under LN_2. Consideration should be given to the potential for cross-contamination of samples stored in this manner via the liquid. There are a number of reports in the literature that indicate that contaminants, including viruses, can survive in LN_2 and there is at least one report of fatal viral transmission through this route. A formal risk assessment should be carried out of sample containment (i.e., primary and secondary containers), and alternatives to such conditions considered. Leakage of LN_2 into the sample container also represents an explosive hazard when samples are removed from storage.

Storage in the gas phase above liquid nitrogen (often referred to as vapour-phase storage) has been recommended. Such storage, while reducing the risk of cross-contamination, increases the likelihood for temperature instability from the inherent temperature gradient between bottom and top of the LN_2 refrigerator. This temperature gradient may be reduced or eliminated by modification to, or purchase of, tanks designed to reduce this temperature gradient. Storage refrigerators are available that exclude LN_2 from the storage compartment altogether (referred to as isothermal vessels) or restricted it to areas below the sample containers, for example by the use of vapour-phase platforms. Temperature gradients are reduced or eliminated either through jacketing the vessel with LN_2 (the isothermal approach) or through the use of a heat-shunt device within the tank or through design of low-loss access to the vessel.

7.1.5 Recovery of frozen or vitrified materials

Cells can be damaged through inappropriate thawing and CPA elution protocols. In general, rapid warming (at 37–40°C) is considered more effective in preventing cell damage from intracellular ice formation or solution effects of the CPA during rewarming. Rapid warming is especially important for vitrified material; however, care must be taken to prevent thermal runaway and exposure of the thawed material to elevated temperatures where the temperature-dependent toxic effects of the CPA may damage the cells. In designing or applying a cryopreservation protocol consideration should be given to the method of rewarming and the freezing/vitrification protocol optimized to that particular rewarming procedure.

Consideration should also be given to the method of eluting the CPA to prevent osmotic damage. The use of non-permeating compounds such as sucrose or mannitol to prevent excessive swelling may be considered. Recipients should be provided with validated thawing and elution protocols and a mechanism for adverse event/adverse incident reporting.

7.2 Shipment

In Europe there is specific legislation for the import and export of tissues [88], which also has technical annexes which prescribe aspects of cell and tissue procurement, processing, storage and testing. However, the situation is highly variable around the world. In some countries such as Israel, a simple statement of commercial worth is required, whereas in Taiwan there are specific import and export regulations, and in some countries such as Singapore, these issues are still under consideration (to the best of the authors' knowledge at the time of publication).

Competent couriers are critical to efficient shipment, and it is best that repositories take responsibility for using couriers that have good knowledge of local requirements for import. It is also important for stem cell repositories to have service level agreements with couriers that identify standards of service and emergency procedures where cryogens become depleted.

Cells cryopreserved by slow cooling may be transported in dry ice. Vitrified material should not be transported in dry ice (solid CO_2) at -79°C, to avoid de-vitrification and cell damage. Cells cryopreserved by either method may be transported in LN_2 dry-shippers which are probably the most secure form for transport. Repositories should identify transportation companies with the required technical expertise to undertake such shipments. Where this is likely to involve shipments outside of the country of origin, repositories should be familiar with the regulatory requirements pertaining to the safe shipment of cells in dry shippers. Use of air freight couriers that avoid transportation on commercial passenger airlines may reduce problems associated with a lack of knowledge of shipping in dry shippers or dry ice shippers. Where cells are transported in the absence of temperature data-loggers, consideration should be given to the use of chemical or other indicators to provide information on temperature during transportation.

8. Future applications of human pluripotent stem cell lines

8.1 Evaluation of human stem cell lines for production of biological medicines

Apart from cell therapy, stem cells or cell lines derived from stem cells can be envisaged for use as substrates for the production of biological

medical products such as recombinant proteins (e.g., growth factors or monoclonal antibodies), vaccines and conditioned media. A ISCBI sub-group including representatives from the pharmaceutical industry, reviewed the requirements for cells used to manufacture such products and provided the following summary.

Guidelines for the testing of diploid cells, continuous cell lines and stem cells for cell seed, MCB, WCB and end of production cells have been provided by Part B of the document, *"WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products"* [3]. In cases where a stem cell line has a finite lifespan (senescence) and a diploid profile, the ISCBI manufacturing sub-group recommended assessment of the basic characteristics of a stem cell line by following the criteria of other accepted diploid cell lines such as MRC-5 for biologics production. In the case of a stem cell line with a continuous cell line profile (unlimited capacity for population doubling), the group considered that the stem cell line can be included in the continuous cell line classification. As stated by WHO, this proposal can be applied to any animal stem cell lines including human stem cell lines.

Depending on the product that is made, the sub-group also proposed reference to the guidelines described in Table 4.

In addition, specific recommendations for the testing of each product type should be tailored to the origin and the derivation process of the stem cell line and to the functions of the product on a case by case basis. The risks related to contaminants from the stem cell line have to be considered in the testing of each product, that is, viruses, retroviruses and other transmissible agents, cellular DNA, cellular proteins (growth-promoting proteins).

8.2 Preparation of pluripotent stem cell lines for use in toxicology assays

The capability of human stem cell lines to create tissue-like cultures *in vitro*, could provide valuable information on the toxicity of medicines and hopefully avoid some of the serious chronic toxic effects of drugs which were not detected by standard assays [103,104]. The principles of GCCP [63] are directly relevant to the use of the undifferentiated hPSC lines used in the development of toxicology assays. As part of the EC funded multi-consortium cluster SEURAT-1 [214] consideration has also been given to the kinds of specific quality control measures needed for hPSC lines and their development [105]. A diverse range of differentiation protocols are being used to develop these assays and the establishment of assay control parameters, and possibly reference preparations of toxicants to provide quality control of the differentiated cultures. This will be vital to ensure reproducibility in assay data and will be paramount for the successful utilization of stem cell-based models in toxicology and drug discovery.

