

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Ex vivo expanded mesenchymal stromal cell minimal quality requirements for clinical application

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1561107> since 2016-05-09T11:10:44Z

Published version:

DOI:10.1089/scd.2014.0299

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)



UNIVERSITÀ DEGLI STUDI DI TORINO

This is an author version of the contribution published on:

[STEM CELLS AND DEVELOPMENT ,Vol. 24 issue 6 , 2015, 10.1089/scd.2014.0299]

The definitive version is available at:

[\[http://online.liebertpub.com/doi/10.1089/scd.2014.0299\]](http://online.liebertpub.com/doi/10.1089/scd.2014.0299)

Ex-vivo expanded mesenchymal stromal cell minimal quality requirements for clinical application

Maria Luisa Torre^{1*}, Enrico Lucarelli^{2*}, Simona Guidi³, Maura Ferrari⁴, Giulio Alessandri⁵, Laura De Girolamo⁶, Augusto Pessina⁷ and Ivana Ferrero^{8§}, on behalf of the Gruppo Italiano Staminali Mesenchimali (GISM).

¹Department of Drug Sciences, University of Pavia, Via Taramelli 12, Pavia, Italy.

²Osteoarticular Regeneration Laboratory, Rizzoli Orthopaedic Institute, Bologna, Italy.

³CTP Tecnologie di Processo S.p.A. Advanced Therapy Division, Poggibonsi (SI), Italy.

⁴Cell Culture Centre, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia, Italy.

⁵Laboratory of cellular Neurobiology, Department of Cerebrovascular Disease, IRCCS Neurological Institute, Carlo Besta, Milan, Italy.

⁶Orthopaedic Biotechnology Laboratory, IRCCS Istituto Ortopedico Galeazzi, Milan, Italy.

⁷Department of Biomedical, Surgical and Dental Sciences, University of Milan, Italy.

⁸Paediatric Onco-Hematology, Stem Cell Transplantation and Cellular Therapy Division, City of Health and Science of Turin. Department of Public Health and Paediatrics, University of Turin, Italy.

*These authors contributed equally to this work.

Running title

MSC requirements for clinical use

§Corresponding Author

Ivana Ferrero

Paediatric Onco-Hematology, Stem Cell Transplantation and Cellular Therapy
Division, City of Health and Science of Turin. Department of Public Health and
Paediatrics, University of Turin.

Piazza Polonia 94 - 10126 Turin, Italy.

Tel. +39 011 3135420

Fax +39 011 3135596

ivana.ferrero@unito.it

List of abbreviations

ATMP: Advanced therapy medicinal products

BM: Bone marrow

CFU-F: Fibroblastic colony-forming unit

CGH: Comparative Genomic Hybridization

CPD: Cumulative population doubling

EMA: European Medicine Agency

EuPh: European Pharmacopoeia

GCP: Good Clinical Practices

GISM: Gruppo Italiano Staminali Mesenchimali (Mesenchymal Stem Cells Italian
Group)

GMP: Good Manufacturing Practice

ISCT: International Society for Cellular Therapy

LAL: Limulus amoebocyte lysate

MSC: Mesenchymal Stromal Cells

NAT: Nucleic acid amplification test

PCR: Polymerase Chain Reaction

STR: Short Tandem Repeat

Abstract

Mesenchymal Stromal Cells (MSC), as advanced therapy products, must satisfy all the requirements for human use of medicinal products, aiming to maintain the quality and safety of the cells. The MSC manufacturing process for clinical use should comply with the principles of Good Manufacturing Practice (GMP). This ensures that cell preparations are produced and controlled, from the collection and manipulation of raw materials, through the processing of intermediate products, to the quality controls, storage, labelling and packaging, and release.

The objective of this document is to provide the minimal quality requirements for the MSC production and its delivery for clinical use, so the safety of the final cell therapy product will not be compromised. For this purpose, the document evaluates the most important steps of GMP-compliant MSC production: the isolation and expansion process; the validation phase of the process, including all quality controls for the characterization, functionality, potency and safety of MSCs; the quality control at the batch release to guarantee the safety of patient infusion.

This opinion paper reflects the consensus viewpoint of the authors and scientists participating the GISM Working Group*.

* GISM Working Group includes the following individual investigators:

Biagi E, Del Bue M, Frigerio S, Lisini D, Marazzi M, Mareschi K, Nava S, Parolini O, Riccobon A, Romagnoli L, Viganò M.

Introduction

Until 2008 gene and cell therapy products were regarded as medicinal products.

Thereafter, based on the European Regulation 1394/2007, a new classification came into place [1]. The Regulation introduced additional provisions to those laid down in Directive 2001/83/EC, and a new class of medicines was defined: the tissue engineered products.

For reasons of clarity, complex therapeutic products such as the Advanced Therapy Medicinal Products (ATMPs) require a precise legal definition. The Regulation defines ATMPs as the gene therapy medicinal products, the somatic cell therapy medicinal products and the tissue-engineered products. Each of these products has specific pharmacologic, metabolic and immunologic activities and the potential for treating a variety of disorders. For these reasons, cellular products for ATMPs, prepared on a routine basis, must meet the same stringent conditions required for drugs before they are placed on the market. In particular, their activity, efficacy, safety and required dose must be defined. Furthermore, they must be manipulated according to the Good Manufacturing Practice (GMP) [2], and they require preclinical testing in Good Laboratory Practice (GLP) and clinical trials conducted in Good Clinical Practice (GCP) before being commercialized [3].

In the last decade one cell type in particular, the mesenchymal stromal cells (MSCs), have attracted great interest due to the numerous applications proposed for their use [4]. Thanks to their intrinsic properties, MSCs represent an attractive candidate for clinical applications [5–7].

However, as ATMPs, they must satisfy all the above-mentioned requirements.

Therefore, the quality and safety of the expanded MSCs must be maintained throughout their production and quality control cycle, ensuring their safe use in the

patient.

The MSC manufacturing process for clinical use should comply with the principles of GMP. This ensures that cell preparations are produced and controlled, from the collection and manipulation of raw materials, through the processing of intermediate products, to the quality controls, storage, labelling and packaging, and release.

During the whole production process, critical steps should be known and described.

A thorough risk analysis during all phases of production and control ensures a final product with the expected quality, both for patients and for the Regulatory Authorities responsible for controls.

