INTERNATIONAL STANDARD

ISO 23033

First edition
2021-08

Biotechnology — Analytical methods — General requirements and considerations for the testing and characterization of cellular therapeutic products

Biotechnologie — Méthodes analytiques — Exigences et considérations générales pour les essais et la caractérisation de produits de thérapie cellulaire

Reference number
ISO 23033:2021(E)

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Published in Switzerland

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Foreword

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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 documents 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*.

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

The emergence of cellular therapeutic products has increased the need for high quality, robust, and validated measurements for the characterization and testing of products containing cells as the active substance. These products are regulated by regional health authorities who evaluate product quality in terms of their quality attributes (QAs) via appropriate biological, physical and chemical assays (analytical methods).

Analytical methods are performed on cellular starting materials, in in-process testing and as a part of product conformance testing, comparability studies, and stability testing. These analytical methods are used to assess attributes associated with product quality features and manufacturing controls (in-process controls), and are performed to establish identity, purity, cell count, viability, potency, and stability in all phases of clinical study and commercialization. Quality attributes are used to ensure that only product lots that meet defined specifications are released. Quality attributes are also used for stability testing and trending purposes as well as in-process indicators.

Analytical methods also underpin the development of new cellular therapeutic products by providing insight into biological mechanisms of action and facilitating the research and development that advances manufacturing. In addition, analytical methods are used to evaluate and compare cellular therapeutic products from different batches that have, for example, been produced on different days, at different locations, or via a changed manufacturing process.

Quantitative measurement of a cellular therapeutic product is challenging due to the complex and highly dynamic nature of viable cells and cell samples, the varying vulnerability of cell types and processing steps, a lack of understanding of fundamental cell biology, and the large number of parameters associated with bioprocessing and measurement processes. Biological variability further complicates measurements. Additionally, different donor samples can have different susceptibilities to processing steps, making the need for in-process controls during the measurement process even more critical. As such, analytical methods are key to evaluate cellular therapeutic products, as well as the cellular starting material and intermediate, although the specific performance criteria can be different from those of the final cellular therapeutic products.

This document provides a general approach to design fit for purpose analytical methods to measure and assess quality attributes of a cellular therapeutic product. Aspects of this document can also be applicable to the testing and characterization of cells used in viruses, exosome, and antibody production. The general process to select and design fit for purpose analytical methods can be applied to cellular starting material, intermediates, cell end products, control cells, feeder cells, and cells used in assays (e.g. target cells). It also provides general approaches to understand, minimize, and monitor sources of variability. Acceptable levels of accuracy and precision are guided by the biological implications of the measurement result and the practical limitations of the measurement process.

This document also provides general considerations for setting specifications for the testing of a final cellular therapeutic product. General considerations are also provided for establishing analytical methods and analytical strategies (including analytical method matrix approaches) for common categories of critical quality attributes (CQAs) (i.e. attributes used to establish identity, cell count, purity or impurity, potency or relevant biological activity, viability, sterility, stability, and maturation profile).

This document was developed to provide additional technical guidance on cell characterization and specifically outlines approaches for strategic development of analytical methods cellular therapeutic product characterization and testing (see Annex A for schematic outline of concepts presented in this document).

Biotechnology — Analytical methods — General requirements and considerations for the testing and characterization of cellular therapeutic products

1 Scope

This document provides general requirements for the testing of cellular therapeutic products intended for human use.

This document also provides considerations for the characterization of cellular therapeutic products, including approaches to select and design analytical methods that are fit for purpose.

Such considerations can be used to establish critical quality attributes for a cellular therapeutic product.

This document is applicable to cellular starting materials (including those for tissue engineered products) and intermediates of cellular therapeutic products.

This document is not applicable to tissues used in transplantation.

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 http://www.electropedia.org/

3.1
acceptance criteria
numerical limits, ranges, or other suitable measures for acceptance of the results of analytical procedures which the product or materials at other stages of manufacture is intended to meet

3.2
adventitious agent
microorganisms unintentionally introduced into the manufacturing process

Note 1 to entry: Microorganisms can include bacteria, fungi, mycoplasma or spiroplasma, mycobacteria, rickettsia, protozoa, parasites, transmissible spongiform encephalopathy (TSE) agents and viruses.

Note 2 to entry: Adventitious agents are a subset of impurity for *cellular therapeutic product* (3.15).

3.3
analytical method
investigative procedure for qualitatively or quantitatively measuring or assessing the presence, amount, or functional activity of a target entity (the analyte)

3.4
analytical target profile
ATP
predefined objective that stipulates the *performance criteria* (3.34) for a *test method* (3.52)

Note 1 to entry: The ATP states the required quality of the results produced by a *test method* (3.52).

3.5
ancillary material
AM
material that comes into contact with the cell or tissue product during cell-processing, but is not intended to be part of the final product formulation

Note 1 to entry: AMs exclude non-biological consumables (e.g. tissue culture flasks, bags, tubing, pipettes, needles) and other plastic ware that come into contact with the cell or tissue but include consumables which can have a biological component (e.g. coated dishes or beads).

Note 2 to entry: AMs exclude feeder cells (cells that are used in the manufacturing process but are not a part of the cellular starting material).

Note 3 to entry: In some cases, AMs are described as raw materials.

[SOURCE: ISO/TS 20399-1:2018, 3.1, modified — Note 2 was reduced to the exclusion of feeder cells and the example was adjusted accordingly.]

Note 4 to entry: For the purposes of this document, the final product formulation is a *cellular therapeutic product* (3.15).

Note 5 to entry: Nature of both biological and synthetic material as well as their impact on cells can be highly complex. Thus, making assumptions as to that a synthetic material is less variable or complex than biological material would be wrong.

3.6
area density
<cells> *cell count* (3.14) of adherent cells on a surface, typically expressed as number of cells per unit area

[SOURCE: ISO 20391-1:2018, 3.4]

3.7
analytical method matrix
set of two or more complementary *analytical methods* (3.3) to measure different aspects of a *quality attribute* (3.38)

3.8
attribute
physical, chemical, biological, or microbiological property or characteristic

[SOURCE: ISO 20391-1:2018, 3.5]

3.9
attribute component
quantity (3.39) used to derive a *quality attribute* (3.38)

3.10
biological activity
specific ability or capacity of the product to achieve a defined biological effect

Note 1 to entry: The biological activity is potentially modified by stimulations including for example chemical, physical, or mechanical stimuli as well as other changes in environment and with time.

3.11
biological property
biological phenomenon that is evaluated to assess the *quality attribute* (3.38)

3.12
calibration
operation that, under specified conditions in a first step, establishes a relation between the *quantity* (3.39) values with measurement uncertainties provided by measurement standards and corresponding indications with associated measurement uncertainties and, in a second step, uses this information to establish a relation for obtaining a measurement result from an indication

[SOURCE: ISO/IEC Guide 99:2007, 2.39, modified — The notes were deleted.]

3.13
cell concentration
cell count (3.14) per volume

Note 1 to entry: Typically used for cells in suspension.

[SOURCE: ISO 20391-1:2018, 3.6]

3.14
cell count
discrete number of cells

Note 1 to entry: Cell count is typically expressed as *cell concentration* (3.13) or *area density* (3.6).

[SOURCE: ISO 20391-1:2018, 3.7]

3.15
cellular therapeutic product
product containing cells as the active substance

[SOURCE: ISO/TS 20399-1:2018, 3.5, modified — Example deleted.]

Note 1 to entry: The following are examples of cellular therapeutic product:

a) a cell therapy medicinal product;

b) a tissue engineered product.

3.16
cellular starting material
living and functional cellular material present at the beginning of a *cellular therapeutic product* (3.15) manufacturing process

Note 1 to entry: There are other types of starting materials also relevant for *cellular therapeutic products* (3.15).