Table 4. **Documents providing guidelines for manufacture of biologics from stem cells.**

Guidelines	Vaccines	Recombinant proteins	Conditioned media
WHO/ DRAFT/ 4 May 2010: Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (proposed replacement of TRS 878, Annex 1). See reference WHO 2010a	√	√	√
International Conference on Harmonization, Q5D, Derivation and Characterization of Cell Substrates Used for Production of Biotechnological/Biological Products, 1997. www.ich.org/LOB/media/MEDIA429.pdf	√	√	
International Conference on Harmonization, Q5A, Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin. www.ich.org/LOB/media/MEDIA425.pdf	√ (a)	√	
International Conference on Harmonization, Q5B, Quality of Biotechnological Products: Analysis of the Expression Construct in Cells Used for Production of r-DNA Derived Protein Products. www.ich.org/LOB/media/MEDIA426.pdf	√ (b)	√	
CBER Guidance for Industry, Characterization and Qualification of Cell Substrates and Other Biological Starting Materials Used in the Production of Viral Vaccines for the Prevention of Infectious Diseases, 2010. www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulation	√		

(a) Applies to recombinant subunit vaccines. Inactivated vaccines, all live vaccines containing self-replicating agents, and genetically engineered live vectors are excluded from the scope of this document.
(b) Applies to subunit vaccines only.

9. Acknowledgements

All contributors who have provided comments as part of ISCBI discussions and meetings, and also the regulatory agencies who provided informal feedback during the development of the manuscript. We would also like to thank the ISCF members [204] *who have continued to support the ISCBI activity. We would also like to acknowledge Dr L Healy who has had significant input in the co-ordination of this manuscript*

9. Financial & competing interests disclosure

The authors acknowledge grants from: T Chinese FZeng, Korean Y Kim; ISCF grant. The authors also acknowledge the following for their support in this publication: The Ministry of Science and Technology of China (grant numbers 2014CB964700 and 2013DFG30680), The Korean National Institute of Health (Intramural grant 4861-307-210-13) and a grant from the Translational Research of the Ministry of Health, Labor and Welfare of Japan. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Complete list of contributors

Andrews PW[1], Baker D[2], Benvinisty N[3], Miranda B[4], Bruce K[5], Brüstle O[6], Choi M[7], Choi Y-M[8], Crook JM[9], de Sousa PA[10], Dvorak P[11], Freund C[12], Firpo M[13], Furue MK[14], Gokhale P[1], Ha H-Y[15], Han E[7], Haupt S[16], Healy L[17], Hei DJ[18], Hovatta O[19], Hunt C[17], Hwang S-M[20], Inamdar MS[21], Isasi RM[22], Jaconi M[23], Jekerle V[24], Kamthorn P[25], Kibbey MC[26], Knezevic I[27], Knowles BB[28], Koo S-K[15], Laabi Y[29], Leopoldo L[30], Liu P[31], Lomax GP[32], Loring JF[33], Ludwig TE[34], Montgomery K[34], Mummery C[12], Nagy A[35], Nakamura Y[36], Nakatsuji N[37], Oh S[38], Oh S-K[8], Otonkoski T[39], Pera M[40], Peschanski M[29], Pranke P[41], Rajala KM[42], Rao M[43], Ruttachuk R[25], Reubinoff B[44], Ricco L[30], Rooke H[45], Sipp D[46], Stacey GN[17], Suemori H[47], Takahashi TA[47], Takada K[47], Talib S[32], Tannenbaum S[44], Yuan B-Z[48], Zeng F[49], Zhou Q[50]

1. Department of Biomedical Science, The University of Sheffield, Sheffield, UK

2. Sheffield Diagnostic Genetic Services (National Health Se rvice) / Centre for Stem Cell Biology (University of Sheffield), UK

3. Stem Cell Unit, Department of Genetics, Silberman Institute of Life Sciences, The Hebrew University, Jerusalem, Israel

4. Biobank Andalousian Public Health System, Spain

5. Roslin Cells Ltd, Scottish Centre for Regenerative Medicine, UK

6. Institute of Reconstructive Neurobiology, University of Bonn; German Centre for Neurodegenerative Diseases, Bonn, Germany

7. Ministry of Food and Drugs Safety (MFDS) (KFDA), Oosong, Republic of Korea

8. Institute of Reproductive Medicine & Population, Medical Research Center, Seoul National University, Seoul, Republic of Korea

9. Synthetic Biosystems Laboratory, ARC Centre of Excellence for Electromaterials Science, Intelligent Polymer Research Institute, AIIM Facility, Innovation Campus, University of Wollongong, Illawarra Health and Medical Research Institute, University of Wollongong, Wollongong, Department of Surgery – St Vincent's Hospital, The University of Melbourne, Fitzroy, Australia

10. Centre for Clinical Brain Sciences, University of Edinburgh; Roslin Cells Ltd, Edinburgh, UK

11. Department of Biology, Faculty of Medicine, Masaryk University, Czech Republic

12. Department of Anatomy and Embryology, Leiden University Medical Centre, The Netherlands

13. Stem Cell Institute, Department of Medicine, Division of Endocrinology, University of Minnesota, USA

14. National Institute of Biomedical Innovation, Japan

15. Korea National Institute for Health (KNIH)I, Republic of Korea

16. LIFE & BRAIN GmbH, Bonn, Germany

17. UK Stem Cell Bank, Division of Advanced Therapies, National Institute for Biological Standards and Control, Potters Bar, Hertfordshire, EN6 3QG, UK

18. Waisman Center, University of Wisconsin, Madison, USA

19. Karolinska Institutet, Sweden

20. Bioresource Collection and Research Center, Hinchu, Taiwan

21. Molecular Biology and Genetics Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, India

22. Centre of Genomics and Policy, McGill University and Génome Québec Innovation Centre, Canada

23. Department of Plastic, Reconstructive and Aesthetic Surgery, University Hospitals of Geneva (HUG), Faculty of Medicine, Geneva University, Switzerland