Nevertheless, MSC-based therapy is different from drug-based therapy in which a known chemical entity has known therapeutic effects as well as defined adverse events. For this reason, the simple transfer of the same rules “from drug to cell” may lead to distortions: criteria to establish bioavailability, biodistribution and excretion profiles, potential adverse effects as well as dosage, potency or shelf-life need to be adapted and stated.

There are several papers that discuss standardization of MSC culture for clinical use. As reported in the review by Ikebe S., a total of 47 reports, describing MSC-isolation methods, were found in literature, published from January 2007 to 2013 [8]. Today, numerous MSC preparations from academic institutions and private companies are being investigated in nearly 350 clinical trials (>80% of which are phase 1 or 2; 131 have reached their scheduled completion, and 37 have been reported to be placebo controlled). Clinical trials examining the safety and efficacy of MSC have used both allogeneic (190) and autologous (150) cells [9].

Clinical trials exploring MSC therapy have been driven predominately by US Companies with proprietary allogeneic MSC preparations such as Osiris' (now

Mesoblast's) Prochymal. Smaller academic-investigator-driven trials have also been conducted in medical centres and academic institutions in Europe, showing the safety of allogeneic MSC therapy [10–11].

The Guideline on human cell-based medicinal products, issue by EMA on 2008, is a useful document for stem cell production for marketing authorization application, covering the manufacturing process, quality considerations, process validation and clinical considerations [12]. Manufacturing and quality control issues are frequently under discussion during the regulatory authorization processes. The recent paper of Wuchter summarizes a consensus meeting between researchers, clinicians and regulatory experts on standard quality requirements for MSC production [13].

However, a practical guideline for standardization of isolation, expansion and characterization methods is missing. Moreover, quality controls of starting material, intermediate and final products should also be considered, as MSCs must be produced in compliance with GMP to ensure reproducibility, efficacy and safety of the clinical product.

Even if the Regulations for biological active substances as GMP Annex 2 [14] can help the ATMP's manufacturer to comply with the GMP requirements, the common opinion is that these regulations are still not enough to cover all the Authority requirements for safety, efficacy and potency related to the ATMP's.

However, a specific step-by-step guideline for MSCs is not available to date.

In this context, the Italian Group of Mesenchymal Stem Cells (Gruppo Italiano Staminali Mesenchimali – GISM), that aim to promote changes and informations among researchers, in particular on clinical application, in this opinion paper, proposes the minimal quality requirements for the production and delivery of MSCs for clinical use. This paper can be considered a proposed tentative guideline that

provides indications for standardization and optimization of production time and costs according to the GMP requirements, without compromising the safety of the final cell therapy product.

The aim of this document is to clarify the critical control points that must be considered during the process of expansion and release of MSC as ATMPs. For this purpose, the document will evaluate the most important steps of GMP production for the application of clinical trials with MSCs. These steps include: 1) the isolation, identification and expansion process; 2) the validation phase of the process, including all quality controls for MSC characterization, functionality, potency and safety; 3) the quality control at the batch release to guarantee the safety of patient infusion. This last step is also applied to products that must be cryopreserved, assuming that the cryopreservation process has been validated during the validation phase. The thawing phase is not covered by this document.

Isolation and expansion of mesenchymal stromal cells (MSCs)

The term “mesenchymal stem cells” initially referred to multilineage progenitor cells isolated and culture-expanded from human adult bone marrow (BM), that once in culture display a heterogeneous morphology and are capable of several subpopulations. Horwitz and the International Society for Cellular Therapy (ISCT) [15] better defined them as “mesenchymal stromal cells”.

Although BM is still the most common source of MSCs, during the last two decades alternative and more accessible tissue sources of MSCs have been identified. For instance, MSCs have been isolated from tissues, such as fat, deciduous teeth, placenta and umbilical cord blood, showing comparable features to BM-derived cells [16-17]. In particular, fresh umbilical cord blood (UCB) is the third common source for

isolating MSCs for clinical use, but the success rate in isolating and further expanding MSCs depends on the volume of blood collected, the cell content, and the time between collection and processing [18]. Also MSCs isolated from Wharton's jelly (WJ), are able to maintain their self-renewal capacity in vitro for a long period of time, showing a higher pluripotency capacity as compared to BM-MSCs [19].

MSCs have been isolated from several different placenta regions, although the isolation of cells from this region carries a risk of contamination with maternal decidual cells. Placental MSCs exhibit plastic adherence and fibroblast-like morphology in culture, and a phenotype in line to ISCT Guidelines [20]. They are able to undergo multilineage differentiation, and even have the capacity to differentiate across germinal boundaries outside of their specific lineage [16].

Adipose-derived stem cells (ASCs), if compared to BM MSCs, have been found to be genetically and morphologically more stable in culture [21], to have lower senescence ratio [22], higher proliferative capacity [21], to be able to retain differentiation potential for a longer period in culture [21], to be a more efficient support to hematopoiesis (both in vitro and in vivo) [23] and to have minor immunophenotypical differences [24]. These in vitro properties have allowed this attractive cell populations to be tested in clinical studies for the treatment of a variety of clinical conditions, including Crohn's disease, end-stage coronary disease, acute myocardial ischemia, femoral head osteonecrosis, calvarial defects, breast reconstruction, facial lipoatrophy [25].

For the purpose of this paper, the intended source of MSCs, for which there are scientifically proven clinical applications, are bone marrow, umbilical cord blood, adipose tissue, placenta (www.clinicaltrials.gov).

All the reagents and materials used during the manufacturing of the cell-based

medicinal products should be well defined and controlled, the suppliers should be validated, and all the batches received should be approved by the Quality Control Unit of the manufacturing site, on the basis of specifications and quality control tests. The cells and tissues used as raw material to produce cell-based medicinal products must comply with the Directive 2004/23/EC, Directive 2006/17/EC and Directive 2006/86/EC on the donation, procurement and testing of human tissues and cells [26-27]. In particular, the Annex 1 of the Directive 2006/17/EC defines the selection criteria for donors, and the Annex 2 defines the laboratory test required for donors. So far, the application of GMP reagent in MSC *ex vivo* culture has been limited by the fact that only few are commercial availability, such as PBS, cell culture media and trypsin. The introduction of closed systems for the isolation without density gradient separation, improved the generation of safe clinical protocols. In the case of BM and peripheral blood stem-cell collection, blood samples must be taken for testing within 30 days prior to donation (table 1).

