TECHNICAL SPECIFICATION

ISO/TS 22859

First edition
2022-07

Biotechnology — Biobanking — Requirements for human mesenchymal stromal cells derived from umbilical cord tissue

Biotechnologie — Banques biologiques — Exigences relatives aux cellules stromales mésenchymateuses humaines issues des tissus du cordon ombilical

Reference number
ISO/TS 22859:2022(E)

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Published in Switzerland

Contents

Page

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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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

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

Mesenchymal stromal cells are a heterogeneous cell population that is characterized by multiple functional properties including the ability to secrete paracrine factors,[8] regulate immune effector cells,[9][10] maintain primitive phenotypes of other cell populations[11][12] and support tissue regeneration.[13][14] Mesenchymal stromal cells can contain a sub-population of stem or progenitor cells that demonstrate *in vitro* self-renewal and differentiation, as has been rigorously demonstrated for umbilical cord-derived progenitor cells.[15]

Mesenchymal stromal cells and mesenchymal stem cells are both abbreviated as "MSCs".[16] For the purpose of this document, the abbreviated term "MSCs" refers to mesenchymal stromal cells.

The functional definition of MSCs has evolved over time as the biology of these cells is better understood. Despite these advances, substantial ambiguities persist regarding the nomenclature, nature, identity, function, mode of isolation and experimental handling of these cells. MSCs are not fully defined by the initial minimal criteria[17] proposed by the International Society of Cell and Gene Therapy (ISCT), and as such require careful characterization by a matrix of functional assays.[16]

MSCs have been isolated from umbilical cord[15], bone marrow[18][19] and other tissue sources, and are widely used for non-clinical research. MSCs from different tissue sources have different properties. Different institutions use different practices for isolating, processing and biobanking these MSCs, making it difficult to compare data and results across institutions. Thus, there is a need for standardized approaches to isolate, process, expand and cryopreserve these MSCs from specific tissue sources.

This document provides requirements for biobanking of human mesenchymal stromal cells derived from umbilical cord tissue (Wharton's jelly) (hUC-MSCs) for research purposes. This document is applicable for academic centres, public and private institutions performing a biobanking service of hUC-MSCs for research and development (R&D) and preclinical studies, not for clinical use.

Importantly, this document is focused on MSCs that have been isolated, manipulated and/or propagated from umbilical cord tissue (also called "Wharton's jelly") in culture for research purposes. These cells are different from unmanipulated cells found in human umbilical cord tissue (Wharton's jelly).

ISBT 128[20] provides terminology and abbreviations for all medicinal products including cell therapy, and abbreviates these as "MSC(WJ)" to denote mesenchymal stromal cells from Wharton's jelly. This document recognizes this abbreviation, but uses the more commonly-used convention in research to denote human mesenchymal stromal cells derived from umbilical cord tissue (Warton's jelly) (hUC-MSCs).[21]

Biotechnology — Biobanking — Requirements for human mesenchymal stromal cells derived from umbilical cord tissue

1 Scope

This document specifies requirements for the biobanking of human mesenchymal stromal cells derived from umbilical cord tissue (i.e. Wharton's jelly), further referred to as hUC-MSCs, including the collection of umbilical cord tissue and associated data, isolation, culture characterization, quality control, cryopreservation, storage, thawing, disposal, distribution and transport.

This document is applicable to all organizations performing biobanking of hUC-MSCs used for research and development.

This document does not apply to hUC-MSCs for the purpose of *in vivo* application in humans, clinical applications or therapeutic use.

NOTE International, national or regional regulations or requirements, or multiple of them, can also apply to specific topics covered in this document.

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 8601-1, *Date and time — Representations for information interchange — Part 1: Basic rules*

ISO 20387:2018, *Biotechnology — Biobanking — General requirements for biobanking*

ISO 21709:2020, *Biotechnology — Biobanking — Process and quality requirements for establishment, maintenance and characterization of mammalian cell lines*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 20387:2018, ISO 21709:2020 and the following 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
authenticity
quality of being genuine or true

3.2
biobank
legal entity or part of a legal entity that performs *biobanking* (3.3)

[SOURCE: ISO 20387:2018, 3.5]

3.3
biobanking
process of acquisitioning and storing, together with some or all of the activities related to collection, preparation, preservation, testing, analysing and distributing defined biological material as well as related information and data

[SOURCE: ISO 20387:2018, 3.6]

3.4
biorisk
effect of uncertainty expressed by the combination of the consequences of an event (including changes in circumstances) and the associated "likelihood" (as defined in ISO Guide 73) of occurrence, where biological material is the source of harm

Note 1 to entry: The harm can be the consequence of an unintentional exposure, accidental release or loss, theft, misuse, diversion, unauthorized access or intentional unauthorized release.

[SOURCE: ISO 35001:2019, 3.17]

3.5
cell culture
growth of cells dissociated from the parent tissue by spontaneous migration, mechanical or enzymatic dispersal for propagation under *in vitro* conditions

3.6
cell master file
complete dossier of all procedures and records used to generate a cell

3.7
cell morphology
form and structure of the cell

Note 1 to entry: Morphology can be represented by a single parameter or a combination of two or more parameters.

[SOURCE: ISO 21709:2020, 3.3]

3.8
cell population purity
percentage of a particular cell type in a population, of which has the same specific biological characteristics, such as cell surface markers, genetic polymorphisms and biological activities

3.9
colony forming unit fibroblast
CFU-F
typical *in vitro* assay to demonstrate *self-renewal* (3.23) potential of progenitor cells plated at low frequencies that results in a formation of a colony of fibroblast-looking cells

Note 1 to entry: A count of these colonies is instructive of the colony forming potential or *in vitro* self-renewal capacity of these cells.

3.10
cryopreservation
process by which cells are maintained in an ultra-low temperature in an inactive state so that they can be revived later

[SOURCE: ISO 21709:2020/Amd 1:2021, 3.6]

3.11
differentiation
process to bring the cells into a defined cell state or fate

3.12
differentiation potential
ability that refers to the concept that stem and progenitor cells can produce daughter cells which are able to further differentiate into other cell types

3.13
flow cytometry
methodologically oriented subdiscipline of analytical cytology that measures cells in suspension in a liquid vehicle as they pass, typically one cell at a time, by a measurement station

Note 1 to entry: The measurement represents transformations of changes in the output of a detector (or detectors) due to changes in scattered light, absorbed light, light emitted (fluorescence) by the cell, or changes in electrical impedance, as the cell passes through the measuring station.

Note 2 to entry: Flow cytometry allows simultaneous evaluation of morphological characteristics of cells (size and internal complexity) with membrane or intracellular antigens.

[SOURCE: CLSI H44-A2:2004, Clause 4, modified — Note 2 to entry has been added.]

3.14
heterogeneity
<cells> non-uniformity of composition, quality or structure of a population of cells

3.15
homogeneity
<cells> uniformity of composition, quality or structure of a population of cells

3.16
human mesenchymal stromal cell derived from umbilical cord tissue
hUC-MSC
heterogeneous cellular population isolated from *umbilical cord* (3.25), which has the ability to modulate the immune response, secrete paracrine factors and undergo adipogenesis, osteogenesis and chondrogenesis *in vitro*

Note 1 to entry: Without any manipulation, "culture-adapted MSCs" is an alternate term used to denote cells that are different from cells that are found *in vivo*. It is increasingly clear that these cell types have different properties in terms of gene expression, functionality and phenotype.

