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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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Foreword

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

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. 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.

NOTE 2 It is reported that stocked HeLa cells originated from different laboratories show notable variability in genome, steady-state mRNA expression, protein expression and protein turnover rates at uniform culture condition. Moreover, progressive divergence can be observed within a specific HeLa cell line after three months of continuous culture. The *in vitro* cell manipulating procedures, such as transfection and gene editing, can also lead to genetic heterogeneity.[15]

Detection of genomic changes depends on cell line-specific markers, which can include:

a) genetic sequence (e.g. Sanger sequencing or MPS of DNA);

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

c) transcription (e.g. mRNA);

d) karyotype.

4.4.1.2 The detection methods of cell line gene mutations can include high-throughput sequencing (e.g. WGS) along with karyotype analysis. The COSMIC database provides list of cell line somatic mutations found in various human cancers.[16]

4.4.2 Detection of cellular differentiation

4.4.2.1 Both pluripotent and multipotent stem cells can differentiate, either spontaneously or with external stimuli into certain cell types *in vitro*. Cellular differentiation within a given cell line can be detected by various gene expression markers, which can include, but are not limited to:

a) cell surface markers;

b) transcription factors;

c) signalling pathway-related intracellular markers;

d) enzymatic markers.

4.4.2.2 Flow cytometry analysis, immunofluorescent staining and enzyme-linked immunosorbent assays can be used to detect differentiated cell-specific gene expression, along with gene expression assays, indicating cellular differentiation status.

NOTE Measurement of cellular differentiation can provide useful supporting data as part of broader cell line identification but is not suitable for authentication testing when used alone as this relies on genome-based methods.

5 Application scenarios of cell line authentication

To avoid cell line misidentification and cross-contamination, cell line authentication should be used in the following scenarios:

a) authentication and characterization of newly established cell lines;

b) routine inspection of cell lines in culture and in stock, especially for rapid growing cell types, cells in extended culture, cells with unusual phenotype and cells after a selection/sorting process;

c) validation of cell line origin upon receiving from other facilities, before sending the material to other facilities and prior to banking;

d) validation of a cell line origin after the preparation of a cell bank (i.e. seed stock);

e) authentication of cell lines used in both basic and clinical research when abnormalities were found in the cultured cells, or after multiple passaging.

6 Sample preparation

6.1 Samples utilized for cell line authentication can include whole cells, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Identification errors can arise due to errors when labelling or handling samples, or cross-contamination of cells or DNA. Care to reduce the possibility of error(s) shall always be taken when handling or labelling cells or DNA.

6.2 Cells used for sampling should be fully mixed and representative of culturing status. The cells used for karyotype analysis should be undergoing active proliferation so that cells in metaphase of mitosis are present for analysis.

6.3 DNA should be of sufficient quality and quantity for downstream assays.

NOTE Requirements can vary with the authentication methods.

6.4 For DNA extraction kits, the supplier's technical manual or other instructions shall be followed. A validation is recommended in order to define the ideal quantity of DNA to be used in the test so that the true peaks are well defined. ISO 20395 can be used for information on more detailed procedures.

7 Method options for cell line authentication

7.1 General

Each method for cell line authentication has its intrinsic deficiencies, which can affect applicability and accuracy (as described in Annex A). Users can select one or more methods depending on their information of cell type, application scenario, sample preparation or potential contaminating sources.

NOTE 1 Historical methods such as human leukocyte antigen (HLA) profiling by PCR and isozyme analysis have previously been used for cell line authentication, but are no longer used due to their limitations in applicability, sensitivity and accuracy. Currently, DNA-based methods are most widely used for cell line authentication, which include STR profiling, SNP profiling, HLA sequencing, DNA barcoding and multiplex PCR. With technology innovation, state-of-the-art methods, such as WGS, have started to play increasingly important roles in the authentication field.

NOTE 2 Karyotype analyses and optical genome mapping technique can detect large chromosomal structure changes. Generally, karyotype analysis cannot give results that are specific to individual of origin unless certain cell lines have marker chromosomes that can be readily recognized, such as diploid cells and stem cells.

NOTE 3 HLA profiling is only applicable to cross-contamination detection of human cell lines derived from different individuals. HLA profiling, by serotyping or PCR, can be relatively informative, and is currently only used for analysis and comparison of historical samples and data.

NOTE 4 Cells derived from different species have different isozyme distribution. Isozyme analysis can have relatively low sensitivity. Isozyme types, quantity and subjective judgement can affect detection accuracy and sensitivity. Reagents for isozyme analysis are difficult to acquire and this method is rarely used anymore.

