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Cell Lines as Biological Models: Practical Steps for More Reliable Research

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Abstract

Research in toxicology relies on *in vitro* models such as cell lines. These living models are prone to change and may be described in publications with insufficient information or quality control testing. This article sets out recommendations to improve the reliability of cell-based research.

Keywords

authentication; cell culture; cell lines; cross-contamination; misidentification; reproducibility

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Author Contributions

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Notes

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The Use of Cell Lines as Biological Models

To understand the effects of chemicals on living organisms, one needs to study living models. Traditionally, toxicology research has relied on human volunteers or laboratory animals, but there is an increasing move towards *in vitro* models. This can be illustrated using recent publications in *Chemical Research in Toxicology*. We examined the Methods sections in five recent issues (volume 31, issue 12 and volume 32, issues 1-4), comprising 84 articles and rapid reports. Of these 84 publications, 58 (69%) used one or more mammalian models. Seven (8%) used human specimens (tissue, blood, or urine samples) and 21 (25%) used animal models. Cell cultures were used in 37 (44%) publications, with 42 different cell lines listed in these 37 publications.

Cell lines are popular in research laboratories because they are widely available and easy to handle, provided the user has access to appropriate facilities and training. The cell lines used in the publications mentioned above include some of the most widely used cell lines, such as HeLa, HEK293, and their derivatives. These cell lines are immortal (capable of unlimited proliferation), allowing a continuous supply of what is commonly assumed to be the “same” material. However, this assumption is not supported by scientific evidence. Cell lines are prone to genetic and phenotypic change, due to alterations in their growth conditions or handling, evolution of clonal populations over time, or simply due to the stresses associated with the culture environment.

Cell Lines Can Come with Identity Surprises

There are many instances when a cell line can no longer be traced to the culture or donor from which it was first established. Such a misidentified cell line, instead of corresponding to the original donor, is derived from a completely different source – often from more aggressive tumor cell lines such as HeLa. This is frequently caused by cross-contamination, which is the accidental introduction of cells from one culture to another. Cross-contamination may occur through lapses in aseptic conditions e.g., if a bottle of medium is shared between two cell lines, allowing droplet spread. Initially, this results in a mixture of the different cultures. However, tumor cell lines such as HeLa typically grow more rapidly and are more resilient at low density compared to many other cultures. The more rapidly growing contaminating cell can overgrow the original culture within several passages, resulting in its complete replacement. In many cases, this occurs without the user being aware of the change.

In the 1960s, scientists discovered that many widely used cell lines were misidentified [ATCC SDO Workgroup ASN-0002 2010]. Many of those misidentified cell lines continue to be used today and others have joined their ranks. The International Cell Line Authentication Committee (ICLAC) curates a Register of Misidentified Cell Lines, which currently lists 486 misidentified cell lines with no known authentic stock (https://iclac.org/databases/cross-contaminations/) [Capes-Davis et al. 2010]. In other cases, authentic stocks have been found for cell lines that were originally thought to be misidentified. These advances were made possible through the development of standardized authentication

testing methods that allow different laboratories to compare their cell lines. Short tandem repeat (STR) profiling is the consensus technique for human cell lines because it can establish identity to the individual donor level (provided suitable reference samples are available), allows interlaboratory comparisons, is inexpensive and easily interpreted, and is available globally [ATCC SDO Workgroup ASN-0002 2010].

Scientists are now generating biological data from cell lines at an exponential rate. It was recently estimated that approximately two million publications have made use of cell lines [Bairoch 2018]. Unfortunately, despite the increasing reliance on cell and tissue culture in biomedical research, there is a tendency to take these living materials for granted. Many laboratories obtain their cell lines from colleagues without confirming the identity of the cultures received, under the assumption that cultures continue to maintain their properties regardless of how they are handled and how they were previously maintained. Misidentified cell lines may, in this way, be passed from laboratory to laboratory without knowledge of the issue. This problem is further compounded by inadequate reporting of cell line attributes in publications. Cell lines may be described by name without considering whether that name is sufficient to uniquely identify them. In reality, a cell line's name may be shared by multiple cultures or may itself evolve over time. Other essential information such as the cell line's source, its passage number, and the culture conditions may not adequately be described.

