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Biotechnology — Biobanking — Requirements for human mesenchymal stromal cells derived from bone marrow

Biotechnologie — Biobanking — Exigences relatives aux cellules stromales mésenchymateuses dérivées de la moelle osseuse

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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).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

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, regulate immune effector cells,[8][9][10][11] maintain primitive phenotypes of other cell populations[12][13] and support tissue regeneration.[14][15] 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 bone marrow-derived progenitor cells[16].

Mesenchymal stromal cells and mesenchymal stem cells are both abbreviated as "MSCs"[17]. 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,[18] proposed by the International Society of Cell and Gene Therapy (ISCT), and as such require careful characterization by a matrix of functional assays[19][20].

MSCs have been isolated from bone marrow,[12][21][22][23][24] umbilical cord[25] 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 bone marrow (hBM-MSCs) for research purposes. This document is applicable for academic centres, public and private institutions performing a biobanking service of hBM-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 in culture for research purposes.

ISBT 128[26] provides terminology and abbreviations for all medicinal products including cell therapy, and abbreviates these as "MSC(M)" to denote mesenchymal stromal cells from bone marrow. This document recognizes this abbreviation, but uses the more commonly used convention in research to denote human mesenchymal stromal cells derived from bone marrow (hBM-MSCs)[27].

Biotechnology — Biobanking — Requirements for human mesenchymal stromal cells derived from bone marrow

1 Scope

This document specifies requirements for the biobanking of human mesenchymal stromal cells derived from bone marrow (hBM-MSCs), including the collection of bone marrow and associated data, isolation, culture, characterization, quality control, cryopreservation, storage, thawing, disposal, distribution and transport.

This document is applicable to all organizations performing biobanking with hBM-MSCs used for research.

This document does not apply to hBM-MSCs for the purpose of *in vivo* application in humans, cell therapy, clinical applications, tissue engineering 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

[SOURCE: ISO/TS 22859:2022, 3.1]

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
bone marrow
bone marrow tissue
soft, sponge-like tissue in the centre of most bones which produces white blood cells, red blood cells and platelets

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

[SOURCE: ISO/TS 22859:2022, 3.5]

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

[SOURCE: ISO/TS 22859:2022, 3.6]

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

[SOURCE: ISO/TS 22859:2022, 3.8]

3.9
colony forming unit fibroblast
CFU-F
typical *in vitro* assay to demonstrate *self-renewal* (3.22) 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.

[SOURCE: ISO/TS 22859:2022, 3.9]

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

[SOURCE: ISO/TS 22859:2022, 3.11]

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

[SOURCE: ISO/TS 22859:2022, 3.12]

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

[SOURCE: ISO/TS 22859:2022, 3.14]

3.15
human mesenchymal stromal cell derived from bone marrow
hBM-MSC
heterogeneous cellular population isolated from *bone marrow* (3.4), 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.

3.16
licensing
<mesenchymal stromal cells> act of stimulating *hBM-MSCs* (3.15) using inflammatory cytokines to become more immunosuppressive

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

[SOURCE: ISO/TS 22859:2022, 3.17, modified — "hBM-MSCs" has replaced "hUC-MSCs" in the definition.]

3.17
passage
subculture
process of further culturing of cells in a new culture vessel to provide higher surface area/volume for the cells to grow

[SOURCE: ISO/TS 22859:2022, 3.18, modified — "new" added to the definition. Note 1 to entry deleted.]

3.18
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.]

3.19
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.]

3.20
primary culture
culture started from cells, tissues, or organs taken directly from an organism, and before the first subculture, propagation and consecutive *passages* (3.17) *in vitro*

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

3.21
proliferation
cell number expansion by cell division

3.22
self-renewal
ability of *stem cells* (3.23) 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.

[SOURCE: ISO/TS 22859:2022 3.23]

3.23
stem cell
non-specialized cells with the capacity for *self-renewal* (3.22) 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.

[SOURCE: ISO/TS 22859:2022, 3.24]

3.24
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]

3.25
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

ACAN	aggrecan
AHR	aryl hydrocarbon receptor
ALP	alkaline phosphatase
ANGPT2	angiopoietin 2
AP2	adipocyte protein-2
BCL-2	B-cell lymphoma 2
CCL2	chemokine C-C motif ligand 2
CCL7	chemokine C-C motif ligand 7
CCR7	C-C chemokine receptor type 7
CCR10	chemokine receptor type 10
CD	clusters of differentiation
CEBPα	CCAAT/enhancer-binding protein alpha
CFSE	carboxyfluorescein succinimidyl ester
CFU-F	colony forming unit fibroblast
CIITA	class II major histocompatibility complex trans activator
CO_2	carbon dioxide
COL10	collagen type X
COL2A1	collagen type 2A1
COX-2	cyclooxygenase 2
CX3CR1	CX3C 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	chemokine receptor type 1
CXCR4	chemokine receptor type 4
CXCR6	chemokine receptor type 6
DMEM	Dulbecco's modified eagle medium
EDTA	ethylenediaminetetraacetic acid
FBS	fetal bovine serum