7.2 DNA-based cell line authentication methods

7.2.1 Short tandem repeat profiling

7.2.1.1 Principle

STR profiling is widely used in cell line authentication, by analysis and comparison of specific STR loci. STR loci consist of two to five nucleotides with different numbers of repeats in a row. Each STR locus can be PCR amplified, labelled with fluorophores of different wavelengths, and distinguished by size and wavelength. STR profiling measures the exact number of repeating units.

7.2.1.2 Scope of application

STR profiling mainly involves three major steps, which include target STR locus amplification, profiling and data interpretation. As for data interpretation, the standard 10 STR loci are recommended for cell line authentication; the 16 STR loci with higher detection accuracy are also recommended (see Table 1). STR loci shall be selected to minimize release of donor-specific information. For the choice of STR loci, the laboratory shall be aware of the relevant data and personal protection scheme in the country(-ies) of use.

Table 1 — Summary of STR profiling

Elements	Methods/ parameters	Application/characteristics
STR loci	PCR/10 STR loci[a]	Standard loci recommended for general human cell line cross-contamination detection
	PCR/16 STR loci[b]	Recommended to detect cross-contamination with higher accuracy and sensitivity
STR loci	PCR/capillary electrophoresis	Standard profiling method for intra-species cross-contamination detection
	Next-generation sequencing	Recommended for high-throughput genotyping analysis of STR loci for detection

[a] The 10 STR loci include D13S317, TH01, D5S818, D16S539, DYS391, TPOX, D7S820, DS21S11, CSF1PO, vWA and Amelogenin. DYS391 is on the Y chromosome and is used for male confirmation.

[b] The 16 STR loci include D18S51, D21S11, TH01, D3S1358, FGA, TPOX, D8S1179, vWA, CSF1PO, D16S539, D7S820, D13S317, D5S818 and Amelogenin. Commercially available kits also include assays either for the Penta E and Penta D loci, or for the D2S1338 and D19S433 loci.

7.2.1.3 Characteristics of the method

STR profiling is currently mainly applicable for human cell line cross-contamination detection. With a comprehensive collection of STR data, this method can also be used for broader cell line intra-species cross-contamination detection (with species-specific probes or primers) and for individual donor identification.

NOTE Available databases for STR profiling are mainly contributed by the American Type Culture Collection (ATCC), Cellosaurus (CLASTR), Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Istituto Di Ricovero e Cura a Carattere Scientifico (IRCSS), Ospedale Policlinico San Martino (CLIMA), Japanese Collection of Research Bioresources (JCRB), Rikagaku Kenkyusho: Institute of Physical and Chemical Research (RIKEN), Short Tanden Repeat DNA Internet Data Base (STRBase) and Register of Misidentified Cell Lines database from International Cell Line Authentication Committee (ICLAC).

When sharing STR profile information, the researchers shall be aware of the relevant data protection scheme in the country(-ies) of use.

Cell line STR profiles should be verified by comparing to other samples from the original donor and to cell line STR profile databases. Match criteria should be used for interpretation of these comparisons as described in ANSI/ATCC ASN-0002[11].

Cancer cells contain a wide range of aberrations in their genome, with frequent gains and losses of alleles at different genomic locations. Mismatch repair (MMR)-deficient cell lines have microsatellite instability, which can cause more marked allelic drift. Thus, cell lines can be misclassified by STR profiling. In this case, analysis of increased number of loci and selection of a suitable set of match criteria shall be used for authentication testing (e.g. list of EXAMPLE 1 ATCC STRBase,[17] 2 CLASTR STRBase,[18] 3 DSMZ STRBase includes data sets of 2455 cell lines from ATCC, DSMZ, JCRB and RIKEN[19]).

ANSI/ATCC ASN-0002:2021[11] is recommended as a reference for STR profiling of human cell lines.

7.2.1.4 Measurement procedure

STR profiling is able to analyse partially degraded and intra-species mixes with at least a 10 % contamination or lower, depending on kits or laboratories, or both.

STR profiling is inherently limited in detecting detailed genetic changes, as it cannot detect large chromosomal structural changes, cell contamination with adventitious agents, or the presence of somatic mutations occurred during extended *in vitro* cell culturing.

7.2.2 Single nucleotide polymorphism profiling

7.2.2.1 SNP refers to single nucleotide variation at a specific position in an individual genome. SNPs within a specific locus are conserved during evolution and can be used for cell line authentication. SNP profiling can be performed by next-generation sequencing, DNA microarray, PCR and mass spectrometry techniques. MPS platforms for SNP profiling have high throughput capacity and high accuracy.

