	Serum type and concentration					
	Batch no.					
	Other additives					
Matrix coating	CO ₂ concentration					
	e.g. fibronectin, Matrigel, collagen					
Other parameters						

² This number, incorporating a letter code for the institution and culture type, may, ultimately, become the cell line designation.

seeding density then a higher split may be required, 1:4 (2 generations), 1:8 (3 generations) or 1:16 (4 generations). If these split ratios are adhered to, then counting generations becomes easy. However, it is important that the cells are examined and counted at each subculture to confirm that the yield remains constant from a given seeding density, to ensure (a) that the cells do not enter plateau before subculture, due to the split ratio being too low, or (b) that the yield does not decline gradually, due to the split ratio being too high,

reducing the seeding density below the optimum. The cell count at subculture is a valuable datum and should be recorded. It confirms consistency of growth and may be the first indicator of problems with medium or technique, or of a cell line entering senescence.

Validation of cell lines

Validation of a cell line implies that it is fit for use, i.e., that its identity is known, it performs the func-

Table 2. Suggested donor consent form*

CONSENT TO REMOVE TISSUE FOR DIAGNOSIS AND RESEARCH

This form requests your permission to sample one or more small pieces of tissue to be used for medical research. This tissue, or cell lines or other products derived from it, may be used by a number of different research organisations, or it may be stored for an extended period awaiting use. It is also possible that it may eventually be used by a commercial company to develop future drugs. We would like you to be aware of this and that, by signing this form, you give up any claim that you own the tissue or its components, regardless of the use that may be made of it.

I am willing to have tissue removed for use in medical research and development and, hereby, waive all property rights to this tissue or any derivative from it. (If the donor is too unwell to sign, a close relative should sign on their behalf).

Name of donor..... Name of relative

Signature.....

Date.....

This material will be coded and absolute confidence will be maintained. Your name will not be given to anyone other than the person taking the sample.

Do you wish to receive any information relating to your state of health?

Yes/No

Signature..... Date.....

Would you like any information relating to your state of health to be given to your doctor?

Yes/No

If yes, Name of Doctor

Address.....

.....

.....

* Table reproduced from Culture of Animal Cells, 4th ed, Freshney (2000), Table 11.1, by permission of Wiley-Liss, a division of John Wiley & Sons, New York.

Table 3. Feeding record

Date:						
Time:						
Operator:						
Cell line	Designation					
	Generation or pass no. ³					
Status	Phase of growth cycle					
	Appearance of cells					
	Density of cells					
	pH of medium (approx.)					
	Clarity of medium					
Medium	Type					
	Batch no.					
	Serum type and concentration					
	Batch no.					
	Other additives					
	CO ₂ concentration					
Other parameters						

³ Generation number should be used for finite cell lines and passage number since last thawed for continuous cell lines. Table modified from Table 12.5, *Culture of Animal Cells*, 4th ed., Freshney (2000), and reproduced by permission of Wiley-Liss, a Division of John Wiley & Sons, New York.

tions required of it, and that it has been shown to be free of microbial contamination. It also means that it can be frozen as a secure resource and that it can be transferred with confidence to another laboratory or cell bank.

Authentication

For a cell line to be authentic it must display characteristics that confirm its identity beyond question. When this is done, then the provenance can be applied to the cell line and it can be used with confidence. Ideally,

authentication, with the provenance, should connect the cell line with its origin. In some cases, however, cell lines established many years ago may lack some aspects of their provenance and their origin may be unknown. In this case it is essential to be able to confirm that the cells in current use can be validated against a previously authenticated stock, either in a cell bank or in the laboratory of the originator.

The major concern, particularly with continuous cell lines, is that the cell line may have been cross-contaminated (see Masters Chapter). A large number of cell lines in common use, e.g., KB, Hep-2, and

Chang liver (see table Masters chapter) are known to have been cross-contaminated with HeLa at some stage after the original cells were isolated (Nelson-Rees et al., 1981; Stacey et al., 2000). However, as long as this fact is acknowledged, the cell line is not necessarily invalid. KB cells have particular phenotypic properties which may set them aside from HeLa, in spite of the clear indication from DNA fingerprinting and chromosome analysis that they are HeLa. It would be foolhardy to discard these cells, and their several different drug resistant variants (Fojo et al., 1985), because of the error in identity. However, the mistaken identity must be acknowledged in all reports and publications so that it is clear that these cells do not represent an example of orolaryngeal carcinoma, which was the derivation of the original cell line. Reporting these cells as HeLa-KB would seem to be the obvious choice, effectively describing them as a subline of HeLa.