3.17
licensing
<mesenchymal stromal cells> act of stimulating *hUC-MSCs* (3.16) using inflammatory cytokines to become more immunosuppressive

Note 1 to entry: Licensing is a biological term and not a regulatory or legal term.

3.18
passage
subculture
process of further culturing of cells in a culture vessel to provide higher surface area/volume for the cells to grow

Note 1 to entry: A passage can be performed by harvesting an aliquot from the parent vessel and reseeding it into another vessel.

3.19
passage number
number of subculturing that occurred

Note 1 to entry: For this document, P_0 is understood as the starting population of the cells.

[SOURCE: ISO 21709:2020, 3.13, modified — Note 1 to entry added.]

3.20
population doubling time
PDT
doubling time
time taken for cultured cell count to double

Note 1 to entry: The time is measured in hours.

[SOURCE: ISO 21709:2020, 3.8, modified — "population doubling time" and "PDT" added as the preferred term. Note 1 to entry added.]

3.21
primary culture
culture started from cells, tissues, or organs taken directly from an organism, and before the first subculture, propagation and consecutive *passages* (3.18) *in vitro*

[SOURCE: ISO 21709:2020, 3.16, modified — Note 1 to entry deleted.]

3.22
proliferation
cell number expansion by cell division

3.23
self-renewal
ability of *stem cells* (3.24) to divide symmetrically, forming two identical daughter stem cells

Note 1 to entry: Adult stem cells can also divide asymmetrically to form one daughter cell, which can proceed irreversibly to a differentiated cell lineage and ultimately lead to focused functional differentiated cells, while the other daughter cell still retains the characteristics of the parental stem cell.

3.24
stem cell
non-specialized cells with the capacity for *self-renewal* (3.23) and *differentiation potential* (3.12), which can differentiate into one or more different types of specialized cells

Note 1 to entry: Most adult stem cells are multipotent stem cells.

3.25
umbilical cord
umbilical cord tissue
UC
soft, gelatinous connective tissue (i.e. Wharton jelly), excluding umbilical arteries, umbilical vein and placenta

3.26
viability
attribute of being alive (e.g. metabolically active, capable of reproducing, have intact cell membrane, or have the capacity to resume these functions) as defined based on the intended use

[SOURCE: ISO 21709:2020, 3.17]

3.27
viable cells
cells within a sample that have an attribute 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

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

4 Abbreviated terms and symbols

2D1	clone of anti-human CD45 antibody
4F2	clone of anti-human CD98 antibody
561	clone of anti-human CD34 antibody
581	clone of anti-human CD34 antibody
58XB4	clone of anti-human CD104 antibody
63D3	clone of anti-human CD14 antibody
A20	tumour necrosis factor alpha-induced protein 3 (TNFAIP3)
ACAN	aggrecan
AD2	anti-human CD-73 (Ecto-5'-nucleotidase) antibody
AHR	aryl hrdrocarbon receptor
ALP	alkaline phosphatase
ANGPT2	angiopoietin-2
AP2	adipocyte protein-2
α-SMA	alpha-smooth muscle actin
B7RP2	B7-related protein 2
BCL-2	B-cell lymphoma 2
BJ18	clone of anti-human CD44 antibody
BM	bone marrow
C44Mab-5	clone of anti-human CD44 antibody
CCL2	C-C Motif Chemokine ligand 2
CCL5	C-C Motif Chemokine ligand 5
CCL7	C-C Motif Chemokine ligand 7
CCR7	C-C Motif Chemokine receptor 7
CCR10	C-C Motif Chemokine receptor 10
CD	clusters of differentiation
CD9	clusters of differentiation 9
CD13	clusters of differentiation 13
CD14	clusters of differentiation 14
CD29	clusters of differentiation 29
CD31	clusters of differentiation 31

CD34	clusters of differentiation 34
CD44	clusters of differentiation 44
CD45	clusters of differentiation 45
CD46	clusters of differentiation 46
CD55	clusters of differentiation 55
CD73	clusters of differentiation73
CD90	clusters of differentiation 90
CD98	clusters of differentiation 98
CD104b	clusters of differentiation 104b
CD105	clusters of differentiation 105
CD 146	clusters of differentiation146
CD276	clusters of differentiation 276
CEBPα	enhancer-binding protein alpha
CFSE	carboxyfluorescein succinimidyl ester
CFU	colony forming units
CIITA	human gene which encodes a protein called the class 2, major histocompatibility complex, transactivator
CO_2	carbondioxide
COL2A1	collagen type 2 alpha 1
COX-2	prostaglandin-endoperoxide synthase 2 (cyclooxygenase-2)
CX3CR1	C-3X-C Motif Chemokine receptor 1
CXCL9	C-X-C Motif Chemokine ligand 9
CXCL10	C-X-C Motif Chemokine ligand 10
CXCL11	C-X-C Motif Chemokine ligand 11
CXCL12	C-X-C Motif Chemokine ligand 12
CXCR1	C-X-C Motif Chemokine receptor type 1
CXCR4	C-X-C Motif Chemokine receptor type 4
CXCR6	C-X-C Motif Chemokine receptor type 6
DCN.70	anti-CD276 (B7-H3) antibody
DMEM	Dulbecco's modified eagle medium
DMEM-LG	Dulbecco's modified eagle medium low glucose
DMSO	dimethyl sulfoxide

DRAP-24	clone of anti-human CD9 antibody
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
FABP4	Fatty acid-binding protein 4
FACS	fluorescence activating cell sorter
FBS	fetal bovine serum
FRP-1	frizzled-related protein
GAL-1	galactose-1
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HCD14	clone of anti-human CD14 antibody
HCsAg	hepatitis C surface antigen
HCV	hepatitis C virus
HEL113	gene of vimentin
HGF	hepatocyte growth factor
HI30	clone of anti-human CD45 antibody
HI9a	clone of anti-human CD9 antibody
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HLA-Class I	human leukocyte antigen – Class I
HLA-Class II	human leukocyte antigen – Class II
HLA-DR	human leukocyte antigen DR
HO-1	haem oxygenase-1
HSP70A	heat shock protein 70A
HSP70B	heat shock protein 70B
hUC-MSC	human umbilical cord mesenchymal stromal cell
ICAM-1	intercellular adhesion molecule-1
IDO	indoleamine 2,3-dioxygenase 1
IFN-γ	interferon-gamma
IFU	instructions for use
IL-1	interleukin-1

IL-1RA	interleukin-1 receptor antagonist
IL-6	interleukin-6
IL-8	interleukin-8
INCAM-110	inducible cell adhesion molecule 110
ITGB1	integrin 1 antibody
ITGB4	integrin 4 antibody
KGF	keratinocyte growth factor
L243	clone of anti-human HLA-DR antibody
LN3	clone of anti-HLA-DR antibody
LPL	lipoprotein lipase
MCAM	melanoma cell adhesion molecule
MEM	minimum essential media
MEM-108	minimum essential media-108
MIH42	molting inhibits hormones-42
MPR-1	mannose 6-phosphate receptor-1
MR106	clone of anti-rat CD106 antibody
mRNA	messenger RNA
MSCs	mesenchymal stromal cells
My10	anti-CD34 antibody
N	count of cells harvested
N_0	count of cells seeded
O91D3	clone of anti-vimentin antibody
O92E4	clone of anti-human CD31 antibody
OPN	osteopontin
P_0	starting culture passage
P1H12	anti-CD146 antibody
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PDL-1	programmed death-ligand 1
PDL-2	programmed death-ligand 2
PDT	population doubling time

