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Points to consider in the development of seed stocks of pluripotent stem cells for clinical applications: International Stem Cell Banking Initiative (ISCBI)

1. Background and utility of this document

In 2009 the International Stem Cell Banking Initiative (ISCBI) contributors and the Ethics Working Party of the International Stem Cell Forum published a consensus on principles of best practice for the procurement, cell banking, testing and distribution of human embryonic stem cell (hESC) lines for research purposes [1], which was broadly also applicable to human induced pluripotent stem cell (hiPSC) lines. Here, we revisit this guidance to consider what the requirements would be for delivery of the early seed stocks of stem cell lines intended for clinical applications. The term ‘seed stock’ is used here to describe those cryopreserved stocks of cells established early in the passage history of a pluripotent stem cell line in the lab that derived the line or a stem cell bank, hereafter called the ‘repository’. The seed stocks should provide cells with suitable documentation and provenance that would enable them to be taken forward for development in human therapeutic applications. WHO recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biologicals and for the characterization of cell banks were updated in 2010 and provide a number of definitions and guiding principles that may apply to stem cells. The term ‘cell bank’ is used to describe a stock of vials or other containers of cells with consistent composition aliquoted from a single pool of cells of the same culture history (for other specific definitions see PAS 84 [2] and WHO [3]).

Three important assumptions have been made in the preparation of this document. First, that seed stocks of hPSCs are used as starting materials to make cell banks for use in clinical trials. The cell banks made within a clinical trial would need to be established according to Good Manufacturing Practice (GMP) in a facility with a relevant product manufacturing license. These banks would need additional risk assessment focused on the new banking process/reagents and the specific intended clinical application.

Second, it has been assumed that undifferentiated pluripotent stem cells would not be inoculated into patients. Third, where feeder cells are used to culture hPSC lines, their cellular nature and intimate contact with the therapeutic cells means that they should be subject to similar risk assessment and banking procedures as applied to the hPSC cells.

It is important to note that responsibility for establishing and updating national regulations for medicinal products relies on National Regulatory Authorities. Therefore, national requirements for cell therapy may vary considerably. Accordingly, it is not intended that this international consensus provides comprehensive guidance that will ensure compliance with requirements in any given jurisdiction. Rather, it is designed to aid the development of clinical grade materials by providing points to consider in the preparation of seed stocks of stem cell lines for use in cell therapy. It may arise that there are circumstances where it is not reasonably possible to meet specific procedures presented in this document. Where this is the case any alternative procedures should be justified and mitigate against any adverse consequences. Finally, this document could also serve as a useful reference to assist in the evaluation of potential sources of candidate cell lines for the development of cell-based medicines, and provide the links necessary to identify some of the key differences in regulatory requirements between countries.

2. Governance and ethics

■ 2.1 General principles

Centers banking stem cell lines (hereafter called repositories) should adopt transparent and harmonized protocols for the collection, storage, access, and use of the cell lines that they curate. As part of a comprehensive governance structure, repositories should establish robust mechanisms for the authentication of *bone fide* users and should strive for equitable and transparent conditions of access and of material transfer (Appendices 1a, 1b and 2). Such protocols

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should be adopted according to internationally accepted principles for research ethics and in compliance with applicable legal, ethical and regulatory requirements (Appendix 3, 4 and 5). Furthermore, repositories should establish a system for documenting and monitoring performance with respect to such principles and requirements.

■ 2.2 Key issues in determining provenance of pluripotent stem cell lines

Repositories should ascertain the provenance (source/origin) of the human biological specimens from which the pluripotent stem cell lines have been derived. International guidance exists for documenting the provenance of the cell lines [1,4–7].

Important issues to consider when evaluating provenance include:

- Evidence of free and voluntary informed consent, for the proposed research use, in conjunction with independent review and oversight, with particular attention given to disclosure of potential clinical and commercial applications.
- The extent to which reimbursement (e.g., expenses, financial incentives, monetary payments) were provided for donation of biological samples.
- The ability of the donor to withdraw original specimens, derived cell lines, data or otherwise to discontinue participation in research.
- The possibility that derived cell lines may be used for a wide range of research, possibly through a public repository.
- The establishment of robust systems for data security and traceability.
- The implementation of mechanisms for the protection of donor privacy and confidentiality. Particular attention should be given to the generation and use of genome sequence data.

Many national and more local jurisdictions have explicit policies governing the acquisition and use of human biospecimens for pluripotent stem cell derivation, particularly with regard to embryonic sources (Appendix 3 and 4). Prior to accepting a pluripotent stem cell line, a repository should determine its provenance by first documenting that the biospecimen was collected and the cell line derived in a manner broadly consistent with international standards for

research ethics [4,5,6,7,201]; and second, to make a positive determination that the biospecimen was obtained in a manner consistent with applicable laws in the country of origin.

2.2.1 Provenance determination and international standards

Providers of cells should be able to demonstrate to the repository that they have met all applicable legal and ethical requirements associated with the procurement of a human biospecimen from which a pluripotent stem cell line was derived. Given the heterogeneity of national laws and regulations governing research and clinical applications, the depositor of a cell line should provide information that enables the repository governance structure to determine whether the conditions of derivation, use and distribution are broadly consistent with the repository's national regulation. Moreover, repositories should have in place a mechanism (e.g., 'horizon scanning', advisory board) to track changes in the legal and regulatory frameworks. In addition, repositories should verify and retain sufficient documentation to support a determination that each cell line has been obtained in accordance with international standards for research ethics.

Key principles include the following:

Independent review and oversight

The protocol for procurement of tissues, gametes or embryos for the purpose of generating a pluripotent stem cell line should be subject to independent scientific and ethical review. Review bodies include ethics committees, licensing bodies or committees responsible for oversight of research involving human subjects.

Voluntary informed consent

In addition to verifying appropriate informed consent, the repository should ascertain additional details regarding donor's disclosure when available (Appendix 1b). Numerous bodies and national policies recommend or require the disclosure of specific information to donors (particularly for hESC derivation). A number of jurisdictions have consent requirements that include, but are not limited to, disclosure of possible human transplantation, genetic modification, international sharing and commercial potential. Documentation of a robust informed consent process that addresses these requirements can serve to support wide distribution and utilization of the cell lines (Appendix 3 and 4). Informed consent requirements for stem cell derivation, use and banking have evolved over time and jurisdictional

variations may exist for different sources of bio-specimens. In addition, standards for evaluating informed consent processes may need to be flexible and allow for context-specific considerations. For example, agreement to banking could include broad consent to future unspecified research (subject to appropriate security mechanisms and governance); whereas some protocols may be intended to develop a specific cell product. Donors should be notified of the possibility of future use in cellular therapies, commercialization of eventual products and of the international sharing of samples and of stem cell lines. Moreover, donors should also be informed of the limitations in privacy protection (see section 3.2 & 5.1) given the need to assure traceability for safety reasons (see section 6.9).

Gratuitous donation

Donors should not be paid to provide somatic cells, gametes or embryos for stem cell derivation, nor should they be reimbursed for any costs, such as tissue storage, prior to the decision to donate.

2.2.2 Compliance determination and access policies

Mechanisms should be in place to make a positive determination of compliance with both the ethical and legal requirements of the jurisdiction of biospecimen's origin, together with those of the jurisdiction where the cell line was derived, deposited, and will be used in research (Appendix 3 and 4). Furthermore, it is important to consider that there may be jurisdictional or funding agency restrictions on the types of cell lines eligible for research use as may be the case for hESC lines. To the extent feasible the repository should strive to compile complete provenance information for evaluation; however, it is ultimately incumbent on the end user of the cell line to determine that its provenance meets local ethics and legal requirements.

Repositories should also adopt transparent, flexible and equitable access policies. Given the importance of international collaboration, such policies should include procedures for deposit of cell lines of foreign origin, and for the distribution of cell lines to researchers in other jurisdictions. Among the policy criteria to be considered are the following:

- Mutual recognition via 'reciprocal policy agreements' allowing for transnational sharing of cell lines provided that the cell lines were derived by, or approved for use by, a licensing

entity formally recognized as having adopted consistent ethical and legal requirements.

- 'Substantial equivalency' whereby criteria for cell line derivation, use, and banking in different jurisdictions involve ethical and legal requirements that are deemed to be 'broadly' or 'substantially' acceptable to the repository management and under applicable regulation.

3. Provenance and selection of donor tissue

3.1 Donor selection, screening and medical records

Eligibility criteria for embryo, cell or tissue donors intended for human transplantation are subject to national regulatory frameworks and institutional protocols in the jurisdiction of origin. As a general rule, donor eligibility determination requires screening for risk factors associated with infection and communicable disease. These are typically focused on serum human viral blood-borne pathogens (e.g., HIV, hepatitis B virus, hepatitis C virus) and may also include other pathogens endemic to the donor's origin (e.g., human T-cell lymphotropic virus I&II, Chaga's disease, malaria). Donor testing for these agents may be required to be carried out under national licensed facilities.

For hESCs there are a number of considerations pertaining to donor screening protocols for assisted reproduction treatment (Appendix 4). For hiPSC evaluation, inclusion and exclusion criteria represent a starting point for risk-assessment or risk mitigation. In some cases, inclusion criteria may call for the collection of cells and tissues from patient groups with specific clinical (disease) indications. Any information regarding known disease indication should be associated with specific cell and tissue samples to support risk evaluation (see section 6.3). While cell lines derived from patients with inherited disease have been recognized as having potential scientific utility, they are unlikely to be suitable for development of general clinical applications.

Finally, regulatory authorities responsible for the evaluation of biological products consistently emphasise the value of a donor medical history. It is important to note that rules adopted in some jurisdictions may require a review of donors' relevant medical records and or a medical history screening; considerations for extended medical histories have been published by Murdoch *et al.* [8].

■ 3.2 Allogeneic cell transplantation

The establishment of hiPSC repositories for human leukocyte antigen (HLA) haplotype representation to facilitate immune-genetic matching is a proposition already being pursued. Of particular interest are individuals who will be homozygous for common HLA haplotypes to maximise prospective histocompatibility matching, although it is important to note that rejection will also be mediated by other non-HLA associated molecules. In the establishment of these resources, health screening, medical history and life style documentation will be important sources of information the help assure the prospective patient safety as described below. However, defining what constitutes a fully functional and 'safe' genetic state is more problematic and may not be resolved by development of autologous hiPSC lines as observed in mouse models. For hESCs derived from surplus IVF embryos, the risk of carrying genetic deficiencies has largely been presumed minimal. This is based on two presumptions: that the infertility of the donors is not in fact a congenital deficiency, and that the culture and manipulation of embryos *in vitro* does not result in genetic and epigenetic perturbations. For hPSC lines in general, it is not possible to screen for cell inheritable genetic or epigenetic conditions that are not known, and these risks are thus tolerated (Advisory Committee on the Safety of Blood, Tissues and Organs [SaBTO] [9]). In the case of some homozygous HLA haplotypes there are also disease associations (see section 4.4).

There is a reasonable prospect that in the near future there will be affordable access to personalized genomic sequence information. If genomic sequence information of banked hiPSC lines were also made openly available to research, then anonymized, or de-identified, donors could ultimately identify cell lines derived from them, or conversely be potentially identified by others [10]. Banking of hiPSC lines may, therefore, require greater attention to systems for preserving donor privacy [11].

■ 3.3 Ongoing donor traceability and management of post-donation disease and adverse events in patient treatment

Ideally, there should be a mechanism that allows a link to be made between cell line and donors, but only in exceptional circumstances such as seeking re-consent or to facilitate reporting of serious post-donation disease e.g., hepatitis C virus, variant Creutzfeldt–Jakob Disease (CJD).

While this should be considered, a risk–benefit analysis should also be carried out taking into account the administrative costs, together with ethical and policy considerations that such a system could impose. Of course, the repository should ensure that there is an effective tracking system for the cellular materials, from reception of tissue to the point of release to users to support internal troubleshooting and to enable management of adverse events in clinical trials (section 6.9). To this end, the donor's informed consent should ideally allow for linkage to medical history and permission to re-contact. Linkage and re-contact will also raise, however, the possibility of donor(s) withdrawal (see section 2 and Appendices 3 & 4).

In cases where the institution that creates the seed stock is a separate entity from the procurement institution, the repository should retain sufficient records to allow traceability to the initial sample, while detailed information relating to procurement process and donor identity may remain with the organisation responsible for procurement (see section 6.8).