GAL-1	galectin-1
hBM-MSCs	human mesenchymal stromal cells derived from bone marrow
HBV	hepatitis B virus
HCV	hepatitis C virus
HGF	hepatocyte growth factor
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HLA-DR	human leukocyte antigen DR
HO-1	heme oxygenase-1
HSP70A	heat shock protein 1
HSP70B	heat shock protein 70B
ICAM-1	intercellular adhesion molecule 1
IDO	indoleamine 2,3-dioxygenase 1
IFN-γ	interferon-gamma
IL-1RA	interleukin-1 receptor antagonist
IL-6	interleukin-6
KGF	keratinocyte growth factor
LPL	lipoprotein lipase
MSCs	mesenchymal stromal cells
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
N	count of cells harvested
N_0	count of cells seeded
OCN	osteocalcin
OPN	osteopontin
P_0	starting population of the cells
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PDL-1	programmed death-ligand 1
PDT	population doubling time
PPAR-γ	peroxisome proliferator-activated receptor gamma
QC	quality control

R&D	research and development
RUNX2	Runt-related transcription factor 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 beta
TIMP-1	tissue inhibitor of metalloproteinases 1
TIMP-2	tissue inhibitor of metalloproteinases 2
TLR-4	toll-like receptor 4
TP	treponema pallidum
TSG-6	tumour necrosis factor-inducible gene 6 protein
ULBP-3	UL16 binding protein 3
VCAM-1	vascular cell adhesion molecule 1
VEGF	vascular endothelial growth factor

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 hBM-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 hBM-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 hBM-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 authenticity and properties of hBM-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 hBM-MSC generation, characterization, culture, cryopreservation, thawing and transport.

hBM-MSC services provided by external operators shall demonstrate relevant professional knowledge, experience and corresponding skills, and regularly conduct documented personnel training and assessment.

The biobank shall ensure that facilities and environmental conditions do not adversely affect hBM-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 hBM-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 hBM-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 related data of hBM-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 hBM-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 bone marrow samples and associated data

6.1 Information about the bone marrow 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 bone marrow donor (e.g. statement of donor health or suitability, disease type, concomitant disease, demographics such as age and sex);

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

d) the negative test result for hepatitis B and C, HIV, TP and toxoplasmosis 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.

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.

The isolation of hBM-MSCs should not be performed from donors with medical contraindications for extraction of bone marrow aspirate, sedation or anaesthesia based on a related risk assessment.

It has been shown that the status of bone marrow donors such as age, viral infections, neoplastic and immunological disorders affect some of the characteristics of MSCs[28].

6.2 Anatomical collection site

With regard to the anatomical collection site, recommendations are given by the International Council for Standardization in Hematology (ICSH) protocols[29].

If the bone marrow is collected by puncturing into the intramedullary canal, the collection can be made from the head of the femur[12][21][30].

The anatomical collection site shall be documented for each procedure.

6.3 Collection volume

It has been shown that the volume of bone marrow collection influences the number of isolated mononuclear cells, and therefore plays an important role in the efficient isolation of hBM-MSCs from bone marrow.[21] Thus:

a) for bone marrow aspirates, a minimum volume of 10 ml should be collected and shall be documented[24][31][32][33];

b) for samples obtained by puncturing into the intramedullary canal, a minimum volume of 15 ml should be collected and shall be documented[12][13][21][30].

6.4 Collection procedure

6.4.1 General

ISO 20387:2018, 7.2, shall be followed.

The biobank shall establish, implement, validate and document a procedure for the collection of bone marrow for each used collection method (see 6.4.2 and 6.4.3).

All reagents and materials used to collect the bone marrow shall be sterile.

The biobank should conform to ISO 35001 or the WHO's *Laboratory Biosafety Manual*[34] 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.

6.4.2 Obtaining bone marrow by puncturing into the intramedullary canal

The sample should be collected into a disposable sterile container, and filled with an anticoagulant.

Internationally recognized and validated anticoagulants should be EDTA (ethylenediaminetetraacetic acid) or heparin at 15 IU/ml to 25 IU/ml.