PECAM-1	platelet endothelial cell adhesion molecule-1
PI9	blast resistance gene PI 9
Poly29291	poly sulfuryl dichloride
PPAR-γ	peroxisome proliferators-activated receptor-gamma
PREF-1	preadipocytokine 1
QA17A19	clone of anti-human CD45 recombinant antibody
QC	quality control
SHM-57	anti-human CD146 (MUC18, Mel-cam) antibody
SOX2	SRY-related HMG box 2
SOX9	SRY-related HMG box 9
T	end time point of incubation, in hours
T_0	starting time point of incubation, in hours
TGF-β	transforming growth factor-β
Thy-1	thymus antigen
TIMP-1	tissue inhibitor of metalloproteinases 1
TIMP-2	tissue inhibitor of metalloproteinases 2
TLR3	toll-like receptor-3
TLR4	toll-like receptor-4
TNF	tumour necrosis factor
TP	Treponema pallidum
TRAIL	tumour necrosis factor-related apoptosis-inducing ligand
TS2/16	clone of anti-human CD29 antibody
TSE	transmissible spongiform encephalopathies
TSG-6	tumour necrosis factor-inducible gene 6 protein
TSP-1180	thrombin-sensitive protein-1180
UC	umbilical cord
UCHL1	ubiquitin carboxy-terminal hydrolase L1
ULBP-3	UL16 binding protein 3
VCAM-1	vascular cell adhesion molecule-1
VEGF	vascular endothelial growth factor
W16220A	clone of anti-vimentin antibody

W6/32	clone of anti-HLA class I antibody
WM15	CD13 antibody
WM59	CD31 antibody
WNTS	Wnt family

5 General requirements

5.1 General

The biobank shall follow ISO 20387 and ISO 21709, in addition to this document. ISO/TR 22758 can be used as additional reference for the implementation of ISO 20387.

The biobank shall establish criteria and procedures for the isolation, culture, storage, thawing and transport of hUC-MSCs.

A data analysis procedure shall be established, documented, implemented, regularly reviewed and updated.

The biobank shall use validated and/or verified methods and procedures for activities pertaining to hUC-MSCs in accordance with ISO 20387:2018, 7.9.2 and 7.9.3, at all stages of the biological material life cycle (as defined in ISO 20387:2018, 3.29).

According to the characteristics of hUC-MSCs, procedures, QC documents for collection, separation, expansion, storage, transportation and testing, and data analysis shall be established, documented, implemented, regularly reviewed and updated.

The donor's health status and characteristics, tissue harvest conditions as well as culture methods can influence the properties of hUC-MSCs. Workflow steps cannot always be controlled. Thus, their impact on the hUC-MSCs properties for biobanking shall be investigated, and mitigation measures shall be established to enable the required quality control. In these cases, quality risk management (QRM) should be taken into account.

The biobank shall assess biorisks of umbilical cord and hUC-MSCs at the facilities and implement appropriate biosafety measures for the protection of personnel and environment.

The authenticity and properties of hUC-MSCs shall be monitored throughout the complete biobanking process from isolation to distribution.

5.2 Personnel, facilities and equipment

ISO 20387:2018, Clause 6, and ISO 21709:2020, 4.3, 4.4, 4.7, shall be followed.

The biobank personnel shall be appropriately and specifically trained in hUC-MSC generation, characterization, culture, cryopreservation, thawing and transport.

The biobank shall ensure that external operators providing hUC-MSC services demonstrate relevant knowledge, experience and corresponding skill.

The biobank shall ensure that facilities and environmental conditions do not adversely affect hUC-MSC quality attributes or invalidate the test results.

Equipment management procedures should be established, including the use of equipment and maintenance plan.

The biobank shall control the operating environment and conditions (e.g. temperature, humidity, cleanliness) according to the relevant characteristics of hUC-MSCs and the need for aseptic processing.

5.3 Reagents, consumables and other supplies

ISO 21709:2020, 4.5, shall be followed.

The biobank shall establish acceptance criteria for materials, including reagents and consumables, necessary for hUC-MSC isolation, culture, storage, thawing and transport.

5.4 Management of information and data

ISO 20387:2018, 7.8.3 and 7.10 shall be followed.

The biobank shall manage and maintain associated data of hUC-MSCs, including but not limited to the following:

a) the technical information: methods used in the derivation of cells, culture conditions, passage data including the passage number, characterization and microbiological test data;

b) the preservation and storage information;

c) the safety testing data.

Certain data retention times, data integrity and security of data storage shall be ensured.

For hUC-MSCs, a minimum period of retention of records shall be established. Special requirements for storage and retention times can apply for future applications. Personal data of each human donor shall be held in a protected location and shall be handled in accordance with ISO 20387:2018, 4.3.

The cell master file shall be kept to enable review of the data and records for specific applications.

6 Collection of umbilical cord and associated data

6.1 Information about the umbilical cord donor

A risk assessment shall be performed and documented.

To protect the private data of the donor, the biobank shall establish donor data protection methods in accordance with ISO 20387:2018, 4.3.

The documentation of the donor information shall be performed. Where possible, the documentation shall be performed prior to sample collection. The documentation shall include but is not limited to:

a) the donor reference, which can be in form of a code (e.g. pseudonymized, anonymized);

b) the relevant health status of the umbilical cord donor (e.g. statement of donor health or suitability, disease type, concomitant disease, demographics such as gestational age and age);

c) the information about medical treatment and special treatment prior to umbilical cord collection (e.g. date, terms of treatment, medication, conclusion of medical specialist);

d) the negative test result for hepatitis B and C, HIV and TP unless a positive test result is needed for a specific research purpose;

NOTE 1 Additional virus testing can be considered where relevant.

e) where applicable, information about the informed consent given by the donor (e.g. copy of the signed informed consent signature form with details of the donor's name redacted); see ISO 20387:2018, 7.2.3.4;

f) the absence of congenital abnormalities in the neonates, when appropriate.

Documentation of the donor information should include the geographical region of the donor as needed based on the purpose of research.

Unless related to a specific research purpose, donors shall not be considered suitable for donation, if they:

— were unhealthy at the time of donation;

NOTE 2 Deferral periods for specific infections can exist and/or vary in accordance with local regulatory guidelines for other cell and tissue products.

— have tested positive for at least one infectious disease, see 6.1 d).

During the collection process for human cells, measures shall be taken to protect donor and biobank personnel health and safety.

6.2 Collection procedure

ISO 20387:2018, 7.2, shall be followed.

The biobank shall establish, implement, validate and document a procedure for the collection of umbilical cord.

Umbilical cord tissue collection should be operated under the aseptic conditions to avoid sample contamination.

