TECHNICAL SPECIFICATION

ISO/TS 23511

Biotechnology — General requirements and considerations for cell line authentication

Biotechnologie — Exigences et considérations générales relatives à l'authentification de la lignée cellulaire

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This document was prepared by Technical Committee ISO/TC 276, *Biotechnology*.

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Introduction

Cell line authentication is a critical quality control (QC) procedure, which aims to verify a cell line's identity and show that it is free of contamination from other cell lines. It has been estimated that a considerable proportion of the cell lines stored in the United States, Europe and Asia are misidentified or cross-contaminated, which results in potentially misleading or non-repeatable data, causing tremendous waste of time and effort.[13] To facilitate proper utilization of a cell line, the standardization of procedures used for cell line authentication is urgently needed. This document elaborates on general requirements for cell line authentication based on the existing national standards and state-of-the-art methods, aiming to represent and provide guidance to stakeholders in life science, biomedicine and other related fields.

Biotechnology — General requirements and considerations for cell line authentication

1 Scope

This document defines terms related to cell line authentication in the field of biotechnology. It describes the general principles, detection strategies and analytical methods for cell line authentication. It specifies requirements and key considerations for method selection, quality control parameters, data analysis and reporting.

This document is applicable to routine inspection of cell lines in culture and in storage in the fields of basic research, translational studies and product manufacturing. It is also applicable to cell line origin validation in academic and industrial laboratories, cell banks and manufacturing sites. It is primarily applicable to mammalian cells, including human cells.

This document does not apply to non-animal cells (e.g. microbial contamination, plant cells), nor to cells in complex matrices (e.g. tissues, organs, organoids, plants).

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO/IEC 17025, *General requirements for the competence of testing and calibration laboratories*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
cell bank
collection of appropriate containers, whose contents are of uniform composition, stored under defined conditions, and where each container represents an aliquot of a single pool of cells

[SOURCE: ICH Q5D[14]]

3.2
cell line
defined population of cells that has been passaged from a primary culture, and can be maintained in culture for an extended period of time, retaining stability of certain initial phenotypes and functions for its intended use

Note 1 to entry: A primary culture is a culture started from cells, tissues or organs taken directly from an organism, and before the first subculture, propagation and consecutive passages *in vitro*.

3.3
cell line authentication
process by which the *cell line* (3.2) identity is verified and shown to be free of contamination from other cell lines

3.4
cell line identification
process by which the *cell line* (3.2) identity is verified, which includes confirmation of cell origin, and identification of cell-specific characteristics

3.5
microbial contamination
presence of exogenous:

a) bacteria and/or fungi;

b) viruses; and/or

c) foreign inter- or intra-species *cell lines* (3.2) in a cell culture

Note 1 to entry: Some cell lines have endogenous virus/viral sequences.

Note 2 to entry: Point c) is commonly known as "cell cross-contamination".

3.6
detection limit
lowest quantity of a substance that can be distinguished from the absence of that substance with a stated confidence limit

[SOURCE: ISO 14687:2019, 3.5]

3.7
DNA barcoding
taxonomic method that uses a short genetic marker in an organism's DNA to identify it as belonging to a particular species

3.8
immunofluorescence
method for studying the distribution of specific protein antigens in cells by combining immunological methods (antigen-specific binding) with fluorescent labelling techniques

3.9
cell line inter-species cross-contamination
contamination of a cell culture by cells derived from different species

3.10
cell line intra-species cross-contamination
contamination of a cell culture by the same type of cells (from different individuals) or different types of cells (from the same or different individuals) derived from the same species

3.11
isozyme analysis
isoenzyme analysis
separation technique based on electrophoresis to generate patterns of enzymatically active polypeptides with identical *specificity* (3.19) but of different molecular structure

3.12
karyotype analysis
chromosomal analysis in each cell to detect aneuploidy, structural abnormalities and ploidy

3.13
MPS
massively parallel sequencing
sequencing technique based on the determination of incremental template based polymerization of many independent DNA molecules simultaneously

Note 1 to entry: Massively parallel sequencing technology can provide millions or billions of short reads per run.

[SOURCE: ISO 20397-2:2021, 3.30]

3.14
cell line misidentification
incidence where the *cell line* (3.2) identity is incorrectly given through mislabelling

3.15
PCR
polymerase chain reaction
enzymatic procedure which allows *in vitro* amplification of DNA

[SOURCE: ISO 22174:2005, 3.4.1]

3.16
sensitivity
quotient of the change in an indication of a measuring system and the corresponding change in a value of a quantity being changed

[SOURCE: ISO/IEC Guide 99:2007, 4.12, modified — Preferred term "sensitivity of a measuring system" and notes to entry deleted. "changed" replaced "measured".]

3.17
STR
short tandem repeat
variable segments of DNA that are composed of multiple adjacent two to five basepair long sequences

3.18
SNP
single nucleotide polymorphism
single nucleotide variation in a genetic sequence that occurs at an appreciable frequency in the population

[SOURCE: ISO 25720:2009, 4.23]

3.19
specificity
property of a method to respond exclusively to the characteristic or analyte under investigation

[SOURCE: ISO 24276:2006, 3.1.4]

3.20
validation
confirmation, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled

[SOURCE: ISO 9000:2015, 3.8.13, modified — Notes to entry deleted.]