Characterisation of a cell line, using the correct parameters, will confirm its identity and may enable confirmation of its origin. It will also reveal any signs of instability and variation occurring in early passage finite cell lines due to selection and overgrowth, and in continuous cell lines due to genetic instability. It is also important that when a new variant cell strain is derived from a cell line following selection, that its relationship to the parental line is confirmed. Some characterisation, such as drug resistance, will arise from experimental use, but other aspects will need to be done purely for reasons of authentication. The procedures used will depend on the type of technology accessible to the user, but certain general recommendations can be made.

Unique characteristics

DNA fingerprinting or profiling are currently the most reliable techniques available (see Masters, this issue). There are now several commercial services if the technology is not available in your own laboratory. Although there may be some problems using cell lines derived from inbred strains of mice, these techniques are extremely reliable for human cell lines and outbred strains of animals.

Human leucocyte-associated antigen (HLA) typing can also be used to characterise human cell lines from different individuals (Hay, 2000; Pollack et al., 1981).

Identification of species

Chromosomal analysis remains one of the best techniques for identifying the species of origin of a normal

cell line as each animal species has a unique karyotype. However, where transformed cells are used, the incidence of aneuploidy and heteroploidy can make species identification difficult unless specific chromosomal markers are used (Hay, 2000; Keith, 2000). For this reason, some laboratories use isoenzyme analysis to differentiate species. A kit for agarose electrophoresis is available from Innovative Chemistry Inc., and, using seven different enzymes, aspartate aminotransferase, glucose-6-phosphate dehydratase, lactate dehydrogenase, malate dehydrogenase, mannose-6-phosphate dehydrogenase, nucleotide phosphorylase, and peptidase B, all species currently cultured can be identified (Hay, 2002).

Species-specific antibodies are also available commercially and these can be used to confirm species of origin by indirect fluorescence or immunoperoxidase staining (Hay, 2002).

Tissue markers

Confirmation of the tissue of origin may be obtained by using tissue specific markers. One group that is particularly useful is that of the intermediate filament proteins, where cytokeratins are found in epithelial cells, vimentin in mesodermally-derived cells, glial fibrillary acidic protein in astrocytes (GFAP), neurofilament protein in neural and some neuroendocrine cells, and desmin in muscle cells. Of these GFAP is probably the most specific and vimentin the least, as it can be expressed in a number of different cell types in culture including epithelial cells.

There are also a number of tissue specific cell surface markers, such as the wide range of leucocyte markers (Villa and Delia, 1988), epithelial membrane antigen (EMA) (Heyderman et al., 1985) and human milk fat globule (HMFG) associated antigen (Burchell et al., 1983) on epithelial cells, and galactocerebroside on oligodendrocytes (Raff et al., 1979). These markers have the advantage that they can be used on unfixed cells, and hence are available for positive sorting by flow cytometry or immunomagnetic beads.

Some tissue-specific markers are only expressed in differentiated cells, e.g., haemoglobin in Friend (Friend et al., 1971) or K562 (Andersson et al., 1979) erythroleukaemia, tyrosine aminotransferase (Ikeda et al., 1998) and albumin (Otsu et al., 2002; Schleger et al., 1997) in hepatocytes, and dome formation in secretory epithelium (Freshney, 2002) and lactalbumin (Soule et al., 1990) in mammary epithelium. These are among the most specific of the tissue markers but may need induction conditions, e.g., soluble inducers

Table 4. Subculture record

Date:						
Time:						
Operator:						
Cell line	Designation					
	Generation or pass no. ⁴					
Status before subculture	Phase of growth cycle					
	Appearance of cells					
	Density of cells					
	pH of medium (approx.)					
	Clarity of medium					
Dissociation agent	Prewash					
	Trypsin					
	EDTA					
	Other					
	Mechanical					
Cell count	Concentration after resuspension (C_i)					
	Volume (V_i)					
	Yield ($Y = C_i \times V_i$)					
	Yield per flask					
Seeding	Number (N) & type of vessel					
	Final concentration (C_F)					
	Volume per flask, dish or well (V_F)					
	Split ratio ($Y / C_F \times V_F \times N$) or number of flasks seeded / number of flasks trypsinized where flasks are of same size					
Medium / serum	Type					
	Batch no.					
	Serum type and concentration					
	Batch no.					
	Other additives					
	CO ₂ concentration					
Matrix coating	e.g. fibronectin, Matrigel, collagen					
Other parameters						