All reagents and materials used to collect the umbilical cord tissue shall be sterile.

The biobank should conform to ISO 35001 or the WHO's *Laboratory Biosafety Manual*[22] when handling biological material contaminated with pathogens.

The risk of microbiological contamination (bacterial, fungal, viral, parasitic) should be mitigated by focusing on those agents which are most likely to be contaminants in relation to the geography, donor cohort and tissue being procured.

Umbilical cord tissue can be collected following vaginal or caesarean delivery. It shall be ensured that collection procedures do not interfere with standard delivery practices or the safety of a newborn or the mother. The collection procedure shall take place at the distal end of the cord.

The required amount of cord should be determined in consideration of the intended purpose of the biobank or user. Both ends of the cord shall be clamped to prevent contamination.

NOTE 1 Length of the cord is discussed in Reference [23].

NOTE 2 Mass can also be used to determine required amount of cord.

NOTE 3 The following biological material can be considered for testing data:

a) cord blood (for detection of certain antigens and/or antibodies against such antigen);

b) cultured mesenchymal stromal cells from umbilical cord (for PCR testing to detect expression of certain gene(s));

c) mother's blood (for detection of certain antigens and/or antibodies against such antigen).

Blood should not be drawn from a newborn.

In case of vaginal delivery, a procedure to minimize bacterial and fungal contamination of collected umbilical cord shall be established, implemented and documented. Such a procedure can include rinsing the collected umbilical cord and content with a rinsing solution, e.g. sterile phosphate-buffered saline solution (1×PBS) containing heparin and/or penicillin, streptomycin and/or Amphotericin B.

The medium for umbilical cord collection should be established in consideration of the intended purpose of the biobank and/or user.

A recommended medium for umbilical cord collection is sterile phosphate-buffered saline solution (1×PBS) containing heparin and/or penicillin, streptomycin and/or Amphotericin B.

7 Transport of umbilical cord or hUC-MSCs and associated data to the biobank

ISO 20387:2018, 7.4, shall be followed. ISO/TS 20658 can be used to consider transport, handling and safety requirements for facilities.

The biobank should conform to ISO 35001 or the WHO's *Laboratory Biosafety Manual*[22] when handling biological material contaminated with pathogens.

The biobank shall determine the appropriate conditions for the transportation of umbilical cord from the collection facility to the biobank. Instructions on the transportation of umbilical cord to the preparation site as well as the transportation of hUC-MSC preparations to the biobank should be included.

EXAMPLE The temperature can be between 2 °C and 8 °C[24].

The following factors shall be taken into account for transportation of umbilical cord tissue:

a) packaging, material, containers and secondary containment;

b) medium or solvent;

c) transportation duration, temperature and temperature monitors.

Biological source material collection medium and conditions shall be established, implemented, documented and validated to ensure maintenance of the viability and other key parameters.

The sample shall be transported under appropriate biosafety conditions.

A procedure for critical control points shall be established, implemented and documented.

8 Reception and traceability of umbilical cord tissue or hUC-MSCs and associated data

ISO 20387:2018, 7.3.1, 7.3.2 and 7.5, shall be followed.

9 Isolation and expansion of hUC-MSCs

9.1 Processes

For establishing hUC-MSCs, ISO 21709:2020, 5.1, shall be followed.

The biobank shall establish, implement, validate, document and maintain procedures for hUC-MSC isolation in primary culture and subculture.

Processes should be performed in a biosafety cabinet or under a laminar flow hood using appropriate aseptic techniques.

9.2 Unique identification

The unique identification of hUC-MSCs shall be established in accordance with ISO 20387:2018, 7.5. This should include a unique cell name or sample number, a biobank batch number and biobank vial number. Cells should be anonymized or de-identified.

9.3 Testing for infectious agents

The cells derived from the donor biological material should be tested for relevant transmittable infectious agents, e.g. HIV, HBV, HCV and TP.

The analytical data and results as well as the associated analyses shall be documented and available to authorized biobank personnel and researchers who process established cells.

9.4 Isolation of hUC-MSCs and primary culture

There are various methods of isolation and establishing a primary culture including explant or enzymatic methods (see Annex B for exemplary methods).

The biobank shall document and provide information about the section of the cord used for the isolation of the cells (e.g. perivascular region, Wharton's jelly).

NOTE Current methods to isolate hUC-MSCs from human umbilical cord (hUC) yield low amounts of cells with variable proliferation potentials. Although hUC is an anatomically-complex organ, differences in MSC properties can appear due to differences in the anatomical regions within the Wharton's jelly[25][26].

It is recommended to mechanically separate the blood vessels from the Wharton's jelly before isolating hUC-MSCs. Umbilical cord tissue should be fragmented small enough (1 mm to 3 mm) to allow the cells to come into contact with gases and nutrients.

Several different culture media have been used to isolate hUC-MSCs such as MEM, DMEM-LG. The biobank shall document and provide the composition or formula of the medium to the hUC-MSCs recipient/user.

9.5 Subculture and limited expansion

A culture can be further expanded for biobanking after successful establishment of the primary culture; this is then known as a "subculture". Each culture expansion is referred to as a "subculture" or "passage".

Cultures should be tested for microbiological contaminants (including bacteria, fungi, yeast, mycoplasma, endotoxins and adventitious viral agents) before any further expansion.

Cell passaging follows the relative protocols after establishment of primary culture. Expansion of hUC-MSCs is recommended for up to three passages to ensure sufficient availability of material while preserving the biological features of the original culture, thus preventing culture-associated adaptations. The biobank shall monitor the expansion for changes in specific biological characteristics (e.g. CFU-F, undifferentiation status and immunophenotyping).

10 Characterization of hUC-MSCs

10.1 General

The biobank shall establish, document and implement procedures to characterize hUC-MSCs and report the relevant data so that users can determine suitability for their intended use.

The biobank shall establish a matrix of assays and a set of markers based at least on Clause 10.

The biobank shall perform ongoing characterization of hUC-MSCs in culture. The characterization shall include but is not limited to:

a) authentication;

b) cell morphology;

c) growth kinetics: can be calculated using PDT;

d) viability;

e) differentiation capability *in vitro*;

f) immunophenotype;

g) functional characterization *in vitro*;

h) being free of microbial contamination.

Exemplary methods for hUC-MSC characterization tests can be found in Annex C.

10.2 Viability

The biobank shall establish, implement and document a procedure to determine cell viability.

Quality control for cell viability test shall be performed using live and dead cells. Cell viability shall be determined and documented.

The biobank shall assess the amount of viable cells in the cell culture at regular intervals and especially after changes of cell culture conditions.

Viability shall be assessed following the thawing after cryopreservation.

The biobank shall limit the acceptable percentage of nonviable cells in the population during the test procedure.

The amount of viable hUC-MSCs should be ≥ 90 % prior to cryopreservation.

The amount of viable hUC-MSCs should be ≥ 70 % immediately post-thaw and determined with a validated method.

NOTE A viability assay is usually performed prior to cryopreservation and immediately after thawing. The post thaw viability is typically an overestimation of viability.

An automated cell viability test should be performed.

The biobank can establish, implement and document a procedure to evaluate apoptosis in the cell culture.