In the case that Bovine Serum is used in the manufacturing of cell-based medicinal products, the viral risk and the risk of transmitting animal spongiform encephalopathy agents must be carefully evaluated according to the EMA Guidance CHMP/BWP/457920/2012 [28]. In order to comply with the Regulation requirements, the manufacturer of FBS should provide the serum user with the EDQM certificate of suitability of the Foetal Calf Serum, and ensure the safety with regard to the risk of transmitting animal agents via veterinary medicinal products [29].

Several different methods for isolating MSCs have been described. The identification of “optimal” conditions is important in order to identify a standard procedure for clinical use. No significant differences among Ficoll, Percoll or whole BM separation methods have been described in terms of morphology, growth rate at the 1st

passage, cumulative population doubling (CPD), immunophenotype or differentiative potential. However, direct selection of MSCs from BM cells by adhesion to culture plastic is a more advantageous method compared to MSCs obtained by gradient separation [30], and results in a significantly higher level of HLA-DR [31]. Human platelet lysate (HPL) is being established as a safe and efficient MSC culture supplement for robust MSC cultivation, thus offering certain advantages as a potential FBS substitute [32-33].

However, as the use of human-derived blood materials leads to immunologic and infectious risks, the application of the Pathogen Inactivation is safe and the inactivated HPL represents a good, GMP-compliant alternative to FBS [34]. This is because it reduces manipulation and haematopoietic contamination (cellular growth) and represents a good procedure for MSC expansion for clinical use [35-36]. The gradient separation cannot be applied to those tissues characterized by a solid matrix, such as fat and placenta, for which the first step to isolate MSCs is the enzymatic digestion. After this, the direct selection of MSCs can be achieved by plastic adhesion to the culture surface. Another important parameter to be considered is the cells' seeding density, which has been demonstrated to be important for the expansion of MSCs in culture. Recent studies showed that directly plated BM offers a more advantageous method in terms of CFU-F number, minimal manipulation, hematopoietic contamination, and cellular growth, showing a minor hematopoietic contamination at the 1st passage with whole BM rather than with Percoll or Ficoll [30, 35-36]. An initial plating density between 10000 and 100000 cells/cm², using flasks for large scale cell culture, significantly improves the expansion growth, providing a high number of cells from a small quantity of BM [30]. For the purpose of this paper, the validation of the better culture condition,

considering the medium, the use of animal-derived supplements, the plastic, and the density and number of passages, should be carried out on at least three representative MSC samples.

Quality control for validation of MSC production

The quality and safety of MSCs must be maintained throughout their production and quality control cycle, ensuring their final use in the patient. According to International Conference on Harmonization Q2 (ICH Q2) Guidelines [37] and the European (EU) Pharmacopoeia [38], the quality control process should be validated to confirm that the analytical procedure employed for a specific test is suitable for its intended use.

The process should follow a validation protocol, also considering instruments, supplies and reagents, and defining roles and responsibilities of each step [39]. A useful scheme of manufacturing and quality control processes of MSC production as a cell therapy medicinal product is represented by Galvez [40]. In figure 1 two flow-charts on validation and production processes are represented. A validation protocol on a minimum of three expansion procedures should be implemented to standardize the best culture condition and to demonstrate the safety and feasibility of the production protocol.

Results from method validation can be used to judge the quality, reliability and consistency of analytical results [39].

The elements of the analytical method requiring proof through validation as contained in the ICH Q2A guidelines are specificity, accuracy, precision, repeatability, linearity and range, as shown in table 2.

Identity

The International Society for Cellular Therapy (ISCT) proposed three minimal criteria to identify BMSCs: the adherence to plastic; the expression of a specific surface antigen (positivity for CD105, CD73, CD90 and negativity for CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA class II); the ability to differentiate into osteoblasts, adipocytes or chondroblasts under standard in vitro culture conditions [41]. These criteria can be also applied to MSCs deriving from other tissue sources, although their immunophenotype can be slightly different, above all in the early phases of culture” [42]. Plastic adherent MSCs are typically visible 5-7 days after plating, appearing at microscopy as fibroblastoid cells with a heterogeneous shape, also including endothelial-like cells. However, during expansion, the adherent cells become less heterogeneous, and fibroblast-like cells become predominant. MSCs surface marker expression may be influenced by the different source of cells and by the method of isolation. Although human MSCs are also positive for several markers, including embryonic stem cell markers, such as Oct-4, Rex-1, and Sox-2 [4] for the purpose of this guideline, the minimal criteria to identify MSCs are the following: positivity for CD105, CD73, CD90 and negativity for CD45, CD34, CD14. In particular, the phenotype of MSCs should at least show positive expression (>90%) for CD105, CD73, CD90, and negative expression (<5%) for CD45, CD34 and CD14. The capacity to differentiate into osteoblasts, adipocytes, and chondrocytes under standard in vitro differentiating conditions is still the most valid demonstration of MSC multipotency [41]. The evidence of osteogenic differentiation should be demonstrated by alkaline phosphatase activity and calcium deposition staining (Van Kossa staining or Alizarin staining). Adipogenic differentiation should be demonstrated through the morphological appearance of lipid droplets stained by Oil Red O staining. Chondrogenic differentiation can be evaluated by culturing MSCs in

complete commercial chondrogenic medium as cellular aggregates floating freely in suspension culture; after inclusion in paraffin, the pellet could be stained with Alcian Blue to identify the presence of hyaluronic acid and sialomucin.

The multipotent capacity should be demonstrated for all three of the differentiative lineages.

MSCs are a promising tool for cell therapies also for their immunomodulatory properties, thanks to their paracrine production of trophic factors together with their broad immune modulatory and anti-inflammatory functions [43].

The recent paper of Menard and Krampera [44] suggested some reproducible immunological assays to quantify the immune-modulatory properties of MSCs produced according to GMP. However, these analyses are recommended in the validation (or in pre-clinical) phase, but not mandatory for the release of MSC.

Growth characteristics

The cellular expansion growth rate of MSCs should be evaluated by counting the cell at each passage and expressed in terms of CPD using the formula $\log N / \log 2$, where N is the cell number of the confluent monolayer divided by the initial number of cells seeded [45].

For clinical safety issues, the expansion should not exceed 4 passages, in an attempt to minimize the administration of senescent cells.

Sterility

For microbiological control, the sterility should be evaluated at each passage on a representative sample of the products, containing cells (EuPh 2.6.27). Due to the

small volume of the cell-based medicinal products, the analysis is commonly performed on the supernatant.