24. European Medicines Agency, London, UK

25. Faculty of Veterinary Science, Chulalogcorn University, Thailand

26. United States Pharmacopeia, Bethesda, USA

27. World Health Organisation, Geneva, Switzerland

28. The Jackson Laboratory, Bar Harbor, Maine, USA and the Siriraj Center of Excellence for Stem Cell Research, Mahidol University, Bangkok, Thailand

29. I-Stem AFM Inserm Évry., France

30. Andalousian Initiative Advanced Therapies (IATA), Spain

31. Wellcome Trust Sanger Institute, Cambridge, UK

32. California Institute for Regenerative Medicine, San Francisco, USA

33. Center for Regenerative Medicine, The Scripps Research Institute, La Jolla, California, USA

34. WiCell Research Institute, Wisconsin, USA

35. Centre for Stem Cells and Tissue Engineering, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Canada

36. Cell Engineering Division, RIKEN BioResource Center, Tsukuba, Japan

37. Institute for Integrated Cell-Material Sciences (WPI-iCeMS), Kyoto University, Japan

*38. Stem Cell Group, Bioprocessing Technology Institute, A*STAR, Singapore*

39. Research Programs Unit, Molecular Neurology and Biomedicum Stem Cell Centre, University of Helsinki, Helsinki, Finland

40. Florey Neuroscience and Mental Health Institute, and Walter and Eliza Hall Institute of Medical Research, University of Melbourne, Australia

41. Hematology and Stem Cell Laboratory, Faculty of Pharmacy, Federal University of Rio Grande do Sul, Brazil

42. Heart group, BioMediTech, University of Tampere, Tampere, Finland

43. Regenerative Medicine, New York Stem Cell Foundation, USA

44. Goldyne Savad Institute of Gene Therapy, Hadassah Hebrew University Medical Center, Jerusalem, Israel

45. International Society for Stem Cell Research, USA

46. RIKEN Center for Developmental Biology, Japan

47. Department of Embryonic Stem Cell Research, The Institute of Frontier Medical Sciences, Kyoto University, Japan

48. National Institutes for Food and Drug Control, Beijing, China

49. Shanghai Institute of Medical Genetics and Shanghai Stem Cell Institute, Shanghai Jiao Tong University School of Medicine, China

50. State key laboratory of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing, China

References

1 ISCBI. International Stem Cell Initiative. Andrews P, Arias-Diaz J, Auerbach J *et al.* Consensus guidance for banking and supply of human embryonic stem cell lines for research purposes. *Stem Cell Reviews and Reports* 2009, 5, 301–314 (2009).

2 PAS 84. Cell Therapy and Regenerative Medicine Glossary. *Regenerative Medicine* 7(3, Suppl. 1) (2012). http://shop.bsigroup.com/Browse-by-Sector/Healthcare/PAS-84

3 World Health Organization. Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks .WHO Technical Report Series 978, Annex 3 (2010). Replacement of WHO Technical Report Series, No. 878, Annex 1. www.who.int/biologicals/vaccines/TRS_978_Annex_3.pdf

4 Isasi R, Knoppers B. Monetary payments for the procurement of oocytes for stem cell research: in search of ethical and political consistency. *Stem Cell Research* 1, 37–44 (2007).

5 Isasi R, Knoppers B, Andrews P *et al.* International Stem Cell Forum Ethics Working Party. Disclosure and Management of Research Findings in Stem Cell Research and Banking: Policy Statement by the ISCF-EWP. *Regen. Med.* 7(3), 439–448 (2012).

6 ISSCR (2006). International Society for Stem Cell Research (ISSCR) Guidelines for the Conduct of Human Embryonic Stem Cell Research (December 2006). Dickens BM. *Med Law* 27(1), 179–190 (2008).

7 ISCF EWP. International Stem Cell Forum Ethics Working Party. Knoppers BM, Andrews PW, Bredenoord A *et al.* Ethics issues in stem cell research. *Science* 312(5772), 366–367 (2006).

8 Murdoch A, Braude P, Stacey G *et al.* The Procurement Working Group of the National Clinical hESC Forum. The Procurement of cells for the derivation of human embryonic stem cell lines for therapeutic use: recommendations for good practice. *Stem Cell Rev. Reports* 8 (1), 91–99 (2011).

9 Advisory Committee on the Safety of Blood, Tissues and Organs (SaBTO). Donation of starting material for cell-based advanced therapies: a SaBTO review, Department of Health, UK (2014).

10 Gymrek M, McGuire AL, Golan D, Halperin E, Erlich Y. Identifying personal genomes by surname inference. *Science* 339(6117), 321–324 (2013).

11 Isasi R, Andrews P, Baltz J *et al.* Identifiability and privacy in pluripotent stem cell research. *Cell Stem Cell* 14(4), 427–30 (2014).

12 Lomax G, Hull S, Lowenthal J, Rao M, Isasi R. The DISCUSS Project: induced pluripotent stem cell lines from previously collected research biospecimens and informed consent: points to consider. *Stem Cells Trans. Med.* 2(10) (2013).

13 ICH Q5D Derivation and Characterization of cell substrates used for production of biotechnological/biological products (1997). www.ich.org/products/guidelines/quality/quality-single/article/derivation-and-characterisation-of-cell-substrates-used-for-production-of-biotechnologicalbiologica.html

14 Malone JH, Oliver B. Microarrays, deep sequencing and the true measure of the transcriptome. *BMC Biol.* 9, 34 (2011).