EXAMPLE Assessment on any cryopreserved and thawed MSCs for apoptosis is typically done using flow cytometry (see Clause B.4).

10.3 Morphology

Cell morphology can be very different depending on growth conditions. There is little knowledge regarding correlation of overall morphology and biological function of hUC-MSC preparations. In addition, at different stages of culture cells have different morphologies reflecting changes in status (e.g. nutritional status, growth profile) and differentiation. Some studies have shown that continuous subculture leads to changes in cell morphology, cell enlargement and ultimately senescence.[27]

A description of cell morphology should include the conditions of culture as well as the stages of culture.

NOTE 1 When growing cells on a tissue culture flask, cells that adhere to the plastic show different morphologies in relation to the stage of culture. At an early stage after starting the *in vitro* culture on plastic flasks, adherent cells with different morphologies can be identified, e.g. tripolar-shaped cells, long spindle-shaped cells, small round cells, short spindle shaped cells, flattened enlarged cells.[27] At later stages, adherent cells show spindle shape similar to that of fibroblast. Colonies have spiral or radial patterns.

NOTE 2 hUC-MSCs can be maintained, and possibly expanded, without adherence under specific culture conditions. But these cells, if maintained under more standardized conditions, would be expected to demonstrate adherence.

The biobank shall document significant cell morphology changes.

10.4 Population doubling time and subculture/passage

10.4.1 PDT

The PDT is the time (measured in hours) required for the replication of the population of hUC-MSCs. The PDT is calculated with Formula (1) using the cell counts obtained before and after harvesting:

$$D = (T - T_0) \times log2 / (logN - logN_0) \quad (1)$$

where

D is the PDT;

$(T - T_0)$ is the incubation time in hours;

N is the count of cells harvested;

N_0 is the count of cells seeded.

NOTE 1 Formula (1) is applicable in a linear range of cell expansion.

The average PDT of hUC-MSCs isolated from human umbilical cord ranges between 20 h and 40 h[28].

NOTE 2 Depending on the culture conditions, culture passage, cell density and characteristics of the donor (e.g. age), the PDT can vary.

The PDT of hUC-MSCs should be determined by the biobank after secondary culture.

PDT can reflect the growth kinetics of hUC-MSCs in culture. The biobank can utilize the PDT of hUC-MSC cultures at different passages to evaluate changes in culture cell growth kinetics.

The PDT shall be documented.

10.4.2 Subculture/passage

P_0, the passage number(s) together with the seeding and final cell density, and the culture vessel surface area shall be documented. When the hUC-MSCs cover the culture vessel surface at 70 % to 80 %, the cells can be passaged.

Passage numbers are frequently used by laboratories. However, the passage number is correlated with the surface area/volume of a culture vessel and how the initial P_0 is defined. It is recommended that the biobank defines P_0 as the initial plating of umbilical cord tissue cells.

Documenting PDT along with passage numbers can facilitate a better understanding of growth dynamics of the hUC-MSCs and the relationship between passages and PDT.

10.5 Cell population purity

The biobank shall evaluate the purity of hUC-MSCs. Unwanted cell populations such as haematopoietic and endothelial cells (see Table 1) shall only be present below defined levels, e.g. ≤ 5 %. Immunophenotyping of hUC-MSCs as described in 10.9 can be used for evaluating and verifying purity and identity. Unwanted microbial contaminants shall be defined and shall be checked, as described in 10.12.

10.6 *In vitro* self-renewal assessment

The biobank shall establish, implement and document a procedure for an *in vitro* self-renewal assessment.

The assay for *in vitro* self-renewal shall be documented including QC criteria as described in Clause 11.

NOTE CFU-F can be used for *in vitro* self-renewal assay (see Annex C).

10.7 Proliferation

The biobank shall establish, implement and document a cell proliferation assay.

The assay for cell proliferation shall be documented including QC criteria as described in Clause 11.

NOTE Cell proliferation can be evaluated by a validated method, e.g. CFSE or by MTT (see Annex C).

10.8 Differentiation capability — *In vitro* multilineage differentiation

10.8.1 General

WARNING — To evaluate *in vitro* multilineage differentiation of hUC-MSCs, the biobank should establish, document and implement procedures and requirements for the specific assays (i.e. hUC-MSCs with ability to undergo *in vitro* multilineage differentiation). The following *in vitro* multilineage differentiation assays are part of the *in vitro* characterization of hUC-MSCs, but do not always reflect the *in vivo* differentiation capacity of these cells.

In vitro multilineage differentiation into osteoblasts, adipocytes and chondroblasts under appropriate conditions is a valuable tool for characterization of hUC-MSCs. This information can be useful, if assessed in a quantitative assay format that is validated and sufficiently sensitive to assess hUC-MSCs grown for different lengths of time, and under different conditions.

More than 95 % of hUC-MSCs exhibit a tri-lineage differentiation potential *in vitro*[29].

10.8.2 *In vitro* adipogenic differentiation

hUC-MSCs can differentiate into adipoblasts *in vitro* in adipogenic induction medium. A generally recommended adipogenic induction medium contains dexamethasone, 3-isobutyl-1-methylxanthine, recombinant human insulin and indomethacin.

hUC-MSC cultures with a confluence ≥ 80 % in the third to fifth culture passage shall be used. hUC-MSC cultures after the fifth culture passage should not be used. Control groups without adipogenic differentiation medium should be incorporated.

Oil Red O staining should be used for adipogenic differentiation detection, typically after three weeks. It stains the formation of neutral lipid vacuoles. Both the staining and washing times should be strictly controlled to avoid false positives.

To further confirm the adipogenic capacity of hUC-MSCs, the expression of genes encoding proteins associated with the adipogenic differentiation status shall be evaluated. A subset of multiple genes associated with adipogenic differentiation such as PPAR-γ[30] (peroxisome proliferator-activated receptor gamma), CEBPα[29] (CCAAT / enhancer-binding protein alpha), FABP4[31] (fatty acid-binding protein 4) and PREF-1[32] (preadipocyte factor 1), AP2[33] (adipocyte protein-2) and LPL[34] (lipoprotein lipase) should be evaluated using quantitative real-time PCR.

In vitro differentiation of hUC-MSC under different conditions including serum-free conditions[35] and from different parts of the Wharton's jelly[36] should be considered.

10.8.3 *In vitro* chondrogenic differentiation

hUC-MSCs can differentiate into chondroblasts *in vitro* in chondrogenic induction medium. A recommended chondrogenic induction medium is composed of TGFβ3, dexamethasone, ascorbic acid, sodium pyruvate, insulin, transferrin, selenous acid, bovine serum albumin and linoleic acid in DMEM-high glucose[37].

In addition to 2D *in vitro* differentiation, chondrocyte differentiation using 3D micromass cultures can also be performed as previously reported for MSCs[38][39].

hUC-MSC cultures with a confluence ≥ 70 % in the third to fifth culture passage shall be used. hUC-MSC cultures after the fifth culture passage should not be used. Control groups without chondrogenic differentiation medium or conditions should be incorporated.

Chondrogenic differentiation shall be demonstrated by the development of a multi-layered, matrix-rich morphology and the extracellular matrix, which, when stained by Toluidine Blue and Heidenhain's azan, indicates sulfated proteoglycan accumulation and collagen fibres, respectively. Other staining methods can be used such as Alician Blue or Safranin O. This is typically done after three weeks in differentiation conditions.