- Bacteriology. For microbiological control, the samples could be tested through rapid microbiology test according to EuPh 2.6.27 [38, 46].
- LAL test. LAL assay should be used as a quantitative method to detect Gram-derived endotoxin in a solution according to EuPh 2.6.14 [38].
- Mycoplasma. Any mycoplasma contamination could be detected through a semi-quantitative PCR reaction. In alternative, culture methods, or indicator cell culture technique have to be performed. All techniques need to be validated and must be performed according to current European Pharmacopoeia chapter 2.6.7 [38].

Tumorigenesis

Although tumour formation during the validation process has not been reported in ongoing clinical trials using MSCs [47], tumorigenesis should be tested during validation.

For the purpose of this guideline, the valid methods to exclude the potential tumorigenicity are as follows:

- Assessment of telomerase activity, considering that it has been documented that non-malignant human MSCs display a low/undetectable level of telomerase activity;
- Soft agar test, with a commercial cell line as the positive control or with the *in vivo* test carried out on suitable animals as reported in European Pharmacopoeia 5.2.3 [38].

Karyotype

In order to demonstrate that the expansion method maintain the genomic stability during the in vitro culture period, the karyotype analysis should be performed. Some studies reported that human MSCs retained chromosomal stability following long-term culture in vitro [47-48]. Tarte showed that MSCs with or without chromosomal alterations, became senescent, without evidence of transformation either in vitro or in vivo, assuming that karyotyping and FISH results are not informative and are thus not adequate controls for the release of MSCs for clinical uses [49]. The analysis of Ben-David et al [50], reveals that MSCs have a 4% probability of acquiring chromosomal abnormalities. However, this study has been argued by Sensebè's group [51].

Recently, Capelli's group performed a large study, by conventional karyotype analysis, on 92 clinical grade BM-derived MSCs, showed the presence of only spontaneous non-clonal anomalies, assuming that the lack of clonal aberrations or the presence of non-clonal anomalies on 10% of the analysed metaphases should be set as the release criteria for MSCs distribution [52]. This is in line with the Cell Products Working Party, in which, it has been proposed, that a karyotype or FISH analysis should be necessary, at batch release, only in cases in which recurrent chromosomal anomalies are found [53].

The demonstration of the absence of genetic instability by karyotype analysis should be assessed by molecular [54] or conventional methods (G banding). At least 20 metaphases should be analysed, and in the presence of aberrations, 20 more metaphases are requested, as defined by the General Guidelines and Quality Assurance for Cytogenetics [47, 55-56]. The karyotyping analysis would be sufficient as a release test with the exclusion of two identical abnormal metaphases on 20

metaphases analysed (10%) [53], comparing the final batch to the donor/patient karyotype status.

Quality control in-process for MSCs

For identity, morphology, immunophenotype and growth characteristics MSCs can be evaluated as described for the validation phase.

For microbiological in-process control, the sterility should be evaluated on cells and on supernatant samples, by rapid microbiology methods (EuPh 2.6.27) or by classical methods (EuPh 2.6.1) by a GMP approved Quality Control laboratory [40].

The quality control analysis and GMP batch release testing, is part of the manufacturing process. For this reason, these tests should be carried out in the same GMP facility, in a dedicated and validated laboratory or as outsource in laboratories providing this service in compliance with GMPs.

Quality control at batch release for MSCs

At batch release, MSCs should be analysed for morphology and immunophenotype, as previously described. In addition, the following tests should be carried out.

Identity

Viability could be evaluated by Trypan Blue exclusion cell counting at microscopy, or by Iodure Propidium exclusion with cytofluorimetric analysis. Both the methods should be validated according to ICH Q2 guidelines. The final batch should have a viability > 90%.

Cross-contamination evaluation, by the analysis of Short Tandem Repeat (STR) DNA profiling to assess the identity of the final product to exclude a cross-contamination

should be assessed only in case it is necessary to share spaces and instruments for the processing of different donors.

Sterility

Bacteriology, LAL test and mycoplasma should be analyzed as previously described. For microbiological control, the sterility should be evaluated on cells and on supernatant samples, according to current European Pharmacopoeia by an accredited laboratory.

The rapid microbiology methods applied to ATMP's are commonly methods to substitute time consuming testing as Sterility testing and Mycoplasma testing. These rapid tests are described in the European Pharmacopoeia and the methods for validation are also described in details. In particular, the chapter EuPh 2.6.27 "Microbiological control of cellular products" recommends the rapid method for the release of the ATMP's in order to evaluate the sterility post-inoculation in a shorter time in comparison to the culture-based methods. The Mycoplasma evaluation on ATMP's should be performed according to the chapter EuPh 2.6.7 "Mycoplasmas" describing both culture methods (Cultural and Indicator cells culture method) and rapid methods as NAT methods qualitative and quantitative.

Since the expanded cells are Medicinal Products, the tools to assess how and which adventitious agents can contaminate the culture should be defined according to the principles of the Quality Risk Management as referred to the ICH Q9 Guideline [57]. In particular, a Failure Mode Effect Analysis (FMEA) tool can be suitable to evaluate the hazardous agents considering the potential source of contamination such as the ancillary material for manufacturing, the adventitious from the manufacturing operators etc.

In the United States Pharmacopoeia, chapter 1050, Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin [58], an exhaustive approach to assess the viral contamination from cell based medicinal products is presented and can be useful to use the suggested sources of contamination also evaluating bacterial, endotoxin, mycoplasma and TSE agents contamination. In particular, the evaluation of the risk associated to the TSE agents contamination is well described in the EMA Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMA/410/01 rev.3) [59].

The evaluation of infections of extraneous agents must be performed either on cell batches used as the feeder-layer or on the final product. One of the following tests must be performed with the aim to detect adventitious viruses:

- Inoculation on selected cell lines;
- Detection of retroviruses;
- Mice inoculation (test in animals);
- Chicken embryos inoculation (test in eggs);
- Nucleic acid amplification test (NAT).

All of the above reported tests must be performed according to current European Pharmacopoeia (chapters 2.6.16; 5.2.3).

Regarding the final product of MSCs, due to the small available amount of cells, only the inoculation on selected cell lines and retrovirus detection test, should be performed.

It must be outlined that animal reagents such as fetal bovine serum used in cell amplification must be tested with the aim to detect contaminants as reported by the EMA document EMA/CHMP/BWP/457920/2012 [28].

Similarly, reagents of swine origin-like proteolytic enzymes have to be tested by NAT techniques or by inoculation in susceptible cell cultures to detect virus contamination. Mycoplasma detection has to be carried out by the methods previously reported. A summary panel of required quality controls is shown in table 3.