■ 3.4 Advantageous capture of biological specimens

In certain jurisdictions it is required that donor blood samples be associated with embryos intended for assisted reproductive treatments. Consequently, there may be blood or other biological specimens associated with some banked embryos and similar arrangements may be in place for some hiPSC lines. While such samples could inform future investigations, they are unlikely to have been consented for this purpose and retention of blood samples from embryo donors may not be the best archive material to use for the purpose of microbial safety testing. In fact samples from the cell line seed stock may be more appropriate for this purpose as proposed by Murdoch *et al.* [8]. For discussion on the consent issues relating to the use of archive tissues for the generation of hiPSC lines see Lomax *et al.* [12].

■ 3.5 Donor medical histories

In a number of jurisdictions a donor medical history may be required that identifies potential hazards in the past of the donor or their family and may also relate to aspects of the donor's lifestyle that may be associated with risk of infection. Repositories may wish to assure themselves that such information is accessible and even collate it in an anonymized, or de-identified, form (i.e., with donor name redacted); however, this may not be possible in some jurisdictions.

If medical histories are not collected at the time of donation, re-contacting donors may be difficult or impossible if, for instance, they have changed location or have become deceased. When establishing requirements for collecting donor medical histories, it is important to decide what information will be useful to collect [8]. This will include risk factors such as sexual activity, drug abuse, cancer and family history of hereditary disease such as familial CJD. Finally, it is important to recognise that the management of donors may vary considerably in different jurisdictions, and in addition, the veracity of information provided by donors on certain risk factors may be difficult to determine. In conclusion, medical histories, in combination with donor virological testing, can be useful to screen out donated tissues carrying higher risk of transmitting certain infections or other disorders, and thereby mitigate against certain risk factors. However, these alone do not necessarily assure safety of cell lines selected for use in clinical products, which will require supplementary risk assessment and testing, as described in sections 4 and 5.

■ 3.6 Disclosure of significant clinical information

In carrying out hPSC research, increasingly large genetic data sets are being generated. These will inevitably contain information on infectious disease and genetic inherited disorders that may be of relevance to the health of the donor and/or their relatives. The return of individual research results and incidental findings should be warranted and supported by informed donor consent, but also by protocols comprehensively detailing the nature of such findings, the mechanisms for disclosure and their management. Ideally, these procedures should be established prior to obtaining informed consent to donate. Moreover, such protocols should be transparent with regard to the conditions for such context-specific and qualified disclosure [5].

■ 3.7 Withdrawal of bio-specimens and/or associated data

Obtaining medical information or other donor information on an ongoing basis constitutes human subjects research, and therefore, the participant has the right to discontinue participation (research withdraw). The extent to which a participant may withdraw will vary depending on the research protocol and applicable laws, but the withdrawal policy should be clearly described in the informed consent document. The following are common examples of withdrawal policies:

- Donors may request that donated embryos for hESC derivation, or somatic cells for hiPSC derivation, may be destroyed. However, it is generally accepted that derived hESC or hiPSC lines may continue to be used, and distributed materials cannot be recalled.
- Donors may request that all individually identifying information be removed from donated samples or resulting cell lines.
- Donors may request that further collection of medical information cease. Policies and legislation vary with regard to the status of medical information already associated with a cell line.
- Donors may request to withdraw consent up to the time their tissue is used to derive a cell line.
- Donors may request that they are no longer to be contacted by researchers.

Any or all of the above provisions may be applicable to a particular hESC or hiPSC line. Typically, donors are offered 'staged' withdrawal options where they may apply one or more of the options above, possibly at different time periods. It is important that the investigator or party responsible for interacting with the donor and the repository have clear procedures and protocols in place to act upon withdrawal requests in a timely and effective manner.

4. Safety assessment of hPSC seed stocks

Whilst microbiological contamination is the most immediately evident hazard from cells intended for human therapy, there are a number of additional factors that should be considered. These include the presence of transformed cells, expression of potentially damaging bioactive molecules and the appearance of novel surface molecules following *in vitro* isolation and culture. The presence of potentially tumorigenic cells is clearly undesirable in a cell culture intended for clinical application. However, the remaining non-microbiological factors are more difficult to evaluate in terms of safety and more experience in the use of hPSC lines will be needed to assess the exact nature of any risk to patients. This section considers the primary biological issues for hPSC lines that will have a critical impact on their safe use in cell-based medicines, and considers approaches to reduce the risk of these hazards employing a risk-based approach.

It is obviously desirable that each stem cell line established for clinical use should be available for use in a broad range of therapies. The specific clinical settings and therapies to be

developed from these seed stocks are unlikely to be known and it is therefore not possible to carry out a full risk analysis that would be needed to determine the testing regime for a cell line used for a wide range of therapies. The testing regime required for release of cell banks will, therefore, inevitably be based on the likely generic hazards associated with cell culture and the specific hazards associated with the origin and specific culture history of each cell line on a case by case basis (see sections 4 & 6). All testing used for release of clinical grade seed stocks should be performed by a qualified and accredited laboratory according to national and/or international regulation and guidance. Similar standards should be applied to any cell banks of partially differentiated or feeder cells.

It is recommended for a manufacturer using a cell line to produce a cell-derived biological product to focus testing and characterization on vials from the master cell bank (MCB) [3]. This practice can make testing regimes more efficient and ensures the MCB is fit, according to current best practice, for production of future working cell banks (WCBs). Additional testing of WCBs should be considered where justified based on science-based risk assessment, such as the risk of an expansion of a viral contaminant from culture reagents or a clonal expansion of karyologically abnormal cells. However, developing guidance [3,14] proposes that alternative strategies may be justified, such as exhaustive testing of each working cell bank as it is produced.

■ 4.1 Microbiological hazards

4.1.1 General considerations on microbiological hazards

A very broad range of microorganisms could potentially contaminate hPSC lines and some may be able to grow in cell culture becoming a permanent and non-cytopathic component of the cell culture. In addition, some of these organisms may have the capacity to transform human cells and present a tumorigenic hazard for clinical use [9]. The primary risk of contamination arises from the donor tissue used to generate the cell line and the associated most likely contaminants will, to some degree, be different for hPSC lines derived from embryos, where contamination from the reproductive tract may need to be considered, compared to hiPSC lines isolated from blood or skin cells. In addition, donor history (section 2) and history of the cell line including storage conditions and detailed records of the reagents used (section), provide the key information to assess risk of contamination for each hPSC line.

This risk assessment can then be used to establish the testing regime for the seed stocks of each cell line. Whilst virological testing of a donor is useful information in risk assessment, it does not guarantee freedom from viral contamination of a cell line derived from that donor's tissue. Thus, in addition to risk mitigation (see section 6.2 & 6.3), microbiological testing of a cell line will provide confidence in its safety for use in humans.

When cells are transferred from supplier to the manufacturer, a different set of conditions and reagents will apply and the appropriate testing regime for MCBs and WCBs established for generating the cell therapy product, will need to be reassessed. Moreover, regulators are likely to expect fully qualified cell banks for manufacturing purposes, as recommended for banks of cells used in other aspects of manufacturing [3,13]. With this in mind some stem cell line repositories may choose to perform testing on seed stock cell banks only for the most serious potential contaminants, whilst others may carry out a broader range of testing on their cells.

Highly sensitive molecular and cell culture based assays have been established and qualified for the evaluation of cells used in the manufacture of vaccines and biotherapeutics [3,13]. However, it is important to recognise that current qualified methods are not sufficiently broad ranging to provide an absolute guarantee of absence of microbial contamination. Deep sequencing technologies and microarray technologies [14–16] offer significant potential advances in the detection of virtually any agent in cell cultures, as has been demonstrated in cells used for vaccine manufacture [17,18]. However, they have yet to be proven and validated for use with cell banks for clinical use. Repositories should keep a 'watching brief' on emerging technologies and engage with their developer to assemble and analyze data that may be useful for clinical validation. Currently, such novel techniques lack appropriate validation for detection of different types of agents. It will be necessary to have widely available control materials and procedures to manage unqualified data as developed by WHO for sequencing [19], and by the Minimum Information About a Microarray Experiment (MAIME) workgroup [20]) to provide minimal datasets from microarrays for interpreting and assessing reproducibility of experiments.

4.1.2 Microbiological testing

The following sections discuss the typical microbiological tests that should be considered for seed stocks of hPSC lines intended for clinical use and an example of a possible core testing regime

for a seed stock of hPSC is provided in Appendix 6 (of note, this is a guide only to key issues and each repository must take responsibility for risk assessment and the final testing regime). ‘Next Generation Sequencing’ (NGS) offers powerful methodologies for the identification of any contaminant including organisms unknown to science. However, care is required in interpreting data as widely available control materials and qualification data are yet to be established. Accordingly, the real value of a negative or a positive result may be uncertain. However, it has proved useful to pick up positive signals which must be verified by standardized and established techniques.

Virological testing

Current established testing regimes do not enable routine release assays for detection of all known viral agents, and a risk assessment should be performed to ensure that tests for the most likely contaminants are applied based on risk associated with the origin and culture history of the cell line (see section 6.8). As already described, the more complete the documentation for the culture history of the hPSC line, the more robust the risk assessment can be and this in turn reduces the dependency on the cell bank safety testing regime.

The risk of contamination of cell therapies by abnormal prion protein can be mitigated by:

- Ensuring that any potentially contaminated culture reagents are traceable to low risk source materials.
- Sequencing of the associated prion gene to identify any cell types with mutations more susceptible to conversion to the abnormal state.
- Testing regimes for particular abnormal proteins of concern.
- Demonstrating failure of prion agents to survive and multiply in cell lines selected for development of cell therapies.

The WHO has published suitable risk assessment procedures to enable selection of source tissue of low risk [21], and this has been reflected in European guidance [22,23].

Repositories should ensure they have access to expert microbiological advice, usually in the form of an expert advisory group, which provides assistance in establishing local testing regimes. It is also beneficial for repositories to coordinate such activities to enable them to keep abreast of developments in emerging diseases and

experience with contamination. It is important for banks to evaluate the risks associated with reagents (e.g., growth factors; see section 6.3) and ensure the appropriate sourcing of components of lowest microbiological risk – especially for reagents such as serum and trypsin, where the reagent cannot be sterilized.

Sterility testing

Standard methods for sterility testing are published by national authorities including the United States Pharmacopeia (USP), and the European Pharmacopeia (EP). Each repository should comply with its own national pharmacopoeia. However, these protocols are aimed to detect breaches in aseptic processing and typically do not use culture conditions that would enable isolation of some more fastidious organisms that could proliferate in the complex media and conditions of cell culture. Additional detection methods may need to be considered to detect such organisms where they are considered to be a special hazard in the local environment or particular reagents. It is important to emphasise that antibiotics should not be used in culture media before sterility or mycoplasma testing is performed. In addition, antibiotics and antifungal agents should not be used in preparation of cells intended for therapy.

Mycoplasma testing

Standard methods based on Vero cell inoculation/DNA stain and culture isolation methods are published in USP, EP and other pharmacopoeia. Polymerase chain reaction (PCR) methods are published and certain assay systems are accepted by the European Pharmacopeia but are not necessarily represented in all national pharmacopoeia [24,25].

Nested PCR may give greater sensitivity of detection, however, it can also give rise to false negatives. Direct quantitative PCR (qPCR) applied to inoculated mycoplasma broths may provide significant advantages regarding sensitivity. Whichever method is selected, as for all analytical methods it will need to be qualified, and in routine testing working reference materials should be established (e.g. DNA preparations, quantified suspensions of organisms) to monitor sensitivity of testing over time.

■ Genetic stability

4.2.1 General considerations on genetic stability

Genetic changes that are known to occur in cultured hPSC lines [26–28] could have a number of deleterious effects including loss of

functional characteristics and transformation into a tumorigenic state [29,30]. Cell lines in culture are known to be karyologically variable, and even human diploid fibroblasts, noted for their karyological stability, show subtle mutations when analysed by single nucleotide polymorphism (SNP) arrays [31–36]. Non-diploid karyotypes are sometimes seen in apparently ‘normal’ tissues. While the significance of such karyologically abnormal cells *in vitro* is yet to be determined they are considered a potentially serious issue for cells intended for implantation into humans. SNP variation in non-pluripotent cells such as fibroblasts, mentioned above, could identify a baseline for genetic stability, but such base-lines may well vary with cell type and culture conditions.

The degree of genetic stability of cultured cell lines intended for cell therapy should be a consideration in their selection, however, as already indicated, no cell line is likely to be absolutely stable in its genetic make-up when passaged *in vitro*. Risk associated with genetic instability can be minimized by limiting the time and number of passages *in vitro* (of note, cumulative population doublings should be used if these can be determined), and risk assessments should include consideration of the influence of any changes or variation in culture conditions.