To further confirm the chondrogenic capacity of hUC-MSCs, the expression of genes encoding proteins associated with chondrogenic differentiation should be evaluated. A subset of genes such as SOX9 (SRY-related HMG box 9), COL2A1 (collagen type 2A1) and ACAN (aggrecan) should be evaluated using quantitative real-time PCR[40][41][42].

10.8.4 *In vitro* osteogenic differentiation

hUC-MSCs can differentiate into osteoblasts *in vitro* in osteogenic induction medium. A recommended osteogenic induction medium contains dexamethasone, ascorbate and β-glycerophosphate.

NOTE The cell density as well as the selection of certain lots of FBS have an impact on the osteoblast differentiation.

hUC-MSC cultures with a confluence ≥ 70 % in the third to fifth culture passage should be used. hUC-MSC cultures after the fifth culture passage should not be used.[43] Control groups without osteogenic differentiation medium should be incorporated.

Osteogenic differentiation should be demonstrated after three weeks of induction by calcium deposition as detected by staining. No osteogenic phenotype should be observed in the control group (without osteogeneic medium).

Alizarin Red S or von Kossa staining should be used for osteogenic differentiation detection. The dyeing time and cleaning should be strictly controlled to avoid false positives.[38]

To further confirm the osteogenic capacity of hUC-MSCs, the expression of genes encoding proteins associated with osteogenic differentiation should be evaluated. These genes, such as ALP (alkaline phosphatase) and OPN (osteopontin), should be evaluated using quantitative real-time PCR. Intracellular staining for protein expression of OPN or other mature bone markers can also be evaluated.

10.9 Immunophenotyping by flow cytometry

hUC-MSCs should be characterized by expressions of antigens listed in Table 1. In addition, markers listed in Table 2 can be used. Additionally, other international guidelines should be followed, where appropriate.

NOTE 1 hUC-MSCs are characterized by the presence of specific cell markers and the absence of others such as those present on the surface of haematopoietic cells. However, there are some discrepancies in the panel of cell surface markers proposed by different studies for characterization of hUC-MSCs. This variability is expressed in terms of number of hUC-MSC markers used to characterize hUC-MSCs at different stages (i.e. in-process and/or lot release testing, frequency and range of expression). These are most likely due to the epigenetic factors caused by various culture conditions. A review of the literature can be found in References [29], [38] and [44].

Table 1 — Antigens and clones of antibodies recommended for immunophenotyping of hUC-MSCs

Antigen [26][45][47][48]	Antibody clones [49][50][51][52][53]	Required detection rate %
CD14-	HCD14, 63D3	≤ 5
CD31-	O92E4, WM59, PECAM-1	≤ 5
CD34-[a]	561, 581, My10	≤ 5
CD45-[b]	2D1, HI30, QA17A19, UCHL1	≤ 5
HLA-Class II-	L243, LN3	≤ 5
CD13+	WM15	≥ 95
CD44+	BJ18, C44Mab-5	≥ 95
CD73+	AD2	≥ 95
CD90+	5E10, Thy-1	≥ 95
CD105+[c]	43A3	≥ 95
HLA-Class I+	W6/32	≥ 95

[a] CD34- subsumes that the cells are negative for endothelial antigens.

[b] CD45- subsumes that the cells are negative for additional haematopoietic antigens including CD11b, CD14 and CD19.

[c] Levels can vary.

Table 2 — Optional antigens and clones of antibodies that can be tested for immunophenotyping of hUC-MSCs in addition to Table 1

Antigen[26][45][46][47][48][54][55][56][57][58][59]	Antibody clones [49][50][51][52][53]	Required detection rate %
CD9+	HI9a, MRP-1, DRAP-24	≥ 95
CD29+	TS2/16, ITGB1	≥ 95
CD98+	MEM-108, 4F2, FRP-1	≥ 95
CD104b+	58XB4, TSP-1180, ITGB4	≥ 95
CD106+ (VCAM1)	MR106, VCAM-1, INCAM-110	≥ 95
CD146+	P1H12, MCAM, SHM-57	≥ 95
CD276+	DCN.70, B7RP2, MIH42	≥ 95
Vimentin	O91D3, W16220A, Poly29291, HEL113	≥ 95

NOTE 2 Cells can be harvested by treatment with 0,25 % Trypsin-EDTA, washed twice with PBS (pH 7,4), and resuspended at 1×10^6–1×10^8 cells/ml in PBS. The cells can be labelled with the selected antibodies in the dark for 30 min at room temperature. Isotype-matched control antibodies can be used as controls. Cells can be analysed in a FACS flow cytometer.

NOTE 3 The cell surface antigen phenotyping between cells obtained by the explant method and those obtained by the enzymatic method show no significant difference.[57]

hUC-MSCs can express HLA-DR under certain conditions (e.g. the presence of IFN-γ but not in an unstimulated state); HLA-DR expression varies with donor. These relevant conditions shall be documented.

NOTE 4 Some studies have demonstrated that UC-MSCs do not express CD105 at all, or until passage 5. Reduction in CD105 expression on UC-MSCs can occur under ischaemic conditions influenced mainly by hypoxia[58].

Clause C.4 includes a general guideline on immunophenotyping by flow cytometry.

10.10 Paracrine secretion/expression (protein-based assay of secretome)

hUC-MSCs are known to secrete/express a host of cytokines, chemokines and growth factors including but not limited to TGFβ, IDO, COX-2, PDL-1, VEGF, WNTS, HGF, IL-6, IL-1RA, KGF, CCL2, CXCL12, exosomes containing microRNA, lipids, mitochondria and other cargo, where relevant. These factors can act on local and distal immune effectors and tissues thereby modulating immune response and tissue repair processes.[59]

A panel of appropriate factors should be pre-defined, documented and tested depending on the research purpose.[59]

NOTE 1 Protein based assays such as ELISAs or multiplex can be used to measure secreted factors by hUC-MSCs.

NOTE 2 Importantly, it has been shown that hUC-MSCs require stimulation to express their immunosuppressive properties. This can be performed by exposure to soluble inflammatory factors such as IFN-γ, TNF, interleukin[60] or by physical contact to inflammatory cells. This method of "licensing" hUC-MSCs is important, and there are several ways of accomplishing this.[61][62]

Unlicensed hUC-MSCs can serve as controls.

mRNA levels should be tested by real-time PCR or multiplex PCR methods. Additionally, protein levels for paracrine secretion/expression should be measured by ELISA or multiplex methods in the cell and culture supernatant.

10.11 Immunoregulation (modulation of immune cells)

hUC-MSCs are known to modulate immune cells. The specific type of immune cell can vary depending on the preclinical model, disease of interest and mechanism of action being studied. It is therefore recommended[59] that specific populations of purified immune effector cells are used in co-culture with hUC-MSCs to measure the effect of the MSC on that population of immune effectors.

Factors to be considered in this functional assay characterizing hUC-MSC immunomodulatory properties include the ratio of hUC-MSCs to immune effector cells, medium conditions, licensing of hUC-MSCs, activation of immune effectors and immune effector donor heterogeneity.

