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Biotechnology — General requirements and considerations for cell line identification and cross-contamination testing

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO document should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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This document was prepared by Technical Committee ISO/TC 276, *Biotechnology*, Subcommittee SC 1, *Analytical methods*.

This first edition cancels and replaces the first edition (ISO/TS 23511:2023) which has been technically revised.

The main changes are as follows:

— Title and Scope revised;

— addition of terms and definitions in Clause 3;

— in 4.1, added Figure 1- “cell line authentication flow chart” and added 4.5 “Best practice in cell culture”;

— in Clause 6, added Table 2 – “Summary of matching criteria” for the method STR profiling, added 6.2.2 profiling for non-human cell lines and Table 3 – “Summary of non-human STR profiling”;

— elaborated on descriptions of current methods, and described their principle, scope of applications, characteristics of the methods, measurement procedures and limitations.

Any feedback or questions on this document should be directed to the user’s national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

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, free of adventitious agents, and expresses cell-specific characteristics (including phenotype, genotype, and function). Each of these methods are necessary for cell line authentication and are only supportive information on their own. It has been estimated that a considerable proportion of the cell lines stored in the biobanks and laboratories in the United States, Europe and Asia are misidentified or cross-contaminated, which results in potentially erroneous or irreproducible data, causing tremendous waste of time, money and effort.[8] To facilitate proper utilization of a cell line and ensure the accuracy and validity of the results of cell research and application, the standardization of procedures used for cell line authentication is urgently needed. This document gives general requirements for cell line identification and cross-contamination testing based on 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 identification and cross-contamination testing

1 Scope

This document specifies general principles, detection strategies and analytical methods for cell line identification of mammalian cells in the field of biotechnology. This document also specifies general requirements and key considerations for method selection, quality control parameters, data analysis and reporting in cell line identification.

This document is applicable to routine cell line cross-contamination testing in the fields of basic research, translational medicine studies and cell therapeutic product manufacturing. This document is also applicable to cell line identity confirmation to prevent cell misidentification in academic and industrial laboratories, cell banks and manufacturing sites. This document is primarily applicable to mammalian cells.

2 Normative references

There are no normative references in this document.

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
allele
member of two or more alternative forms of a DNA sequence found at a particular locus

[SOURCE: ISO/IEC 19794-14:2022, 3.1.6]

3.2
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[16] — adapted.]

3.3
cell line
progeny of a primary culture after it has been passaged beyond crises or beyond senescence either spontaneously or after introduction of immortalizing factors

Note 1 to entry: A cell line is continuous for proliferation.

[SOURCE: ISO 21709:2020, 3.2, modified — Note 2 to entry deleted.]

3.4
cell line authentication
process by which the *cell line* (3.3) is verified by confirming identification, *cell line origin* (3.11), absence of cell line cross-contamination, absence of adventitious agents, and that it expresses cell-specific characteristics (phenotype, genotype, function)

3.5
cell line identification
process by which genomic identity of a *cell line* (3.3) is verified or confirmed

3.6
DNA barcoding
method of identifying organisms at the species level based on short, standardized DNA fragments containing both conserved and variable sequences from a specific region or regions of the genome

Note 1 to entry: The principle of DNA barcoding is that by comparison with a reference database the sequence from these DNA fragments can be used to uniquely identify an organism or link it to a specific taxon.

[SOURCE: ISO 16577:2022, 3.7.3]

3.7
examination reliability
probability that an examined value is the same as a reference value of a nominal property

Note 1 to entry: The nominal property can be the identity of a cell line.

3.8
inter-species cross-contamination
contamination of a *cell line* (3.3) by cells from another species

3.9
intra-species cross-contamination
contamination of a *cell line* (3.3) by cells from the same species

Note 1 to entry: Cells from the same species can include the same type of cells from different individuals or different types of cells from different individuals.

3.10
cell line misidentification
incidence where the *cell line* (3.3) identity no longer corresponds to the donor or species from which it was originally established.

Note 1 to entry: Improper naming of newly established cell lines or cell line derivatives (clones) can lead to cell line misidentification.

3.11
cell line origin
donor attributes and date of *cell line* (3.3) establishment

Note 1 to entry: Donor attributes can include race, sex, age, tissue, and disease status, depending on the species from which the cell line is derived.

3.12
cell type
classification used to distinguish among distinct cell forms

[SOURCE: ISO 21709:2020, 3.5]

3.13
detection limit
lowest quantity of a substance that can be distinguished from the absence of that substance with a stated confidence limit

[SOURCE: ISO 14687:2025, 3.1.5]

3.14
gene mutation
permanent alteration (as by point mutation or frameshift mutation) in the nucleotide sequence of a gene, referred to as alterations in a single-base pair nucleotide or changes in one or more nucleotides altering the open-reading frame

3.15
genetic drift
change in *allele* (3.1) frequency in a cell population, due to a random selection of certain genes

3.16
immunofluorescence
method for detecting the expression of specific protein antigens on cell membrane or in cells by combining immunological methods (antigen-antibody recognition) with fluorescent labelling techniques

3.17
isozyme analysis
isoenzyme analysis
separation technique based on electrophoresis to generate patterns of enzymatically active polypeptides with identical *specificity* (3.26) but of different molecular weight and charge

3.18
karyotype analysis
process of ordering all the chromosomes of each cell and determining chromosomal constitution in *cell lines* (3.3) to reveal changes in chromosome number associated with aneuploidy, ploidy and structural changes, such as deletions, duplications, translocations, or inversions

3.19
massively parallel sequencing
MPS
next generation sequencing
non-Sanger-based high-throughput nucleic acid sequencing

Note 1 to entry: Millions or billions of nucleic acid strands can be sequenced in parallel, yielding substantially more throughput.

Note 2 to entry: NGS (next generation sequencing) is also well recognized as MPS in the ISO 20397 series.

Note 3 to entry: MPS or NGS covers long read sequencing and short read sequencing.

[SOURCE: ISO 20397-3:2025, 3.15]

3.20
misidentified cell lines
cell lines (3.3) that no longer correspond to original donor or species

Note 1 to entry: Misidentified cell lines can arise due to cross-contamination or mislabelling.

3.21
polymerase chain reaction
PCR
enzymatic procedure that allows in vitro amplification of deoxyribonucleic acid (DNA)

[SOURCE: ISO 22174:2024, 3.1.17]