It has been clearly demonstrated that genetic changes occur in the early phase of hiPSC line derivation [37,38] and such changes may give a selective advantage for *in vitro* culture [39,40]. Selection of methods of hPSC line isolation that minimize the risk of such changes should be a significant consideration in cell line development and selection of hPSC lines to be banked for clinical application.

There is also evidence that culture conditions and passaging methods can dramatically influence the genetic stability of stem cells, even over relatively short culture periods [40,41]. Accordingly, a means of monitoring genomic stability is important for cell bank testing. Karyotyping by Geimsa banding is the technique most commonly performed, as this can identify changes in chromosomal numbers as well as translocations and other rearrangements. Demonstration of maintenance of a diploid karyotype at a certain passage number (e.g., every ten passages or equivalent population doublings) will be of value. Array comparative genomic hybridization is now increasingly used in clinical diagnosis and offers significant benefits in terms of the size of genetic lesions that can be detected, although it will not recognise some aberrations such as balanced translocations.

Other genomic information derived from techniques, such as chromosome painting to identify aberrant chromosomes (e.g., spectral karyotyping, fluorescent *in situ* hybridization [FISH]) and deep sequencing can also be considered [42–46], however the sensitivity of these methods should be evaluated alongside the level of resolution of genetic changes and the availability of suitable controls. Analysis of wide ranging gene expression profiles has also been proposed as a means of virtual karyotyping and detection of genetic instability [47].

It may be useful to perform copy number analysis of certain sequences since there is evidence that specific lesions (deletions and duplications) are found repeatedly at specific genomic regions [47]. Copy number analysis can be performed using SNP or comparative genomic hybridization microarray analysis, as well as sequencing across the region of interest. However, the biological significance of gain or loss of small regions of the genome remains to be defined and such changes may arise in the donor population [37].

The epigenetic status of undifferentiated pluripotent stem cell lines has been widely investigated, but it is currently difficult to set standards for stem cells [48,49]. DNA methylation studies have not yielded clear and consistent results with respect to stability. However, it is known that culture conditions can strongly influence DNA methylation [50–53]. Microarrays now allow affordable high-resolution genome-wide DNA methylation analysis [52]. In the case of hiPSCs created from somatic cells, DNA methylation patterns might be an approach to determine whether cells have been completely reprogrammed from parental lines. For a review of epigenetic instability in hPSC lines see [26].

As part of the evaluation of a stem cell line for its suitability to deliver cell therapies, it will also be helpful to demonstrate that it is possible to passage the cell line up to or beyond the number of population doublings under conditions which replicate or simulate the actual production culture expansion process. Such qualification and testing (e.g., phenotype, ultrastructure, virology) is prescribed by the WHO for cell substrates used for the manufacture of therapeutics and vaccines, which also considered the potential requirements for evaluation of stem cell lines for use in humans [3] (see also section 8.1).

4.2.2 Genetic stability testing

The requirement for karyological testing of seed stock may differ from the requirements for final product cells used in the manufacturing process.

The requirement for karyological analysis of seed stocks will depend on the characteristics of the cell line in question (e.g., its degree of genetic stability). It is considered sufficient for seed stocks that data on 20 Geimsa-banded metaphase spreads be provided and to have chromosome counts on a further ten metaphase spreads, as proposed for research grade cell lines [1]. This will enable the detection of karyologically abnormal cells at the level of 5%, although certain abnormalities may not be detected.

Certain levels of genetic abnormality may be acceptable in undifferentiated seed stocks, provided there are procedures that eliminate abnormal cells or any related hazard in cells for final clinical use. The recommended criteria for karyological screening of seed stocks is given in TABLE 1. However, cells to be used in cell therapy products will need to be evaluated on a case by case basis with respect to the karyotype.

Whilst karyology is the current reference method for evaluating genome integrity, it may not be sensitive to small genetic changes. A number of important new techniques for characterising the genome include spectral karyotyping, comparative genome hybridization (CGH) microarray, SNP microarrays, and whole genome sequencing. These offer the opportunity to analyze and understand changes in the genome at different levels of resolution. While these are still essentially research tools, CGH microarray is now becoming qualified for diagnosis of genetic disorders [54] and could be the first of these techniques used for lot release by stem cell repositories. However, it should be noted that this technique does not detect balanced translocations and it is best practice that any genetic aberration detected, is validated using FISH. In general, these techniques could benefit the characterization of stem cell lines intended for clinical use, but would be for 'information only' rather than release criteria.

A better understanding of the levels and types of genetic instability of each type of cell culture and the potential impact on safety of the final product will clearly be important but is still developing. Repositories of stem cell lines should keep abreast of current developments e.g. through recruitment of appropriate experts for their advisory board.

■ 4.3 Tumorigenicity versus pluripotency

General considerations on evaluation of tumorigenicity

The inoculation of cells into an immune-compromised host animal has been used for many

years to evaluate the ability of different cell types to form or cause tumors as an indication of potential risk associated with the use of such cells to make therapeutic products and vaccines. Animal cells have been considered to have two types of capability to cause malignancy: first, tumorigenicity, by which the cells grow in a host organism in an uncontrolled way to create masses of cells; and second, oncogenicity, by which cells or the components of cells are able to induce malignant growth of the host organisms cells. Clear definitions for tumorigenicity and oncogenicity have been established for such testing in cells used for manufacture of products [3] and also proposed for use in cell therapy [2]. The same types of test methods are also used to assess the potential pluripotency of stem cell lines and some methodologies have been proposed as standards for assessing this property of hPSC lines [55]. The reproducibility and standardization of assays has been debated for many years [56], but if they are to be used it is important for the investigator to be absolutely clear on the objective of the test and standardized methodology for the intended purpose (tumorigenicity, oncogenicity or pluripotency), and to have clear criteria for assessment of the results. Of course, it should not be forgotten that the utility of teratoma formation from hPSC lines in mice is not just in the assessment of tumorigenicity, but also in providing potentially valuable tools for investigation of early human development [57].

4.3.1 Tumorigenicity testing

As for pluripotency testing (below), there has been tremendous variation in assays for *in vivo* tumorigenicity testing. The minimum inoculum dose is not standardized, but in many protocols 10^6 – 10^7 cells are injected, in clusters, per animal. It is believed that the preparation of the cells and the site of inoculation could have a significant influence on results [58,59]. The strain of mouse could also influence the outcome of tumorigenicity assays due to differences in physiology and immune status. In the ISCBI survey (see Appendix 9) seven different strains of immune-deficient mice were reported in use, some of which retain certain immune cell functions. For tumorigenicity testing mouse strains with multiple immune deficiencies, including lack of functional T- and B-lymphocytes and NK cells are recommended, including NOG (NOD/Shi-scid/IL2R γ null) [60,61] and also the NGS [263]. In addition, the time period of observation of inoculated animal and its predisposition to develop spontaneous tumors may also affect results of

Table 1. Standard methods, procedures and recommended terms for the reporting of the karyological analysis of undifferentiated human pluripotent stem cells.

Karyological analysis of pluripotent stem cells	
Standard Geimsa-band analysis	Examination of metaphases with eight metaphases analyzed (minimum) and 20 metaphases counted (ISCBI, 2009)
Clonal abnormal findings	Confirmation of clonal chromosome abnormalities in a later cell culture passage or calculated population doublings
Abnormalities observed in single cells	Aneuploidy of chromosomes Aneuploidy of chromosomes can be observed in pluripotent cell lines with most common occurrence for chromosomes 1,8,12,14,17 and X Analysis of a minimum of 30 G-banded cells counted from initial culture (ISCBI, 2009) Follow-up analysis of a further 30 G-banded cells taken from a later passage cell culture in combination with the examination of 100 interphases using fluorescent <i>in situ</i> hybridization (FISH) with a relevant probe Other aneuploidy and structural abnormalities Analysis of a minimum of 30 G-banded cells counted from initial culture
Minimum quality score	Minimal level of G-banding analysis for hESC lines for research purposes was published previously (ISCBI, 2009) and was developed from the International System for Human Cytogenetic Nomenclature (ISCN) in which analysis to Band level 400 was recommended with an expectation that analysis of band level 500 or above would be attempted See also Professional Guidelines for Clinical Cytogenetics General Best Practice Guidelines (2007) v1.04 March 2007
Sub-standard analysis	Failure to attain an ISCN 400 level of banding can be reported with the proviso that the analysis may need to be repeated
Reporting the results	The report should contain: The karyotype description stated using the current ISCN nomenclature 2009 The type of analysis used e.g., fluorescent <i>in situ</i> hybridization, type of banding The average banding level attained Single cells displaying aneuploidy or structural anomalies should be reported. Cells should be analyzed again after extended passaging (or high population doublings) in culture to investigate and interpret the abnormality
Definition of terms (taken from the Association for Clinical Cytogenetics Professional Guidelines for Clinical Cytogenetics, General Best Practice Guidelines [2007] v1.04)	Analyze: To count a metaphase and compare every chromosome, band for band, with its homologue and to verify the banding pattern of the X and Y-chromosomes in male karyotypes. Clone: A cell population originally derived from a single progenitor cell. Such cells will have an identical chromosome constitution. Generally, in cytogenetics, a clone is said to exist if three cells have lost the same chromosome, or two cells contain the same extra or rearranged chromosome. Count: To enumerate the total number of chromosomes in any given metaphase, or in FISH analysis to enumerate the number of signals in an interphase nucleus. Examine: To look for the presence or absence of any abnormality in a case. Score/screen: To check for the presence or absence of abnormalities in a cell or metaphase without full analysis.

Adapted from [1].

tumorigenicity assays. A standardized method was recently published by the WHO for evaluation of tumorigenicity in cells used for vaccine and biotherapeutic manufacture [3], but whatever method is used it will need to be optimized for detection of tumorigenicity in pluripotent stem cell lines.

The role of assays specified to optimise detection of potentially malignant tumorigenic cells has not yet been established for hPSC lines. Teratoma assays established to evaluate pluripotent potential of a culture are not designed to detect low levels of transformed malignant

cells. However, the possibility to detect such cells present at a significant level in *in vivo* pluripotency assays should be born in mind when reviewing teratoma assay data. For *in vivo* tumorigenicity testing it will be important for such analysis to be performed by a qualified histologist familiar with the morphologies of teratoma (benign) and teratocarcinoma (malignant) cytology and tumor formation. In addition, as prescribed for general good cell culture practice (GCCP) [63], it may also be valuable to carry out routine microscopical screening of cultures for abnormal cells.

Specially designed tumorigenicity assays that can detect low levels of tumorigenic cells, will also be important for cell therapy products [64,65]; however, this is out of the scope of the current document.

■ 4.4 Genetic disorders

4.4.1 General considerations on inherited genetic disorders

The genomes of any donor of tissue for generation of hPSCs, will contain sequences that are associated with predisposition to disease. However, it is relatively rare that such sequences become expressed in the individual's phenotype, or otherwise develop (such as disease associated with expansion of DNA microsatellite repeats), and cause disease in the individual carrying the affected sequence. In addition, certain HLA allele haplotypes have autoimmune disease associations (e.g., diabetes, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, celiac disease), but obviously donors with the disease-associated HLA alleles do not necessarily develop disease.

The detection of a genetic attribute or variation in a donor is likely to mean that this is present in the stem cell line. However, as already mentioned, pluripotent stem cell lines are known to acquire genetic and epigenetic changes during derivation and culture, thus, they may have more potential abnormalities than may be found in the donor. The real level of risk from these or other identified disease associated genetic variants to the functionality of cell therapies is uncertain. A possible exception to this may be where tumor suppressor genes, oncogenes or miRNA genes are altered or overexpressed, rendering the host cell potentially tumorigenic [66]. This obviously would need to be considered in safety assessment of the cellular products intended for therapy.