15 Lui L, Li Y, Li S *et al.* Comparison of next generation sequencing systems.*J. Biomed. Biotechnol.* 251364 (2012).

16 Frese K, Katus, H, Meder B. Next-generation sequencing: from understanding biology to personalized medicine. *Biology* 2, 378–398 (2013).

17 Victoria J, Wang C, Jones M *et al.* Viral nucleic acids in live-attenuated vaccines: detection of minority variants and an adventitious virus. *J. Virol.* 84(12), 6033–40 (2010).

18 Gilliland S, Forrest L, Carre H et al. Investigation of porcine circovirus contamination in human vaccines. Biologicals 40(4), 270–277 (2012).

19 World Health Organisation. Community genetics services: report of a WHO consultation on community genetics in low- and middle-income countries. (2011).

20 Brazma A. On the importance of standardization in life sciences. *Bioinformatics* 17(2), 113–114 (2001).

21 World Health Organization. WHO Guidelines on Tissue Infectivity Distribution in Transmissible Spongiform Encephalopathies. WHO, Geneva (2010). www.who.int/bloodproducts/tablestissueinfectivity.pdf

22 EMA. CHMP/CAT position statement on Creutzfeldt–Jakob disease and advanced therapy medicinal products. Committee for Medicinal Products for Human Use (CHMP) and Committee for Advanced Therapies (CAT). London, UK (2011).

23 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). *Official Journal of The European Union.* (2011). 2011/C 73/01.

24 Young L, Sung J, Stacey G, Masters J. Detection of mycoplasma in cell cultures. *Nat. Protoc.* 5(5), 929–934 (2010).

25 Volokhov D, Graham L, Brorson K, Chizhikov V. Mycoplasma testing of cell substrates and biologics: review of alternative non-microbiological techniques. *Mol. Cell Probes.* 25(2–3), 69–77 (2011).

26 Martins-Taylor K, Xu R-H. Concise review: genomic stability of human induced pluripotent stem cells. *Stem Cells* 30, 22–27 (2012).

27 Nguyen H, Geens M, Spits C. Genetic and epigenetic instability in human pluripotent stem cells. *Human Reproduction Update* 19, 187–205 (2013).

28 Ben-David U, Arad G, Weissbein U *et al.* Aneuploidy induces profound changes in gene expression, proliferation and tumorigenicity of human pluripotent stem cells. *Nat. Commun.* 5, 4825 (2014).

29 Lund J, Emani M, Barbaric I *et al.* Karyotypically abnormal ESCs are sensitive to HDAC inhibitors and show altered regulation of genes linked to cancers and neurological diseases. *Stem Cell Res.* 11, 1022–1036 (2013).

30 Sun Y, Yang Y, Zeng S, Tan Y, Lu G, Lin G. Identification of proteins related to epigenetic regulation in the malignant transformation of aberrant karyotypic human embryonic stem cells by quantitative proteomics. *PLoS One* 2014, 9, e85823.

31 Chen Z, Zhao T, Xu Y. The genomic stability of induced pluripotent stem cells. *Protein Cell* 3(4), 271–277 (2012).

32 Hyka-Nouspikel N, Desmarais J, Gokhale PJ *et al.* Deficient DNA damage response and cell cycle checkpoints lead to accumulation of point mutations in human embryonic stem cells. *Stem Cells* 30(9), 1901–1910 (2012).

33 ISCI. International Stem Cell Initiative. Amps K, Andrews P, Anyfantis G *et al.* Screening ethnically diverse human embryonic stem cells identifies a chromosome 20 minimal amplicon conferring growth advantage. *Nat. Biotechnol.* 29(12), 1132–1144 (2011).

34 Laurent LC, Ulitsky I, Slavin I *et al.* Dynamic changes in the copy number of pluripotency and cell proliferation genes in human ESCs and hiPSCs during reprogramming and time in culture. *Cell Stem Cell* 8(1), 106–118 (2011).

35 Steinemann D, Göhring G, Schlegelberger B. Genetic instability of modified stem cells – a first step towards malignant transformation? *Am. J. Stem Cells* 2(1),39–51 (2013).

36 Yang S, Lin G, Tan Y, Deng L, Yuan D, Lu G. Differences between karyotypically normal and abnormal human embryonic stem cells. *Cell Prolif.* 43(3),195–206 (2010).

37 Gore A, Li Z, Fung HL, Young JE *et al.* Somatic coding mutations in human induced

pluripotent stem cells. *Nature* 471(7336), 63–67(2011).

38 Sugiura M, Kasama Y, Araki R *et al.* Induced pluripotent stem cell generation-associated point mutations arise during the initial stages of the conversion of these cells. *Stem Cell Reports* 2, 52–63 (2014).

39 Avery S, Hirst A, Baker D *et al.* BCL-XL mediates the strong selective advantage of a 20q11.21 amplification commonly found in human embryonic stem cell cultures. *Stem Cell Reports* 1, 379–386 (2013).

40 Närvä E, Autio R, Rahkonen N *et al.* High-resolution DNA analysis of human embryonic stem cell lines reveals culture-induced copy number changes and loss of heterozygosity. 28, 371–377 (2010).

41 Chen KG, Mallon BS, McKay RDG, Robey PG. Human pluripotent stem cell culture: considerations for maintenance, expansion, and therapeutics. *Cell Stem Cell* 14, 13–26 (2014).

42 Anguiano A, Wang B, Wang S *et al.* Spectral Karyotyping for identification of constitutional chromosomal abnormalities at a national reference laboratory. *Mol. Cytogenet.* 5, 3 (2012)

43 Das K, Tan P. Molecular cytogenetics: recent developments and applications in cancer. *Clin. Gen.* 84, 315–325(2013).

44 Moralli D, Yusuf M, Mandegar MA, Khoja S, Monaco ZL, Volpi EV. An improved technique for chromosomal analysis of human ES and iPS cells. *Stem Cell Rev. Reports* 7, 471–477 (2011).