A Culture of Change is Needed

What can be done to address these problems in cell line usage? Most problems with cell lines can be traced back to their inherent ability to evolve in culture and to inadequate reporting and quality control testing by the research community. While it is not possible to stop a living cell line from changing, laboratories can minimize the changes using good cell culture practice. Detailed recommendations for good *in vitro* methods have recently been published by the Organisation for Economic Co-operation and Development (OECD), focusing on the appropriate use of *in vitro* systems to evaluate chemical safety [OECD 2018]. However, the need for good practice is not confined to any one application. The entire research community should work to improve the use and reporting of cell lines and other *in vitro* models.

Four practical steps can be taken to make cell-based research more reliable and reproducible:

1) ***Cell lines should be tested for contamination***.

   Misidentified cell lines can be detected using authentication testing [ATCC SDO Workgroup ASN-0002 2010]. STR profiling is recommended as a consensus method for human cell lines and is available for some non-human species. Other genotype-based methods, such as single nucleotide polymorphism (SNP) analysis, are also suitable for authentication and may be used to confirm the species and strain of non-human cell lines. Phenotype-based methods including morphology and immunocytochemistry are not sufficient for authentication; these methods are usually based on a limited set of characteristics and may give atypical results for some cell lines [Kniss and Summerfield 2014].

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Microbiological contamination testing, such as mycoplasma, should also be performed.

2) ***Cell line information should be more visible***.

For manuscripts, a section in the Materials and Methods should be dedicated to "Cell Lines and Culture Reagents". The information could be presented in a tabular form that lists key reagents and resources for experimental work, and many peer-reviewed journals already require this. Each cell line listed should be accompanied by a unique Research Resource Identifier (RRID), which is generated through a collaboration between the Cellosaurus knowledgebase (https://web.expasy.org/cellosaurus/) [Bairoch 2018] and the Resource Identification Initiative (https://f1000research.com/articles/4-134/v2). The RRID enables the cell line to be flagged for searches and data analysis. If a cell line does not have an RRID, a new cell line entry may be proposed by contacting Cellosaurus directly (mailto:cellosaurus@sib.swiss).

3) ***Reporting of cell line information should be more transparent***.

Sufficient information should always be reported for the reader to replicate the conditions of the experiment. The author should verify that a cell line is not on the ICLAC Register of Misidentified Cell Lines and report on the source of their sample (i.e., the laboratory or cell repository from which it was obtained, including a catalog number for the latter), its passage number or range during experimental work, the testing performed, and the culture conditions. ICLAC has developed a checklist to assist with cell line reporting for manuscripts or grant applications, which is available on the committee's website (https://iclac.org/resources/cell-line-checklist/).

4) ***Organizations should develop policy guidelines for cell line use and handling***.

There are a number of excellent guidelines available on good cell culture practice, but how these are put into practice may vary with the application. All journals should include specific instructions for cell line reporting and testing in their author guidelines. Funding agencies and research organizations also have an important part to play in assuring high-quality standards in the biomedical research enterprise. For research organizations, a draft policy that may be useful as a starting point for policy development is available on the ICLAC website (https://iclac.org/resources/cell-line-policy/). Key requirements include authentication by STR profiling for any human cell line accepted into a laboratory, distributed by a laboratory, or used in a publication [Kniss and Summerfield 2014].

A Call to Action

For a real solution, increased engagement and education is needed across the research community. Some organizations will be able to make large steps to address misidentified cell lines and the broader problem of cell line variability. Others may focus on smaller steps to begin with and then assess the impact of these steps and revise their guidelines accordingly.

But all researchers can do something to address the challenges that come with *in vitro* models, using the practical steps that are suggested here as a reference.

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Abbreviations

RRID	Research Resource Identifier
STR	short tandem repeat

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