4.4.2 Genetic screening for disease-associated sequences

As discussed above and in section 3, the final impact of a genetic or epigenetic lesion in the donor in most cases will be unknown and testing for disease associated genetic variations will generally not be helpful, unless the donor comes from a genetic line or population that suffers from a genetically inherited trait [9]. Current experience in therapeutic transmission of disease predisposition is currently limited to cell and tissue transplantation, predominantly from one donor to one recipient. Future experience with single cell lines developed for many patients will be needed to identify any real genetic risk

factors. However, as also briefly discussed in section 3, it may be useful to screen for altered genes (oncogenes, growth factors, etc.) in cell lines. The Center for iPS Research and Application (CiRA) Institute in Kyoto has published a list of oncogenes as a basis for such screening of hPSC lines, and microarray technology provides the means to do this routinely. Whole genome sequencing of cell lines intended for clinical use is generally agreed to be desirable to develop our scientific understanding of these cell types and repositories should seek to develop such data. However, given the issues of potential for donor identification (see above), repositories should establish policies and procedures for release of such data, that will oblige recipients of repository data to use it in a way that would not increase risk of donor identification [11]. Furthermore, in order to avoid presenting misleading data on cells for clinical use, repositories should also seek to assure that best practice has been applied in developing any genetic data they publicise. In particular, whole genome sequencing still requires development of appropriate standardization, without which the data should be considered to be research data for information only and not necessarily relevant at this stage to establish suitability of lines for clinical application.

5. Characterization of hPSC seed stocks

■ 5.1 Cell identity

It is part of GCCP [63] to authenticate cell lines. Cell line authentication is a critical step in the banking process, assuring that a cell line is not cross-contaminated by another line or otherwise misidentified. Methodologies for individual specific genetic identification have been standardized within the field of forensics, and commercial services and kits are readily accessible as described in the guidance on research grade cells [1]. These kits typically comprise primers for up to 16 short tandem repeat (STR) DNA alleles with 5 or more of these alleles in common which can be utilized to facilitate direct comparison of cell line profiles even when generated by different repositories using different kits (see [1] for a comparison of STR alleles shared between commercial kits). Such comparisons are not so readily achieved using other genetic identity testing techniques such as SNP analysis. It is advised that the STR testing be performed in accordance with the Authentication of Human Cell Lines standard ANSI/ATCC ASN-0002–2011 [67,201]. This standard advises the use of 8 STR loci with a match threshold of 80% to ensure

specific identification of the line. Reporting of DNA profile data should be considered carefully as donors could be identified [11,68].

In the case of multiple cell lines isolated from the same embryo or donor tissue, DNA fingerprinting is not likely to discriminate between such cell lines. It is important that such clones are identified clearly in their naming [69]. However, some means of demonstrating their unique identity will be required and if this is not possible by molecular analysis the mechanisms used to ensure the physical isolation of cell lines during culture should manage the risk of lines that have the same identity profile, becoming switched (see section 6.4).

■ Viability and measurement of growth

Special care should be given to choosing the time point at which viability tests are performed, as tests taken immediately after thawing may overestimate viability. It is therefore important for the repository to gain experience in assessing post-thaw viability and survival of colonies under its own culture conditions. Regulators and others have addressed the idea of setting acceptability limits for viability, but this has proven difficult as it may be process and cell type-dependent. A range of other tests such as propidium iodide, neutral red assay, fluorescein diacetate or alamar blue may be used, but each give data on a different aspect of cellular function. Other regulatory guidance on cell substrates used for manufacturing purposes [3], councils that the method of viability testing, and the levels of viability considered acceptable, should be established based on their suitability for the specific cell types in question and scientific knowledge of the cell type. This latter position is especially relevant for stem cell lines. Finally, it is important to recognise that viability does not necessarily predict desired functionality of a cell preparation, which must be demonstrated by other means (see section 5.3 & 5.4).

The nature of growth measurements will depend on whether cells are passaged as single cell suspensions or colony fragments. Single cell suspension passage is the more convenient and more efficient technique, but will require validation in each laboratory to assure that the genetic stability and pluripotent potential of the stem cell lines is not affected. Growth rate is an important characteristic that needs to be monitored using population doublings where possible, as an increase in cell replication rate may indicate transformation. Switching growth medium may affect growth rate, but this would typically

be reversible on return to original medium, if the cells have not become transformed or permanently altered in some other way. Alkaline phosphatase-positive colony-forming assays may also be useful for quantitation of growth of stem cell lines [70].

■ 5.3 Characterization of gene and antigen expression

Characterization of gene and antigen expression provides useful fundamental information on cell state and the variability and consistency of cultures, especially where assays allow many targets to be evaluated simultaneously as in microarrays (e.g., whole genome expression arrays [Illumina, Agilent or Affymetrix], TaqMan™ Low Density Array cards, Scorecard™ [LifeTechnologies]) and the multi-fluorochrome labelling of cells. There are a range of antibody-based markers that are used for identification of different stem cell types [71] and further markers may be useful to qualify the nature and state of pluripotent stem cells [72].

It is well known that pluripotent stem cell cultures vary in gene and antigen expression from one passage to another [73], but a stem cell repository should seek to set acceptable ranges for expression in the culture systems they use. Typical surface antigen markers that may be used to monitor phenotypic stability are indicated in Appendix 6. Control cell cultures are useful to run in parallel with undifferentiated cell lines and in number of settings the 2102Ep embryonal carcinoma cell line has been recommended for this purpose as it shows stable expression of common hPSC markers [73–75]. However, pluripotency assays have greater value in that they provide an indication that the relevant functional capabilities of a pluripotent stem cell line remain unaffected by the banking process (Appendix 6).

To assure the quality of reprogrammed cells it is important to demonstrate that expression of exogenous reprogramming factors has been silenced or removed. In retroviral systems, that are unlikely to be used in cells for clinical application, incomplete silencing is an indicator of partial reprogramming and checks for sustained silencing of exogenous factors may be needed with less optimal vector systems. For non-integrating reprogramming vectors, which in theory are the most promising for clinical applications [76,77], it is important to demonstrate silencing and removal of the original exogenous expression system (episomal viral construct or mRNA). Accordingly, both antibody- and qPCR-based

test methods are available for the commercially available reprogramming kits and qualification of the sensitivity of these methods would be needed if iPSC for lines were to be considered for clinical applications. It should be born in mind that non-integrating virus constructs may persist for a number of passages and testing is typically performed between passage 5–10 after an iPSC line has been established (see also Appendix 6).

■ 5.4 Pluripotency assays

5.4.1 General considerations on pluripotency

Teratoma assays to evaluate the pluripotency of stem cell lines provide a valuable characterization of the key functional feature of these cells (i.e., the benign tumors exhibit tissue representing all three germ layers required to form the human body). However, responses to a survey by the International Stem Cell Initiative (ISCI; see Appendix 9 for details) and other reports [79] have revealed significant variation in methodologies used to perform the teratoma assay, which might be expected to influence the ability to compare data from different Centers directly. The range of parameters that may affect the reliability of teratoma data, including the strain of mouse used, are consistent with those which may influence tumorigenicity assays as discussed in section 4.3.2. An approach to develop a standardized tumorigenicity assay has been proposed by Gropp *et al.* [55].

A number of papers have been published [78–80] proposing assays using a transcriptome-based bioinformatic approach. Alternative ways of analysing the pluripotent properties of cells is an active area of investigation, and methods including gene expression profiling of differentiating cells *in vitro* in embryoid bodies or earlier phases of induced differentiation, or the analysis of epigenetic status [52,81,82] are being considered. Pluripotency can also be characterized by formation of embryoid bodies *in vitro* and gene expression or immunological marking of the three germ layers, or use of directed differentiation protocols. These are also being used in combination with gene expression systems to provide assays that could replace the use of teratomas [56].

5.4.2 Pluripotency testing

Pluripotency assays can be used to give an indication that the cell line has not been altered by *in vitro* culture, although it should be recognised that they are not conclusive for pluripotency in this respect (i.e., demonstrate the cell lines capability to generate all cells of the adult human body or that the cell retains normal

differentiation pathways). Testing using one or a combination of assays for pluripotent potential qualified by the stem cell repository (see Appendix 6) may, therefore, give an indication that the cell line has not been affected by its derivation and culture history and retains a potentially broad range of capability for cell therapy. Conversely, it may be concluded that a purported pluripotent cell line that fails to demonstrate potential pluripotency may have been isolated from cells that were not fully pluripotent or has undergone deleterious changes during isolation and culture. For this reason, and also to assure broad potential applicability in therapy, it is therefore recommended that stem cell lines should be assessed for pluripotency.

At this time it is not possible to make firm conclusions about the most suitable methods to use as a pluripotency assay for seed stocks intended for clinical use. Stem cell line repositories will need to consider what method is most appropriate to confirm the desired characteristics of the cells they release. Ideally, more than one assay type would be used, that in combination reveal different aspects of pluripotency, that is, the ability to show molecular evidence for the ability to commit to all three germ line lineages, but also to create cells representative of certain tissue phenotypes typical of the three germ lineages.

6. Regulation and quality assurance

■ Quality assurance: general principles

Stem cell repositories providing cells intended for use in humans require an established quality assurance (QA) procedure providing a formal methodology and due diligence, designed to afford adequate confidence that the entire operation will fulfil expected and defined requirements for quality of seed stocks of pluripotent stem lines. A quality management system (QMS) should be implemented that describes the organisational structure, responsibilities, policies, procedures, processes and resources required for QA [84]. The QMS should be based on the principles of current good manufacturing practice (cGMP) [83–87], and should consider relevant local regulatory requirements and guidance. However, such systems are not necessarily required to be performed under a GMP manufacturing license, but should meet a certain standard (such as the European Union Tissues and Cells Directive, EUTCD [88], which assures suitability of the stem cell repositories for clinical application and critically establishes traceability for all materials and procedures

used from the point of informed consent for procurement of primary tissue, to the final seed stocks. All critical procedures used in delivery of the seed stocks should be documented as formally recorded standard operating procedures (SOPs), associated forms and higher level documents such as policies, process descriptions covering a number of SOPs, manuals and training documents. All critical records should be controlled to assure that only the correct and current procedures and forms are used and that old versions are archived carefully to allow review and audit in the future. Regulatory requirements will also apply to storage and retention times for the repository's critical records including those for procurement, facilities, staff training, banking, testing, storage and distribution.

Definitions of terms used in QA are important to enable the user to comply with the regulation. Appendix 7 shows examples of such definitions but it should be born in mind that, whilst the terminologies used are broadly consistent, there can be significant differences and the user is advised to check the national or locally applicable terms.

■ 6.2 Risk analysis

Stem cell repositories should adopt an appropriate risk evaluation model to identify and manage risk within the operation. This process usually involves the maintenance of a risk register to ensure the ongoing monitoring of risk. Repositories should use risk management to ensure effort in assessing risk is appropriately focused. While not limited to these items, a risk management system should as a minimum:

- Identify and evaluate risks and compile a risk register (of note, risk assessment of reagents and processes can be managed within the Quality System [see section 6.1]);
- Score and prioritize risks;
- Assess residual risk after application of controls already in place;
- Develop action plans for any unacceptable residual risks;
- Regularly review for change and identify new risks.

New risks may be identified through various routes such as regulatory alerts and reviews of emerging diseases. Stem cell repository scientific advisory boards should be used to help identify new risks as part of their horizon scanning activity.

■ 6.3 Risk assessment of donor tissues and critical reagents

6.3.1 Donor tissues

Key issues and approaches to microbiological risk assessment of donor tissues have already been considered in section 4.1.1. In addition, evidence for lack of susceptibility of stem cells to certain agents can be used to give confidence in suitability for clinical use, but these susceptibility profiles have yet to be established for pluripotent cells and their differentiated progeny.

Recommendations for the evaluation of cell substrates for production of biologicals, including vaccines and biotherapeutics [3,14] have identified key issues for risk evaluation of cell lines, and these may be helpful in establishing testing regimes for seed stocks of hPSC lines. The WHO document [3] has also addressed some of the key issues for evaluation of stem cell lines for the manufacture of biological products (see section 8.1). However, regulatory documents intended for use with the manufacture of different kinds of products should be used with caution to avoid implementation of inappropriate or unnecessary quality control and safety testing procedures.

6.3.2 Critical reagents

Critical reagents in the preparation of seed stocks of hPSC lines, for the purposes of this document, include those materials used in the generation of hPSC lines and the production of cell banks that come in direct contact with, or otherwise could have a critical influence on, the properties and safety of the resulting seed stocks. Process maps, such as that given in FIGURE 1, are valuable in enabling a complete understanding of the derivation and cell banking process (and any other process to which they are applied), including identification of all critical reagents used and key points where cells may be exposed to contamination.

Repositories should establish a specification and acceptability criteria for all raw materials, including the original cell lines if not generated by the repository itself. They should also consider auditing suppliers of raw materials [89,90] to assure compliance with these specifications. This can be an extremely burdensome process and may need to be managed, such that the repositories resource for performing its own audits can then be focused by risk assessment. These should address risk factors such as the absence of formal supplier audit, inappropriate or inadequate QA and suppliers of complex biological reagents of biological origin.