3.17
certified reference material
reference material characterized by a metrologically valid procedure for one or more specified properties, accompanied by an RM certificate that provides the value of the specified property, its associated uncertainty, and a statement of metrological traceability

[SOURCE: ISO Guide 33:2015, 3.2]

3.18
comparable
conclusion that products have highly similar *quality attributes* (3.38) before and after manufacturing process changes and that no adverse impact on the safety or efficacy, including immunogenicity of the drug product occurred

Note 1 to entry: This conclusion can be based on an analysis of product *quality attribute* (3.38). In some cases, non-clinical or clinical data can contribute to the conclusion.

3.19
contaminant
any adventitiously introduced material not intended to be part of the manufacturing process of the drug substance or drug product

Note 1 to entry: Adventitiously introduced materials can be e.g. chemical, biochemical, or microbial species.

3.20
critical quality attribute
CQA
physical, chemical, biological, or microbiological property or characteristic intended to be within an appropriate limit, range, or distribution to ensure the desired quality and consistency of a product

Note 1 to entry: CQA is generally related to the clinical efficacy and safety of the product.

3.21
final cellular therapeutic product
formulated *cellular therapeutic product* (3.15) intended for administration to human subjects

3.22
fit for purpose
fitness for the intended purpose
in line with prearranged requirements for an *intended use* (3.26)

[SOURCE: ISO 20387:2018, 3.24, modified — Note deleted.]

3.23
impurity
any component present in the product which is not the desired product, a product-related substance, or excipient including buffer components

Note 1 to entry: An impurity can be either process- or product-related.

3.24
in-house reference material
non-certified material or substance, produced by one laboratory, one or more of whose property values are sufficiently homogeneous and well established to be used for the *intended use* (3.26)

Note 1 to entry: The use of in-house reference materials can include, but is not limited to, *validation* (3.54), *calibration* (3.12), monitoring of comparability, and *potency* (3.36) and process evaluations.

[SOURCE: ISO 16140-1:2016, 2.32, modified — "validation" replaced with "the intended purpose", note added.]

3.25
installation qualification
IQ
establishing by objective evidence that all key aspects of the process equipment and ancillary system installation adhere to the manufacturer's approved *specification* (3.50) and that the recommendations of the supplier of the equipment are suitably considered

3.26
intended use
intended purpose
use for which a product, process, or service is intended according to the *specifications* (3.50), instructions or information or multiple of them provided by the manufacturer or user

[SOURCE: ISO/IEC Guide 63:2019, 3.4, modified — Added new term, as well as "or multiple of them" and "or user" to the definition; "and" was replaced by "or".]

3.27
intermediate
material in manufacturing process that is nominally between two unit operations

3.28
intermediate precision
measurement *precision* (3.37) under a set of intermediate precision conditions

[SOURCE: ISO/IEC Guide 99:2007, 2.23, modified — "measurement" was deleted from the term, note deleted.]

Note 1 to entry: The intermediate precision condition refers to a set of conditions that includes the same measurement procedure, same location, and replicate measurements on the same or similar objects over an extended period of time, but can include other conditions involving changes (e.g. analyst or instrument).

3.29
limit of detection
lowest amount of analyte in a *sample* (3.46) which can be detected but not necessarily quantitated as an exact value

3.30
limit of quantitation
lowest amount of analyte in a *sample* (3.46) which can be quantitatively determined with suitable *precision* (3.37) and accuracy

Note 1 to entry: The limit of quantitation is a parameter of quantitative analytical methods for low levels of compounds in sample matrices and is used particularly for the determination of *impurities* (3.23) or degradation products or both.

3.31
measurement target
intended object of measurement

Note 1 to entry: A measurement target can denote a feature or complex features of cells that is informative of cellular status or quality. The term is additional to the term analyte or measurand in situations where the use of those terms is not appropriate or possible.

3.32
nominal property
property of a phenomenon, body, or substance, where the property has no magnitude

[SOURCE: ISO/IEC Guide 99:2007, 1.30, modified — Notes and examples deleted.]

Note 1 to entry: The nominal property of a measurement target is one that can be described but not quantified with a magnitude.

3.33
operational qualification
OQ
establishing by objective evidence process control limits and action levels which result in an *analytical method* (3.3) that meets all predetermined requirements

3.34
performance criteria
required functionality and behaviour of the *test method* (3.52)

3.35
performance qualification
PQ
establishing by objective evidence that the *analytical method* (3.3), under anticipated conditions, consistently meets all predetermined requirements

3.36
potency
measure of the *biological activity* (3.10) using a suitably quantitative *analytical method* (3.3), based on the *attribute* (3.8) of the product which is linked to the relevant *biological properties* (3.11)

3.37
precision
closeness of agreement between indications or measured *quantity* (3.39) values obtained by replicate measurements on the same or similar objects under specified conditions

Note 1 to entry: Precision is usually expressed numerically by measures of imprecision, such as standard deviation, variance, or coefficient of variation under the specified conditions of measurement.

Note 2 to entry: The 'specified conditions' can be, for example, repeatability conditions of measurement, intermediate precision conditions of measurement, or reproducibility conditions of measurement.

[SOURCE: ISO/IEC Guide 99:2007, 2.15, modified — Term "measurement precision" was deleted. Notes 3 and 4 were deleted.]

Note 3 to entry: Measured *quantity* (3.39) value refers to the *quantity* (3.39) value representing a measurement result.

3.38
quality attribute
physical, chemical, biological, or microbiological property or characteristic that is an indicator of the quality

3.39
quantity
property of a substance with a magnitude that can be expressed as a number and a reference

[SOURCE: ISO/IEC Guide 99:2007, 1.1, modified — Notes, example, and "phenomenon, body, or" were deleted.]

3.40
reference material
material, sufficiently homogeneous and stable with reference to specified properties, which has been established to be fit for its intended use in measurement or in examination of *nominal properties* (3.32)

[SOURCE: ISO/IEC Guide 99:2007, 5.13, modified — Notes and examples deleted.]

3.41
repeatability
measurement *precision* (3.37) under a set of repeatability conditions of measurement

[SOURCE: ISO/IEC Guide 99:2007, 2.21, modified — Term "measurement repeatability" was deleted.]

Note 1 to entry: Repeatability conditions of a measurement refers to condition of measurement, out of a set of conditions that includes the same measurement procedure, same operators, same measuring system, same operating conditions and same location, and replicate measurements on the same or similar objects over a short period of time.

3.42
representative sample
sample (3.46) that accurately represents or reflects the *attributes* (3.8) of the system

Note 1 to entry: Generally intended to provide information on the system, often to serve as a basis for decision on the system or its production.

3.43
reproducibility
measurement *precision* (3.37) under reproducibility conditions of measurement

[SOURCE: ISO/IEC Guide 99:2007, 2.25, modified — Note and term "measurement reproducibility" deleted.]

Note 1 to entry: Reproducibility conditions of measurement refer to the condition of measurement, out of a set of conditions that includes different locations, operators, measuring systems, and replicate measurements on the same or similar objects.

3.44
robustness
measure of a *test method* (3.52) capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage

3.45
ruggedness
degree of *reproducibility* (3.43) of test results obtained by the analysis of the same *samples* (3.46) under a variety of normal test conditions

Note 1 to entry: Normal test conditions can include for example: different laboratories, different analysts, different instruments, different reagent lots, different analysis days, different elapsed times, different temperatures etc.

3.46
sample
one or more parts taken from a system

3.47
sensitivity
quotient of the change in an indication of a measuring system and the corresponding change in a value of a *quantity* (3.39) being measured

[SOURCE: ISO/IEC Guide 99:2007, 4.12, modified — Notes and term "sensitivity of a measuring system" deleted.]

3.48
specificity
characteristic of a *test method* (3.52) that expresses qualitatively and quantitatively its ability to detect or determine an individual analyte without interferences from accompanying species

Note 1 to entry: Specificity increases with *sensitivity* (3.47) and amount of analyte and decreases with increasing cross-sensitivity and amounts of accompanying species and larger disturbing effects.

