Terms of Reference

What Do We Mean by Cell Line Misidentification?

In the context of cell line authentication, a cell line is considered to be misidentified if its DNA profile (genotype) is no longer consistent with the donor from whom it was first established. Such a cell line is described as a misidentified or false cell line. Investigators who are unaware that they are working with a false cell line may use it in their work, without realising that it comes from an entirely different cell type or tissue – perhaps even a different species – and is likely to be unfit for their application. This lack of awareness leads to unreliable research findings, and the use of those unreliable findings by other scientists in turn.

To judge whether a cell line is authentic or misidentified, it is important to look at its genotype and not its behaviour (phenotype). A cell line's phenotype can vary depending on passage number and growth conditions. Genotype is ideally assessed through comparison to donor DNA. Since donor DNA profiles are seldom known, this ideal is only met by a subset of newer cell lines. In remaining instances, other criteria must be used including corresponding DNA profiles – ideally from independent samples held by different cell banks – and cytogenetic analysis to determine if karyotypes correspond to those originally published.

Although there are many causes of false cell lines, including mislabelling of culture samples, the problem is often caused by cross-contamination (see the Definitions later in this document). Many cell lines have been cross-contaminated during establishment and so all subsequent work based on the false cell line has used the contaminant rather than the correct species, tissue or cell type that was originally present in that culture. Once cross-contaminated, cell lines are often handed from laboratory to laboratory without anyone being aware that their stocks may be unfit for use. This practice can only lead to confusing results and represents a waste of precious resources.

Why Do We Focus on Cell Line Authentication?

Authentication testing is an effective way to combat the use of false cell lines. Authentication testing aims to compare a test sample to other reference samples from that donor, or to a database of reference samples if donor material is not available, to see whether samples correspond to the expected genotype. The test method should distinguish between different species and different individuals within that species, although this will depend on the technology available to the field of authentication testing. When multiple cell lines have been established from a single individual, identification rests on additional test methods and characteristics such as cytogenetics which, although reliable, requires special expertise and is unsuited to automation. In addition, tissue-specific markers, phenotype and morphology are sometimes also used for authentication; the latter methods are unreliable as identity tests and therefore these aspects of authentication are outside the scope of this document.

In 2011, the American Type Culture Collections Standard Development Organization (ATCC SDO) published a standard on authentication testing of human cell lines using short tandem repeat (STR) profiling (ANSI/ATCC ASN-0002-2011). The standard sets out a consensus approach for authentication of human cell lines using DNA genotyping. The International Cell Line Authentication Committee (ICLAC) was formed after publication of the standard to provide guidance and an ongoing focus for improvement in this area.

ICLAC aims to make the use of false cell lines more visible and to promote awareness and authentication testing as effective ways to combat the problem.

Goal 1: Make Cell Line Misidentification More Visible

A number of laboratories and cell line repositories have uncovered false cell lines, in publications dating from the 1960s. More information will come from public databases such as the NCBI cell line database (BioSample), which is currently in development. These online, interactive databases give cell banks and laboratories an effective way to compare samples and share authentication test

results. But it is important that the older reports are not lost and that any incorrect or inaccurate information added to the literature, or to online databases, should be addressed.

To make this information more visible and informative, ICLAC aims to:

1. Review reports of cross-contaminated or otherwise misidentified cell lines
2. Gather information to provide a written response, where needed, to the scientific community
3. Manage a master list of misidentified cell lines for the research community and for feedback to the NCBI cell line database

As a resource for the research community in this area, members of the group have previously developed a single list or database of misidentified cell lines. The list will be used as an initial template for this goal, with ongoing updates and online release of updated information. It can then be used as a tool to help ensure that reports of cell line misidentification are recorded, made accessible to the research community, and any inaccuracies are corrected.

The ICLAC list of misidentified cell lines can be found on the ICLAC website (www.iclac.org/databases/cross-contaminations/).

Goal 2: Promote Authentication Testing

The standard referred to above is an important resource for authenticating human cell lines. However, there is a need for education and resources to help laboratories apply the standard in their own situations.

How this is done will depend on the resources available to the group. Possibilities include:

- Provide advice to scientists planning a project or grant application, starting new work or initiating new cell lines
- Guidelines and protocols on application of the standard and recommended test measures
- Approaches to journals and funding bodies to encourage mandatory authentication testing, with assistance if needed to make such testing recommendations easier to make
- Shared data to provide more effective datasets for shared online databases
- Shared policies on difficult issues
- Develop guidelines for cell line authentication policies at research institutions.

Goal 3: Harmonization of Guidelines and Standards

In addition to the standard referred to above, other standards and guidelines are relevant to authentication testing or – more broadly – to good cell culture practice.

Use of standards and guidelines may vary from country to country because of different regulatory frameworks. Use may also vary from one application to another, depending on the type of cell culture being performed. However, it is important to maintain consistency across all reference documents wherever possible, so that laboratories are not faced with conflicting requirements. ICLAC aims to assist in harmonization of relevant guidelines and standards by providing an independent forum for communication and discussion of such reference documents as they arise.

ICLAC Members and Partners

Members are invited to join ICLAC based on expertise in cell line misidentification, authentication testing, or database applications. Members act in a voluntary capacity and their individual contributions and commitment to addressing the problem of cell line misidentification are respectfully acknowledged.

A full list of committee members and their affiliations can be found on the ICLAC website (www.iclac.org/members/).