6.4.3 Obtaining bone marrow by aspirate

Bone marrow aspiration can be performed in accordance with the International Council for Standardization in Hematology (ICSH) protocols[29].

Bone marrow aspirates should be taken in EDTA or heparinized syringes of 15 IU/ml to 25 IU/ml[29].

7 Transport of bone marrow samples or hBM-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*[34] when handling biological material contaminated with pathogens.

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

The following factors shall be taken into account for transportation of bone marrow:

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 bone marrow or hBM-MSCs and associated data

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

9 Isolation and expansion of hBM-MSCs

9.1 Processes

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

The biobank shall establish, implement, validate, document and maintain procedures for hBM-MSC isolation in primary culture and subculture depending on the method used for collection (see 6.4.2 and 6.4.3).

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 hBM-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, toxoplasmosis 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 hBM-MSCs from bone marrow samples obtained by puncturing into the intramedullary canal and primary culture

For optimal functionality, the hBM-MSC isolation procedure should be started within 2 h but not beyond 8 h after the bone marrow has been collected (see 6.4.2)[21][35].

Procedures for the isolation of hBM-MSCs from bone marrow can vary. The following workflow should be followed:

a) Perform a sterile mechanical disruption of puncturing into the intramedullary canal to create a cell suspension. The cell suspension can be made as described in Annex A, b).

b) Filter the resulting sample, e.g. see Annex A, c).

c) Collect the filtered sample and centrifuge it, e.g. see Annex A, d) and e).

d) Collect cells (buffy coat) and dilute with PBS. Antibiotics should be added. Subsequently, mononuclear cells need to be separated by density gradient centrifugation. See Annex A, f) to j) for exemplary workflow steps.

e) Once the mononuclear cells have been separated, perform a cell count and determine cell viability using a dye exclusion technique (e.g. trypan blue) either manually or using an automated device.

f) It is suggested to initiate the cultures when cell viability is greater than 90 % and the cell count is between 100×10^3 cells/cm^2 to 160×10^3 cells/cm^2. Culture conditions should be documented, e.g. O_2 tension, pH, temperature, humidity conditions and normoxic hypoxic conditions.

g) After 72 h of culture, remove non-adherent cells and add fresh culture medium. Do not remove the culture medium to observe adherent cells (approximately 5 days to 7 days).

h) When a confluency of adherent cells is observed between 70 % to 80 %, passage the cells by trypsinization. Serum free media should be used for culture. Alternatively, the use of culture

medium supplemented with FBS (typically 5 % to 10 %), platelet lysate (typically 5 % to 10 %) or human serum (typically 5 % to 10 %) is also suggested.

9.5 Isolation of hBM-MSCs from bone marrow samples obtained by aspirate and primary culture

In order to maintain the cell viability of the hBM-MSCs, the hBM-MSC isolation procedure shall be performed within a pre-determined time limit.

a) Transfer the bone marrow aspirate into (a) sterile tube(s) and centrifuge them. Then resuspend the cells in a culture medium[35][36][37]. An exemplary workflow can be found in Annex A.

b) Perform a cell count and determine the viability of the cells.

c) Direct seeding: the culture should be started with a cell viability ≥ 90 % and a cell density of 1×10^6 cells/cm^2.

d) Incubate at 36,5 °C ± 0,5 °C, with 5 % CO_2 and 95 % relative humidity. Then, follow 9.4, f) and g).

e) hBM-MSCs obtained from bone marrow aspirate should be grown after the first passage, at a density of 3×10^3 cells/cm^2 to 7×10^3 cells/cm^2.

It is suggested not to use the hBM-MSCs after the fifth passage, because the probability of phenotypic and chromosomal instability increases[38][39][40].

9.6 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 hBM-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 hBM-MSCs

10.1 General

The biobank shall establish, document and implement procedures to characterize hBM-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 hBM-MSCs in culture. These characterizations shall include but are 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.

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 of 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 cells of hBM-MSCs should be ≥ 90 % prior to cryopreservation.

The amount of viable cells of hBM-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.

10.3 Morphology

Microscopically, seeded hBM-MSCs typically display a fibroblastoid like appearance (spindle-shaped and fusiform) and grow by attaching to the bottom of the culture dish, with a cell size/length ≥ 15 µm. Colonies have spiral or radial patterns. The morphological features shall be evaluated and shall be congruent with features of hBM-MSCs isolated from bone marrow.

10.4 Population doubling time and subculture/passage

10.4.1 PDT

The PDT[41] is the time (measured in hours) required for the replication of the population of hBM-MSCs. The PDT is calculated with Formula (1) using the cell counts obtained before and after harvesting:

$$D = (T - T_0) \times \log^2 / (\log N - \log N_0) \quad (1)$$

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 hBM-MSCs isolated from human bone marrow ranges between 40 h and 100 h[30][42][43].