3.49
shelf life
specific time period for which a *cellular therapeutic product* (3.15) can maintain suitability for its *intended use* (3.26)

3.50
specification
list of tests, references to analytical procedures, and appropriate *acceptance criteria* (3.1) that would be expected to be met to demonstrate suitability for its *intended use* (3.26)

[SOURCE: ISO/TS 20399-1:2018, 3.9, modified — Replaced "intended use definition" by "intended use".]

3.51
stability
characteristic of a material, when stored under specified conditions, to maintain a value(s) for stated property(ies) within specified limits for a specified period of time

[SOURCE: ISO/TS 20399-1:2018, 3.10]

3.52
test method
technical procedure used with a specified *analytical method* (3.3)

3.53
therapeutic cells
cells within a therapeutic product required for the therapeutic effect

Note 1 to entry: Sometimes referred to as the cellular active substance.

3.54
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 deleted.]

3.55
verification
confirmation, through the provision of objective evidence, that specified requirements have been fulfilled

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

3.56
viable cells
cells within a *sample* (3.46) that have an *attribute* (3.8) of being alive (e.g. metabolically active, capable of reproduction, possessed of intact cell membrane, or with the capacity to resume these functions) defined based on the *intended use* (3.26)

[SOURCE: ISO 20391-1:2018, 3.29]

4 Cellular starting materials

The provenance and bioprocessing of cells can have significant effects upon the quality attributes of cellular therapeutic products. This is due to both the donor-to-donor variability and differential cell status conditions of the cellular starting material.

Information regarding cellular starting materials that can impact the design of analytical methods and the evaluation of acquired data should be documented. At minimum the following information should be given:

a) the consent form for data and materials from human donors;

b) the donor medical history and the results of analytical methods performed on the donor for the detection of viral and microbial infections.

NOTE 1 The details that document provenance of the cells are not exhaustive. For example, depending on the specific application, documentation that allows for tracking and traceability of the cellular starting material can be made available.

Aspects of the bioprocessing history of cells should be documented. Documentation for cell culture history can include:

1) tissue source of harvested cells;

2) anatomic site of isolation;

3) harvest method used for isolation of cells;

4) number of population doublings, number of passages, and total time of culture;

5) ancillary materials used (e.g. media) and substrate materials;

6) results from analytical methods to asses attributes used to establish identity;

7) results from analytical methods for adventitious (contaminating) agents;

8) preservation history (e.g. freeze and thaw);

9) for expanded cells, additional details such as seeding density and % confluence at subculture;

10) results from karyotype testing (for sex and loss of chromosomes).

A test method, which ensures biosafety, should be specified.

NOTE 2 Infectious substances or toxic substances in cells can affect biosafety of the person conducting the analytical methods.

The person or organization that performs analytical studies should be aware of relevant national, regional and international approved ethics, laws and regulations relating to the cellular starting material.

NOTE 3 Examples of relevant regulations include EU, Directive 2004/23/EC[15], EU Directives 2006/17/EC[16], and EU Directives 2006/86/EC[17].

5 Design of fit for purpose analytical methods for evaluating quality attributes

5.1 General concepts

Cellular therapeutic products should be described by a set of attributes (properties) that are useful to:

a) ensure quality (safety and efficacy);

b) define specifications for the product; and

c) establish identity.

These attributes are often referred to as critical quality attributes (CQAs) and ideally correlate with the intended clinical outcome, mechanism of action (MOA), or safety of the product. Characterization of quality attributes for a given cellular therapeutic product should be undertaken with analytical methods that have been confirmed to be fit for purpose. Where those analytical methods are used as routine test methods for the manufacture of the cellular therapeutic product, they should be validated.

CQAs for a cellular therapeutic product should include a set of quality attributes associated with:

1) identity;

2) cell count;

3) cell viability;

4) purity or impurity;

5) potency or relevant biological activity;

6) stability;

7) microbiological quality.

NOTE 1 Each cellular therapeutic product generally has its own set of CQAs based on the products' intended use or MOA or both. This is due to the fact that cellular therapeutic products are complex, dynamic, and often heterogeneous mixtures of viable and non-viable cells, derivatives of cells (e.g. exosomes and cytokines), and active or inactive substances or both.

In practice, CQAs are defined based on comprehensive research and development as well as pre-clinical and clinical data on quality attributes. The analytical methods for cellular therapeutic product characterization should be refined during product development. Often, the critical nature of a quality attribute is not confirmed until clinical data are established regarding the relevance of the quality attribute to patient outcome. Comparability studies to assess the impact of process changes on product safety and quality, can also call for reconsideration of CQAs and how they are applied.

The identification of relevant quality attributes often requires a risk-based approach to be applied to ensure that the list of attributes to evaluate is manageable and meaningful. Analytical methods are expected to evolve over the development program and are continuously refined in response to new understanding over the entire product life cycle (see Annex A).

NOTE 2 For the purpose of this document, attributes describing a cellular therapeutic product are referred to as quality attributes with the understanding that in some cases those quality attributes can be confirmed as critical during product development.

Product testing and the development of a testing strategy are integral parts of ensuring control of the manufacturing process and consistency (see Annex A).

Testing can be conducted throughout the manufacturing process, including on the manufacture of cell banks, to evaluate the manufacturing process itself and to ensure the quality and consistency of the product.

Quality control requirements can be different at different stages of cellular therapeutic product development and at different manufacturing steps (e.g. cellular starting materials, in-process and final product testing) of the cellular therapeutic product. The selection, design, and optimization of analytical methods should start in the development program for a given cellular therapeutic product. The development program is, in part, the process of gathering data on the characteristics of batches of cellular starting material and corresponding intended purpose in order to establish what information is necessary to ensure the quality of the therapeutic cells in the cellular therapeutic product. This provides data on variability that can be related to the cells themselves and variability that is due to the analytical method measurement process. These data normally form part of the justification for the final product specification range.

The characterization data from the development program should be used to establish quality attributes that are intended to be assessed for each lot of cellular starting material and cellular therapeutic product. The additional characterization undertaken during the development provides a basis for extended characterization to confirm that the change to the cellular starting material (e.g. a new working cell bank or different biopsy location or procedure) has not altered the quality of the final cellular therapeutic product.

5.2 Approach to analytical method design

When possible, a systematic approach to analytical method design should be used to meet the specific objectives of measurements necessary for quality attributes. The systematic approach can include determining specific measurement targets, analytical method selection and qualification, reducing the sources of measurement variability, defining a design space that ensures confidence in the

analytical method, control strategies and continual improvement to increase method robustness and understanding of the analytical method design space.

NOTE This systematic approach to analytical method design is sometimes referred to as quality by design (QbD) for analytical methods. A QbD approach can be beneficial for analytical method improvement and optimization.

The process of analytical method development can be an iterative process involving continuous improvement in different stages of product development. This process benefits from increased understanding of the connection with biological activity, and eventually leads to development of suitable analytical methods that evaluate potency or relevant biological activity. The process also benefits from understanding product heterogeneity and its potential relationship to donor differences and manufacturing process-related effects. Analytical method development begins with qualification of various analytical methods and steadily moves toward validation while also considering the on-going analytical method verification. Instrument hardware qualification is also an integral part of this process, e.g. instrument qualification (IQ) and operation qualification (OQ). The rationale supporting analytical method development should consider the relationship between the characterization method being developed, and how it assesses the intended biological activity of the cellular therapeutic product (see Annex A).

5.3 Defining quality attributes by considering components of a cellular therapeutic product

Quality attributes important for the manufacturing control and product release of the cellular therapeutic product should be defined as early as possible during a product development process. These attributes are likely to be based on the scientific hypotheses that define the efficacy and safety of the cellular therapeutic product.

Appropriate analytical methods and data to assess each quality attribute depend on the components of the cellular therapeutic product. A quality attribute can depend on the quantification of one or more components of the cellular therapeutic product[12].