45 Peterson SE, Westra JW, Rehen SK *et al.* Normal human pluripotent stem cell lines exhibit pervasive mosaic aneuploidy. *PLoS One* 6, e23018 (2011).

46 Riegel M. Human molecular cytogenetics: from cells to nucleotides. *Genetics Mol. Biol.* 37, 194–209 (2014).

47 Ben-David U, Mayshar Y, Benvenisty N. Vurtual karyotyping of pluripotent stem cells on the basis of their global gene expression profiles. *Nat. Protocols* 8, 989–997 (2013)

48 Zhang F, Gu W, Hurles M, Lupski J. Copy number variation in human health, disease and evolution. *Ann. Rev. Hum. Genet.* 10, 451–481 (2009).

49 Liang G, Zhange Y. Embryonic stem cell and induced pluripotent stem cell: an epigenetic perspective. *Cell Res.* 23, 49–69 (2013).

50 Liang G and Zhange Y. Genetic and epigenetic variations in iPSCs: potential consequences and implications for application. *Cell Stem Cell* 13, 149–159 (2013).

51 Frost J, Monk D, Moschidou D, Guillot PV, Stanier P, Minger SL, Fisk NM, Moore HD, Moore GE. The effects of culture on genomic imprinting profiles in human embryonic and fetal mesenchymal stem cells. *Epigenetics* 6(1), 52–62 (2011).

52 Nazor KL, Altun G, Lynch C *et al.* Recurrent variations in DNA methylation in human pluripotent stem cells and their differentiated derivatives. *Cell Stem Cell* 10(5), 620–634 (2012).

53 Tompkins J, Hall C, Chen V *et al.* Epigenetic stability, adaptability, and reversibility in human embryonic stem cells. *Proc. Natl Acad. Sci. USA* 109(31), 12544–12549 (2012).

54 Association for Clinical Cytogenetics: Professional Guidelines for Clinical Cytogenetics Constitutional Postnatal Chromosomal Microarray Best Practice Guidelines. Volume 2 (2011).

55 Gropp M, Shilo V, Vainer G *et al.* Standardization of the teratoma assay for analysis of pluripotency of human ES cells and biosafety of their differentiated progeny. *PLoS One* 7(9), e45532 doi:10.1371/journal.pone.0045532 (2012).

56 Buta C, David R, Dressel R *et al.* Reconsidering pluripotency tests: do we still need teratoma assays? *Stem Cell Res.* 11(3), 1022–1036 (2013).

57 Aleckovic M, Simón C. Is teratoma formation in stem cell research a characterization tool or a window to developmental biology? *Reprod. Biomed. Online* 17, 270–280 (2008).

58 Prokhorova TA, Harkness LM, Frandsen U *et al.* Teratoma formation by human embryonic stem cells is site dependent and enhanced by the presence of Matrigel. *Stem Cells Dev.* 18(1),47–54 (2009).

59 Quintana E, Shackleton M, Sabel MS, Fullen DR, Johnson TM, Morrison SJ. Efficient tumor formation by single human melanoma cells. *Nature* 456, 593–598 (2008).

60 Ito M, Hiramatsu H, Kobayashi K *et al.* NOD/SCID/gamma(c)(null) mouse: an excellent recipient mouse model for engraftment of human cells. *Blood* 100, 3175–3182 (2002).

61 Kanemura H, Go MJ, Shikamura M *et al.* Tumorigenicity studies of induced pluripotent stem cell (iPSC)-derived retinal pigment epithelium (RPE) for the treatment of age-related macular degeneration. *PLoS One* 14, e85336 (2014).

62 Shultz LD, Lyons BL, Burzenski LM. Human lymphoid and myeloid development in NOD/LtSz-scid ILR2 gamma null mice engrafted with mobilised human hemopoetic stem cells. *J. Immunol.* 174, 6477–6489 (2005).

63 Coecke S, Balls M, Bowe G *et al.* Guidance on Good Cell Culture Practice. A report of the second ECVAM Task Force on Good Cell Culture Practice, ATLA, 2005, 33, 1–27.

64 Andrews P. From teratocarcinomas to embryonic stem cells. *Philos. Trans. R Soc. Lond. B Biol. Sci.* 357(1420), 405–17 (2002).

65 Solter D. From teratocarcinomas to embryonic stem cells and beyond: a history of embryonic stem cell research. *Nat. Rev. Genet.* 7(4),319–27 (2006).

66 Croce CM. Oncogenes and cancer. *N. Engl. J. Med.* 358, 502–511 (2008).

67 Kerrigan L,Nims RW. Authentication of human cell-based products: the role of a new consensus standard. *Regen. Med.* 6(2), 255–260 (2011).

68 Knoppers BM, Isasi R, Benvenisty N *et al.* Publishing SNP genotypes of human embryonic stem cell lines: policy statement of the International Stem Cell Forum Ethics Working Party. *Stem Cell Rev.* 7(3), 482–4 (2001).

69 Luong MX, Auerbach J, Crook JM *et al.* A call for standardized naming and reporting of human ESC and iPSC lines. *Cell Stem Cell* 8(4), 357–359.

70 O'Connor MD, Kardel MD, Iosfina I et al.Alkaline phosphatase-positive colony formation is a sensitive, specific, and quantitative indicator of undifferentiated human embryonic stem cells. *Stem Cells* 26, 1109–1116 (2008).

71 Calloni R, Cordero EA, Henriques JA, Bonatto D. Reviewing and updating the major molecular markers for stem cells. *Stem Cells Dev.* 22, 1455–1476 (2013).

72 Zhao W, Ji X, Zhang F, Li L, Ma L. Embryonic stem cell markers. *Molecules* 17, 6196–6236 (2012).

73 ISCI. International Stem Cell Initiative. Adewumi O, Aflatoonian B, Ahrlund-Richter L *et al.* Characterization of human embryonic stem cell lines by the International Stem Cell Initiative. *Nat. Biotechnol.* 25, 803–816 (2007).