⁴ Generation number should be used for finite cell lines and passage number since last thawed for continuous cell lines. Table modified from Table 12.6, Culture of Animal Cells, 4th ed., Freshney (2000), and reproduced by permission of Wiley-Liss, a Division of John Wiley & Sons, New York.

like sodium butyrate, matrix components like laminin and collagen, homologous and heterologous cell interaction, and a high cell density, polarised culture, such as is available in a filter well insert, for complete expression.

Transformation markers

While most normal human finite cell lines are genetically stable, cell lines from mice and a number of other species are prone to transformation in vitro. There are two main elements to transformation, immortalisation and the development of aberrant growth control, which usually correlates with the cells becoming malignant

Figure 2. Serial subculture and the relationship between passage number, generation number and split ratio. (Figure modified from Fig. 12.5, Culture of Animal Cells, 4th ed, Freshney (2000) and reproduced by permission of Wiley-Liss, a division of John Wiley & Sons.)

if transplanted *in vivo*. Rodent cells can immortalise spontaneously *in vitro*, and, under the correct conditions of low cell density and using donor bovine serum rather than foetal bovine serum, can retain normal growth characteristics, i.e., sensitivity to contact inhibition of cell motility and density limitation of cell proliferation, inability to clone in agar, and lack of tumorigenicity *in vivo*, as is found with mouse 3T3 cells. However, in high cell density, particularly in fetal serum (Parkinson and Yeudall, 2002), these cells can undergo further transformation, lose contact inhibition and density limitation, clone in agar, and become tumorigenic.

Although finite human cell lines rarely, if ever, undergo such spontaneous transformation, it is possible that immortalised cell lines may do so as many have abnormal tumor suppressor gene expression, even when induced by telomerase (Parkinson and Yeudal, 2002).

Markers used to monitor cells for transformation include the following (Freshney, 2000):

- Development of aneuploidy and heteroploidy.
- Overexpression of oncogenes such as *ras*, *myc*, *erbB* and deletion, or mutation, of suppressed genes, such as p53 or Rb.
- Loss of contact inhibition of cell motility.

- Increased proliferation at high cell density resulting in a higher saturation density.
- Reduced serum dependence in regular media such as Eagle's MEM.
- Ability to clone in suspension in agar.
- Elevated protease activity, such as urokinase-like plasminogen activator.
- Tumorigenicity *in vivo*, e.g. in nude or SCID mice.

Contamination

Part of the validation process is required to ensure that the cells are free of contamination. Cross-contamination with other cell lines remains a serious problem (Stacey et al., 2000; MacLeod et al., 1999) (see above and Masters, this issue). Discarding the cells and thawing fresh stock is the best solution, but is not an option if the culture has not been frozen. If detected early enough, before complete overgrowth, it is sometimes possible to recover the original cells by cloning. Most physical separation techniques, such as density gradient sedimentation would not give sufficient purity, although a combination of negative sorting with immunomagnetic beads, followed by flow cytometry might work. Usually, however, overgrowth is virtually complete by the time cross-contamination is detected and the only option is to discard the cells.

Microbial contamination

The common contaminants are fungi, yeasts, and bacteria and will usually become apparent by naked eye or microscopic examination, if the cells are cultured in the absence of antibiotics. Antibiotics are not essential for routine culture in laminar flow cabinets and should not be used except in high risk situations, e.g., when initiating a primary culture. Some antibiotics are toxic, e.g. amphotericin B, while others will encourage the generation of cryptic contaminants.

For most purposes, it is sufficient to grow the cells antibiotic-free to demonstrate that they are free from microbial contamination. However, if a microbial contamination is suspected but not evident on visual examination, or more stringent quality control is required, it may be necessary to culture a sample of the cells and/or medium in nutrient broth or on nutrient agar. DNA fluorescent stains, such as DAPI or bisbenzimidazole (Hoechst 33258) will stain microbial DNA, and PCR methods with appropriate primers can also be used to detect low levels of contamination.