A matrix assay approach shall be applied as follows[59]:

a) Quantitative analysis of selected gene products shall be done:

   1) hUC-MSCs should be licensed, and unlicensed MSCs should be used as controls;

   2) quantitative real-time gene expression of at least three genes shall be performed; the following genes (non-exhaustive list) can be measured[59]: IDO, CXCL10, CXCL9, CXCL11, CIITA, ICAM-1, CCL5, TRAIL, TLR3, CCL7, VCAM-1, HLA-DR, HGF, IL-6, CCL2, PI9, CCR7, VEGF, PDL-1, CX3CR1, COX-2, AHR, TSG-6, KGF, TLR4, CXCL12, CD46, PDL-2, TGF-β, CXCR6, CCR10, TIMP-2, CD55, BCL-2, ANGPT2, A20, HSP70A, IL-8, ULBP-3, HSP70B, CXCR1, GAL-1, CXCR4, HO-1,TIMP-1, IL-1RA.

      NOTE These genes are examples and the entire list does not necessarily need to be assayed. It is up to the user to select from this list (or other lists in the literature) to come up with a panel of secreted/expressed factor readouts that is appropriate for the pre-clinical research context.

b) Protein expression of selected secreted/expressed factors [see 10.11, a) 2)] shall be done.

c) Immunoregulation of hUC-MSCs shall be assessed by testing their effects on *in vitro* proliferation of total peripheral lymphocytes or certain lymphocyte subpopulations during co-incubation of hUC-MSCs with the peripheral blood mononuclear cells (PBMC)[63] or purified subsets of T-cells, monocytes/macrophages or other immune cells.

10.12 Microbial contamination

Procedures for microbial contaminant testing of hUC-MSCs shall be established, validated, implemented and documented throughout the whole process.

Throughout the whole process from donation and procurement, preparation of culture reagents and equipment, to maintenance and cryopreservation of cultures, it is important to take a holistic view and establish microbiological testing at all critical points of the process. In addition, procedures to minimize risks to other established cultures should be in place. It is good practice to maintain QC procedures for primary tissues or cells newly brought into the biobank. Such cultures should be maintained in a dedicated area and in segregated equipment until sufficient data are available to justify their relocation.

Test methods used for microbiological testing shall be validated. It is important to be sure that appropriate levels of sensitivity, specificity and robustness are being used in respect of testing cell cultures.

Microbial contamination shall be assessed by risk management throughout the process.

hUC-MSCs used for R&D shall be free of contaminants. These contaminants include but are not limited to bacteria, yeast, fungi and mycoplasma.

a) Tests for the presence of bacteria, yeast fungi, and mycoplasma shall be conducted routinely. The use of antibiotics should be eliminated as soon as possible. However, where they are used in the culture medium, these should be removed prior to sampling.

b) It is also necessary to be aware of the impact that some contamination can have on the biological characteristics of the cell population being cultured. For example, a low level of viral infection will probably not have a significant impact on cell death, but can dramatically influence the biological activity. This type of contamination can impact any research data being generated.

EXAMPLE Mycoplasma is recognized as a common contaminant of cell cultures due to the risk of contamination from numerous sources. Mycoplasma can be very difficult to remove from cell culture as their small size limits filtration and they can be difficult to detect without establishing routine testing procedures.

The risks posed by transmissible spongiform encephalopathies (TSEs) should be considered irrespective of the origin or history of the cells. There are a number of TSE diseases across the globe showing their ability of transmitting to humans.

11 Quality control

ISO 20387:2018, 7.8, and ISO 21709:2020, 5.5, shall be followed.

The biobank shall establish, implement and document a QC procedure which shall include the testing of biological characteristics related to the *in vitro* functionality of hUC-MSCs as given in Clause 10.

QC of biological characteristics (see Clause 10) of hUC-MSCs shall be performed for all critical procedures, from isolation to thawing. A recommended QC procedure for biobanking of hUC-MSCs is given in Annex A.

The biobank shall establish, implement and document QC acceptance criteria for all the biological characteristics of hUC-MCSs included in Clause 10.

The biobank shall establish, implement and document QC acceptance criteria for all critical control points, e.g. culture media, reagents, equipment.

Throughout the biobanking processes, the culture media shall be periodically tested for *Mycoplasma* spp.

QC shall be established with a risk-based approach related to laboratory safety.

12 Storage

ISO 20387:2018, 7.5, 7.7, Clause A.6, and ISO 21709:2020, 5.3.4, shall be followed.

Optimization of the cryopreservation procedure and method(s) to minimize damage to cells during freezing and thawing is critical to ensure reliable availability of viable cells.

NOTE Controlling the freezing rate and the use of appropriate cryoprotectant, as well as stable storage temperature, can minimize the negative effects on the cell viability.

For cryopreserved hUC-MSCs, the following information shall be documented:

a) the cell name;

b) the preserved hUC-MSC batch number;

c) the date of preservation in accordance with ISO 8601-1;

d) the culture conditions;

e) the passage number;

f) the operator name.

Each stored vial derived from the same batch of cultured cells shall have a unique identification reference number (i.e. a biobank or batch number), which is traceable throughout the processes of collection, separation and expansion in accordance with ISO 20387:2018, 7.5.

Prior to freezing, cell morphology (see 10.3), immunophenotype, immunoregulatory functionality (see 10.11), viability (see 10.2) and paracrine secretion (see 10.10) shall be tested and the results shall be consistent with features of MSCs isolated from umbilical cord. Representative samples should be taken for assessment of the differentiation capability (10.8), growth kinetics and self-renewal assay (see 10.6), and the assessment results can be available after the cryopreservation. The results of these assays should be documented and included into the cell master file.

The amount of viable cells shall be ≥ 80 % and they should be free of contamination(s).

Cells should be in the growth phase, centrifuged and resuspended in cryopreservation medium. The cell count should be between 500 000 cells/ml to 5 000 000 cells/ml.

Important factors for the cryopreservation procedure are as follows:

— Cryovials shall be appropriate for cryopreservation.

— For cryoprotectant reagent(s), the following applies:

  — Cryoprotectant formulas (containing DMSO or other constituents) should be optimized by biobanks to preserve cell viability and function. The most common cryopreservation medium to store hUC-MSCs is a solution of 5 % to 10 % (v/v) DMSO and up to 90 % to 95 % (v/v) complete medium or animal serum (e.g. FBS). DMSO facilitates entry of organic molecules into tissues. Hazardous materials shall be handled by using appropriate safety practices.

  — The time that cells are in contact with DMSO should be minimized until frozen. If using controlled-rate freezing, additional considerations can apply.

    NOTE 1 Exposing cells to DMSO at high concentrations or for prolonged periods of time is damaging to the cells.

    NOTE 2 hUC-MSCs have been cryopreserved using both DMSO and FBS free systems, comprising different polymers either alone or in combination with ethylene glycol, 1,2-propylene glycol, trehalose, sucrose and/or glucose.

— There are two procedures to achieve the efficient cryopreservation of hUC-MSCs: conventional controlled-rate cooling and vitrification (rapid cooling):

  — controlled-rate cooling allows a controlled and reproducible means of cryopreserving hUC-MSCs;

  — if using a rapid freezing protocol, the freezing box can be stored at −80 °C for at least 16 h prior to moving to long-term storage between −135 °C and −210 °C (e.g. nitrogen gas or liquid phase).