74 Josephson R, Ording CJ, Liu Y *et al.* Qualification of embryonal carcinoma 2102Ep as a reference for human embryonic stem cell research. *Stem Cells* 25, 437–446 (2007).

75 Loring JF, Rao MS. Establishing standards for the characterization of human embryonic stem cell lines. *Stem Cells* 24(1), 145–50 (2006).

76 Durruthy-Durruthy J, Briggs S, Awe J *et al.* Rapid and efficient conversion of integration-free human induced pluripotent stem cells to GMP-grade culture conditions. *PLoS One* 204, 9, e94231.

77 Goh PA, Caxaria S, Casper C, Rosales C, Warner TT, Coffey PJ, Nathwani AC. A systematic evaluation of integration free reprogramming methods for deriving

clinically relevant patient specific induced pluripotent stem (iPS) cells. *PLoS One* 8(11), e81622 (2013).

78 Müller FJ, Goldmann J, Löser P, Loring JF. A call to standardize teratoma assays used to define human pluripotent cell lines. *Cell Stem Cell* 6, 412–414 (2010).

79 Müller FJ, Schuldt BM, Williams R *et al.* A bioinformatic assay for pluripotency in human cells. *Nat. Methods* 8(4), 315–317 (2001).

80 Müller FJ, Brändl B, Loring JF. Assessment of human pluripotent stem cells with PluriTest. StemBook [Internet]. Cambridge (MA, USA), Harvard Stem Cell Institute, 2008–2012.

81 Bock, C, Kiskinis E, Verstappen G *et al.* Reference maps of human ES and iPS cell variation enable high-throughput characterization of pluripotent cell lines. *Cell* 144(3), 439–52 (2001).

82 Sheridan SD, Surampudi V, Rao RR. Analysis of embryoid bodies derived from human induced pluripotent stem cells as a means to assess pluripotency. *Stem Cells Int.* 738910 (2012).

83 US FDA. Guidance for Industry: Current Good Tissue Practice (CGTP) and Additional Requirements for Manufacturers of Human Cells, Tissues and Cellular and Tissue-Based Products (HCT/Ps). (2011)

84 EudraLex. EudraLex Volume 4, Chapter 1 (2011). http://ec.europa.eu/health/files/eudralex/vol-4/pdfs-en/2008_02_14_gmp-part1-chapter1_q9_en.pdf

85 MHRA. Rules and Guidance for Pharmaceutical manufacturers and Distributors. 2014. http://www.mhra.gov.uk/Publications/Regulatoryguidance/Medicines/CON2030291

86 World Health Organization (2006). Annex 4 Supplementary guidelines on GMPs: validation, WHO Technical Report Series, No. 937, (2006). http://apps.who.int/prequal/info_general/documents/TRS937/WHO_TRS_937-Annex4.pdf

87 World Health Organisation (2007). Quality assurance of pharmaceuticals: A compendium of guidelines and related materials, Volume 2, 2nd Updated Edition, Good Manufacturing Practices And Inspection. (2007). www.who.int/vaccines-documents/DocsPDF/www9666.pdf

88 EUTCD (2004). Directive 2004/23/EC of the European Parliament and of the Council of 31 March 2004 on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells. (2004). http://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX:32004L0023

89 Beck G, Schenerman M, Dougherty J *et al.* Raw material control strategies for bioprocesses. *BioProcess International* 18–33 (2009).

90 Melton L, and Wahlgren C. Participating in the Rx360 pilot joint audit programme: a suppliers perspective. *BioProcess International* 10(10), 20–23 (2012).

91 Unger C, Skottman H, Blomberg P, Dilber MS, Hovatta O. Good manufacturing practice and clinical-grade human embryonic stem cell lines. *Hum. Mol. Genet.* 17(R1), R48–53(2008)

92 US FDA 2008. Guidance for FDA reviewers and sponsors: content and review of chemistry, manufacturing, and control (CMC) information for human somatic cell therapy investigational new drug applications (INDs).

93 PAS 93. *Characterization of Human Cells for Clinical Applications.* BSI Standards Limited. (2011).

94 Sheridan B, Stacey G, Wilson A, Ginty P, Bravery C, Marshall D. Standards can help bring cell therapy products to market. *BioProcess International* 10(4), 18–20 (2012).

95 Andrews P, Cavagnaro J, Deans R *et al.* Harmonizing standards for producing clinical-grade therapies from pluripotent stem cells. *Nat. Biotechnol.* 32, 724–726 (2014).

96 Benson EE, Betsuo F, Fuller BJ, Harding K, Kofanova O. Translating cryobiology principles into transdisciplinary storage guidelines for biorepositories and biobanks: a concept paper. *Cryoletters* 34, 277–312(2013).

97 Pegg D. *Cryopreservation and Freezedrying Methods.* Day DG, Stacey GN (Eds). Humana Press, Totowa, USA, 39–58 (2007).

98 Pegg D. Viability assays for preserved cells, tissues, and organs. *Cryobiology* 26, 212–231 (1989).

99 Fahy GM, Wowk B, Wu J, Paynter S. Improved vitrification solutions based on the predictability of vitrification solution toxicity. *Cryobiology* 48(1), 22–35 (2004).

100 Hunt CJ. Cryopreservation of human stem cells for clinical application: a review. *Transfus. Med. Hemother.* 38(2), 107–123 (2011).

101 Day JG, Stacey GN. *Cryopreservation and Freeze-drying Protocols.* Day DG, Stacey GN (Eds). Humana Press, Totowa, USA (2007).

102 Lawrence D. Stem cells in toxicology. *Curr. Protocol. Toxicol.* 47, 22:22.0.1 (2011).

103 Inoue H, Yamanaka S. The use of induced pluripotent stem cells in drug development. *Clin. Pharmacol. Therap.* 89 (5), 655–661 (2011).