3.21
WGS
whole genome sequencing
methods that approach determination of the entire nucleotide sequence of the nuclear DNA of eukaryotic organisms

3.22
verification
confirmation, through the provision of objective evidence, that specified requirements have been fulfilled

[SOURCE: ISO 9000:2015, 3.8.12, modified — Notes to entry deleted.]

4   Principles of cell line authentication

4.1   General

Multiple test methods that rely on genomic analysis combined with phenotypic characteristics can be used as part of the process of cell line authentication. Purposes of genomic analysis include:

a)   confirmation of cell origin;

b)   examination of cell species, to ensure that no cell line inter- or intra-species cross-contamination exists in cell cultures;

c)   either identification or confirmation, or both, of certain cell line-specific characteristics. Cell line-specific characteristics such as gene mutations can be useful supporting evidence for the cell line authentication. However, many "cell-specific" characteristics are related to tissue type or disease status and are not unique.

4.2   Confirmation of cell origin

For a newly established cell line, a liquid or solid tissue sample from which a cell line is derived, or a liquid or solid tissue sample from the same donor from whom the cell line was derived, should be stored for origin confirmation. The baseline DNA profile of the original sample should be used in cell line authentication by comparing it to the DNA profiles of subsequent passages. If the source tissue or blood, or both, are not available, the DNA profile of an early passage stock can be used as the baseline. DNA-based profiling methods intended for routine genotype analysis include:

a)   short tandem repeat (STR) analysis with polymerase chain reaction (PCR) assays followed by fragment size analysis or by Sanger sequencing;

b)   single nucleotide polymorphism (SNP) analysis by single-base extension assay or SNP genotyping qPCR assays;

c)   latest DNA profiling technologies, such as massively parallel sequencing (MPS).

SNP databases of targeted panels are now available for analysis. However, there are no central databases or universally accepted SNP markers, so any SNP comparison shall be in-house or have similar usage limitations. The whole genome sequencing (WGS) data of newly or already established cell lines should be provided as a further information source.

4.3   Detection of cross-contamination

4.3.1   Detection of cell line inter-species cross-contamination

4.3.1.1   Cell line inter-species cross-contamination occurs when a cell line is contaminated by undesired cells from different species. Cell lines derived from different species have different characteristics, not all of which are suitable for authentication.

Cell line authentication should be performed with consideration for various characteristics, including:

a)   genetic characteristics (e.g. CO1, CytB and ND5 genes);

b)   cytogenetic characteristics (e.g. chromosome karyotype, marker chromosome);

c)   biochemical characteristics (e.g. enzyme type);

d)   cell markers (e.g. proteins, lipids, glycosylation, histocompatibility antigen, tissue-specific antigens);

e)   cell kinetics (e.g. differences in cell division frequency or cell generation time);

f)   morphological characteristics (e.g. round, long spindle).

4.3.1.2   Methods based on different measurement principles should be used for the detection of cell line inter-species cross-contamination. For genetic and cytogenetic characteristics, detection methods include DNA barcoding, PCR assays and karyotype analysis. DNA barcoding can be used to investigate the mitochondrial gene sequences associated with species-specific cytochrome c oxidase subunit 1 (CO1) gene. PCR assays utilize either species-specific or degenerate primers, which can amplify DNA fragments for species identification and can detect lower levels of cross-contamination than Sanger sequencing-based DNA barcoding. Karyotype analysis can directly reveal cross-contamination by comparing species-specific chromosomes. Morphological characteristics, cell kinetics, biochemical characteristics and phenotype are useful to provide supporting data for occurrence of cell cross-contamination, but are not suitable for authentication testing when used alone.

4.3.2   Detection of cell line intra-species cross-contamination

4.3.2.1   Cell line intra-species cross-contamination occurs when a cell line is contaminated by cells of the same type (from different individuals) or of different types (from the same or different individuals) within the same species. Detection of cell line intra-species cross-contamination depends on individual cell line-specific characteristics, which can include:

a)   genetic characteristics (e.g. STR profiling, SNP profiling);

b)   genetic sequence (e.g. WGS);

c)   cell markers (e.g. proteins, lipids, glycosylation, histocompatibility antigen, tissue-specific antigens);

d)   morphological characteristics (e.g. round, long spindle);

e)   histology (e.g. extracellular cellular markers).

Morphological characteristics and cell markers are useful to provide supporting data for occurrence of cell cross-contamination, but are not suitable for authentication testing when used alone.

4.3.2.2   Sequence-specific STR or SNP profiles can be used to discriminate among individuals within the same species. STR or SNP profiling-based Sanger sequencing or MPS technologies should be used for detection of cell line identity but they also can provide data about intra-species cross-contamination. Attention should be given to contamination at an early stage, which can go unnoticed even by these techniques.

4.4   Identification of cell line-specific characteristics

4.4.1   Detection of cell line genome heterogeneity

4.4.1.1   During extended *in vitro* cell culturing, cell lines can acquire additional genomic changes and evolve into multiple genetically, transcriptionally, proteotypically or phenotypically different sub-clones (e.g. the genetic instability and cell heterogeneity of cancer cell lines).

NOTE 1   Detection of cell line genome heterogeneity is not an authentication test method. Methods for cell line authentication can be used for detecting cell line genome heterogeneity.

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