7.2.2.2 SNP profiling involves major steps of primer design, amplification, detection and data analysis. Multiple sites joint analysis can be used to obtain specific SNP maps of different cell lines, and for detection of cell line intra-species cross-contamination. With species-specific probes or primers, SNP profiling can also be applicable for detection of cell line inter-species cross-contamination.

7.2.2.3 SNP profiling has certain advantages over STR profiling. SNP profiling can be used to analyse heavily degraded DNA fragments. A low mutation rate makes SNPs good genetic markers. Multiplexing and automation of SNP profiling can also be more accessible compared with STR profiling. However, the displayed sensitivity of SNP profiling depends on the copy number at locus of interest. SNP profiling is not suitable for authentication testing of mixed DNA samples.

7.2.2.4 For cell lines with microsatellite instability, SNP profiling can be used as an alternate method for authentication testing.

NOTE 1 With more SNP loci, the authentication accuracy can be higher.

NOTE 2 SNP data have been collected for some cell lines.[20]

7.2.3 DNA barcoding

7.2.3.1 The cytochrome c oxidase subunit 1 (CO1), a mitochondrial gene, is usually conserved within a given species and exhibits polymorphism between species. Standardized methods of CO1 DNA barcoding for species-specific animal cell line authentication have been developed. This method is widely used for cell line species identification by Sanger sequencing of specific conserved regions of the CO1 gene and by data analysis with the Barcode of Life Data System (BOLD) and National Center for Biotechnology Information (NCBI) databases.

7.2.3.2 The major steps of DNA barcoding include a PCR amplification of the barcode region, using a portion of the PCR product to run a gel confirming amplification, purifying the remaining amplicon not used on the gel, Sanger sequencing the purified template, collecting the sequence data from a genetic analyser, and comparing the analysed sequence data to database reference sequences. The species level identification is verified by database DNA sequence comparison (e.g. BOLD).

7.2.3.3 DNA barcoding is mainly used to find cell lines having a misidentified species for which the barcode sequence is available as a database reference. Since Sanger sequencing analysis only detects a dominant sequence, DNA barcoding is not suitable for authentication testing of a mixed DNA sample from multiple species.

7.2.3.4 ANSI/ATCC ASN-0003-2015[12] is recommended as a reference for inter-species cell line cross-contamination testing with the CO1 DNA barcoding method.

7.2.4 Multiplex PCR

7.2.4.1 Multiplex PCR is a widely used technique in the molecular biology field. It can simultaneously amplify multiple loci within a single PCR reaction by using different primer sets for multiple targets.

NOTE ISO 20395 and ISO 21474-2 are available for further information.

7.2.4.2 Multiplex PCR can be used for inter-species cross-contamination detection of frequently used cell lines.

7.2.4.3 Multiplex PCR provide information of multiple loci in a time- and cost-effective manner. However, primer design for multiplex PCR is complex. Assay design and optimization should also be performed. A development validation (or complete validation) shall be applied for in-house products. This method is suitable for laboratories with technical sufficiency and those who routinely perform assays with the same set of targets.

NOTE ISO 20395 and ISO 21474-2 are available for further information.

7.2.5 Whole genome sequencing

7.2.5.1 The genome of a cell line encodes the complete genetic information representing species- or individual-specific characteristics. WGS is used to decode an individual's complete genomic landscape, which includes detailed information of genetic polymorphism at CO1, HLA, STR, SNP, and other loci, as well as genetic drift, spontaneous mutations and large chromosomal variation.

7.2.5.2 The workflow for WGS includes DNA extraction, DNA shearing, library construction, library sequencing, raw data output and data analysis. WGS is suitable for cell line authentication in applications of cell origin confirmation, cross-contamination detection and cell line-specific characteristic identification. Due to its broad coverage, increasingly high throughput and decreasing cost, WGS is recognized as a state-of-the-art method for cell line authentication.

7.2.5.3 High-quality, intact and non-degraded DNA at a sufficient amount (around 1 µg of DNA is required as starting material for sequencing) is required for WGS, particularly of long-insert size libraries.

NOTE ISO 20397-1 and ISO 20397-2 are available for further information on massive parallel sequencing.

7.3 Related methods for cell line identification

Certain cell lines present specific antigens and receptor markers and secrete exclusive hormones and proteins. Immunofluorescence is performed by labelling the antibody with a fluorochrome marker and a specific fluorescent reaction can be observed by the binding of the antibody to its corresponding antigen (or antibody). Therefore, immunofluorescence technology can be used to determine cell line-specific characteristics.

NOTE 1 With some commercially available species-specific antibodies, the species of cell lines can also be determined.