The components of a cellular therapeutic product that are important to consider when defining quality attribute measurements can be broadly characterized as therapeutic cells, residual and other impurities, and excipients or suspension medium (including transfer medium) (see Figure 1)[12]. It is also important to consider the complexity of cell populations and their different origins, which can directly relate to the safety and efficacy (or potency) of the product when determining attribute measurements.

Each category of a cellular therapeutic product's components can be broken down into further sub-categories.

The cellular component within a cellular therapeutic product can be further categorized into user defined cell populations based on properties of the cells. These properties can include, but are not limited to:

a) genomic characteristics;

b) function;

c) morphology;

d) stage of cell cycle;

e) viability;

f) specific biomarkers;

g) cell kinetics;

h) phenotype.

The properties measured should be considered to exist on a spectrum as there is a heterogeneity of cells, which can affect variation in properties.

Inclusion and exclusion criteria for a user-defined cell population in a cellular therapeutic product shall be specified and documented.

The suspension medium can include biologically-derived or chemically-derived materials or both, as well as other additives (e.g. preservatives).

Additional definitions and considerations for ancillary materials are provided in ISO/TS 20399-1, ISO/TS 20399-2, and ISO/TS 20399-3.

NOTE 1 Residuals from ancillary material can affect the safety, potency, and purity of the final product.

Potential impurities can include, but are not limited to, biological impurities (e.g. unintended cells and cellular components), adventitious agents (e.g. endotoxin, bacterial and viral contaminants), dissolved gases and volatiles, and non-biological impurities (e.g. extractables, leachables, and other non-biological contaminants).

NOTE 2 Biological impurities can arise as byproducts from the manufacturing process.

NOTE 3 Criteria for identifying different cell populations are both user-defined and dependent on limitations of the sensitivity and selectivity of analytical methods. Potential overlap can exist between cell sub populations due to the continuous nature of heterogeneity in cell populations.

NOTE 4 Populations can be highly heterogeneous and exhibit plasticity.

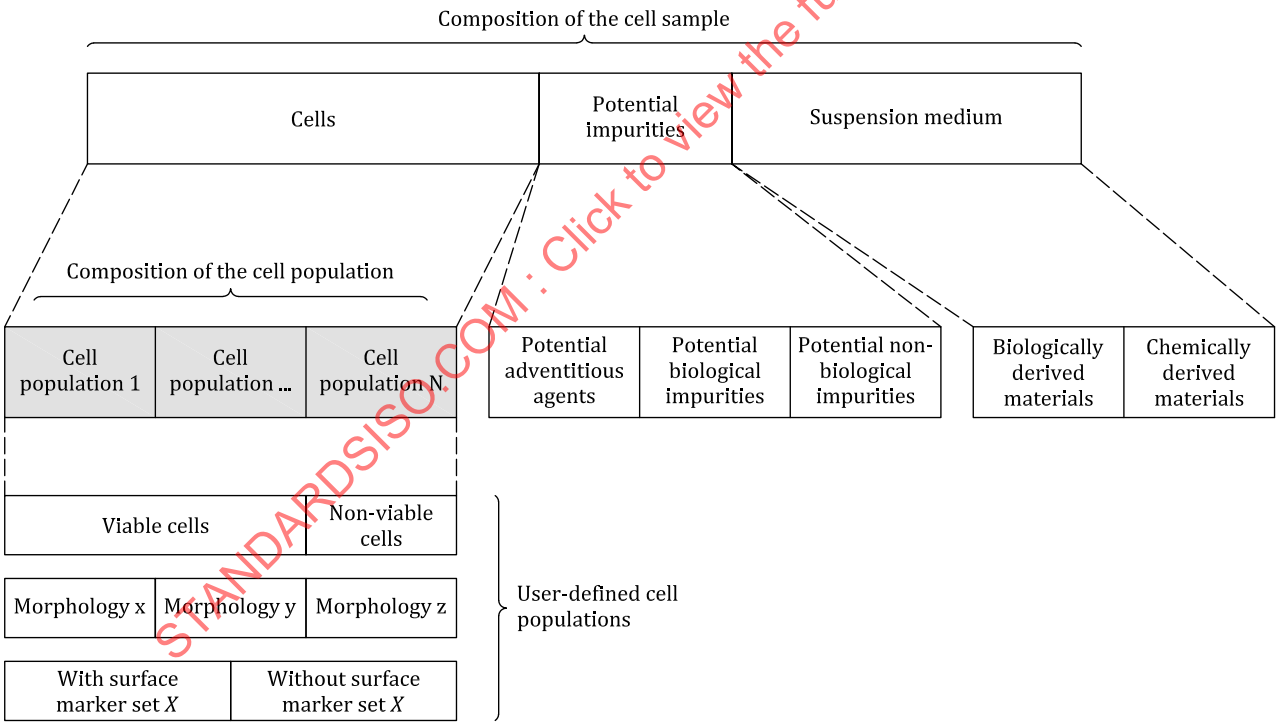

Figure 1 — Example of components and sub-components for a cellular therapeutic product at a given time

Quality attributes are generally expressed as a quantification of a specified component of a given cellular therapeutic product. For example, attributes used to establish identity can be defined by one or more components, including user-defined cell population(s), and further allows the quantification of the named component(s). Likewise, purity or impurity can be measured by assessing components that have been identified as potential impurities.

Components of a cellular therapeutic product should be listed and considered during the design and selection of appropriate analytical methods.

It is helpful to consider the intended analytical method purpose and the appropriateness of the analytical method performance in the analytical method design and selection.

The process to establish quality attributes and their test methods should include:

1) stating the specific quality attribute of interest;

2) stating the intended use of the quality attribute (e.g. in-process testing, release testing);

3) listing the quality attribute components (if the specified quality attribute is derived from multiple quantities);

4) identifying the biological property(ies) that are intended to be used to evaluate the quality attribute;

5) describing the analytical method(s) established to evaluate the selected biological property(ies);

6) describing the analytical target profile:

   — listing the measurement target(s) for the selected analytical method(s);

   — describing performance criteria of the analytical method.

An example for establishing a quality attribute for cell viability is shown in Table 1.

Table 1 — Example of a process to establish quality attributes and their analytical methods

Quality attribute	Quality attribute description	Intended use	Attribute components	Biological property(ies)	Analytical method(s)	Analytical target profile	
						Measurement target	Performance criteria
% Cell viability	$V = 100 \times \left(\frac{N_t - N_n}{N_t} \right)$ where V is the cell viability, in %; N_t is the total cell number; N_n is the non-viable cell number.	Release testing	Total cell number	Cells that contain nuclear materials	Direct cell counting; imaging-based acridine orange and DAPI dye exclusion method	Cells stained with acridine orange	Sensitivity Precision Proportionality Specificity
			Non-viable cell number	Cells with membranes permeable to viability dye		Cells stained with acridine orange and DAPI	

5.4 Design of a matrix of analytical methods

In some cases, a single analytical method cannot provide an adequate measure of a given QA. Multiple complementary analytical methods that measure different aspects of a quality attribute should be used, if one analytical method is not sufficient.

Such a collection of analytical methods (or analytical method matrix) can consist of a combination of biological and non-biological measurements, or non-biological measurements alone. The analytical method matrix can include analytical methods that give a quantitative readout (e.g. concentration) or nominal readout (e.g. pass or fail) or both.

EXAMPLE 1 An analytical method matrix for viability can include one or more membrane permeability dye(s) and a metabolic activity assay.

Analytical methods included in an analytical method matrix should be conducted on comparable samples to ensure interoperability of the data generated.

Results within an analytical method matrix are not always expected to be linearly correlative. If two analytical methods are measuring different measurement targets probing slightly different biological properties, then the result can be interpreted as cumulative rather than confirmatory.