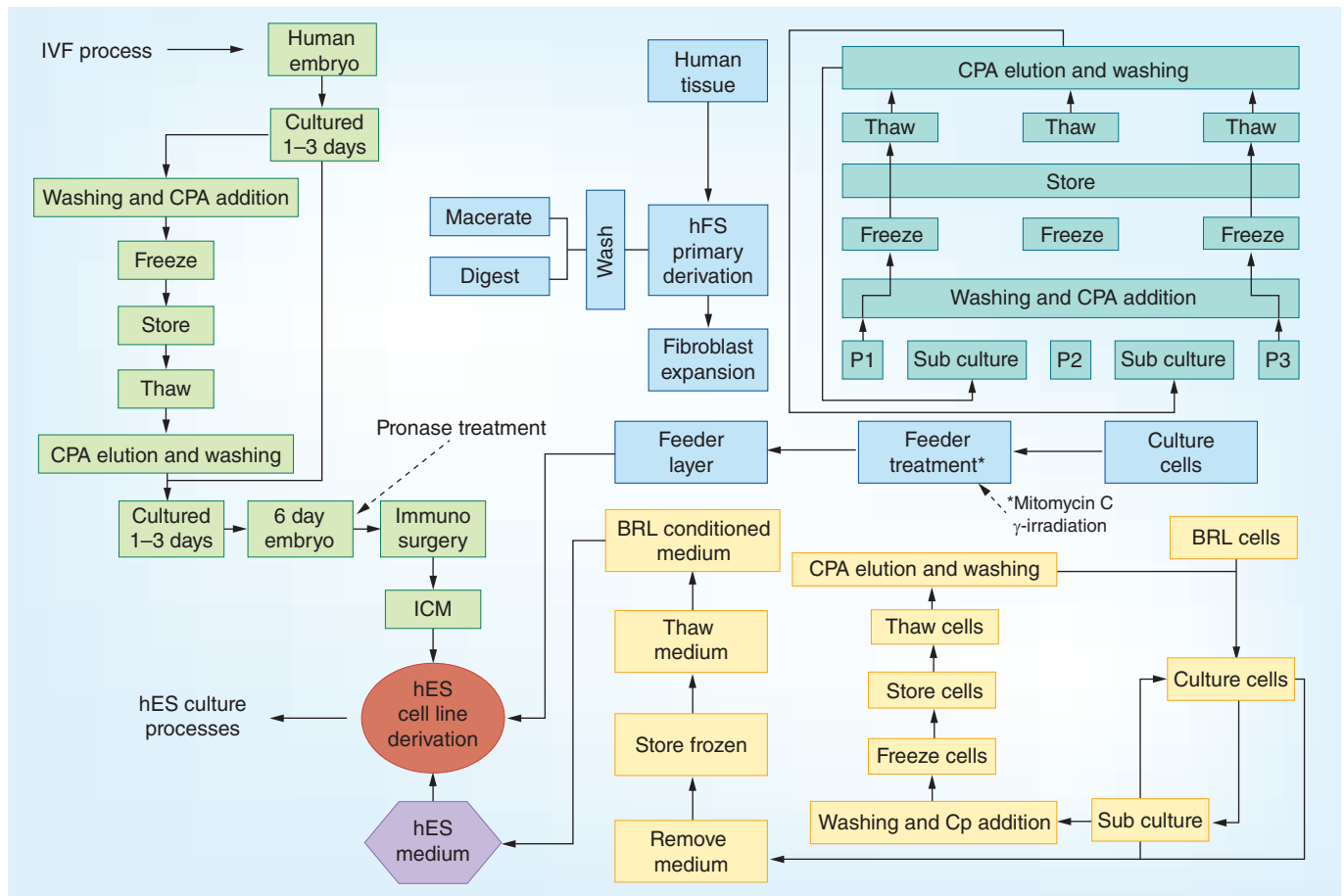


Figure 1. Example of a process map for derivation of a human embryonic stem cell line.

Courtesy of C Hunt, UK Stem Cell Bank, NIBSC, 2013.

It is important to establish a 'document trail' for critical reagents. The documents should be available from the supplier, who ideally should be able to trace the source of raw materials used, how they are processed, treated and shipped. However, this may not always be the case. Accordingly, the development of a supplier questionnaire should be considered. With this in mind the following list, while not exhaustive nor necessarily sufficient for any particular regulator process, is intended as a guide to the kinds of issues that may need to be addressed when soliciting information from a supplier (section 6.8) and assist in prioritizing the need for a repository to audit suppliers as discussed above:

- Details of the supplier: name, address, telephone number, principal contact and position;
- General information:
 - Description of function e.g., manufacturer, distributor etc.
 - Does the supplier sub-contract, and if yes,

how is control of the subcontract and materials achieved?

- Is there a supplier audit programme or vendor rating scheme in place, and how is this monitored?
- Are customers informed of changes to their products and how is this information transmitted?
- Quality Management System (QMS):
 - Is there a QMS in place?
 - Is there an internal audit programme in place?
 - Is there a document control system in place?
 - Is quality documentation issued with the product (e.g., Certificates of analysis)?
 - Where applicable, are certificates for animal derived/origin products provided?
 - Are there procedures in place for calibration, verification and maintenance of equipment.
 - Is there a procedure to communicate regulatory alerts to customers?
- Product Specification questions:
 - Name of product/catalogue number
 - Is QC performed on the product and is this

- carried out by the supplier themselves?
- What type of QC is carried out and what are the pass/fail criteria?

In order for the questionnaire to be of value it should also include the date it was completed and details of the person completing it on behalf of the supplier and any relevant documentary evidence to support the answers to the questions.

Supplies of cells used to facilitate the culture of hPSC lines (e.g., feeder cells, cells used to make cell-conditioned medium or other product) should also be subjected to similar evaluation and risk assessment.

■ 6.4 Seed stock and clinical trial cell bank production and labelling

The suggested structure for an appropriate two-tier cell banking system (MCBs and WCBs, see section 4) is outlined in ISCBI [1]. Sufficient vial numbers should be established to meet anticipated demand for seed stock cell supply and testing that may be required in the near future (i.e., next 5–10 years). Contingency to allocate seed stock vials for additional testing that may be needed will be important. Furthermore, past experience in cell banking for cell lines used to manufacture vaccines and biotherapeutic products, has shown that it can be extremely valuable to allow for some additional production contingency vials. While it is difficult to prescribe numbers of these additional vials, some contingency will enable immediate response to a sudden increased demand for testing or for production cells and avoid delays caused by re-banking in the future.

If repositories are providing cell banks that are to be used to provide material direct into a clinical application (e.g., clinical trial, EU hospital exemption) they would usually be expected to do so under a Manufacturing License with GMP accreditation. This requires careful environmental controls [91] and other more specific requirements, depending on the local jurisdiction [84,87,92]. A glossary of terms commonly used in GMP production can be found in Appendix 7. However, it is important to note that precise definitions of particular words in this glossary may vary between regulators, accordingly, Appendix 7 is provided as an example only. Repositories should be aware of local and international regulatory requirements, which will apply to all aspects of the facility, including movement of staff and materials, staff health status and other activities or services which in particular, could introduce contamination.

It is essential to assure that cell lines do not become switched or transmit microbial contamination to other cells used in the banking and storage facility. Accurate labelling (see below) and documentation of cell handling processes are clearly vital to this and in addition preparation of cell banks of different cell lines on a 'campaign' basis (i.e., one cell line per laboratory at any one time with qualified cleaning completed between banking events).

All repository systems and equipment that may affect the final seed stock quality must be monitored for operation between limits established for validation (section 6.5), and alarmed to warn when out of specified conditions. Where temperature limits are key to the process (e.g., to prevent storage at inappropriate temperatures) the equipment should be alarmed and upper (and where appropriate lower) limits set. Alarms for other parameters, (e.g., low liquid nitrogen [LN₂] levels, failure of LN₂ supply) should also be in place.

Importantly, stem cell lines and products incorporating viable cells cannot be terminally sterilized, and it is therefore vital that the conditions of cell banking do not introduce microbiological contamination or permit growth of any microorganisms that might already be present. Cell culture rooms must be operated to ensure environmental contamination is controlled to acceptable levels prescribed in appropriate legislation [83,84,86,88]. In addition, documented procedural controls will be required to reduce the risk of introducing or spreading contamination and cell banking records should be able demonstrate that the appropriate procedures were used in each case. Both physical and chemical means of disinfection may be employed as appropriate for specific facilities and equipment. The cleaning and disinfection procedures should also be validated to show they are effective against likely contaminants.

Labelling is a critical element in assuring traceability of materials. Repositories should aim to adopt appropriate labelling systems to fit the developing norms for supply of cells for clinical use. The Information Standard for Blood and Transplant (ISBT) 128 system [202] developed in the USA by the American Association of Tissue Banks, is now being considered as a model in other countries and whilst unmodified hPSCs are not intended to be used directly as therapeutic products, this example could be considered as the basis of best practice for labelling containers of individual release lots of stem cell lines.

■ 6.5 Validation

All repository processes, equipment and facilities should be validated to demonstrate they are fit for their intended purpose. Validation is the documented act of ensuring that any procedure, process, equipment, material, activity or system actually gives the expected results with adequate reproducibility [87]. This approach should include implementation of the key elements of validation including a user requirement specification (URS), impact/risk assessments, and a series of qualification stages for equipment (i.e. design qualification [DQ], installation qualification [IQ], operational qualification [OQ], and performance qualification [PQ]). Repositories may also use a validation master plan that describes the overall philosophy, strategy, and methodology for validation, and which equipment, processes and other items require validation. A validation matrix or schedule of validation will also be useful to document which organisation or contractor is responsible for each item subjected to validation. It is important that risk assessments are performed in advance of validation to ensure critical areas are targeted and that any validation performed is appropriate and optimised in terms of use of resource. Due to commonality of operations this is an area where exchange of learning experiences between repositories can help to reduce the burden of QA.

Validation should be considered for any equipment used that may impact on the suitability of the cell banks for clinical use, such as that used in processing, cleaning, environmental monitoring, storage and shipment. Equipment such as controlled-rate freezers, mechanical refrigerators, LN₂ storage refrigerators and dry-shippers will require appropriate monitoring, such as continuous temperature monitoring and recording when in use, to demonstrate that the required conditions are maintained. Shipment devices, such as 'dry shippers', will also require validation to assure fitness for purpose. Critical equipment such as heating, ventilation and air conditioning (HVAC), biological safety cabinets, particle counters, incubators and cold storage should be validated. The Pharmaceutical Inspection Cooperation Scheme [203] and WHO [87] both provide guidance on related validation, and compliance with national regulation.

Process validation in particular should be considered on a case-by-case basis. Validation of routine expansion and banking of cell lines will need to take many factors into account, including the number and type of interventions required, the culture format being used (e.g., open or closed system), transfers between

processing areas and incubators, and the impact of different operators and different cabinets/rooms. Within the banking process, the cryopreservation process itself should be validated to demonstrate that cells recovered from cryopreservation have the characteristics set out in the repository's cell bank release specification for cell lines.

■ 6.6 Qualification and standardization of test methods and reagents

Establishing the testing regime for seed stock banks has been described and discussed in section 4 and Appendix 6. All tests used to establish suitability of hPSC seed stocks for clinical use should be qualified for use. This qualification should address requirements, including but not necessarily restricted to, sensitivity, specificity and also potential for effects (such as test inhibition) by the hPSC sample components. This is most readily achieved by supplying samples to testing laboratories accredited for the tests in question. Where such accredited testing is not available the repository should be able to provide qualification data for the tests performed. Accredited services may be available that can provide tests that meet multiple or harmonised pharmacopoeia requirements and these may be required where the cell line is to be used internationally [93].

Well established surface markers and a wide range of gene markers are used in stem cell characterization, and selected reference materials for their assay may be useful (e.g., fixed cell preparations, RNA preparations). Standardized functional assays will need to be developed, and in particular standardized pluripotency assays will be important to progress in the field as assays and reagents vary between laboratories. The ISCI has focused on a number of relevant issues in this area, including the initial identification of standard markers for hESC lines [73]. This group has also begun to work on determination of pluripotency in hPSC lines and further international collaborative effort is required in this important aspect of pluripotent stem cell research, which is fundamental to supporting high-quality research data (see www.stem-cell-forum.net). For an overview on standards in the cell therapy area see Sheridan *et al.* [95] and for an overview on cell characterization for cell therapy see PAS 93 [93].