Mycoplasma

Mycoplasmas are DNA-containing organisms too small to be detected by the naked eye or regular microscopy. Contamination with mycoplasma has a number of serious consequences for cultured cells (see Drexler, this issue) including alteration in growth characteristics, metabolism, and cell surface modification. Detection is most easily achieved by fluorescence staining with Hoechst 33258 or by PCR (see Drexler, Stacey, Chapters). Proper cell line validation requires regular monthly checks for mycoplasma by one of the recognised detection methods (see Drexler).

Viruses

Viral contamination presents an even greater detection problem than mycoplasma because of the diversity of potential viral contaminants (see Merten Chapter). PCR and immunostaining are probably the most useful methods. Proper validation should ensure that the culture is virus-free, but that has to be qualified by the availability of the correct primers or antibodies, and presumes that the likely identity of contaminants is predictable.

There is as yet no known way of eliminating viral contaminants, other than by discarding stocks. As for other forms of contamination, prevention is better than cure, and screening incoming biologicals is essential. Most reliable suppliers will screen serum and other biologically derived reagents but individual

laboratories must carry the responsibility for screening incoming cell lines or biopsies.

Various effects of viral contamination can be expected (see Merten chapter). Cytopathological effects (CPE) may be evident under the microscope, particularly if a known host indicator cell strain is exposed to contaminated medium. The effect of transforming viruses can also be detected using the criteria in *Transformation Markers*, above. However, many viruses may not have any detectable effects, other than viral replication, and hence tend to be ignored.

Good cell culture practice

Maintenance regimes

Good aseptic technique is essential (Freshney, 2000). It will protect the cell line against microbial and cross contamination, provide consistency in handling, and minimize variations in culture behaviour. This should be confirmed by regular observation, by eye and on the microscope, and any variations noted and, hopefully, accounted for. Records should be kept (see above).

Frozen stocks

While a token stock may be frozen on initial acquisition or isolation, once a cell line is validated a seed stock should be frozen in liquid nitrogen. If the cell line is to be used, a distribution stock should be prepared from the seed stock, and a sample passed on to the user, who, in turn, should prepare a user stock which is not propagated beyond that user. Cultured stock should be replaced regularly, usually every three months, from user stocks, so enough user stock should be frozen to last the duration of the project.

Transfers

Cell lines should not be transferred to another laboratory without proper validation, and then the whole provenance, or an abbreviated form of it, should be sent with the cells. It is preferable to make the initial transfer to a cell bank (see below) and let them attend to any further distribution, with the originator's approval as required. Even well validated cell lines run the risk of losing this validation by cross contamination or microbial contamination, or by undergoing phenotypic or genotypic alteration, if passed on from one laboratory to another. If you do transfer cells to another laboratory, insist that they do not pass it on,

but refer any future requests to you, the originator, or to your nominated cell bank.

Cell banks

Once a newly established or newly acquired cell line has been properly validated it is advisable to submit it to a recognised cell bank. Distribution of this line can be agreed with the depositor, and the cells need not be distributed, other than to the laboratory of the originator, if so desired. Lodging cells in a cell bank, not only protects the depositor against accidental loss, but also provides authenticated stock for future reference. Once authorised, it can also allow easier and more rapid, validated, distribution to other laboratories.

Existing banks include the following:

- American Type Culture Collection (ATCC): www.atcc.org
- Coriell Cell Repository: www.locus.umdj.edu/ccr
- Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ): www.dsmz.de
- European Collection of Cell Cultures (ECACC): www.ecacc.org.uk/
- Japanese Collection of Research Bioresources (JCRB): www.cellbank.nihs.go.jp
- RIKEN: www.rtc.riken.go.jp

Conclusions

It is important to realise that such events as cell line cross-contamination and mycoplasma contamination can and do happen, often with disastrous consequences. Too many laboratories are unaware of the extent of these problems because of inadequate screening procedures. It is too late to check out your cell lines when you are about to submit a publication or grant application, or prepare a PhD thesis. Correct procedures must be instigated at the start of a project and should be in place as routine in every laboratory.

All that you do to a cell line, from routine maintenance through validation procedures, to the generation of experimental data, adds to the provenance of the cell line. Like an original painting or a piece of furniture, the more complete the provenance, the more valuable the cell line.

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