Batch control sample

A sizable aliquot of the final batch should be cryopreserved as a backup in order to further analyze the cells in case of a clinical need.

Conclusion

MSC production according to GMP requirements is an important issue that has emerged in recent years. Currently there is no consensus on quality standards for the production of MSC for clinical application. In this paper the GISM Working Group has identified the minimal quality criteria for MSCs at different production stages.

As the recent work of a group of German researchers pointed out the manufacturing and quality control issues [13], we hope that our work could be a significant first step forward the awareness of the Regulatory Authority that a wider agreement on this matter is the need.

Acknowledgements

The authors are grateful to Ms. Alexandra Dyer for editorial assistance.

Author Disclosure Statement

The authors declare that no conflicting financial interests exist.

References

1. Regulation (EC) N. 1394/2007 of the European Parliament and of the Council of 13 November 2007 on advanced therapy medicinal products and amending Directive 2001/83/EC and Regulation (EC) N. 726/2004.
2. EudraLex. Volume 4: Good manufacturing practice (GMP) Guidelines for medicinal products for human and veterinary use, laid down in Commission Directives 91/356/EEC, as amended by Directive 2003/94/EC, and 91/412/EEC.
3. European Commission. Detailed guidance on the request to the competent authorities for authorisation of a clinical trial on a medicinal product for human use, the notification of substantial amendments and the declaration of the end of the trial (CT-1). Official Journal of the European Union 2010/C 82/01.
4. De Girolamo L, Lucarelli E, Alessandri G, Avanzini MA, Bernardo ME, Biagi E, Brini AT, D'Amico G, Fagioli F, Ferrero I, Locatelli F, Maccario R, Marazzi M, Parolini O, Pessina A, Torre ML, Italian Mesenchymal Stem Cell Group. (2013). Mesenchymal stem/stromal cells: a new cells as drugs" paradigm. Efficacy and critical aspects in cell therapy. *Curr Pharm Des* 19:2459–73.
5. Patel DM, Shah J, Srivastava AS. Therapeutic potential of mesenchymal stem cells in regenerative medicine. (2013). *Stem Cells Int* 2013:496218.
6. Motaln H, Schichor C, Lah TT. Human mesenchymal stem cells and their use in cell-based therapies. (2010). *Cancer* 116:2519–30.
7. Yi T, Song SU. Immunomodulatory properties of mesenchymal stem cells and their therapeutic applications. (2012). *Arch Pharm Res* 35:213–21.

8. Ikebe C, Suzuki K. Mesenchymal stem cells for regenerative therapy: optimization of cell preparation protocols. (2014) *BioMed Res Int* 2014:951512.
9. Ankrum JA, Ong JF, Karp JM. Mesenchymal stem cells: immune evasive, not immune privileged. (2014) *Nat Biotechnol* 32:252–60.
10. Le Blanc K, Rasmusson I, Sundberg B, Götherström C, Hassan M, Uzunel M, Ringdén O. (2004). Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. *Lancet* 363:1439–41.
11. Von Bahr L, Sundberg B, Lönnies L, Sander B, Karbach H, Hägglund H, Ljungman, B Gustafsson, H Karlsson, K Le Blanc and O Ringdén. (2012). Long-term complications, immunologic effects, and role of passage for outcome in mesenchymal stromal cell therapy. *Biol Blood Marrow Transplant J Am Soc Blood Marrow Transplant* 18:557–64.
12. Guideline on Human Cell-based Medicinal Products. (2008). EMEA/CHMP/410869/2006.
13. Wuchter P, Bieback K, Schrezenmeier H, Bornhäuser M, Müller LP, Bönig H, Wagner W, Meisel R, Pavel P, Tonn T, Lang P, Müller I, Renner M, Malcherek G, Saffrich R, Buss EC, Horn P, Rojewski M, Schmitt A, Ho AD, Sanzenbacher R, Schmitt M. (2014). Standardization of Good Manufacturing Practice-compliant production of bone marrow-derived human mesenchymal stromal cells for immunotherapeutic applications. *Cytotherapy* May 20.
14. EU guidelines for Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use. Annex 2. (2013). *Manufacture of Biological active substances and Medicinal Products for Human Use.*

15. Horwitz EM, Le Blanc K, Dominici M, Mueller I, Slaper-Cortenbach I, Marini FC, Deans RJ, Krause DS, Keating A; International Society for Cellular Therapy. (2005). Clarification of the nomenclature for MSC: The International Society for Cellular Therapy position statement. *Cytotherapy* 7:393-5.
16. Parolini O, Alviano F, Bagnara GP, Bilic G, Bühring HJ, Evangelista M, Hennerbichler S, Liu B, Magatti M, Mao N, Miki T, Marongiu F, Nakajima H, Nikaido T, Portmann-Lanz CB, Sankar V, Soncini M, Stadler G, Surbek D, Takahashi TA, Redl H, Sakuragawa N, Wolbank S, Zeisberger S, Zisch A and Strom SC. (2008). Concise review: isolation and characterization of cells from human term placenta: outcome of the first international Workshop on Placenta Derived Stem Cells. *Stem Cells Dayt Ohio* 26:300–11.
17. De Coppi P, Bartsch G Jr, Siddiqui MM, Xu T, Santos CC, Perin L, Mostoslavsky G, Serre AC, Snyder EY, Yoo JJ, Furth ME, Soker S and Atala A. (2007). Isolation of amniotic stem cell lines with potential for therapy. *Nat Biotechnol* 25:100–6.
18. Sharma RR, Pollock K, Hubel A, McKenna D. (2014) Mesenchymal stem or stromal cells: a review of clinical applications and manufacturing practices. *Transfusion* 54:1418-37.
19. Troyer DL, Weiss ML. (2008). Concise review: Wharton's jelly-derived cells are a primitive stromal cell population. *Stem Cells* 26: 591-9.
20. Caruso M, Evangelista M, Parolini O. (2012). Human term placental cells: phenotype, properties and new avenues in regenerative medicine. *Int J Mol Cell Med* 1:64-74.

