Terms of Reference

The International Cell Line Authentication Committee (ICLAC) is a voluntary, independent scientific committee that aims to make cell line misidentification more visible and promote authentication testing to combat this problem. ICLAC's rationale, goals, and ground rules are set out below.

ICLAC's Rationale

Why do we Focus on Cell Line Misidentification?

Cell lines are essential models for life sciences research. However, problems can arise during cell culture that compromise their validity as research models. Cell lines must undergo routine testing to confirm identity, ensure purity, and guard against contamination so that cell-based research is reliable and reproducible. Failure to perform these simple quality assurance tests may lead to publications that are based on invalid models and wasted research funding.

Cell line misidentification is a problem that can result from cross-contamination, where cells from one culture are accidentally introduced into another without the researcher being aware of the mistake. If the contaminating cells divide more rapidly than the original culture, they will overgrow and replace the original, authentic material. The end-result is a "misidentified" or "falsely identified" cell line that no longer corresponds to its original donor, but instead corresponds to another cell line. A similar outcome can occur if a culture is mislabeled or accidentally substituted through some other mechanism.

The first misidentified cell lines were discovered more than 50 years ago (1). Stanley Gartler, Walter Nelson-Rees and their colleagues used isoenzyme and cytogenetic analysis to demonstrate that more than 80 widely used cell lines had been cross-contaminated by HeLa, the first human cell line to be established. Ongoing testing by cell line repositories and other laboratories has uncovered many new cases of misidentification. More than 500 misidentified cell lines are now reported where no authentic material is known to exist (2).

Cell line misidentification continues to be a common problem, largely due to ingrained poor practices in the research community. Laboratories commonly obtain their cell lines from colleagues, rather than obtaining tested material from a cell line repository or other validated primary source. Cell lines from colleagues or other secondary sources are rarely tested and there is often no documentation of, or adherence to, good laboratory practices or standard operating procedures. A recent re-appraisal of testing data, generated from 848 leukemia-lymphoma cell lines over 25 years, demonstrated that the incidence of misidentification remains high when cell lines are obtained from secondary sources. This risk was estimated at 1:6 (3).

Why do we Focus on Cell Line Authentication?

Authentication testing enables the research community to detect cell line misidentification and is an important part of good cell culture practice (4). Such testing is required for all cell-based models, including stem cell cultures and patient-derived xenografts (5,6), and should be a mandatory requirement for policy development, publication, and research funding (7,8). Identity testing is also specified in the quality

requirements that are set out for the establishment, maintenance, and characterization of mammalian cell lines in The International Organization for Standardization (ISO) document 21709:2020 (9)

Clearly, it is important to use a test method that is standardized and proven to be effective for authentication. Inappropriate methods are frequently used to demonstrate whether a cell line is authentic or misidentified. A cell line is often assumed to be a valid model if it expresses tissue-specific markers that are consistent with its reported origin. However, a cell line's phenotype can vary depending on its passage number and growth conditions. For example, the "Chang liver" cell line is known to be misidentified and is actually HeLa (from cervical cancer), despite reports that the cell line expresses liver-specific markers (10). Although phenotype can be informative, it does not prove that a cell line is authentic.

Cell line authentication requires a method that is based on genotype, using genetic or chromosomal variations that are specific to the cell line or the original donor. In 2011, the American National Standards Institute (ANSI) published a written standard on cell line authentication which was recently updated in 2021 (11,12). This document developed a consensus approach for testing human cell lines using short tandem repeat (STR) profiling. Researchers can compare their STR profile to reference samples – ideally, to donor tissue or an early passage of the cell line held by a cell line repository. If donor material is not available, a database of reference samples from other cell lines can be used to exclude cross-contamination by many classic tumor cell lines, which are the most likely contaminants (13). Interpretation of STR profiling results requires careful consideration, especially when genetic drift is present (14), and is an important part of the standard document (11,12).

Other genotype-based methods can be highly effective for authentication testing and provide important complementary data alongside the STR profile. These test methods include cytogenetic analysis, cytochrome c oxidase 1 (CO1) barcoding, and single nucleotide polymorphism (SNP) analysis. All such testing data must be assessed alongside the cell line's provenance (its recorded origin and history), to determine whether the data matches its reported origin or whether there are discrepancies that would indicate the cell line is misidentified.

An increasing number of standards now set out consensus approaches for testing of human and non-human cell lines (15). ICLAC was formed in 2012 to provide guidance on implementation of these standards and an ongoing focus for awareness and improvement. ICLAC can also act as a forum for discussion and harmonization of resources where needed.

ICLAC's Goals

1) Make Cell Line Misidentification More Visible

Many laboratories and cell line repositories have discovered misidentified cell lines, in publications that date back to the 1960s. Publicly available databases and resources have been developed to make this information available (13), including the Cellosaurus knowledge resource (16) and the NCBI BioSample database (17). Such resources enable the research community to compare samples and share

authentication testing data. But it is important that older reports should not be lost, and that inaccurate information in the scientific literature or 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 Register of misidentified cell lines for the research community and for feedback to Cellosaurus, BioSample, and other resources.

As a resource for the research community in this area, members of the group have previously compiled a list of reported misidentified cell lines in one location (2). This list, which is now known as the ICLAC Register of Misidentified Cell Lines, continues to be reviewed and updated as new reports of misidentification are published. Updates are versioned and made freely available online. The ICLAC Register serves as a resource to help ensure that reports of cell line misidentification are recorded, made accessible to the research community, and any inaccuracies are corrected.

The ICLAC Register of Misidentified Cell Lines can be found on the ICLAC website (2).