EXAMPLE 2 One cannot necessarily expect identical viable cell count determined by the number of membrane intact cells and by metabolic activity. Rather, combined results provide a more comprehensive understanding of different aspects of cell viability on a continuum spectrum.

If qualitative methods are used as part of an analytical method matrix to determine a quality attribute for lot release, stability or comparability studies, they should be accompanied by one or more quantitative methods although in some cases, such as mycoplasma PCR or PCRs for viral agents, qualitative results can be sufficient.

5.5 Design of a fit for purpose analytical method

Well-defined quality attributes and a clear understanding of their intended uses guide the design of analytical methods with biological relevance and appropriate performance criteria (e.g. selectivity, sensitivity, precision, accuracy, robustness, range) to enable subsequent decision-making, resulting in fit for purpose analytical methods.

The intended use of the analytical method guides the fit for purpose requirements of the measurement.

The measurement target of an analytical method generally represents a surrogate measure for assessing the biological property(ies) associated with a quality attribute.

When an analytical method that directly assesses the biological property(ies) is not available, an analytical method(s) should be chosen such that the measurement target is as closely related to the biological property(ies) as possible.

The analytical method should have high specificity for the measurement target without significant interference from other components in the cell sample.

The analytical method should be sufficiently sensitive to detect the smallest relevant quantity to discern relevant changes in the quality attribute using statistical methods.

The analytical method should be sufficiently robust such that the results are not significantly affected by small changes in the measurement process (e.g. temperature fluctuations, minor sample handling fluctuations) as defined by the user for the intended purpose.

The analytical method should also be sufficiently robust for the measurement target such that the results are not significantly affected by small changes in other components of the cell sample (e.g. serum concentration, presence of cryo-preservation agents, different batches of analytical reagents).

NOTE Cell samples from different points in the manufacturing process can have very different compositions. In this case, the cell samples can be qualified and validated separately due to the differences in the cell sample components. Alternatively, different analytical methods can be used to evaluate the quality attributes of cell samples from different points in the manufacturing process.

Appropriate analytical method design should include performance criteria for the test method and strategies to ensure measurement quality. This can include incorporating replicate measurements, using sample randomization to reduce biases, and using appropriate measurement controls and measurement control strategies.

5.6 Selection of instruments

Instruments, including hardware and software, that are applicable to the test method shall be selected.

Instruments shall be capable of carrying out the selected test method.

Specifications documented for the instruments, including software, can be evaluated and serve as a basis for selection.

Instruments should be evaluated by the user. The performance of an analytical method should be evaluated by defining the limit of detection, limit of quantification and dynamic range of the analytical method using replicated measurements of appropriately prepared calibrants or reference materials. Analytical method calibration should demonstrate an appropriate dynamic range such that measured results are proportional to an intended measurement target. Test methods should be conducted on test materials that represent the cell samples intended to be measured (i.e. representative samples).

Performance criteria should include:

a) specificity;

b) sensitivity;

c) range;

d) linearity or proportionality;

e) repeatability;

f) accuracy;

g) available proficiency tests.

Evaluation can also be based on comparability of results to existing methods or reference measurement procedures or both.

NOTE If the accuracy of the existing method is unknown or if the precision of the existing method is poor or both, comparability of analytical method results to an existing method does not ensure the quality of the method.

Additional considerations for instrument selection can include, but are not limited to, the following:

1) instrument cost;

2) cost per measurement;

3) required sample volume;

4) ease of use;

5) analytical method execution time;

6) (meta)data recording or availability;

7) information on the compliance with regional regulatory requirements [e.g. current good manufacturing practices (cGMP)[21]];

8) reagent requirements or availability;

9) consumable requirements or availability;

10) versatility for different sample types;

11) versatility to run different analytical methods;

12) ability to optimize analysis parameters;

13) sample preparation requirements;

14) built-in control strategies;

15) instrument-to-instrument variability;

16) operator-to-operator variability;

17) readiness for translation (e.g. automation friendliness);

18) historical precedence or trust in the method;

19) calibration and maintenance needs;

20) customer support.

5.7 Instrument qualification and maintenance

The selected instruments, including hardware and software, should be qualified based on pre-determined procedures with documented verification of the instruments' ability to meet requirements.

This includes, but is not limited to:

a) installation qualification (IQ);

b) operational qualification (OQ);

c) performance qualification (PQ).

IQ and OQ should be conducted by the instrument manufacturer.

PQ should be conducted with representative test samples.

Re-qualification should be performed after major maintenance or when the instrumentation is modified.

Instruments shall be properly maintained.

Instruments and software should be planned for periodical maintenance and, when necessary, calibration.

NOTE 1 Calibration, under specified conditions, firstly, establishes a relationship between the quantity values with measurement uncertainties provided by measurement standards and corresponding indications with associated measurement uncertainties. And secondly, uses this information to establish a relation for obtaining a measurement result from an indication.

NOTE 2 Calibration can be expressed by a statement, calibration function, calibration diagram, calibration curve, or calibration table. In some cases, it can consist of an additive or multiplicative correction of the indication with associated measurement uncertainty.

Records of calibrations, qualifications and maintenance shall be maintained.

Supply of consumables for the instruments and analytical methods should be ensured.

5.8 Managing sources of measurement variability for cell measurements

5.8.1 General

A generalized cell measurement process is shown in Figure 2. The process consists of three general phases:

a) The pre-analytical phase can include sample handling that occurs prior to the time the sample is received in the analytical laboratory.

b) The analytical phase generally refers to the "actual" laboratory testing procedures, processes, and products that ultimately provide results.

c) The post-analytical phase culminates in the production of a final value, result, or report or multiple of them.

The pre-analytical phase can include sample selection and collection, as well as sample processing, storage and transportation. The analytical phase can include mixing, diluting, and staining of cells, sampling as well as data collection. The post-analytical phase can include data analysis that can or cannot require additional user input for setting parameters. Each phase can introduce sources of variability to affect the measurement outcome.

Control strategies should be implemented to manage sources of variability from each analytical phase. Control strategies can include, but are not limited to, analytical method validation, incorporation of in-process measurement controls, the use of reference materials, and the use of experimental design to systematically examine the quality of a measurement process[13],[14].

Strategies used to identify and manage sources of variability shall be documented.

NOTE 1 The listed strategies are not intended to be complete, universally applicable, or required for all cell measurement processes.

NOTE 2 Upstream activities can significantly impact the measurement results such as the collection of cells and their subsequent processing, storage and transportation steps. Time and temperature for processing as well as storage and transportation of test samples can impact the measurement.

NOTE 3 Reagents, consumables, and testing kits can affect the measurement.

NOTE 4 The three phases of the cell measurement process are illustrated; other aspects such as the composition of donor samples, reagent sourcing, and sample handling can contribute additional variability to the cell measurement process.