21. Izadpanah R, Trygg C, Patel B, Kriedt C, Dufour J, Gimble JM and Bunnell BA (2006). Biologic properties of mesenchymal stem cells derived from bone marrow and adipose tissue. *J Cell Biochem* 99:1285-97.
22. Kern S, Eichler H, Stoeve J, Klüter H, Bieback K. (2006). Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. *Stem Cells*. May 24:1294-301.
23. De Toni F, Poglio S, Youcef AB, Cousin B, Pflumio F, Bourin P, Casteilla L and Laharrague P. (2011). Human adipose-derived stromal cells efficiently support hematopoiesis in vitro and in vivo: a key step for therapeutic studies. *Stem Cells Dev* 20:2127-38.
24. Strioga M, Viswanathan S, Darinskas A, Slaby O, Michalek J. (2012). Same or not the same? Comparison of adipose tissue-derived versus bone marrow-derived mesenchymal stem and stromal cells. *Stem Cells Dev* 21:2724-52.
25. Sousa BR, Parreira RC, Fonseca EA, Amaya MJ, Tonelli FM, Lacerda SM, Lalwani P, Santos AK, Gomes KN, Ulrich H, Kihara AH, Resende RR. (2014). Human adult stem cells from diverse origins: an overview from multiparametric immunophenotyping to clinical applications. *Cytometry A* 85:43-77.
26. European Commission Directive 2006/17/EC of 8 February 2006 implementing Directive 2004/23/EC of the European Parliament and of the Council as regards certain technical requirements for the donation, procurement and testing of human tissues and cells.
27. Commission Directive 2006/86/EC of 24 October 2006 implementing Directive 2004/23/EC of the European Parliament and of the Council as regards traceability requirements, notification of serious adverse reactions and events

and certain technical requirements for the coding, processing, preservation, storage and distribution of human tissues and cells.

28. Committee for Medicinal Products for Human Use (CHMP). Guideline on the use of bovine serum in the manufacture of human biological medicinal products. (2013). EMA/CHMP/BWP/457920/2012 rev 1.
29. European Commission. (2011). Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products. EMA/410/01 rev.3. Official Journal of the European Union C 73/01.
30. Mareschi K, Rustichelli D, Calabrese R, Gunetti M, Sanavio F, Castiglia S, Risso A, Ferrero I, Tarella C, Fagioli F. (2012). Multipotent mesenchymal stromal stem cell expansion by plating whole bone marrow at a low cellular density: a more advantageous method for clinical use. *Stem Cells International* 2012:920581.
31. Tarte K, Gaillard J, Lataillade JJ, Fouillard L, Becker M, Mossafa H, Tchirkov A, Rouard H, Henry C, Splingard M, Dulong J, Monnier D, Gourmelon P, Gorin NC, Sensebé L; Société Française de Greffe de Moelle et Thérapie Cellulaire. Clinical-grade production of human mesenchymal stromal cells: occurrence of aneuploidy without transformation. *Blood*. 2010 Feb 25;115(8):1549-53.
32. Schallmoser K, Bartmann C, Rohde E, Reinisch A, Kashofer K, Stadelmeyer E, Drexler C, Lanzer G, Linkesch W, Strunk D. Human platelet lysate can replace fetal bovine serum for clinical-scale expansion of functional mesenchymal stromal cells. *Transfusion*. 2007 Aug;47(8):1436-46.
33. Trojahn Kølle SF, Oliveri RS, Glovinski PV, Kirchhoff M, Mathiasen AB, Elberg JJ, Andersen PS, Drzewiecki KT, Fischer-Nielsen A. (2013). Pooled human

- platelet lysate versus fetal bovine serum-investigating the proliferation rate, chromosome stability and angiogenic potential of human adipose tissue-derived stem cells intended for clinical use. *Cytotherapy* 15:1086-97.
34. Castiglia S, Mareschi K, Labanca L, Lucania G, Leone M, Sanavio F, Castello L, Rustichelli D, Signorino E, Gunetti M, Bergallo M, Bordiga AM, Ferrero I, Fagioli F. (2014) Inactivated human platelet lysate with psoralen: a new perspective for mesenchymal stromal cell production in Good Manufacturing Practice conditions. *Cytotherapy* 16:750-63.
35. Capelli C, Salvade A, Pedrini O, Barbui V, Gotti E, Borleri G, B Cabiati, D Belotti, P Perseghin, P Bellavita, A Biondi, E Biagi, A Rambaldi, J Golay and M Introna (2009). The washouts of discarded bone marrow collection bags and filters are a very abundant source of hMSCs. *Cytotherapy* 11:403–13.
36. Lucchini G, Introna M, Dander E, Rovelli A, Balduzzi A, Bonanomi S, Salvade A, Capelli C, Belotti D, Gaipa G, Perseghin P, Vinci P, Lanino E, Chiusolo P, Orofino MG, Markt S, Golay J, Rambaldi A, Biondi A, D'Amico G and Biagi E (2010). Platelet-lysate-expanded mesenchymal stromal cells as a salvage therapy for severe resistant graft-versus-host disease in a pediatric population. *Biol Blood Marrow Transplant J Am Soc Blood Marrow Transplant* 16:1293–301.
37. International Conference on Harmonization (ICH) Harmonised Tripartite Guideline. Validation of analytical procedures: text and methodology Q2 (R1). 2005.
38. European Pharmacopoeia. 8th edition: European Directorate for the Quality of Medicines & HealthCare (EDQM); 2013.

39. Rustichelli D, Castiglia S, Gunetti M, Mareschi K, Signorino E, Muraro M, Castello L, Sanavio F, Leone M, Ferrero I and Fagioli F (2013). Validation of analytical methods in compliance with good manufacturing practice: a practical approach. *J Transl Med* 11:197.
40. Gálvez P, Clares B, Bermejo M, Hmadcha A, Soria B. (2014). Standard Requirement of a Microbiological Quality Control Program for the Manufacture of Human Mesenchymal Stem Cells for Clinical Use. *Stem Cells Dev* 23:1074-83.
41. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop D and Horwitz E (2006). Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 8:315–7.
42. Sousa BR, Parreira RC, Fonseca EA, Amaya MJ, Tonelli FM, Lacerda SM, Lalwani P, Santos AK, Gomes KN, Ulrich H, Kihara AH, Resende RR. (2014). Human adult stem cells from diverse origins: an overview from multiparametric immunophenotyping to clinical applications. *Cytometry A* 85:43-77.
43. Menard C, Pacelli L, Bassi G, Dulong J, Bifari F, Bezier I, Zanoncello J, Ricciardi M, Latour M, Bourin P, Schrezenmeier H, Sensebé L, Tarte K, Krampera M. (2013). Clinical-grade mesenchymal stromal cells produced under various good manufacturing practice processes differ in their immunomodulatory properties: standardization of immune quality controls. *Stem Cells Dev* 22:1789-801.
44. Krampera M, Galipeau J, Shi Y, Tarte K, Sensebe L; MSC Committee of the International Society for Cellular Therapy (ISCT). (2013). Immunological characterization of multipotent mesenchymal stromal cells-The International