Cell line-specific surface and intracellular markers can also be used for cell characterization (e.g. haematopoietic stem cells express CD34 surface marker, and cytokeratin 8 is an intracellular marker of epithelial cells).

NOTE 2 Immunofluorescence staining, isozyme analysis and flow cytometry are phenotype-based methods, which can provide supportive data, but are not suitable for authentication testing.

8 Authentication method selection

8.1 General

For the purpose of cell line authentication, appropriate methods should be chosen depending on cell origin, cell type, authentication purpose and the laboratory's expertise.

8.2 Cell origin

8.2.1 Confirmation of cell line origin

8.2.1.1 For newly established cell lines, extra tissue, along with its histopathological data as well as either donor or patient consent or ethical review permit, or both, should be stored for origin confirmation. In cases where the original tissue is not accessible, a comparison with the genetic profile of the earliest possible cell lineage, such as from data of original cell stock, established public database and from reputable suppliers that provide STR profile during cell line purchase, shall be done. For origin confirmation, methods of STR profiling, SNP profiling, either multiplex PCR or DNA sequencing, or both, are recommended as appropriate (intra-species identification assays are still unavailable for many species).

8.2.1.2 Cell lines should be routinely identified both in culture and in stock, upon receiving from other facilities, when newly established, after manipulation or extended *in vitro* culture (such as cloning experiments).

8.2.2 Identification of cell line gene mutations

Cell line evolution can occur during extended *in vitro* culture with genetic and phenotypic instability. Genomic alterations, such as DNA insertions, deletions, point mutations and some chromosome rearrangements, can be detected by WGS.

WGS can address most mutational metrics provided there is sufficient bioinformatics support for the interrogation of genomic sequence databases.

8.2.3 Identification of cell line-specific properties

During cell line establishment, targeted cells are often contaminated by undesired cell populations.

EXAMPLE A primary lung cancer cell line is often contaminated by lung fibroblasts.

Methods based on reporter gene expression and genetic markers, such as flow cytometry and immunofluorescent staining, as well as methods based on functional properties, such as doubling time, cytokine secretion and differentiation capacity, are recommended for cell line-specific analysis.

Phenotype-based methods can provide useful supporting data as part of broader cell line identification, but are not suitable for authentication testing, which relies on genome-based methods.

8.3 Species types of cross-contamination

8.3.1 Cell line inter-species cross-contamination

8.3.1.1 Cell biology laboratories can simultaneously culture or operate cells originated from different species for research or manufacturing. This can increase the risk of misidentification and cell line inter-species cross-contamination.

8.3.1.2 WGS, DNA barcode analysis, karyotype analysis or PCR-based detection of conserved gene sequences, such as that of mitochondrial cytochrome b or cytochrome c oxidase subunit 1 (CO1), or of

a method based on cytochrome b PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) are recommended to detect cell line inter-species cross-contamination.

8.3.2 Cell line intra-species cross-contamination

8.3.2.1 Laboratories can simultaneously culture cell lines from different individuals of the same species. Cell lines can also be misidentified and arise from the same species even, if this is not known to be the case. Detection of cell line intra-species cross-contamination shall be performed routinely for all cell lines of human origin (where testing is widely accessible) and for cell lines of rodent origin if testing is available. Also, intra-species strain identification shall be done for animal facilities, particularly for inbred mouse and rat colonies and xenograft models.

8.3.2.2 STR profiling or next-generation sequencing are available methods for detection of cell line intra-species cross-contamination with high accuracy.

8.4 Cell culture methods

8.4.1 Authentication for co-cultured cells

8.4.1.1 Certain cell lines shall be co-cultured with feeder cells derived from different species.

EXAMPLE Human embryonic stem cells are usually co-cultured with mouse embryonic fibroblast (MEF) cells, which leads to high cross-contamination risk between these two cell types.

8.4.1.2 A species-specific PCR method based on detection of conserved gene sequences (such as that of mitochondrial cytochrome b or CO1) should be used for authentication testing of co-cultured cells from different species.

8.4.2 Authentication for *ex vivo* cell culture

8.4.2.1 The patient-derived xenograft (PDX) model is widely used for the establishment of human cancer cell lines. Cross-contamination risk can occur by using host animals. The detection of cell line inter-species cross-contamination and cell line-specific characteristics should be performed.

8.4.2.2 Species-specific PCR-based methods, MPS or DNA barcode analysis should be used for the detection of cell line inter-species cross-contamination. Flow cytometry and immunofluorescence staining, as well as PCR and sequencing-based methods, can be used for cell line-specific characteristic analysis.