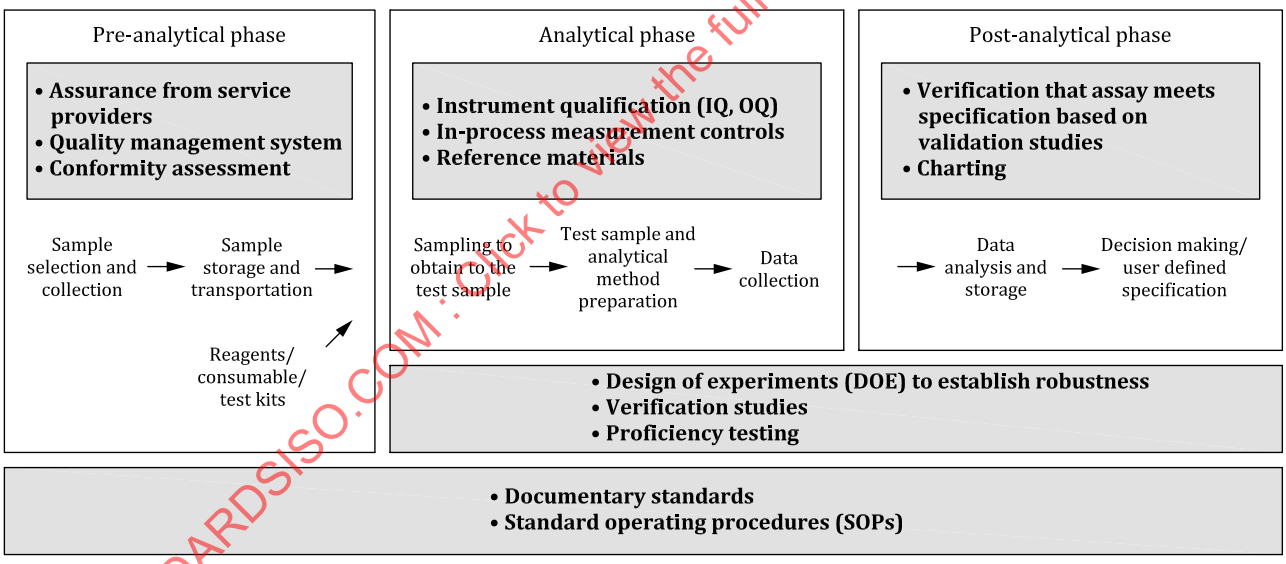

Figure 2 — Example of a generalized cell measurement process where examples of potential controls, practices and standards for managing and minimizing sources of variability are highlighted in the grey boxes

Annex B shows sources of variability that can be considered when designing and validating a cell analytical method via Ishikawa (Fishbone) diagram. Other approaches, such as failure mode and effects analysis (FMEA), can also be used to identify possible failures in an analytical method design.

Frequently, the use of control or reference material should be implemented to ensure the quality at each or several steps of the cell measurement process, in the case control and reference material is not sufficient to ensure the quality of the entire cell measurement process. Instead, the use of multiple

controls and reference material(s) can be implemented to ensure quality at each or several steps of the measurement process[13],[14].

NOTE 5 Some strategies to reduce the sources of variability are intended to be implemented prior to routine measurements, while others are meant to serve as in-process controls or comparators during measurement of the samples.

5.8.2 Sampling and sample preparation

A sampling plan shall be developed.

The sampling plan should ensure that samples taken for measurement (test samples) are representative.

The state of the cells for testing should be compatible with the sample requirements for the test method.

Sample handling for the test method, such as sampling and sample preparation, should minimize damage to the cells with regards to properties that affect the measurement. This is to sustain characteristic of the cells during measurement. A test sample preparation method should be established, taking into account the effects of contaminants (e.g. DNA contamination in RNA measurement) that can affect the measurement results.

The test sample should be stable enough to carry out the test method. The stability of the test sample during its preparation should be evaluated. When the test sample does not have sufficient stability, the preparation procedure, including manipulation method, should be reviewed.

In carrying out an analytical method using a cell suspension, it is sometimes desirable that the cells are prepared in a single and uniform suspension. Cell characteristics, however, can be deteriorated by the stress (e.g. physical, chemical, or temporal) applied to the cells during the preparation process used to make the cell suspension. Therefore, sample preparation factors that can affect the cell sample should be considered.

NOTE Careful pipetting operations are considered to be useful in preparing uniform test samples. This method, however, is not always appropriate for cells, as it is time consuming and can damage characteristics of the cells.

A sufficient amount of cells should be reserved for the analytical method. When the amount of cells is limited, the test sample or performance criteria (e.g. accuracy) or both should be modified based on the risk-based approach.

The measurement target (e.g. cytoplasm, cell membrane, supernatant) should be identified and its appropriate fraction should be sampled for the test method.

5.8.3 Reference materials

Reference materials can be used to ensure measurement traceability, enable comparison, and verify a measurement process.

A reference material should be used for its intended purpose based on its reference value(s).

Appropriate reference materials should be used for instrument qualification, validation, and verification.

Appropriate reference materials should be used for analytical method calibration.

Reference materials can also be used for training or proficiency testing.

When available, an appropriate cell-based reference material should be used for its intended purpose.

Suitable certified reference materials should be used, when available.

Certified reference materials should be stored according to supplier instructions and used within its expiry date.

Certified reference materials should be used in combination with reference methods, where applicable.

NOTE 1 Use of certified reference materials can help to ensure a traceability chain.

NOTE 2 Certified reference materials can be used in combination with other reference materials (e.g. a property value of an in-house reference material can be linked to a certified value of a certified reference material) to reduce unnecessary use of limited supplies.

NOTE 3 In some cases, reference materials are not yet available for certain analytical methods for cell-based measurements. Many aspects of characterization and testing of cellular therapeutic products can benefit from new reference material development, such as fixed cell reference materials for flow cytometry; however, validation for use in cell therapy manufacturing is still necessary. In some cases, bespoke reference materials can be considered for the release of final cellular therapeutic product batches, once the reference materials are validated for their intended use.

In-house reference materials should be evaluated for their purpose in a cell measurement process. Reference values for in-house reference materials should be measured with associated uncertainties or demonstrated to be fit for intended use in an examination of nominal properties.

In-house reference materials should be prepared from a suitable material (e.g. process sample or cellular therapeutic product batch material). In-house reference materials should be homogeneous and stable with appropriate shelf life. The reference values of an in-house reference materials should be verified regularly, and storage conditions should be established. A certificate of analysis (CoA) should be issued for in-house reference materials.

5.8.4 Analytical reagents

The quality and consistency of analytical reagents can influence the results of the analytical method.

Analytical reagents used in sample preparation for measurement should be selected considering their possible effects on the sensitivity, selectivity, and robustness of the analytical method.

Some analytical reagents (e.g. fluorescent dye, buffer) are not stable over time or under certain environmental conditions or both. Cell measurements should be carried out within the accepted stability range of the analytical reagents.

Formulation errors of some analytical reagents can cause either overestimation or underestimation of measurement. Acceptable analytical reagent concentration ranges should be determined.

Supply of analytical reagents and its stability in storage should be ensured.

It is possible that some analytical reagents (e.g. antibodies) are not consistent by lot-to-lot or over different suppliers or both. Acceptable specifications should be determined prior to using these analytical reagents.

5.9 Documentation of procedure

The established analytical method shall be documented.

The documented analytical method should include, but is not limited to:

a) instrument used and instrument settings;

b) analytical reagents used;

c) sample handling procedures, including for example:

   1) sample mixing methods (e.g. mode, speed, duration) as well as wait or hold time in between processes;

   2) sample transfer procedures (e.g. pipetting);

3) containers and transferring apparatus;

d) specified time limit for operation;

e) data analysis procedures;

f) use of any calibration, control and reference materials;

g) data to be acquired.

NOTE Background information leading to the test method can be useful. Examples are:

— effect of instrument on the analytical method;

— effect of consumables on the analytical method;

— information on instrument qualification.

6 Analytical method qualification, validation and continued verification

6.1 General

A plan for qualification or validation of test method should be made according to application, development phase, sample availability, experience, and its circumstance.

6.2 Analytical method qualification

Analytical method qualification is conducted to confirm that an analytical method performs to a reasonable degree of reproducibility and is suitable for its intended use, under controlled conditions.

Controlled conditions for an analytical method include, but are not limited to, the use of a specific instrument, specific reagents, and other conditions that can affect the analytical method.

The analytical method qualification should demonstrate the capability of the analytical method, under controlled conditions, with regards to the characteristics of:

a) accuracy;

b) repeatability;

c) specificity;

d) sensitivity;

e) linearity or proportionality;

f) range.

NOTE Specificity is often evaluated in lieu of selectivity. Selectivity, in general, characterizes the ability of the analytical method to detect or determine several given species without interference, i.e. independently and undisturbed by each other and by additional constituents in the sample when more than one species is analysed in a multicomponent system[19].

The analytical method qualification should confirm that the analytical method meets pre-determined performance criteria. Performance criteria can be set based on the intended use of the analytical method and historical data.

