INTERNATIONAL STANDARD

**ISO 24651**

First edition
2022-08

# 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*



Reference number
ISO 24651:2022(E)



ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

# Contents

Page

**Foreword** ..... **v**
**Introduction** ..... **vi**
**1 Scope** ..... **1**
**2 Normative references** ..... **1**
**3 Terms and definitions** ..... **1**
**4 Abbreviated terms and symbols** ..... **5**
**5 General requirements** ..... **7**
5.1 General ..... 7
5.2 Personnel, facilities and equipment ..... 7
5.3 Reagents, consumables and other supplies ..... 8
5.4 Management of information and data ..... 8
**6 Collection of bone marrow samples and associated data** ..... **8**
6.1 Information about the bone marrow donor ..... 8
6.2 Anatomical collection site ..... 9
6.3 Collection volume ..... 9
6.4 Collection procedure ..... 9
6.4.1 General ..... 9
6.4.2 Obtaining bone marrow by puncturing into the intramedullary canal ..... 10
6.4.3 Obtaining bone marrow by aspirate ..... 10
**7 Transport of bone marrow samples or hBM-MSCs and associated data to the biobank** ..... **10**
**8 Reception and traceability of bone marrow or hBM-MSCs and associated data** ..... **10**
**9 Isolation and expansion of hBM-MSCs** ..... **11**
9.1 Processes ..... 11
9.2 Unique identification ..... 11
9.3 Testing for infectious agents ..... 11
9.4 Isolation of hBM-MSCs from bone marrow samples obtained by puncturing into the intramedullary canal and primary culture ..... 11
9.5 Isolation of hBM-MSCs from bone marrow samples obtained by aspirate and primary culture ..... 12
9.6 Subculture and limited expansion ..... 12
**10 Characterization of hBM-MSCs** ..... **12**
10.1 General ..... 12
10.2 Viability ..... 13
10.3 Morphology ..... 13
10.4 Population doubling time and subculture/passage ..... 13
10.4.1 PDT ..... 13
10.4.2 Subculture/passage ..... 14
10.5 Cell population purity ..... 14
10.6 *In vitro* self-renewal assessment ..... 14
10.7 Proliferation ..... 14
10.8 Differentiation capability — *In vitro* multilineage differentiation ..... 15
10.8.1 General ..... 15
10.8.2 *In vitro* adipogenic differentiation ..... 15
10.8.3 *In vitro* chondrogeneic differentiation ..... 15
10.8.4 *In vitro* osteogenic differentiation ..... 16
10.9 Immunophenotyping by flow cytometry ..... 16
10.10 Paracrine secretion/expression (protein-based assay of secretome) ..... 17
10.11 Immunoregulation (modulation of immune cells) ..... 17
10.12 Microbial contamination ..... 18

**11 Quality control** ..... **19**
**12 Storage** ..... **19**
**13 Thawing** ..... **20**
**14 Disposal** ..... **20**
**15 Distribution of hBM-MSCs — Information for users** ..... **21**
**16 Transport of hBM-MSCs** ..... **21**
16.1 General ..... 21
16.2 hBM-MSCs frozen in ampoules or cryovials ..... 22
16.3 Living hBM-MSC cultures ..... 22
**Annex A** (informative) **Preparation of human bone marrow mononuclear cells (hBM-MNCs)** ..... **23**
**Bibliography** ..... **24**

# Foreword

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

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO 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 |