Of note, where reagents of biological origin are clinical products in their own right, standardization of their biological activity is

often performed under the auspices of WHO and its Expert Committee on Biological Standardization [205]. Most of the WHO International Reference Materials (IRMs) are made and distributed by the National Institute for Biological Standards and Control (a center of the Medicines and Health-care Products Regulatory Authority [MHRA]) and a listing of these materials can be found on the National Institute for Biological Standards and Control website [206].

Standardization of certain reagents such as growth factors used in cell culture may also be helpful to enhance reproducibility of cultures of hPSC lines. This can in part be achieved by the repository establishing specifications and acceptance criteria for the properties of complex cell culture components. In addition, cell culture assays and control materials can be established to determine batch consistency in supplies of such factors. Where such reagents are used widely it may be feasible to establish international reference materials (see previous paragraph). Furthermore, for certain reagents there are Pharmacopeia reference methods for their characterization.

■ 6.7 Auditing suppliers and service providers

An important element in assuring traceability, safety, and thus suitability for repositories of hPSCs, is the performance of audits of suppliers of critical reagents and services that would impact on the final quality of the cell lines offered for clinical use. Such audits may range from a paper-based audit (which may be justified where suppliers operate under relevant and independently inspected quality standards) to a detailed on-site inspection of procedures and documentation. The sharing of such audits between repositories could provide both cost- and time-saving benefits. However, implementing such a scheme would be challenging and repositories would need to be confident in the ability of any third party auditor and in the consistency of the auditing procedure between repositories. Recruiting a common auditor with appropriate training and expertise using a common audit protocol is a possible solution. Such an auditor should have previous experience with inspecting similar facilities and operations and should have a regulatory background. Alternatively, repositories may decide only to use suppliers who are registered and inspected by a recognised regulatory body; however, this should be done using a risk-based approach.

■ 6.8 Cell line ‘history file’

Careful evaluation of the information associated with a stem cell line is necessary to determine its suitability for developing a clinical product. Where the repository has derived the hPSC line it can collate this information directly under its own QMS. However, where this is not the case it is important to avoid wasting time and resource on unsuitable cell lines, thus, stem cell repositories should request relevant historical information from the depositor and continue to build a documented history pertaining to each cell line as it is processed and banked. This compiled documentation, sometimes called a cell line ‘history file’, should provide all information necessary to enable traceability of cell line establishment and processing, from the derivation and original transport to the repository, through banking, testing, storage and any subsequent distribution. This history file should also include evidence that the cell banking was performed under principles of GMP or other suitable conditions where a GMP manufacturing license is not applicable (i.e., early seed stocks where a final product is not identified, whereas MCBs and WCBs for specific clinical applications in a clinical trial or under Hospital Exemption arrangements, would probably be required to be prepared under a GMP manufacturing license). For example, the EU directive on tissues and cells for use in humans [88] is based on the principles of GMP, but a manufacturing license under EU GMP is not required for cells and tissue intended for human application including seed stocks of hPSC lines. Some of the key aspects that should be considered for inclusion in a cell line history file are given in TABLE 2. Whilst it is unlikely to be feasible to include all raw data and original information, the history file should at least facilitate traceability to that information. Where the cell repository receives the cell line from a depositor working under a suitable quality system, the repository may decide that a documented audit (physical site audit or paper based) along with traceability (typically an anonymized link) to the donor and appropriate informed consent may be sufficient. Where such links are not possible the repository will need to carry out a risk assessment with respect to the acceptability of that line within its own jurisdiction and if contingencies cannot be put in place to resolve significant risks then the repository may decide not to receive the line or supply it for restricted purposes such as for laboratory research only.

Table 2. Examples of information that may be required in a cell line history file.

Section	Typical content
Depositor information	Name of owner of cell line Address (registered company and manufacturing sites where applicable) Primary contact Telephone number(s) Evidence of ownership*
Shipping records	Signed records of inventory shipped and cross check of received goods, including 'chain of custody' documentation Records of temperature monitoring data Record of courier used Record of arrival at repository including transport time/temperature and condition on receipt
Provenance	Donor information related to the donation of primary tissue** Original, anonymized donor consent and medical history (this may not always be available depending on national laws and regulations)
Culture/banking details	Description of the culture conditions related to (where applicable): tissue or embryo culture; cell line derivation; cell line expansion; reagent documentation, traceability and cryopreservation. This should include, for example, passage number (or population doublings where possible) of seed lots and subsequent banks that were created up to the point of manufacture relevant to the material being received by the repository
Quality control test results	Characterization and safety test results both provided by the depositor and generated by the repository and given with associated passage or population doubling levels
Facility and equipment details	Qualification records: records of use, maintenance, calibration, validation, re-verification, repair
Environmental monitoring records	Records of and trends in scores of contamination for testing applied to the environmental conditions, which may include: viable and non-viable particle counts; active air sampling, air pressures, temperature, relative humidity, operator finger dabs, ambient temperatures in critical storage areas
Deviations from standard procedures (SOPs)	Records of deviations from normal procedure, which may affect the specific cell line, for example failure of an incubator in which the line was processed
Change controls	Records of change control investigations relevant to the cell line, for example impact of changes to QC test specifications or moving storage location of cryopreserved material
Records of staff training and illness of an infectious nature	Records of training and return to work procedures to ensure staff infectious status is not a risk to cell cultures
<p>*There is a risk to final clinical utility of a particular cell line if all potential owners are not identified at an early stage. Thus, it is important to obtain accurate information from the cell provider, about all parties with a potential interest in ownership of the cell line (e.g., sponsors of research, host organisation, principle investigator) and to confirm, first, that they are in agreement with the repository receiving and distributing the cells, and second, whether they need to be a signatory party to the deposit of the cell line in the repository.</p> <p>**Detailed donor information may be held by the repository, but special care will obviously need to be taken (and may be a legal requirement) for its control and security. For example, in the UK the Caldicot Principles apply to the management of sensitive patient data [215].</p>	

Over long periods of time, after the seed stocks of cells have been released, quality control data may become summarized and/or archived by suppliers and service providers, which means that its retrieval from the original source is not practicable or not possible. It is therefore important to endeavor to anticipate the kinds of critical information that may be required many years into the future (e.g., details of quality control, information on production processes, safety testing data), and obtain and store copies of this from the respective sources (e.g., raw material manufacturers, testing companies) when the cell line is banked, to form part of the cell line 'history file' whether the cells are stem cell lines or some other propagatable cell type.

■ 6.9 Serious adverse reaction (SAR) and serious adverse event (SAE) reporting

Events may arise during the provision of cells for therapy that indicate potential risk to patients. Whenever such events are identified, they are required to be investigated for impact on the patient and if necessary action taken to minimise the impact and prevent re-occurrence. Two kinds of event are generally recognised, a serious adverse reaction (SAR) and a serious adverse event (SAE). Whilst definitions of these may vary significantly between regulators, a SAR usually refers to a serious adverse reaction related to treatment of a patient receiving the therapy and a SAE refers to any other occurrences that might have an impact on patients receiving the

therapy. Repositories clearly need to be aware of the regulatory definitions that apply to them.

Most countries have established systems for reporting post-donation disease and adverse events in clinical trials. Repositories supplying cells that may be used for human application should be coordinated within these systems to ensure that SARs and SAEs related to subsequent final products can be traced back through the repository and ultimately to the primary tissue donor to enable full investigation of the potential causes. Establishment of mechanisms to assure traceability are critical in the development of seed stocks, as already discussed extensively throughout the earlier sections of this document.

Stem cell repositories supplying cells for clinical use will be expected in the first instance to identify, investigate and report SAEs occurring in the banking process, which might affect the suitability of the cells for clinical use. Second, they will also be expected to submit to regulatory investigations when SARs or SAEs occur in clinical applications using cells they have supplied. In such cases, they will be expected to demonstrate full traceability on the procurement, banking, testing, storage and supply for the cells in question. It is vital that stem cell repositories understand their responsibilities in these situations and how to manage them through appropriate elements of their QMS.

Within Europe, the Rapid Alert system for human Tissues and Cells (RATC) has been implemented whereby manufacturers (including 'tissue establishments' providing cells and tissue as starting materials for cell therapies) and distributors of medicinal products (including advanced therapy medicinal products [ATMPs]) are required to report all SARs for medicinal products (licensed, unlicensed and clinical trial products) to their national competent authority within a defined time period under RATC [207].

In the EU each national competent authority reports incidents to the Europe-wide pharmacovigilance web-based AE/AR collection system EudraVigilance which is managed by the European Medicines Agency (EMA). In the USA, the FDA runs MedWatch [209] for reporting and monitoring adverse reactions. This includes specific guidance for human cell- and cellular-based tissue products. EU member states are also required to report all adverse incidents to the WHO international drug monitoring programme and this is done by the national competent authority. The WHO maintains an international system for monitoring adverse

reactions to drugs using information derived from Member States within and beyond the EU. The system is run and coordinated by the Uppsala Monitoring Center (UMC) in Sweden (www.who.umc.org). Similar requirements apply in other jurisdictions and a list of notified bodies in different countries is given in

TABLE 3 [208].

Stem cell repositories should consider the International Conference on Harmonisation (ICH) guidance on efficacy, which includes guidance for pharmacovigilance planning and definitions and standards for preparing and submitting safety reports [209]. Guidance can also be obtained from the Council for International Organisations of Medical Sciences (CIOMS) [210], which was jointly established by the WHO and the United Nations Educational Scientific and Cultural Organisation.

■ 6.10 Disaster recovery, contingency planning and legacy management

It is necessary that procedures for disaster recovery are in place to manage unforeseen events that may severely impact on repository critical operations (e.g., fire, flood, loss of power, failure of liquid nitrogen supply). Repositories should at least maintain some local backup storage system such as splitting storage of stocks over different equipment and locations. Such backups must be maintained under the same conditions as the main stocks. Where possible repositories should encourage and advise depositors to secure their own cell stocks for backup in this way. Records of banking inventories should also be backed up and other critical repository documentation on cell bank production either backed up or adequately secured. In addition, it is necessary to ensure that contingency plans are in place to secure the continued availability of stored cell lines for appropriate periods of time in the event of normal repository operations being discontinued. These procedures can be delivered within a risk management system as outlined in section 6.2.

A course of action should also be defined in the event of a planned termination of the repository (such as an orderly wind-down when the facility is transferred elsewhere) or an emergency termination (including loss of key resources, funding or regulatory approval). It will also be important to distinguish between obligations regarding cells intended for human application and cells held for research, since the standards and conditions required for both cells and associated records will be different for each.

Table 3. National competent authorities for serious adverse event and serious adverse reaction reporting.

Country	National competent authority	Program/website
Australia	Therapeutic Goods Administration	www.tga.gov.au
Brazil	ANVISA	http://portal.anvisa.gov.br/wps/portal/anvisa-ingles
Canada	Health Canada	www.hc-sc.gc.ca/index-eng.php
China	National Institutes for Food and Drug Control National Centre for ADR Monitoring	www.nicpbp.org.cn/en/CL0309
European	European Commission Rapid Alert system for human Tissues and Cells	http://ec.europa.eu/health/blood_tissues_organ/docs/ratc_report_2008_2012_en.pdf
Finland	Finnish Medicines Agency	www.fimea.fi/frontpage
France	French National Agency of Medicine and Health Products Safety, ANSM	ansm.sante.fr/Produits-de-sante/Medicaments
Germany	Federal Institute for Drugs and Medical Devices	www.bfarm.de www.bfarm.de/EN/Home/home_node.html (English)
India	Indian Pharmacopoeia Commission	www.ipc.gov.in
Israel	Israeli Ministry of Health	www.health.gov.il/english
Japan	The Pharmaceuticals and Medical Devices Agency	www.pmda.go.jp/english
Netherlands	Pharmacovigilance Centre Lareb	www.lareb.nl
Singapore	Health Sciences Authority	www.hsa.gov.sg
South Korea	MFDS	www.mfds.gov.kr
Spain	Spanish Medicines and Health Products Agency	www.aemps.gob.es/en
Sweden	Medical Products Agency	www.lakemedelsverket.se
Taiwan	Bureau of Medical Affairs, Department of Health and Center for Drug Evaluation	www.fda.gov.tw
Thailand	US FDA, Drug Information Centre and NADRM	www.fda.moph.go.th
UK	Medicines and Healthcare Regulatory Agency	www.mhra.gov.uk
USA	US FDA	www.fda.gov

■ 6.11 Regulation in different countries

The regulation for cell-based therapies is still at an early stage of development, and progress in establishing formal regulatory frameworks varies across jurisdictions [96]. As cell therapy products are being developed, manufacturers will aim to market their products in different countries, making knowledge of the differences in regulatory frameworks of vital importance. A comparison of the regulatory frameworks in the EU and the USA has been published by the British Standards Institute (PAS 83) [94]. The ISCF section on the ISCF website has also developed information on the national regulatory bodies (TABLE 3) and donor selection procedures in different countries (see Appendix 4), and provides relevant policy statements by the ISCF Ethics Working Party on cell banking procedures [5,11]. Some countries have developed regulatory route maps to help national cell/tissue repositories, hospitals, and industry negotiate the regulatory landscape, and a toolkit used in the UK for stem cell therapy [211]. A route map regarding the Canadian regulatory framework for the development of stem cell-based therapies has been developed under the auspices of the Canadian Stem Cell Network [212].