104 Pistollato F, Bremer-Hoffmann S, Healy L, Young L, Stacey G. Standardization of pluripotent stem cell cultures for toxicity testing. *Exp. Opin. Drug Metab. Toxicol.* 8(2), 239–257 (2012).

105 ICH Q 5 A (R1). Quality of Biotechnological Products: Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin. Geneva (1997). www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002801.pdf

106 ICH Q 3A-3D: Impurities. Geneva. 2006–2014. www.ich.org/products/guidelines/quality/quality-single/article/impurities-in-new-drug-substances.html

107 Code of Federal Regulations PART 1271 – Human cells, Tissues, and Cellular and Tissue-based Products FDA, 21 CFR 1271.3(y). Revised as of April 1, 2013. www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=1271.3

108 International Conference On Harmonisation Of Technical Requirements For Registration Of Pharmaceuticals For Human Use. ICH Harmonised Tripartite Guideline. Clinical Safety Data Management: Definitions And Standards For Expedited Reporting E2A. www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E2A/Step4/E2A_Guideline.pdf

109 Wang L, Pegg DE, Lorrison J, Vaughan D, Rooney P. Further work on the cryopreservation of articular cartilage with particular reference to the liquidus tracking (LT) method. *Cryobiology* 55(2),138–47 (2007).

110 Wowk B, Leitl E, Rasch CM, Mesbah-Karimi N, Harris SB, Fahy GM. Vitrification enhancement by synthetic ice blocking agents. *Cryobiology* 40(3), 228–236 (2007).

111 Morris GJ, Acton E. Controlled ice nucleation in cryopreservation – a review. *Cryobiology* 66, 85–92 (2013).

112 Morris GJ1, Acton E, Faszer K *et al.* Cryopreservation of murine embryos, human spermatozoa and embryonic stem cells using a liquid nitrogen-free, controlled rate freezer. *Reprod. Biomed. Online* 13(3), 421–426 (2006).

113 Morris GJ, Acton E, Collins D, Bos-Mikich A, de Sousa P. Optimisation of current Good Manufacturing Practice (cGMP) compliant controlled rate freezing for human embryonic stem cells. *Cryobiology* 61, 406 (2010).

Websites

201 International Compilation of Human Research Standards (2013). www.hhs.gov/ohrp/international

202 International Cell Line Authentication Committee. http://iclac.org

203 Information Standard for Blood and Transplant (ISBT) 128.

www.iccbba.org

204 The Pharmaceutical Inspection Co-operation Scheme.
www.picscheme.org

205 International Stem Cell Forum.
www.stem-cell-forum.net

206 WHO Expert Committee on Biological Standardization.
www.who.int/biological/expert_committee/en

207 National Institute for Biological Standards and Control.
www.nibsc.org

208 Rapid Alert system for human Tissues and Cells (RATC).
http://ec.europa.eu/health/blood_tissues_organs/docs/ratc_report_2008_2012_en.pdf

209 MedWatch.
www.fda.gov/Safety/MedWatch

210 International Conference on Harmonisation (ICH), (E1– E2F).
www.ich.org/products/guidelines/efficacy/article/efficacy-guidelines.html

211 Council for International Organisations of Medical Sciences (CIOMS).
www.cioms.ch

212 UK toolkit for stem cell therapy.
www.sc-toolkit.ac.uk/home.cfm

213 Canadian regulatory framework for the development of stem cell-based therapies.
www.stemcellethics.ca/sites/default/files/roadmap_june_2011.pdf

214 US Pharmacopeial Forum.
www.usp.org/usp-nf/pharmacopeial-forum

215 SEURAT-1 EU Framework 7 project.
www.seurat-1.eu

216 UK Caldicot Principles.
www.bcf.nhs.uk/about_us/information/_governance/caldicott

Appendices

Appendix 1

Appendix 1 (a). Compliance and provenance determination.

(1) Embryo provenance determination	Code	Considerations
(a) Independent review and oversight	B	The protocol for obtaining gametes and embryos from living donors should be subject to independent review. Review and approval of the hESC derivation protocol may be required in some jurisdictions, but is not an essential requirement
(b) Voluntary informed consent	B	hESC-specific consent requirements may exist or subsequent users of hESC lines may be required to obtain lines for which comprehensive consent has been obtained. Bank should seek to obtain documentation of consent protocol
(c) Gratuitous donation	B	Banks should receive assurance that donors were not paid for embryos or storage costs
(2) Compliance determination	**Code**	**Considerations**
(a1) Embryo was donated in a jurisdiction with no explicit prohibition on hESC derivation	B	Accepting embryos from jurisdictions where hESC research is restricted may incur legal liability
(a2) Derivation protocol confirms to any unique legal requirements in jurisdiction where hESC derived	B	Jurisdiction may have unique requirements in addition to international standards for research ethics (e.g., embryo research oversight or licensing); Consistent with 1a
(b) Any line derived using IVF for research purposes, parthenogenesis or SCNT is identified	B	The use of hESC lines derived from embryos created for research purposes are prohibited by some jurisdictions and funding bodies
(c) Consent requirement for third-party gamete donors	A	Some donated embryos may have been created using gametes from someone other than the embryo donor; Some jurisdictions require consent from third-party donors
(c1) hESC lines derived from embryos intended for reproductive use where a third-party donor(s) was contracted to provide gametes	A	Bank or entity performing hESC derivation should review donor/recipient contract for any conditions that would restrict research use Policies regarding the use of such embryos or resulting hESC lines are variable. Bank should review egg-sharing contract or exchange policies Certain end-users may not be able to utilize lines derived from embryos for which gamete donors were paid or where egg sharing, exchange or anonymous donation has taken place. Documentation serves to enable end user to perform use eligibility determination
(c2) hESC lines derived from embryos for which gamete donor(s) participated in egg sharing or exchange programs are identified	A	
(c3) hESC lines derived from embryos created with anonymous gamete donation are identified	A	
(d) Donor medical history	A/PU	Requirement for medical history may vary depending on relationship between donor and recipient of embryo for IVF. If embryos are created specifically for research, gamete donor medical history should be obtained