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 hBM-MSCs should be determined by the biobank after secondary culture.

PDT can reflect the growth kinetics of hBM-MSCs in culture. The biobank can utilize the PDT of hBM-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 hBM-MSCs cover the culture vessel at approximately 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 bone marrow cells.

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

10.5 Cell population purity

The biobank shall evaluate the purity of hBM-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 hBM-MSCs as described in 10.9 can be used for evaluating and verifying purity and identity. Unwanted microbial contaminants shall be defined and checked, as described in 10.12.

NOTE Identity verification is part of the process of verifying the authenticity of a cell line in which the cell origin is genetically confirmed.

10.6 *In vitro* self-renewal assessment

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

Nucleated cells isolated from bone marrow aspirate shall be used for colony formation assays prior to any manipulation.

Incubate cells for 10 days to 14 days at 36,5 °C ± 0,5 °C in humidified 5 % CO_2[44][45][46].

EXAMPLE Nucleated cells isolated from bone marrow aspirate are cultured for 14 days at 5 % CO_2. Cells can be set up in a range of cell concentrations ranging from 0,5 ×10^6 cells to 2,5 × 10^6 cells. Cells are typically cultured in Alpha Medium supplemented with 20 % FBS, but other medium and FBS concentrations can be used. Medium exchange is performed after day 4 and day 7. Cells are fixed in methanol and stained with 1 % crystal violet for microscopic enumeration of colonies with fibroblast morphology (CFU-F)[47].

10.7 Proliferation

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

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

NOTE Cell proliferation can be evaluated by a validated method, e.g. CFSE or by MTT.

10.8 Differentiation capability — *In vitro* multilineage differentiation

10.8.1 General

WARNING — To evaluate *in vitro* multilineage differentiation of hBM-MSCs, the biobank should establish, document and implement procedures and requirements for the specific assays (i.e. hBM-MSCs with ability to undergo *in vitro* multilineage differentiation). The following *in vitro* multilineage differentiation assays are part of the *in vitro* characterization of hBM-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 hBM-MSCs. This information can be useful, if assessed in a quantitative assay format that is validated and sufficiently sensitive to assess hBM-MSCs grown for different lengths of time, and under different conditions.

10.8.2 *In vitro* adipogenic differentiation

hBM-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.

hBM-MSC cultures with a confluence ≥ 80 % in the third to fifth culture passage shall be used. hBM-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 hBM-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 (peroxisome proliferator-activated receptor gamma), CEBPα (CCAAT/enhancer-binding protein alpha), FABP4 (fatty acid-binding protein 4) and PREF-1 (preadipocyte factor 1), AP2 (adipocyte protein-2) and LPL (lipoprotein lipase) should be evaluated using quantitative real-time PCR[48][49][50].

10.8.3 *In vitro* chondrogeneic differentiation

hBM-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[48][51][52].

In addition to 2D *in vitro* differentiation, chondrocyte differentiation using 3D micromass cultures can also be performed as previously reported for MSCs[53].

hBM-MSC cultures with a confluence ≥ 80 % in the third to fifth culture passage shall be used. hBM-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.

To further confirm the chondrogenic capacity of hBM-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), COL10 (collagen type X) and ACAN (aggrecan) should be evaluated using quantitative real-time PCR[48][50][51].

10.8.4 *In vitro* osteogenic differentiation

hBM-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.

hBM-MSC cultures with a confluence ≥ 80 % in the third to fifth culture passage should be used. hBM-MSCs after the fifth culture passage should not be used. 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.

To further confirm the osteogenic capacity of hBM-MSCs, the expression of genes encoding proteins associated with osteogenic differentiation should be evaluated. These genes, such as ALP (alkaline phosphatase), RUNX2 (Runt-related transcription factor 2), OCN (osteocalcin) and OPN (osteopontin), should be evaluated using quantitative real-time PCR.[48][50] Intracellular staining for protein expression of OPN or other mature bone markers can also be evaluated.

10.9 Immunophenotyping by flow cytometry

hBM-MSCs should be characterized by a panel that includes at least the markers (antigens) and expressions of antigens listed in Table 1. Additionally, other international guidelines should be followed, where appropriate.

NOTE Antigens: CD146 and CD271 have been identified in hBM-MSCs.