Records of qualification can be useful to validate the analytical method.

Points to consider during analytical method qualification can include:

1) interfere or synergy between active substances;

2) stability of cells in the cell sample;

3) possible aggregation of cells;

4) heterogeneity of the cell population.

6.3 Analytical method validation and continued verification

6.3.1 Validation

Analytical method validation is conducted to confirm that an analytical method is suitable for its intended purpose.

Analytical method validation should be conducted when a new analytical method is implemented, or an existing analytical method is used for a new purpose.

Analytical methods should be demonstrated to meet performance criteria.

Performance criteria of the analytical method should address the following validation characteristics:

a) accuracy;

b) precision:

— repeatability;

— intermediate precision;

c) specificity;

d) sensitivity;

e) limit of detection;

f) limit of quantitation;

g) linearity or proportionality;

h) range.

NOTE 1 Intermediate precision refers to variability due to within-laboratory variations: different days, different analysis, different equipment, etc.[20].

NOTE 2 The limit of detection is sometimes referred to as the detection limit and the limit of quantitation is sometimes referred to as the quantitation limit[20].

NOTE 3 The list of selected validation characteristics can be dependent on the type and purpose of the analytical method being validated. For example, while the limit of quantitation can be important for a test for impurities, it is possible that it is not appropriate for a test for identification.

In addition to these validation characteristics, the following characteristics should be determined in the case that the analytical method is aimed for routine measurement:

1) robustness;

2) ruggedness;

3) reproducibility.

NOTE 4 Generally, robustness testing evaluates the influence of small deliberate changes in "procedure-related" method parameters and provides an indication of a methods reliability during normal usage while ruggedness testing is performed under different test conditions to examine the effects of "non-procedure-related' factors and can be expressed as a lack of influence on test results of operational and environmental variables on the analytical method. The purpose of ruggedness testing is to determine the variables (external experimental factors) that strongly influence the measurements provided by the method and to determine how closely these variables are to be controlled. After examining reasonable ranges of factors in an experimental method, if it is said that the factors do not strongly (statistically) influence the measurements or results, then we say that the method is rugged for the factors over the ranges tested. Ruggedness is considered a measure of reproducibility of test results under the variation in conditions normally expected from laboratory to laboratory and from analyst to analyst[22].

A multi-variate approach to evaluate effects of various factors on method performance can be used.

EXAMPLE Design of Experiment can be used to find ranges for instrument operating parameters, to understand sample preparation variations, and variations of method precision. Method validation characteristics are response variables in the design of experiment approach.

Representative samples should be used for analytical method validation.

Appropriate statistical methods for the design and analysis of laboratory experiments should be used.

In defining the analytical method control space, cell measurements can have the following properties, which can require further consideration:

— availability of appropriate reference materials;

— limited availability of samples for testing;

— stability of the measurement target;

— ambiguity in defining cell states.

The results of validation studies should address the selected validation characteristics, factors that can influence the validation characteristics, and their conformance to test method performance criteria.

Potential sources of analytical method variability and variations from replicates should be taken into account when reporting results.

Documentation of validation should include justification and rationale and contain sufficient information to permit independent statistical analysis and evaluation of the results.

NOTE 5 Laboratory records that include complete data derived from all analytical methods necessary to ensure compliance with established specifications and standards can be maintained.

Use of reference materials in validation shall be documented.

NOTE 6 Cases can exist where no suitable reference material is available for validation. In these cases, alternative approaches can be applied. For example, for cell counting, practices described in ISO 20391-2 can be applied[8].

NOTE 7 See 5.8.3 for additional information on reference materials.

6.3.2 Continued verification

During routine execution of the analytical method, continued verification should be conducted. Continued verification is the continual assurance that the analytical method remains in a state of control (or in the validated state)[21].

A system(s) for detecting unplanned deviations from the validated state should be developed. The collection and evaluation of information and data about the performance of the analytical method over time can allow the detection of undesired process variability. Evaluating the performance of the analytical method can help to identify problems and determine whether action needs to be taken to correct, anticipate, and prevent problems, so that the analytical method remains in a validate state.

Data gathered through continued verification can also suggest ways to improve and optimize the analytical method.

When new cellular samples with unknown characteristics are to be measured, re-qualification or re-validation of the test method should be carried out. Measurement precision or variability or both of examined nominal properties observed during re-qualification or re-validation should be within predefined limits.

While continued verification is performed, the variability can be extracted, and the method used in the validation can be determined.

6.4 Test method performance criteria

The test method performance criteria shall be consistent with the intended use of the test method.

Test method performance criteria can be predefined by external requirements. Examples are tests for:

— endotoxin level;

— sterility.

Test method performance criteria can be:

— either quantitative or qualitative;

— defined by performance index or boundary/limit/criteria samples or both.

Test method performance criteria shall be determined before validation.

Performance criteria shall be documented.

Documentation should include justification and rationale for performance criteria and contain sufficient information to permit evaluation of the results.

When the test method does not meet performance criteria, the test method should be reconsidered. Considerations can include, change of instruments, reagent, or protocols or multiple of them.

7 Testing of cellular therapeutic products

7.1 Considerations for specifications and release criteria for cellular therapeutic products

7.1.1 General

The specification for the cellular therapeutic product should be developed in sufficient detail to ensure:

a) consistency of the cellular therapeutic product when manufactured on a routine basis; and

b) that the cellular therapeutic product being produced for use in commercial lots is comparable to that used for safety tests, clinical trials or previous commercial lots or multiple of them.

Specifications should include:

1) a list of analytical methods;

2) references to analytical procedures; and

3) appropriate acceptance criteria[23] that are expected to be met to demonstrate suitability for its intended use.

NOTE Specifications can be established such that they are appropriate to the stage of the product development, and acceptance criteria are typically refined and tightened as the product development progresses towards licensure.

Specifications should also be described for intermediate product acceptance criteria.

Quality attributes and specifications important for the manufacturing process (intermediates) can be different than for the final product.

7.1.2 Considerations for release criteria for the final cellular therapeutic product

Release criteria for the final cellular therapeutic product should be based on scientific evidence and manufacturing experience obtained during development of the product. Release criteria are acceptance criteria for final product testing and release for use.

NOTE 1 The development program for the release criteria can be guided by a risk analysis and be used to establish limits on CQAs identified during product development or clinical testing or both.

The final product testing shall be performed on each lot of product manufactured.

NOTE 2 Depending on the manufacturing process, each dose can be considered a single lot.

Testing required for release of the cellular therapeutic product should fit within the timescale of the products useable shelf life.

The results from final product release criteria testing should be available prior to administration to a human subject.

If results from final product testing are not expected to be available prior to release or within the useable shelf life of the cellular therapeutic product or both, an investigational plan should be developed that can address the actions to be taken in the event that the cellular therapeutic product did not meet the specified criteria (e.g. in the case of some types of sterility testing).

7.2 General requirements for the testing of the cellular therapeutic product

Testing of cellular therapeutic products should include the evaluation of quality attributes related to identity, cell count, cell viability, purity or impurity(ies), potency or relevant biological activity, stability, as well as microbiological quality.

Claims to evaluate quality attributes shall be made based on sufficient data obtained using validated or qualified analytical methods.

Analytical methods for testing should be selected based on scientific evidence and manufacturing experience.

Analytical methods required for testing shall at minimum be qualified.

Analytical methods required for testing should be validated.

An analytical method matrix may be used in the testing of cellular therapeutic products.

7.3 Testing to evaluate identity of a cellular therapeutic product

Testing of attributes used to establish an identity is important to ensure that the contents of the vial or container of the cellular therapeutic product are labelled appropriately.

The analytical method(s) to test attributes used to establish an identity shall be specific for the intended measurement purpose for a given cellular therapeutic product.