7. Preservation and storage

■ 7.1 Cryopreservation of hPSC lines

Cells can be stored in a stable state through the application of appropriate cryopreservation protocols [96]. Cryopreservation includes a number of processing steps both before low-temperature storage and again at thawing and culture of the cryopreserved material. In addition, material must be stored and transported under conditions that maintain material stability. Cryopreservation protocols generally fall into two types: those that incur the formation of ice within the system, whether intracellular or extracellular (i.e. freezing) and those that avoid ice formation (i.e. vitrification). For a review of cryopreservation and vitrification methods [97].

In applying or designing an effective cryopreservation process, there are a number of key technical issues that should be considered:

- Methods for assessing recovery of cells from the cryopreservation process
- Choice of cryoprotective agent (CPA)
- Choice of container and packaging
- Mode of cryopreservation (i.e., freezing vs vitrification)

- Method of cooling (passive vs controlled rate cooling)
- Storage conditions
- Transportation of cryopreserved material
- Recovery process (i.e., rewarming and elution of cryoprotectant)

7.1.1 Assessing recovery from cryopreservation

In order to design or optimise any cryopreservation protocol, an assessment of recovery is required. Tests using trypan blue or fluorescent compounds such as acridine orange/ propidium iodide are often referred to as 'viability tests', but are more truly membrane integrity tests [98]. The accuracy of these tests in indicating normal function of the cell, particularly the complex requirements of hESCs in culture, is arguable. Such tests may over- or under-estimate the ability of cells to survive, attach, proliferate and maintain the undifferentiated state and differentiate into the required cell type. Furthermore, cells that still show membrane integrity at the time of thawing may die later by apoptosis. Such tests should not be employed in isolation. It may be necessary to consider evaluation and quantification of the viable material at a point sometime after thawing, such as 24 or 48 h post-thaw. Consideration should also be given to use of a range of tests, including appropriate functional assays, when assessing recovery from cryopreservation [98].

7.1.2 Choice of cryoprotectant

In choosing an appropriate CPA, consideration should be given to any known specific effect on the cells e.g., cytoskeleton effects, membrane effects, induction of cell differentiation. In order to provide protection, cells must be equilibrated in the CPA solution prior to the application of cooling. CPAs can be toxic to cells and consideration must be given to the intrinsic toxicity of standard compounds which is time, temperature and concentration dependent, whether using a controlled rate freezing method or vitrification [99]. Additives to the solution (e.g., serum) should be assessed for their ability to mitigate these and other effects.

Cryoprotectant solutions will exert an osmotic effect during their addition to and elution from the cells. If uncontrolled, such effects can be damaging and compromise cell survival. Osmotic damage can be reduced or eliminated by the use of step-wise addition and elution protocols. Single step protocols (e.g., centrifugation and

re-suspension in medium containing cryoprotectant) should be assessed for their effect on survival. Step-wise or slow addition or elution protocols should take into account the likelihood of incurring damage from CPA toxicity.

7.1.3 Choice of primary container

For cell suspensions, the choice of primary container will generally be conditional on the mode of cryopreservation. The most practical and generally acceptable options currently available are straws, vials and bags. Each option should be assessed for its suitability not only for the mode of cryopreservation (e.g., whether or not the required cooling rate is achievable) but also its ability to prevent or reduce contamination (primarily during cooling and storage), and its compliance with regulatory guidelines (such as requirements for labelling of the primary container). The use of open systems is not considered best practice and represents a hazard to stored cells (see below).

The primary techniques and methods available for preservation of hPSC lines are described by Hunt [100] in Appendix 8. Further expert opinion on preservation technologies can be found in Day and Stacey [101] and the recently published informational general chapter 'Cryopreservation of Cells available in Pharmacopeial Forum section 39(2)' [213].

7.1.4 Storage conditions

Scientific evidence suggests that storage at ultra-low, sub-zero temperatures (generally accepted to mean storage in or above liquid nitrogen) does not result in significant deterioration of material over extended periods of time (measured in decades, for a review see [102], provided that the temperature remains stable and uniform. This may be extended to mechanical refrigeration at temperatures at or below -160°C. Storage in mechanical freezers at -80 to -85°C is acceptable for short periods of time if the sample is to be, or has been, preserved by freezing, but is likely to result in potentially damaging ice formation in vitrified samples. If storage at this temperature is considered necessary, the period of storage should be validated to show that the cells do not demonstrate any adverse effects. Storage above -80°C is not recommended. For vitrified material, temperatures above, or repeated cycling through, the glass transition temperature (approximately -130°C) should be avoided to prevent progressive formation of ice crystal nuclei.

The most stable conditions for storing cells at ultra-low temperatures are provided by storage

under LN₂. 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 LN₂ 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 LN₂ 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 LN₂ 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 LN₂ 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 LN₂ (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 cryogenics become depleted.

Cells cryopreserved by slow cooling may be transported in dry ice. Vitrified material should not be transported in dry ice (solid CO₂) at -79°C, to avoid de-vitrification and cell damage. Cells cryopreserved by either method may be transported in LN₂ 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.			

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Appendices

Appendix 1

Appendix 1 (a). Compliance and provenance determination.		
(1) Embryo provenance determination	Code	Considerations
<ul style="list-style-type: none"> ▪ (a) Independent review and oversight 	<ul style="list-style-type: none"> ▪ B 	<ul style="list-style-type: none"> ▪ 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
<ul style="list-style-type: none"> ▪ (b) Voluntary informed consent 	<ul style="list-style-type: none"> ▪ B 	<ul style="list-style-type: none"> ▪ 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
<ul style="list-style-type: none"> ▪ (c) Gratuitous donation 	<ul style="list-style-type: none"> ▪ B 	<ul style="list-style-type: none"> ▪ Banks should receive assurance that donors were not paid for embryos or storage costs
(2) Compliance determination	Code	Considerations
<ul style="list-style-type: none"> ▪ (a1) Embryo was donated in a jurisdiction with no explicit prohibition on hESC derivation 	<ul style="list-style-type: none"> ▪ B 	<ul style="list-style-type: none"> ▪ Accepting embryos from jurisdictions where hESC research is restricted may incur legal liability
<ul style="list-style-type: none"> ▪ (a2) Derivation protocol confirms to any unique legal requirements in jurisdiction where hESC derived 	<ul style="list-style-type: none"> ▪ B 	<ul style="list-style-type: none"> ▪ Jurisdiction may have unique requirements in addition to international standards for research ethics (e.g., embryo research oversight or licensing); Consistent with 1a
<ul style="list-style-type: none"> ▪ (b) Any line derived using IVF for research purposes, parthenogenesis or SCNT is identified 	<ul style="list-style-type: none"> ▪ B 	<ul style="list-style-type: none"> ▪ The use of hESC lines derived from embryos created for research purposes are prohibited by some jurisdictions and funding bodies
<ul style="list-style-type: none"> ▪ (c) Consent requirement for third-party gamete donors 	<ul style="list-style-type: none"> ▪ A 	<ul style="list-style-type: none"> ▪ Some donated embryos may have been created using gametes from someone other than the embryo donor; Some jurisdictions require consent from third-party donors
<ul style="list-style-type: none"> ▪ (c1) hESC lines derived from embryos intended for reproductive use where a third-party donor(s) was contracted to provide gametes 	<ul style="list-style-type: none"> ▪ A 	<ul style="list-style-type: none"> ▪ Bank or entity performing hESC derivation should review donor/recipient contract for any conditions that would restrict research use
<ul style="list-style-type: none"> ▪ (c2) hESC lines derived from embryos for which gamete donor(s) participated in egg sharing or exchange programs are identified 	<ul style="list-style-type: none"> ▪ A 	<ul style="list-style-type: none"> ▪ Policies regarding the use of such embryos or resulting hESC lines are variable. Bank should review egg-sharing contract or exchange policies
<ul style="list-style-type: none"> ▪ (c3) hESC lines derived from embryos created with anonymous gamete donation are identified 	<ul style="list-style-type: none"> ▪ A 	<ul style="list-style-type: none"> ▪ 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
<ul style="list-style-type: none"> ▪ (d) Donor medical history 	<ul style="list-style-type: none"> ▪ A/PU 	<ul style="list-style-type: none"> ▪ 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 derivatives.

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 derivatives, 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

Appendix 3. Compliance determination: specific issues to consider for hESCs

Prior to initiation of hESC derivation protocol or intent to bank a hESC line, the following compliance issues should be considered (see Appendix 1 to 3).

Appendix 3. Compliance determination: specific issues to consider for hESCs.	
Embryo donation/ hESC derivation	Some jurisdictions explicitly prohibit the derivation of hESC from human embryos. It is not uncommon for individuals residing in prohibitive jurisdictions to inquire about research donation to outside research centers or banks. Embryos originating from prohibitive jurisdiction should not be used for the derivation of hESC lines if an explicit prohibition is/was effective at the time of donation.
IVF for research purposes & parthenogenesis	Some national, sub-national jurisdictions or funding organizations impose limits on hESC line eligibility. For example, certain jurisdictions have adopted explicit policies determining which hESC lines may be used in research, including requiring that such lines only be derived from embryos that were created using <i>in vitro</i> fertilization for reproductive purposes and were no longer needed for this purpose. This reproductive use requirement prevents the use of IVF to develop hESC lines specifically for clinical application or the use of parthenogenetic lines. Consequently, lines derived from oocytes (parthinodes) or embryos created for non-reproductive use should be identified as such.
Special considerations for third-party gametes	<p>Most established hESC lines have been derived from embryos that were created using <i>in vitro</i> fertilization for reproductive purposes and were no longer needed for this purpose. Gametes used in the creation of reproductive embryos frequently come from intimate partners. There are, however, a proportion of embryos created with gametes from third-party donors. The conditions surrounding the procurement of third-party gametes may influence the compliance determination and should be documented to the extent feasible. Potential factors to consider include the following:</p> <ul style="list-style-type: none"> • Paid gamete donation: oocyte and sperm donors are routinely financially compensated. Some policies limit the use of hESC lines derived from embryos for which gamete donors were paid [4]. Banks should be aware of any payment or financial compensation restrictions in their jurisdiction. In addition, it should be noted that certain funding organizations have restrictions on the use of hESC lines derived from embryos where gamete donors were financially compensate beyond the reimbursement of expenses. • Use restrictions: it is also advisable to review the donor contract to support provenance determination and ensure there is no clause in the contract that the resulting embryos be used exclusively by the couple to which they were donated or otherwise restricting research use. • Oocyte sharing/exchange programs: various mechanisms exist for the financing of fertility treatment. One mechanism is 'egg sharing' where fertility treatment costs are reduced for the donor who consents to donating a portion of her oocytes to other women seeking treatment for infertility. Jurisdictional variations exist in the interpretation of this kind of arrangement as a financial incentive, compensation or payment. <p>It is important to note that the applicability of the above factors relating to third-party gametes will vary by national, local or supra-national jurisdictions. For instance, all embryos created using <i>in vitro</i> fertilization for reproductive purposes and no longer needed for this purpose are potentially eligible in some jurisdictions regardless of third-party donor payment or exchange. However, in other jurisdictions hESC lines derived from embryos for which a gamete donor(s) were paid are not eligible for research or funding. Documentation of the factors above by the banking entity will enable end users to determine if specific lines are eligible for use in their jurisdiction, but such documentation should not be viewed as essential prerequisite for banking.</p>

Appendix 4. Donor screening protocols for assisted reproductive treatments

The majority of existing hESC lines have been derived from embryos intended for assisted reproductive treatments (ART). Cells differentiated from hESC lines have been utilized in clinical trials after extensive safety evaluation by national regulatory bodies. These evaluations incorporate the donors' medical history and tests that are required in the context of ART treatments for screening low-risk donors of gametes. Consequently, there is no evidence at this time to support the need for further screening of donors of embryos used to derive clinical grade hESC lines [8].