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

Antigen	Antibody clones[12][13][54][55][56][57][58][59]	Required detection rate %
CD31-	Insufficient information to suggest clone	≤ 5
CD34-[a]	581, Q-BEN 10, 8G12, AC136, 4H11	≤ 5
CD45-[b]	2D1, t29 / 33, HI30	≤ 5
HLA-DR± [c]	L243	Depends on the culture medium
CD44+	MEM-85, G44-26	≥ 90
CD73+	AD2	≥ 90
CD90+	Thy-1, F15-42-1, 5E10	≥ 90
CD105+	SN6	≥ 90
CD146+	Insufficient information to suggest clone	≥ 90

[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] HLA-DR-: Although unstimulated hBM-MSCs are negative for HLA-DR, this varies with donor. Importantly, hBM-MSCs express HLA-DR upon stimulation with interferon-gamma (IFN-γ).

Table 1 *(continued)*

Antigen	Antibody clones[12][13][54][55][56][57][58][59]	Required detection rate %
CD271+	Insufficient information to suggest clone	≥ 90
uncertainty		≤ 5

[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] HLA-DR-: Although unstimulated hBM-MSCs are negative for HLA-DR, this varies with donor. Importantly, hBM-MSCs express HLA-DR upon stimulation with interferon-gamma (IFN-γ).

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

hBM-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, IL6, IL1RA, 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[19][60][61][62].

A panel of appropriate factors should be pre-defined, documented and tested depending on the research purpose[61].

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

NOTE 2 Importantly, it has been shown that hBM-MSCs require stimulation to express their immunosuppressive properties. This can be performed by exposure to soluble inflammatory factors such as IFN-γ TNF, interleukin or by physical contact to inflammatory cells. This method of "licensing" hBM-MSCs is important, and there are several ways of accomplishing this[63].

Unlicensed hBM-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 culture supernatant.

10.11 Immunoregulation (modulation of immune cells)

hBM-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 that specific populations of purified immune effector cells are used in co-culture with hBM-MSCs to measure the effect of the MSC on that population of immune effectors.

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

A matrix assay approach shall be applied as follows[19]:

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

   1) hBM-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: IDO, CXCL10, CXCL9, CXCL11, CIITA, ICAM-1, CCL5, TRAIL, TLR-3, CCL7,VCAM-1, HLA-DR, HGF, IL-6, CCL2, PI9, CCR7, VEGF, PDL-1, CX3CR1, COX-2, AHR, TSG-6, KGF, TLR-4, 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[61][64].

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 MSCs should be assessed by testing their effects on *in vitro* proliferation of immune effector cells. To this aim, incubation of MSCs should be carried out with either total peripheral lymphocytes from peripheral blood mononuclear cells (PBMCs) or purified lymphocyte subpopulations. It is preferable to use standardized assays that can validate MSC-based clinical approaches as a potentially useful treatment for immunological diseases, as described elsewhere[62].

In addition, a matrix assay approach is recommended as follows:

— quantitative analysis of selected gene products;

— flow cytometry analysis of functionally relevant surface markers;

— protein-based assay of secretome for testing immunomodulation functionalities of MSCs[19].

Studies have shown that the functional heterogeneity of hBM-MSCs depends on the culture conditions, number of passages and activation status, among others. Therefore, it is recommended to perform functional evaluations, in addition to *in vitro* multilineage differentiation, related to the immunomodulatory capacity of the hBM-MSCs to confirm their functional status.

10.12 Microbial contamination

Procedures for microbial contaminant testing of hBM-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.

hBM-MSCs used for research 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) in culture media (bovine serum) 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 hBM-MSCs as given in Clause 10.

QC of biological characteristics (see Clause 10) of hBM-MSCs shall be performed for all critical procedures, from isolation to thawing.

The biobank shall establish, implement and document QC acceptance criteria for all the biological characteristics of hBM-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, using an appropriate cryoprotectant, and maintaining a stable storage temperature can minimize negative effects on the cell viability.

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

a) the cell name;

b) the preserved hBM-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, the cell morphology (see 10.3), immunophenotype, immunomodulatory 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 bone marrow. Representative samples should be taken for assessment of the differentiation capability (see 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.

Controlled-rate cooling of hBM-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 shall 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 hBM-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 hBM-MSCs shall be conducted in accordance with applicable environmental, biosafety and ethical requirements.

15 Distribution of hBM-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 hBM-MSCs should be provided to hBM-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 hBM-MSC users.

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

The characterization and microbiological test data from the depositor for hBM-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 hBM-MSCs, this is usually considered to be the date the cells were isolated from the bone marrow 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 hBM-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 hBM-MSCs and their associated data.

Unnecessary exposure to radiation should be avoided during shipment.

hBM-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 hBM-MSCs;

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

   3) instructions for secondary storage conditions;

   4) medium or serum required;