8.4.3 Authentication for laboratory operation

To avoid cross-contamination, an aseptic technique should be strictly applied during long-term cell culture.

Cell cross-contamination can occur by simultaneous manipulation of multiple samples or by public sharing of equipment and reagents. In this case, the contamination risk should be determined depending on laboratory protocols. Detection methods should be chosen accordingly based on cell line authentication principles.

8.5 Authentication purpose

8.5.1 Cell lines should be re-authenticated upon receiving a sample from external cell banks or institutions, after extended *in vitro* culture or after the preparation of a cell bank. Cell line authentication should be performed according to standard procedures including confirmation of cell origin, detection of cross-contamination and identification of cell line-specific characteristics.

NOTE A formal report provided by accredited providers such as companies/laboratories that are accredited for STR profiling can be used for cell line authentication purposes upon receiving a sample.

8.5.2 When it is necessary to detect whether contamination by a specific cell line (e.g. HeLa cell line) has occurred, a genotype-based authentication method should be used (e.g. STR profiling). Methods that are considered cell line-specific, such as flow cytometry or immunofluorescent staining, detect tissue- or disease-specific characteristics. These methods can also be misleading when used alone, due to changes in phenotype in culture.

9 Quality control

9.1 Operator training

9.1.1 The personnel in charge of QC, e.g. technicians, should be specialized in molecular biology, cell biology or other related research areas. They should finish career training and get institute certification with competence assessment reviews.

9.1.2 Prior to the independent processing of samples, the QC technician should have proficient skills in cell culturing, as well as preventing and detecting cell line cross-contamination.

9.1.3 Each test method has specific QC requirements; therefore, the operator shall develop proficiency in each relevant method separately. ASN-0002[11] and ASN-0003[12] should be used for reference of detailed information on QC requirements for STR profiling and DNA barcoding.

9.1.4 The training content for the QC technician should include, but is not limited to:

a) cell culture and other related aseptic techniques;

b) optimization of cell growth;

c) the characteristics of the cultured cell lines;

d) knowledge for detection and prevention of cell contamination and misidentification;

e) the laboratory environment control, keeping the laboratory safe, clean and free of contaminations;

f) the extraction of nucleic acids from cell samples;

g) PCR using primer pairs and multiplex techniques;

h) other molecular biology experience;

i) the use of cell line and molecular biology databases.

9.2 Instruments and equipment

9.2.1 Instruments and equipment should possess the appropriate installation qualification, operational qualification and performance qualification. The operator should follow the standard operating procedures (SOP) of instruments and equipment. The SOP of advanced instruments should be documented.

9.2.2 The performance of instruments and equipment should be examined and calibrated at regular intervals.

9.2.3 Measures to control facilities shall be implemented, monitored and periodically reviewed, and shall include, but are not limited to:

a) the access to and use of areas affecting laboratory activities;

b) the prevention of contamination, interference or adverse influences on laboratory activities;

c) the effective separation between areas with incompatible laboratory activities.

9.3 Reagents

9.3.1 The reagents used for cell line authentication should be purchased from a qualified supplier, who should provide an attached quality inspection report.

9.3.2 The reagents should be used according to the user manual.

9.3.3 The reagent consumption should be documented with the reagent label, the time of first use and the final expiration date. The expired reagents should be removed from the laboratory and only non-expired reagents should be used. For in-house preparations, the identity of the person preparing the reagent should be documented.

9.3.4 The reagents should be handled in separate containers by certified technicians to avoid cross-contamination during transportation, storage and utilization.

9.4 Validation and verification of methods

9.4.1 General

Either validated methods or verified methods, or both, shall be used according to 9.4.2 and 9.4.3.

9.4.2 Validation

9.4.2.1 When methods are used, it shall be ensured that these methods have been validated, in order to ensure fitness for the intended purpose. When the validation is performed by the laboratory, it shall document and retain for a defined period of time the results obtained, the procedure used for the validation and a statement as to whether the method is fit for purpose.

9.4.2.2 When changes are made to a validated method, the impact of such changes shall be documented and, when appropriate, a new validation shall be carried out. The assessment contents of the modified method should include accuracy, sensitivity, specificity, variability and detection limit, and should meet the needs of a given application quantitatively and qualitatively.

9.4.2.3 Validation can include procedures for sampling, handling and determination of tested biological samples, and items for calibration.

ASN-0002[11] and ASN-0003[12] contain details on validation requirements for STR profiling and DNA barcoding.

9.4.3 Verification

9.4.3.1 Validated methods used without modification shall be subject to verification before being used.

9.4.3.2 The procedure used for the verification and the results obtained shall be documented.