- Society for Cellular Therapy (ISCT) working proposal. *Cytotherapy* 159:1054-61.
45. Stenderup K, Justesen J, Clausen C, Kassem M. Aging is associated with decreased maximal life span and accelerated senescence of bone marrow stromal cells. (2003) *Bone* 33:919–26.
46. Thorpe TC, Wilson ML, Turner JE, Di Guiseppi JL, Willert M, Mirrett S, Reller LB. (1990). BacT/Alert: an automated colorimetric microbial detection system. *J Clin Microbiol* 28:1608–12.
47. Bernardo ME, Zaffaroni N, Novara F, Cometa AM, Avanzini MA, Moretta A, Montagna D, Maccario R, Villa R, Daidone MG, Zuffardi O and Locatelli F (2007). Human bone marrow derived mesenchymal stem cells do not undergo transformation after long-term in vitro culture and do not exhibit telomere maintenance mechanisms. *Cancer Res* 67:9142–9.
48. Zhang ZX, Guan LX, Zhang K, Wang S, Cao PC, Wang YH, Wang Z, Dai LJ. (2007). Cytogenetic analysis of human bone marrow-derived mesenchymal stem cells passaged in vitro. *Cell Biol Int* 31:645-8.
49. Tarte K, Gaillard J, Lataillade JJ, Fouillard L, Becker M, Mossafa H, Tchirkov A, Rouard H, Henry C, Splingard M, Dulong J, Monnier D, Gourmelon P, Gorin NC, Sensebé L; Société Française de Greffe de Moelle et Thérapie Cellulaire. (2010). Clinical-grade production of human mesenchymal stromal cells: occurrence of aneuploidy without transformation. *Blood* 115:1549-53.
50. Ben-David U, Mayshar Y, Benvenisty N. (2011). Large-scale analysis reveals acquisition of lineage-specific chromosomal aberrations in human adult stem cells. *Cell Stem Cell* 9:97-102.
51. Sensebé L, Tarte K, Galipeau J, Krampera M, Martin I, Phinney DG, Shi Y;

- MSC Committee of the International Society for Cellular Therapy. (2012). Limited acquisition of chromosomal aberrations in human adult mesenchymal stromal cells. *Cell Stem Cell* 10:9-10.
52. Capelli C, Pedrini O, Cassina G, Spinelli O, Salmoiraghi S, Golay J, Rambaldi A, Giussani U, Introna M. (2014). Frequent occurrence of non-malignant genetic alterations in clinical grade mesenchymal stromal cells expanded for cell therapy protocols. *Haematologica* 99:e94-7.
53. Barkholt L, Flory E, Jekerle V, Lucas-Samuel S, Ahnert P, Bisset L, Büscher D, Fibbe W, Foussat A, Kwa M, Lantz O, Mačiulaitis R, Palomäki T, Schneider CK, Sensebé L, Tachdjian G, Tarte K, Tosca L, Salmikangas P. (2013). Risk of tumorigenicity in mesenchymal stromal cell-based therapies--bridging scientific observations and regulatory viewpoints. *Cytotherapy* 15:753-9.
54. Neri S, Bourin P, Peyrafitte J-A, Cattini L, Facchini A, Mariani E. (2013). Human adipose stromal cells (ASC) for the regeneration of injured cartilage display genetic stability after in vitro culture expansion. *PloS One* 8:e77895.
55. Borghesi A, Avanzini MA, Novara F, Mantelli M, Lenta E, Achille V, Cerbo RM, Tzialla C, Longo S, De Silvestri A, Zimmermann LJI, Manzoni P, Zecca M, Spinillo A, Maccario R, Zuffardi O, Stronati M. (2013). Genomic alterations in human umbilical cord-derived mesenchymal stromal cells call for stringent quality control before any possible therapeutic approach. *Cytotherapy* 15:1362–73.
56. Hastings RJ, Cavani S, Bricarelli FD, Patsalis PC, Kristoffersson U, ECA PWG Co-ordinators. (2007). Cytogenetic Guidelines and Quality Assurance: a common European framework for quality assessment for constitutional and acquired cytogenetic investigations. *Eur J Hum Genet EJHG* 15:525–7.

57. International Conference on Harmonization (ICH) Harmonised Tripartite Guideline. Quality Risk management. Q9. (2005).
58. Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin. United States Pharmacopeia, general chapter 1050. (2013).
59. Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMA/410/01 rev.3). (2011/C 73/01).
- 60.

Figure legends.