2) Promote Authentication Testing

The authentication standard referred to above is an important resource to standardize the testing of human cell lines (11,12,15). However, there is a need for education and additional resources if laboratories are to apply testing requirements to their own situations.

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

- Provide advice to scientists planning a project or grant application, starting new work, or initiating new cell lines
- Develop guidelines and protocols on application of the standard and recommended test measures
- Approach journals and funding bodies to encourage mandatory authentication testing, with assistance if needed to make such testing recommendations easier to make
- Share data to provide more effective datasets for shared online databases
- Share policies on difficult issues
- Develop guidelines for cell line authentication policies at research institutions
- Publish articles on best practice requirements and ways in which testing can be improved.

3) Act as a Forum for 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.

Ground Rules for Committee

Correspondence

The committee meets every 3-4 months by teleconference, or on request from any ICLAC member to discuss ICLAC-related business. Correspondence and other business are carried out by email as far as possible, to allow members from different time zones to contribute.

Committee 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.

Voting on Cell Line Entries

A new finding of cell line misidentification can be reported by any member or by the public. New reports will be distributed to the ICLAC members, providing an opportunity to vote, give feedback, and potentially share testing data where available. If members agree with the initial report, the cell line will be added to the ICLAC Register of Misidentified Cell Lines. If members challenge the initial report, the cell line will be reviewed 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 requested by any member or by the public. Requests are assessed using the same process as new entries, based on available data. If the committee concludes that authentic stock has been found, the cell line is moved from Table 1 (no known authentic stock) to Table 2 (authentic stock known). If the committee decides that more data are needed to conclude that a cell line is misidentified, the cell line is moved to a separate More Data Needed table

until more information becomes available. If the committee decides that the entry is invalid (e.g., if found to be legitimately derived from its parental cell line), it is moved to a separate Withdrawn table.

Ground Rules for Members and Partner Organizations

ICLAC Members

ICLAC members may also be referred to as "full members" to distinguish from associate members (see below). Members have expertise in cell line misidentification, authentication testing, or database applications. All members act in a voluntary capacity; their individual contributions and commitment to addressing the problem of cell line misidentification are respectfully acknowledged.

Members are required to contribute to the committee's work as determined by Goals 1, 2 and 3 above. Voting on new entries for the list of misidentified cell lines is considered an important priority. However, there are many ways in which members can contribute, depending on each person's expertise.

Members are appointed by invitation, following review of each request by the full committee via email. A list of members and their affiliations can be found on the ICLAC website (18).

Associate Members

Associate members may include individuals with related expertise – for example, an associate member may have expertise in database applications but minimal knowledge of cell line misidentification or authentication testing. Associate members may also include past members who no longer have time to contribute regularly to ICLAC's work. This enables consultation when ICLAC requires additional expertise for specific issues and allows past members to remain aware of ICLAC activities.

Associate members receive email correspondence but have no obligation to attend teleconferences and do not vote on new entries for the ICLAC Register of Misidentified Cell Lines.

Associate members are listed following the member list on the ICLAC website (18).

ICLAC Chair and Vice-Chair

The ICLAC Chair and Vice-Chair are jointly responsible for coordinating the activities of the committee, including scheduling teleconferences and making changes to the ICLAC membership and associate membership. The Chair and Vice-Chair can delegate ICLAC activities to other members as required, such as preparing teleconference minutes or modifying the ICLAC Register of Misidentified Cell Lines.

The Chair and Vice-Chair must both be ICLAC members. The roles of Chair and Vice-Chair extend for two years with the option for extension. When the Chair or Vice-Chair resigns, nominations are sought to

fill the role from amongst the ICLAC membership. Members are informed that they are nominated by email and have the option of declining the nomination. If more than one person accepts the nomination for the role of Chair or Vice-Chair, the ICLAC membership will vote to determine who shall hold the position.

Membership Upper Limit and Duration

An upper limit of 25 full members has been set to facilitate teleconference discussion and general logistics. There is no limit to the number of associate members.

Members are asked to renew their membership every two years. A call to renew is sent by email to all full members, followed by a reminder email. If a member does not renew their membership, their name will be removed from the ICLAC membership list. Members are able to resign their membership, or request to be transferred to the associate member list, at any time.

Partner Organizations

Partner organizations are invited to support ICLAC in various 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 the organizations who have supported the work of the committee in different ways.

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.

A full list of partner organizations and funding sources can be found on the ICLAC website (19).

Position on Product and Service Endorsement

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 partner organizations 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.

Definitions

A number of cell culture-related terms are used in this document. Many of these terms are defined in cell culture-related textbooks and resources (4,20). Key terms for ICLAC's work are defined below.

Authentication. The aim of authentication is to confirm or verify the identity of a cell line, demonstrating 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 their genotypes 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. Where unambiguous identification is not possible, species verification using methods such as CO1 barcoding (21), 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, either human cells or non-human cells arising from species such as mouse or rat. 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.

DNA (CO1) Barcoding. A method used for species-level identification of animal cell lines that is based on the mitochondrial gene that encodes the cytochrome C oxidase subunit 1 (CO1) (21). This method involves the amplification of a 648 base-pair region of the CO1 target using universal primers. The PCR amplicon is sequenced and compared to a reference database (GenBank and Barcode of Life Data systems (BOLD)) of sequences of known species identity. This method is useful for animal species that do not have an established STR profiling method in place for cell line authentication.

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 is considered to be a falsely identified or misidentified 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 or genotyping. 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 analyzing a set of STR loci (thirteen 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 (donor). STR profiling is useful for authentication of human, mouse, and dog cell lines.

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 (donor). An exception applies to inbred populations such as laboratory rodents, where it may be concluded that samples come from the same strain.

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