The analytical methods to establish identity(s) should be able to:

a) confirm that the manufacturing process has delivered the intended therapeutic cells;

b) distinguish between multiple cell types, if a cellular therapeutic product is known to require more than one cell type for the therapeutic effect;

c) distinguish the therapeutic cells from other cells that can reasonably be present in the cellular therapeutic product;

d) distinguish a cellular therapeutic product from other products processed in the same facility.

NOTE 1 Depending on the product, analytical methods for identity can test for donor identity (e.g. STR, HLA haplotype) or tests for phenotypes or both.

The specification for the identity of a cellular therapeutic product should include one or a matrix of analytical method(s) to test attributes used to establish identity as necessary to confirm the presence of the therapeutic cells.

EXAMPLE Many cell types can appear similar under different microscope conditions, and the same cell type can exhibit different morphologies depending on culture and plating conditions (substrate and media) as well as cell cycling status (i.e. active mitosis or senescence), so morphology alone is often not a specific confirmation of identity.

The demonstration of attributes used to establish identity can include an evaluation of the genotype, phenotype, and other markers of the therapeutic cells.

A phenotype analytical method can confirm the presence or absence of established surface or intracellular markers or both, which are characteristic of certain cells. The phenotypic evaluation should also include testing for markers of other cell types that can reasonably be expected based on the origin of the cells.

NOTE 2 Although this aspect of characterization falls more properly within the scope of purity of the population, purity and attributes used to establish identity are related and the characterization strategy can consider the likelihood of undesirable cell types that could have been introduced through the cellular starting material or other aspects of manufacturing.

NOTE 3 Pre-analytical steps, including prolonged sample storage, can change the phenotype of cells.

7.4 Testing to evaluate cell counts within cellular therapeutic products

The cell count in predefined cell populations shall be documented. In general, cell count can include:

a) total cell count;

b) viable cell count;

c) count of live or dead cells or both identified as the therapeutic cells;

d) count of user defined cell population.

The biological activity of the final cellular therapeutic product is generally dependent upon the various activities of the cells, and therefore the measurement of viable cell concentration can be useful.

Release criteria shall be established for count of viable cells. See 7.5 for more information on defining viability.

Release criteria shall be established for the count of cells identified as the therapeutic cells.

NOTE 1 The discrete number of cells, or cell count is often expressed as cell concentration (i.e. cell count per volume) when in suspension and area density of cells (i.e. cell count per unit area) when adhered to a surface.

NOTE 2 Standards are available for general guidance on cell counting methods[7] and an experimental design and statistical analysis approach to quantify counting method performance[8].

7.5 Testing to evaluate viability of cells within a cellular therapeutic product

The presence of non-viable cells in a cellular therapeutic product can be a concern. Therefore, the ratio of viable cells to the total number of cells in the cellular therapeutic product should be evaluated.

NOTE 1 Non-viable cells are generally considered as an impurity except for the situation in which the therapeutic cells themselves are composed of non-viable cells.

Viability testing shall demonstrate the ratio of viable cells to the total number of cells in the population.

NOTE 2 Viability is usually expressed as a percentage (i.e. % viability).

Release criteria for viability of cells in cellular therapeutic products shall be established and documented.

The definition of a viable cell can be dependent on the intended use of the viability measurement.

NOTE 3 The definition of a viable cell can sometimes be made such that it expresses the state of cell health. Cell health can be considered as a continuum.

NOTE 4 Measurements of viability can be biased due to continual degradation processes within a cell culture, meaning that measurements of total cell count, of live or dead count are continually in fluctuation.

The user shall select definitions for viable and non-viable cells based on their intended use. The biological property(ies) selected to distinguish viable from non-viable cells shall be documented.

Viability should be evaluated for all identified cell populations in the cellular therapeutic product.

7.6 Testing to evaluate potency of a cellular therapeutic product

7.6.1 General

Potency is a concept that describes the ability or capacity of the product to produce a predefined result. Analytical methods that evaluate potency shall demonstrate the bioactivity of the therapeutic cells for the intended use.

7.6.2 Importance of potency as a CQA

Potency measurements are important parts of product release testing as well as comparability studies, in the development of stability protocols and to establish consistently manufactured products during all phases of clinical investigation.

NOTE An analytical method that evaluates potency can quantify the therapeutic cells within a product or the overall relevant biological activity of the product. As such, some analytical methods in an analytical method matrix for potency can be the same as those used to measure identity and quantity.

7.6.3 Assessment of potency

The common approach for assessing the potency of biological products is to develop a quantitative biological assay (bioassay) that measures the bioactivity of the product related to its specific ability to produce a given result. Bioassays can provide a measure of potency by evaluating a product's active ingredient(s) within a living biological system. Bioassays can include *in vivo* animal studies, *in vitro* organ, tissue or cell culture systems, or any combination of these.

NOTE 1 *in vitro* or *in vivo* bioassays or both can be used to measure the biological activity of the cellular active substance. In cases where development of a suitable bioassay is not feasible for product release (e.g. bioassays that would take too long to use for release, or are not amenable to validation), surrogate measurement of biological activity can be used to demonstrate potency, if the surrogate measurement(s) can be substantiated by correlation to a relevant product specific biological activity(s).

EXAMPLE 1 A non-biological analytical method, that is practical and demonstrates adequate performance characteristics for lot release, can provide extensive product characterization data by evaluating immunochemical, biochemical, or molecular attributes of the product or multiple of these attributes.

EXAMPLE 2 Biological activity can include for example, the cells‘ ability to produce required functional proteins, change their phenotype, exert effects on other cells, or respond to stimulus by performing a biochemical function or multiple of these abilities.

The relevant biological activity of the therapeutic cells should be quantitatively or qualitatively assessed in terms of the properties or behaviours required to lead to a clinically functional cellular therapeutic product.

NOTE 2 Potency does not necessarily equate to clinical efficacy. A single test is unlikely to adequately measure those product attributes that predict clinical efficacy. Efficacy data from well controlled clinical investigations can provide evidence that a product has a relevant biological activity, and thus is potent. Analytical methods that evaluate potency are still used to quantitatively test for potency for product release.

NOTE 3 Ideally, the analytical methods that evaluate potency represent the product's mechanism of action (i.e. relevant therapeutic activity or intended biological effect). However, many cell therapy products have complex (e.g. rely on multiple biological activities) or not fully characterized mechanisms of action (MOA) or both, making it difficult to determine which product attributes are most relevant to measuring potency.

In the case that the cellular therapeutic product contains more than one active ingredient or cellular active substance or both, more than one analytical method to measure potency should be considered.

7.6.4 Requirements for analytical methods that evaluate potency

Analytical methods that evaluate potency should:

a) reflect the product‘s relevant biological properties;

b) be specifically designed for each product type;

c) be based on individual products attributes and components.

NOTE Potential non-additive effects between active ingredients, such as interference or synergy can affect the design of analytical methods to evaluate potency. In many cases, an analytical method cannot provide an adequate measure of potency. If one analytical method is not sufficient to measure the product attribute(s) that indicates potency, then an analytical method matrix can be used.

7.6.5 Design of analytical methods that evaluate potency

Understanding of attributes that establish a product's potency usually evolves as more knowledge is acquired and can change significantly as a product is developed (see Annex A). An incremental approach to the implementation of analytical methods that evaluate potency can be beneficial. Appropriate understanding of the biological properties of the cellular therapeutic product is necessary in order to develop relevant and meaningful measurements of potency. Considerations for establishing relevant and meaningful analytical methods that evaluate potency can include:

a) evaluation of relevant pre-clinical investigations, proof of concept studies, early clinical studies, available historical experience, and available reference materials and controls;

b) evaluation of characterization data obtained during product development.

NOTE A wide range of cellular therapeutic product attributes can be measured in addition to tests used for routine lot release. These exploratory studies can help to assess which product attribute(s) best correlate(s) with potency. While some of the analytical methods are not practical for lot release, they can provide helpful information about product attributes related to relevant biological activity or clinical effectiveness, or both.