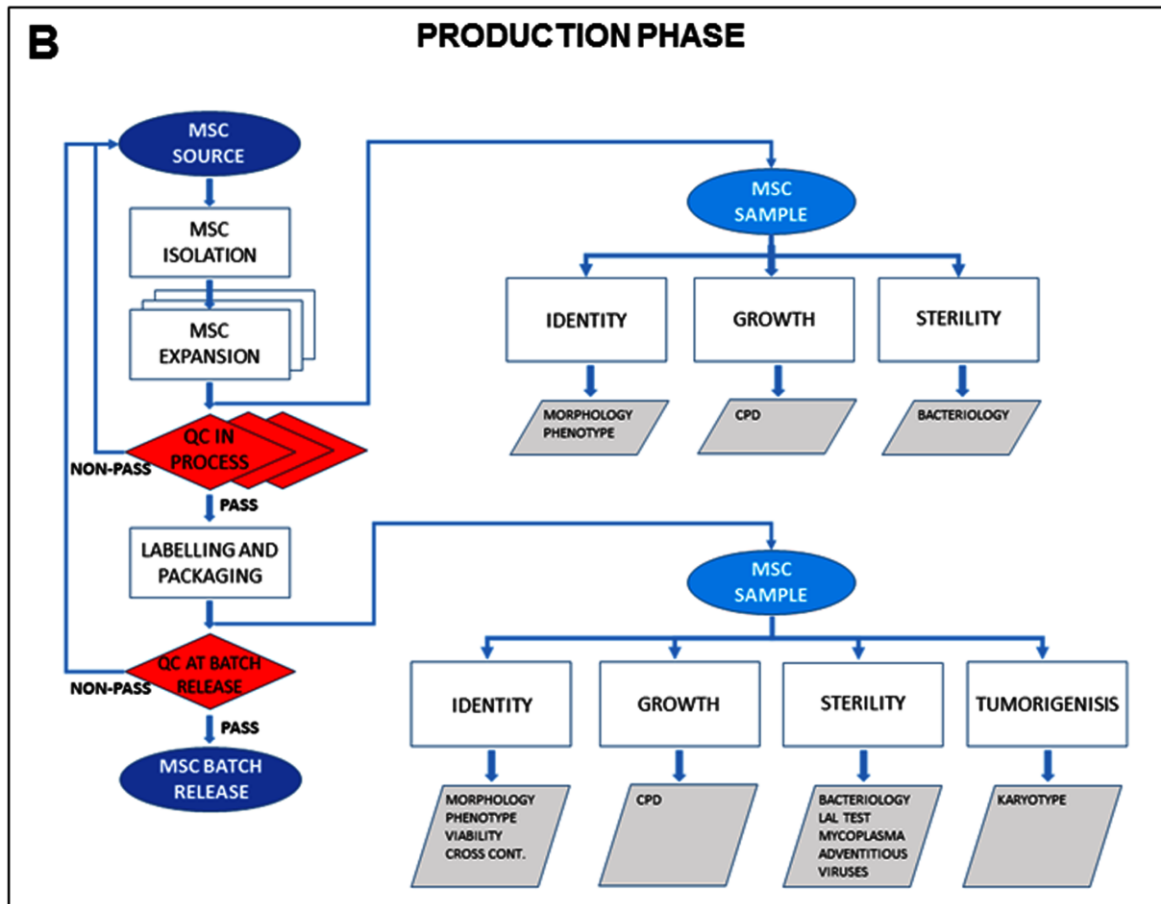
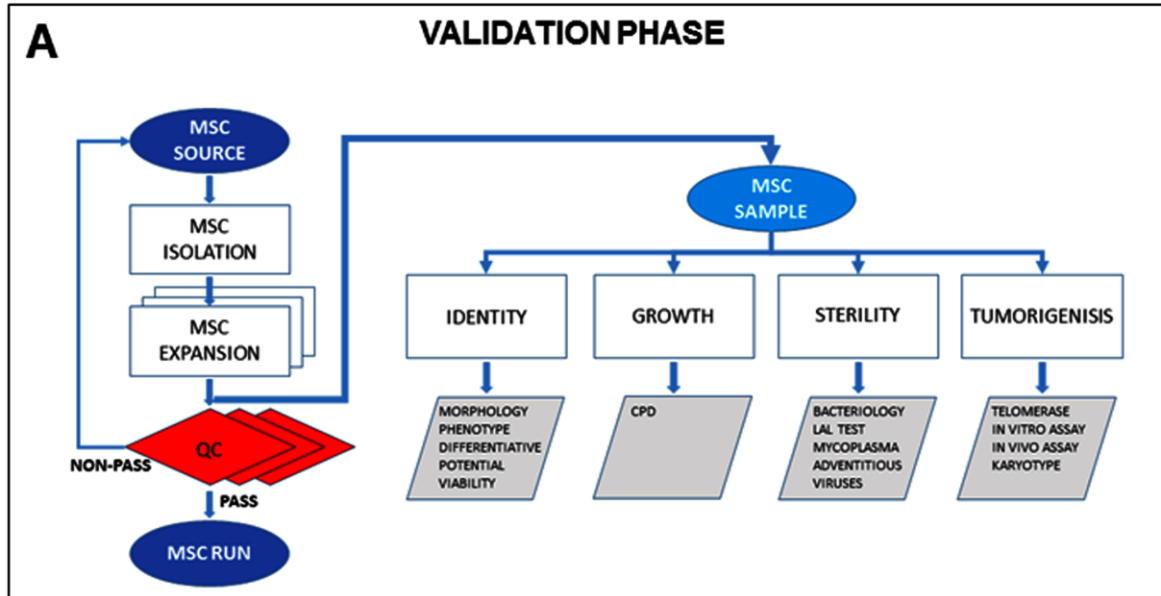


Figure 1. Flow chart representing the workflow and the steps of the validation phase (panel A), and the production process (panel B) of mesenchymal stromal cell as a cell therapy medicinal product.

Table 1. Biological tests required for all donors as a minimum requirement.

HIV 1 and 2	Anti-HIV-1,2
Hepatitis C	Anti-HCV-Ab
HCV-RNA/HIV1-RNA/HBV-DNA	TriNAT
Hepatitis B	HBsAg
	Anti HBc
Syphilis	A validated testing algorithm must be applied to exclude the presence of active infection with <i>Treponema pallidum</i> . A non-reactive test, specific or non-specific, can allow tissues and cells to be released. A donor whose specimen tests reactive on a <i>Treponema</i> -specific test will require a thorough risk assessment to determine eligibility for clinical use.

Table 2. Types of Analytical Procedures to be validated and typical validation characteristics to be considered (24). ✓ Signifies that this characteristic is normally evaluated.

Type of analytical procedure	IDENTIFICATION	TESTING FOR IMPURITIES		ASSAY
		QUANTITATION	LIMIT	
CHARACTERISTICS				
ACCURACY		✓		✓
PRECISION				
REPEATABILITY		✓		✓
INTER PRECISION		✓		✓
SPECIFICITY	✓	✓	✓	✓
DETECTION LIMIT			✓	
QUANTITATION LIMIT		✓		
LINEARITY		✓		✓
RANGE		✓		✓

Table 3. Summary of required quality controls on MSCs. CPD: Cumulative population doubling; LAL: Limulus ameocyte lysate; *: according to European Pharmacopoeia.

	VALIDATION	IN PROCESS	AT BATCH RELEASE
IDENTITY	MORPHOLOGY	MORPHOLOGY	MORPHOLOGY
	IMMUNOPHENOTYPE	IMMUNOPHENOTYPE	IMMUNOPHENOTYPE
	DIFFERENTIATIVE		
	POTENTIAL		
	VIABILITY		VIABILITY
			CROSS-CONTAMINATION
GROWTH	CPD	CPD	CPD
STERILITY	BACTERIOLOGY TEST	BACTERIOLOGY TEST	BACTERIOLOGY TEST*
	LAL TEST		LAL TEST*
	MYCOPLASMA TEST		MYCOPLASMA TEST*
	ADVENTITIOUS		ADVENTITIOUS VIRUSES*
	VIRUSES*		
TUMORIGENESIS	TELOMERASE		
	<i>IN VITRO</i> ASSAY		
	<i>IN VIVO</i> ASSAY		
	KARYOTYPE		KARYOTYPE