Code Key:

A *Advisable (recommended?): Level of attainment recommended at this time by the International Stem Cell Banking Initiative.*

B *Baseline: Minimum level of attainment generally consistent with the current standard of care for clinical grade stem cell lines.*

NR *Not recommended: This option not recommended at this time. Consideration subject to revision based on new information.*

PU *Potentially utility if available but not required: In certain circumstances supplemental information: medical records, biological specimens (e.g., blood or urine specimens) or quality control assays may be available or have been performed. Banks are encouraged to retain access to supplemental information. Absent evidence of utility – safety or clinical efficacy – the acquisition of supplemental information should not be required for the development of clinical grade stem cell lines.*

hESC: Human embryonic stem cell; SCNT: Somatic cell nuclear transfer.

Appendix 1 (b). Informed consent and donor disclosures: compliance determination check list.			
2.1 Did the informed consent process communicate the following elements?	Yes	No	N/A
That the somatic tissue/cells would be used for the purpose of stem cell research, including the derivation of stem cell line(s).			
That genetic tests may be performed, including whole-genome sequencing.			
That research may be conducted on human transplantation.			
That the research is not intended to provide direct benefit to the donor(s) except in the case of autologous donation.			
That the cell lines might be used in research involving genetic manipulation of the cells.			
That the cell lines might be used in research involving the mixing of human and nonhuman cells in animal models.			
That the research entails both foreseeable risks and benefits.			
That any stem cell lines created may be used and stored indefinitely.			
That any stem cell lines created may be used in future unspecified research projects.			
That the decision whether to donate would not affect future medical care.			
That confidentiality will be maintained.			
That the cells would be coded or anonymized (i.e. irreversibly de-linked).			
That donor recontact may be possible (unless anonymized).			
That the donor was informed concerning the disclosure (or not) of general, individuals and/or incidental findings.			
That the donor was informed of the right of withdrawal provided this is not overridden by complete anonymization.			
That the stem cell lines derived will be deposited in a repository for long-term storage and use.			
That once the cells have been used in research, the donor will have no further control over any use of the cells or derived stem cell lines.			
That the cells may be distributed to researchers and institutions within and beyond Canada.			
That the cell lines may be used for commercial purposes but without financial benefit to the donor.			
That the donor was informed of the researchers' actual or potential conflicts of interests.			

Appendix 2. Material transfer agreements

A material transfer agreement (MTA) is a contract that governs the transfer of tangible research materials between two organizations (the provider, who is the owner/custodian or the authorized licensee of the material and associated data, and the recipient), thereby defining the contractual rights and obligations with respect to the materials and any derivates.

Important issues to consider when drafting or evaluating an MTA include:

- Ownership of the materials.
- Definition and legal status of original/biological materials, modifications of materials and derivates, progeny;
- Definition of commercial purposes, non-profit organizations, investigator or researcher
- Intellectual property rights;
- Publication rights;
- Royalty fees
- Confidentiality;
- Scope of use and restrictions (e.g., non-commercial/academic vs. commercial research; ethical limitations on types of research to be conducted (e.g., limitations on research aimed at the generation of gametes);
- Use of materials in sponsored research (e.g., industry vs. industry/academic sponsored research);
- Transferability of cell line , cell products or data derived from cell products (e.g., genetic sequencing data);
- Conflicts with existing agreements;
- Compliance with laws and ethical guidelines;
- Processing, cost-recovery and other fees
- Warranties;
- Liability;
- Indemnification.

Model material transfer agreements.

UK Stem Cell Bank	Clinical/Commercial use http://www.ukstemcellbank.org.uk/cell_lines/eutcd_grade_stem_cell_lines/depositing_eutcd_stem_cell.aspx Research Use http://www.ukstemcellbank.org.uk/legal_agreements.aspx
USA National Institutes of Health (NIH), Center for Regenerative Medicine (CRM)	Master Agreement Regarding Use of the Uniform Biological Material Transfer Agreement http://www.crm.nih.gov/researchTools/uniform_transfer_agreement.asp CRM Induced Pluripotent Stem (iPS) Cell Material Transfer Agreement http://www.crm.nih.gov/researchTools/material_transfer_agreement.asp Public Health Service Biological Materials License Agreement http://www.crm.nih.gov/researchTools/bio_mats_agreement.asp
International Society for Stem Cell Research (ISSCR)	ISSCR Sample Material Transfer Agreement http://www.isscr.org/home/publications/guide-clintrans/sample-material-transfer-agreement
ATCC	General MTA http://www.atcc.org/Documents/Product%20Use%20Policy/Material%20Transfer%20Agreement.aspx Research Use http://www.atcc.org/en/Documents/Product_Use_Policy/Research_Use.aspx Commercial Use http://www.atcc.org/en/Documents/Product_Use_Policy/Commercial_Use.aspx
California Institute for Regenerative Medicine (CIRM)	http://www.cirm.ca.gov/our-funding/stem-cell-regulations-governing-cirm-grants BioTimes hESC Lines http://www.cirm.ca.gov/our-funding/biotime-stem-cell-lines-agreement
WiCell	iPS Wisconsin MTA http://www.wicell.org/media/WiCellAgreements/WiCell-iPS-MTA.pdf UCSF MTA http://www.wicell.org/media/WiCellAgreements/WiCell-UCSF-Material-Agreement.pdf
Wisconsin Alumni Research Foundation (WARF)	Agreements http://www.warf.org/home/for-industry/Agreements/agreements.cmsx