NOTE 3 Both of these methods can lead to cell damage during loading/unloading of the cryoprotectant agents (CPAs), freezing and thawing steps.

NOTE 4 The controlled-rate cooling procedure allows the preparation of large amounts of vials at one time. Cryopreservation by vitrification has shown higher cell survival and it has been recognized as a promising strategy for long-term cell banking.

For long-term biobanking of hUC-MSCs (e.g. more than 12 months), the vapour phase nitrogen method should be used.

Controlled-rate cooling of hUC-MSCs should be performed as follows:

— document the date and time of the start of the cryopreservation process in accordance with ISO 8601-1;

— begin fast cooling from room temperature down to −10 °C at 0,5 °C/min to 1 °C/min;

— hold at −10 °C for 5 min; this step is critical to ensure the formation of the ice crystal seed;

— cool down to −80 °C at 1 °C/min (target temperature);

— transfer into liquid nitrogen for subsequent storage.

The biobank shall maintain records of the cryopreservation process, including the cell density, viability and temperature control.

13 Thawing

In the cell thawing process, frozen cells should be thawed at 36,5 °C ± 0,5 °C, or processed for culture with drop-wise addition of medium, put into culture and then transferred into an incubator with appropriate gas atmosphere and humidity. To optimize the process, the incubator should be set to an appropriate culture temperature, which is typically 36,5 °C ± 0,5 °C.

The frozen cells should be quickly thawed by warming and transferred directly to pre-warmed culture medium at 36,5 °C ± 0,5 °C to ensure maximal hUC-MSC viability and biological activity.

For cells preserved by vitrification methods, this can be more critical and expert advice should be sought.

The following information should be clearly documented, including but not limited to:

a) the batch number for the set of frozen vials;

b) the cell name;

c) the passage number;

d) the culture condition;

e) the operator name;

f) the thawing date of thaw operation in accordance with ISO 8601-1;

g) the thawing time in accordance with ISO 8601-1 as the time point when frozen cells leave liquid nitrogen to the time point when the cells are put into culture;

h) the date in accordance with ISO 8601-1 at which the culture reaches sufficient colony density to be passaged.

Cell viability shall be tested after thawing.

14 Disposal

For managing waste disposal, ISO 20387:2018, 4.1.8, 7.1.1, 7.5.3, 8.4.2, Clause A.7, and ISO 21709:2020, 5.3.6, shall be followed.

Any disposal of hUC-MSCs shall be conducted in accordance with applicable environmental, biosafety and ethical requirements.

15 Distribution of hUC-MSCs — Information for users

ISO 20387:2018, 7.12, shall be followed.

Instructions for use (IFU) and/or standard operational procedures for isolation, culture, preservation, storage and transport of hUC-MSCs should be provided to hUC-MSC users. The IFU should typically contain information prescribing general culture, preservation methods and procedures.

Batch numbers, traceable to the batch or biobank, and a statement or material safety data sheet on hazards for the cells shipped shall be provided to hUC-MSC users.

Terms and conditions or a warranty, which qualifies hUC-MSC potential and characteristics based on testing performed by the biobank, should be provided.

The characterization and microbiological test data from the depositor for hUC-MSCs in the biobank shall be available for users.

The biobank should have a documented policy for the quality and sourcing of raw materials that can impact the quality of cell preparations, subject to national or international restrictions, e.g. fetal bovine serum, trypsin, growth factors.

The biobank should provide information to facilitate the efficient selection of suitable cells. Information should include but is not limited to:

a) the date of collection and preservation of tissue in accordance with ISO 8601-1;

b) the date, in accordance with ISO 8601-1, of attempted isolation (for hUC-MSCs, this is usually considered to be the date the cells were isolated from the umbilical cord or plated *in vitro*);

c) whether fresh or frozen biological source material has been used;

d) where applicable, relevant information regarding informed consent obtained from the human donor for use of the original tissue for research;

e) any associated constraints on the use of the derived cells;

f) the data and interpretation resulting from characterization and QC.

16 Transport of hUC-MSCs

16.1 General

ISO 20387:2018, 7.4, and ISO 21709:2020, 5.4.4, shall be followed.

The biobank shall establish, implement and document procedures for the transport and handling of hUC-MSCs and their associated data.

Unnecessary exposure to radiation should be avoided during shipment.

hUC-MSCs can be transported as frozen ampoules/vials or as living cultures; in either case:

a) advise the recipient as to when the cells are to be shipped;

b) provide written instructions on the following:

   1) instructions upon reception of hUC-MSCs;

   2) instructions for thawing and reconstitution of hBM-MSCs;

   3) instructions for secondary storage conditions;

   4) medium or serum required;

   5) any special supplements;

   6) subculture regimen;

c) tape the cells' data sheet and a copy of the instructions to the outside of the package so that the recipient knows what to do before opening it.

Each frozen ampoule/vial or living culture container (primary container) shall be introduced into a pre-sterilized self-adhesive seal package. The packaging shall be labelled with:

— the sample's data;

— the production and expiration date;

— the name and contact information of the entity that performs biobanking.

16.2 hUC-MSCs frozen in ampoules or cryovials

For long distance transport, hUC-MSCs frozen in ampoules or cryovials should be shipped in but not limited to dry ice or liquid nitrogen dewar.

NOTE 1 The appropriate amount of dry ice or liquid nitrogen dewar depends on the condition of the transport, e.g. duration.

An outer container with thermal insulation, e.g. a thick-walled polystyrene foam container, should be used for shipping the ampoules, cryovials or both. If an outer container is used, the outer container shall be disinfected, shall be filled with appropriate cold charge (e.g. dry ice), and should include a data logger thermometer for temperature control.

NOTE 2 If hUC-MSCs thaw slowly, their viability will decline rapidly.

Ampoules/cryovials shall be hermetically closed to prevent leakage.

16.3 Living hUC-MSC cultures

hUC-MSCs can be transported as a living culture in culture media in appropriate culture flasks, typically over short durations, i.e. ≤ 2 h. In such cases, hUC-MSCs in the middle and late stages of the growth curve should be used for shipping. The temperature during handling and shipping shall be ≤ 37 °C and > 2 °C.

As much culture medium as possible should be used (e.g. fill the flask to the top with medium).

NOTE 1 Medium touching the lid of the flask can cause contamination.

NOTE 2 Confluent or post confluent cultures will exhaust the medium nutrients more rapidly and can tend to detach in transit.

Annex A
(informative)

Exemplary quality control test procedure for biobanking of hUC-MSCs

Table A.1 — Exemplary quality control test procedure for biobanking of hUC-MSCs

Quality test	Isolation and primary culture	Subculture	Cryopreservation	After thawing
Cell morphology	+	+	—	+
CD marker	+	+	—	+
Growth kinetics	—	+	—	+
Count and viability	—	+	+	+
Differentiation capability *in vitro*	—	+	—	+
Immunoregulation capability	—	+	—	+
Paracrine secretion	—	+	—	+
In vitro self-renewal	—	+	—	+
Microbial contamination	+	—	+	+
Key +: test in procedure —: test not in procedure				