Partners are invited to support ICLAC in a number of ways, including administrative support, website resources, sourcing of cell line samples, and sample testing. Partner organizations also enable staff to contribute their time as ICLAC members. We wish to acknowledge all of the organizations who have supported the work of the committee in different ways.

A full list of partner organizations and funding sources can be found on the ICLAC website (www.iclac.org/partners/).

Contributions from members are subject to the policies of their individual institutions. All shared policies and sharing of data must be approved by the contributing organization.

Contributions from members and partner organizations are based on clear scientific evidence showing that cell line misidentification is an important cell culture problem that is best addressed through authentication testing. ICLAC does not endorse or recommend specific products or services offered by its members or partner organizations. All funding sources are declared on the ICLAC website.

Members and partners are welcome to indicate their ICLAC affiliation on their websites, or through other forms of communication such as newsletters. All website references must be accompanied by a link to the ICLAC website. Other forms of communication such as press releases and newsletters must be referred to the ICLAC Chair and the wording agreed prior to public release.

Concerned individuals are welcome to submit publicly available material that refers to ICLAC to the committee for review. If the members conclude that the material refers to ICLAC inappropriately – for example, as promoting specific products or services – the person or organization responsible will be asked to modify or withdraw that material. Failure to do so, or repeated release of inappropriate material, will be grounds for the member or partner responsible to be removed from the committee.

Ground Rules for Committee

The committee meets every three months by teleconference. Correspondence and other business are carried out by email as far as possible, to allow members from different time zones to contribute.

A new finding of cell line misidentification can be reported by any member. New reports will be distributed to the members with an opportunity to give feedback and contribute further data where available. If members agree with the initial report, the curator of the list of cross-contaminated or misidentified cell lines will add that entry to the list. If any member challenges the initial report, the cell line will be reviewed by the members and a decision made on its status based on available data. A lack of a response from a member will be taken as agreement to the cell line being added to the list.

A change in status for cell lines already entered on the list can be reported using a similar process. For example, if authentic stock is found, the cell line can be moved from Table 1 (no known authentic stock) to Table 2 (authentic stock known).

Duration and Terms of Reference

No duration has been set for the committee. To promote effective use of resources, the committee will review its purpose and goals on an annual basis via teleconference. The Terms of Reference can be modified by the group following teleconference discussion. All changes must be reviewed by the members via email before being adopted.

Definitions

Some words are used in many different ICLAC documents and resources. They include:

Authentication. The aim of authentication is to confirm or verify the identity of a cell line, ensuring that it is derived from the correct species and donor. Testing involves comparison of a test sample to other reference samples from that donor, or to a database of reference samples if donor material is not available, to see whether samples correspond. Ideally, the test method should distinguish between different species and different individuals within that species, although this will depend on the technology available to the field of authentication testing. Not all currently used test methods have the power of discrimination of STR profiling or SNP testing; therefore authentication may not in all cases lead to unambiguous identification of cells to a specific donor or donor tissue. Where unambiguous identification is not possible, species verification is used as the best alternative currently available.

Cross-contamination. The term contamination refers to introduction of foreign material into a cell culture. Cross-contamination occurs when that foreign material consists of cells from another culture. Cross-contamination initially results in a mixed culture, containing cells from the authentic culture and the contaminant. If the contaminant has a survival advantage – for example, if it proliferates more rapidly – it will overgrow and replace the authentic cells within the culture. A contaminant usually comes from a different donor or species and so can be detected by authentication testing.

Misidentification. A misidentified cell line no longer corresponds to the donor or species from which it was originally established. Misidentification may arise due to cross-contamination. It may also arise from a variety of errors, including mislabelling of samples. If it happens early – for example, during cell line establishment – there will be no authentic material retained, and the cell line will be considered to be a false cell line. If misidentification happens late – for example, after the cell line is established and distributed to other locations – then authentic material may still exist and only some stocks may be false.

Misidentification does not refer to problems with the technical procedure of authenticating cell lines. It also does not typically extend to other characteristics such as tissue type, cell type or disease state. If the tissue type, cell type or disease state of a cell line is incorrectly attributed, the cell line is considered to be misclassified.

Short tandem repeat (STR) profiling. STR profiling is an experimental procedure that is currently used to authenticate cell samples. Short tandem repeats are regions (loci) of repeated DNA, typically 3-5 bases long, located throughout the genome. STR loci vary throughout the population and are inherited by individuals in unique patterns. STR profiling is performed by taking a DNA sample and analysing a set of STR loci (eight STR loci are accepted as the minimum number for cell lines). The resulting STR profile is compared to other results from that individual or from other cell lines. If STR profiles correspond, it is concluded that samples come from the same individual.

STR profiling is a form of DNA fingerprinting or genotyping. It examines STR loci only and focuses on the genetic markers that discriminate between individuals. This method is effective for authentication because when cultures become misidentified, the contaminant typically comes from a different individual. STR profiling is different from other forms of profiling such as expression profiling.

Single nucleotide polymorphism (SNP) analysis. SNP analysis is an experimental procedure that is increasingly used to authenticate cell samples. Single nucleotide polymorphisms are variations in single nucleotides located throughout the genome. SNP testing is performed by taking a DNA sample and sequencing a set of SNP markers that are known to vary throughout the population. As with STR profiling, SNP analysis is a form of DNA fingerprinting or genotyping where the result is compared to other results from that individual or other cell lines. If SNP results correspond, it is concluded that samples come from the same individual. An exception applies to inbred populations such as laboratory rodents, where it may be concluded that samples come from the same strain.