Screening assays occurring prior to hESC derivation should be documented. It is sufficient to verify testing was performed in accordance with prescribed regulatory requirements. For instance, gamete donation (from non-intimate partners) is generally regulated as a biological product and, therefore, subject to both donor infectious disease testing and sample screening (21 CFR part 1271, subpart C, 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). Verifying tests performed (as opposed obtaining quantitative results) is sufficient. Testing and screening regulations have evolved over time, so the bank should seek to document the specific screening requirements in place at time of gamete donation.

ART embryos created with anonymous gametes donors should be acceptable for clinical use provided that first, the donor contract is sufficient to support provenance determination (see section 1 Governance and Ethics); and second, gametes and/or gamete donor were subject to any required screening and testing for relevant communicable disease agents and diseases (see section 4).

Appendix 4. hESC lines: additional donor screening and medical records.

Third-party (allogeneic) donation from ART	In the case of gamete donation for the purpose of embryo creation, medical history requirements may vary depending on the relationship between the donors and the individuals undergoing ART treatment as well as jurisdictional policy. A third-party gamete donor would typically undergo medical screening and a medical history will be obtained. Entities deriving hESC lines have demonstrated the ability to obtain third-party medical history information (www.cirm.ca.gov/CIRMCeLLines). Researchers deriving new hESC lines should inquire about the availability of medical history information. Due to privacy and contractual considerations it is generally not possible to re-contact third-party donors. Again, it should be noted that donor-screening requirements have evolved over time, so it is critical to document the time when gamete donation occurred.
Self (autologous) donation from ART	Embryos created from the gametes of sexually intimate partners for self-reproductive use are not necessarily subject to the same screening requirements as third-party (allogeneic) donation. Resulting ART embryos are generally regulated in a manner consistent with requirements for autologous human transplantation. In this case, the individual(s) donating the embryo(s) for hESC derivation are the gamete donors. A medical history is generally performed in the context of ART treatment and may be available. A medical screening and history may also be obtained at time of embryo donation with donor consent. There is evidence from hESC derivation protocols that donors may consent to (1) being re-contacted in the future or (2) allow linkage to their medical records [8]. Consequently, entities deriving or banking clinical grade lines should examine the possibility of donor re-contact and record linkage options when possible.
Gamete donation for research purposes	Blastocysts may also be made specifically for research using assisted reproductive technologies. In this case, it is recommended to obtain a medical history at the time of gamete donation to inform risk assessment. When available, banks should associate anonymous medical history with the banked hESC lines. Banks may also seek to determine whether the donor(s) of gametes used to derive the hESC line underwent a previous medical screening or history consistent with requirements for tissue intended for allogeneic human transplantation. The nature and extent of such screening should be documented.

Appendix 5. Donor selection, eligibility, release criteria and screening procedures: normative and institutional documents

Appendix 5.	
CANADA	<p>Standard Z.900.1 "Cells, Tissues and Organs for Transplantation: General requirements". Canadian Standards Association. (2nd edition under review)</p> <p>Safety of Human Cells, Tissues and Organs for Transplantation Regulations (SOR/2007–118) (Enabling Statute is the Food and Drug Act)</p> <p>http://www.laws.justice.gc.ca/en/SOR-2007-118/FullText.html</p> <p>Guidance Document for Cell, Tissue and Organ Establishments (Safety of Human Cells, Tissues and Organs for Transplantation- April 6th, 2009)</p> <p>http://www.hc-sc.gc.ca/dhp-mps/alt_formats/hpfb-dgpsa/pdf/brgtherap/cell/cto_gd_ld-eng.pdf</p> <p>Transplantation Registration Application Form</p> <p>http://www.hc-sc.gc.ca/dhp-mps/alt_formats/hpfb-dgpsa/pdf/compli-conform/frm_0171-eng.pdf</p> <p>Annex E (normative) Exclusionary Criteria for Risk Factors Associated with HIV, HBV, and HCV</p> <p>http://www.hc-sc.gc.ca/dhp-mps/alt_formats/hpfb-dgpsa/pdf/brgtherap/cto-reg-annexe-eng.pdf</p> <p>Regulations Amending the Food and Drug Regulations (1024—Clinical Trials) (Division 5: Drugs for Clinical Trials Involving Human Subjects)</p> <p>http://www.hc-sc.gc.ca/dhp-mps/compli-conform/clin-pract-prat/reg/1024-eng.php</p> <p>Canadian Institute for Health Research Updated Guidelines for Human Pluripotent Stem Cell Research 2010.</p>
FRANCE	<p>Bioethics Law (2004)</p> <p>Arrêté du 21 décembre 2005 pris en application des articles R. 1211–14, R. 1211–15, R. 1211–16 et R. 1211–21 du code de la santé publique</p> <p>http://www.legifrance.gouv.fr/affichTexte.do?cidTexte=JORFTEXT000000456466&dateTexte=Dcret_n_2005-1618_du_21_d%C3%A9cembre_2005_relatif_aux_r%C3%A8gles_de_s%C3%A9curit%C3%A9_sanitaire_portant_sur_le_pr%C3%A9l%C3%A8vement_et_l%27utilisation_des_%C3%A9l%C3%A9ments_et_produits_du_corps_humain_et_modifiant_le_code_de_la_sant%C3%A9_publique_(partie_r%C3%A9glementaire)</p> <p>http://www.legifrance.gouv.fr/affichTexte.do?cidTexte=JORFTEXT000000636261&dateTexte=Arr%C3%AAt%C3%A9_du_11_avril_2008_relatif_aux_r%C3%A8gles_de_bonnes_pratiques_cliniques_et_biologiques_d%27assistance_m%C3%A9dicale_%C3%A0_la_procr%C3%A9ation</p> <p>http://www.legifrance.gouv.fr/affichTexte.do?cidTexte=JORFTEXT000018829426&dateTexte=Dcret_n_2008-588_du_19_juin_2008_transposant_en_mati%C3%A8re_de_don_de_gam%C3%A8tes_et_d%27assistance_m%C3%A9dicale_%C3%A0_la_procr%C3%A9ation_la_directive_2004/23/CE_du_Parlement_europ%C3%A9en_et_du_Conseil_du_31_mars_2004</p> <p>http://www.legifrance.gouv.fr/affichTexte.do?cidTexte=JORFTEXT000019060568&dateTexte=</p>
UNITED STATES	<p>Guidance for Industry. Eligibility Determination for Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps)</p> <p>http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Tissue/ucm091345.pdf</p> <p>International Compilation of Human Research Standards (2012)</p> <p>http://www.hhs.gov/ohrp/international/intlcompilation/intlcompilation.html</p>
SINGAPORE	<p>Guidelines for Healthcare Institutions Providing Tissue Banking: Regulation 4 of the Private Hospitals and Medical Clinics Regulation</p> <p>http://www.moh.gov.sg/mohcorp/uploadedFiles/Publications/Guidelines/institutions_providing_tissue_banking_guidelines.pdf</p> <p>Medicines Act (Chapter 176, ss. 18 and 74) Medicines (Clinical Trials) Regulations</p> <p>http://www.hsa.gov.sg/publish/etc/medialib/hsa_library/health_products_regulation/legislation/medicines_act.Par.41439.File.dat/MEDICINES%20(CLINICAL%20TRIALS)%20REGULATIONS.pdf</p> <p>Medical (Therapy, Education and Research) Act</p> <p>http://statutes.agc.gov.sg/non_version/cgi-bin/cgi_retrieve.pl?actno=REVED-175&doctitle=MEDICAL%20%28THERAPY%2c%20EDUCATION%20AND%20RESEARCH%29%20ACT%0a&date=latest&method=part</p> <p>Human Organ Transplant Act</p> <p>http://statutes.agc.gov.sg/non_version/cgi-bin/cgi_retrieve.pl?actno=REVED-131A&doctitle=HUMAN%20ORGAN%20TRANSPLANT%20ACT%0a&date=latest&method=part&sl=1</p>

Appendix 5.	
SPAIN	<p>Real Decreto 1301/2006 (10 Noviembre, 2006) por el que se establecen las normas de calidad y seguridad para la donación, la obtención, la evaluación, el procesamiento, la preservación, el almacenamiento y la distribución de células y tejidos humanos y se aprueban las normas de coordinación y funcionamiento para su uso en humanos.</p> <p>Ley 14/2006 (26 Mayo, 2006) sobre técnicas de reproducción humana asistida.</p> <p>Real Decreto 65/2006 (30 Mayo, 2006) por el que se establecen requisitos para la importación y exportación de muestras biológicas.</p> <p>Plan Nacional de Sangre de Cordón Umbilical. http://www.ont.es/infesp/DocumentosDeConsenso/plannscu.pdf</p> <p>Programa de Garantía de Calidad en el proceso de donación. Organización Nacional de Trasplantes. http://www.ont.es/infesp/Paginas/ProgramadeGarantiadeCalidad.aspx</p> <p>Real Decreto 2132/2004 begin_of_the_skype_highlightingend_of_the_skype_highlighting, de 29 de octubre, por el que se establecen los requisitos y procedimientos para solicitar el desarrollo de proyectos de investigación con células troncales obtenidas de preembriones sobrantes (BOE 30 octubre).</p> <p>Ley 14/2007, de 3 de julio, de Investigación biomédica.</p> <p>ORDEN SCO/393/2006, de 8 de febrero, por la que se establece la organización y funcionamiento del Banco Nacional de Líneas Celulares.</p> <p>Banco Nacional de Líneas Celulares requisitos para depósito y acceso http://www.iscii.es/htdocs/terapia/terapia_bancocelular.jsp</p> <p>Requisitos que debe cumplir la Hoja de Información a los Participantes y el Consentimiento Informado para investigaciones que impliquen la generación de células pluripotentes inducidas (iPS) http://www.iscii.es/htdocs/terapia/terapia_comiteetica.jsp</p> <p>Real Decreto 1527/2010 (noviembre, 2010) por el que se regulan la Comisión de Garantías para la Donación y Utilización de Células y Tejidos Humanos y el Registro de Proyectos de Investigación http://www.boe.es/boe/dias/2010/12/04/pdfs/BOE-A-2010-18654.pdf</p>
INDIA	<p>Guidelines for Stem Cell Research and Therapy. Department of Biotechnology and Indian Council for Medical Research (2013)</p> <p>The Assisted Reproductive Technologies (Draft Regulation), Rules – 2010. Ministry of Health and Family Welfare, Government of India.</p> <p>The Assisted Reproductive Technologies (Draft) Bill. Ministry of Health and Family Welfare, Government of India</p>
AUSTRALIA	<p>Therapeutic Goods (Charges) Amendment Act 2010 (No. 53, 2010). An Act to amend the Therapeutic Goods (Charges) Act 1989, and for related purposes.</p> <p>Australian code of good manufacturing practice for human blood and blood components, human tissues and human cellular therapy products (2013)</p> <p>National Statement on Ethical Conduct in Human Research (2007), developed jointly by National Health and Medical Research Council, Australian Research Council and Australia Vice-Chancellors' Committee</p> <p>Ethical Guidelines on the use of assisted reproductive technology in clinical practice and research (June, 2007), National Health and Medical Research Council.</p> <p>NHMRC Embryo Research Licensing Committee, Information Kit, National Health and Medical Research Council (2008).</p>
UNITED KINGDOM	<p>UKSC Bank, MRC, Code of Practice for the use of Human Stem Cell Lines (April, 2010)</p> <p>HFEA Code of Practice (8th edition), HFEA (2009)</p> <p>The Human Fertilisation and Embryology Act (2008)</p> <p>UK Stem Cell Tool Kit http://www.sc-toolkit.ac.uk/home.cfm</p> <p>Data and Tissues Tool Kit http://www.dt-toolkit.ac.uk/home.cfm</p> <p>HTA Code of Practice on Research (2009)</p> <p>Human Tissue Act (2004)</p> <p>Human Tissue (Quality and Safety for Human Application) Regulations 2007</p> <p>British Standards Institute (BSI) Publicly Available Specification PAS 83:2012 Developing human cells for clinical applications in the European Union and the United States of America. Guide</p> <p>BSI Publicly Available Specification PAS 84:2012 Cell therapy and regenerative medicine. Glossary</p> <p>BSI Publicly Available Specification PAS 93:2011. Characterization of human cells for clinical applications. Guide</p>

Appendix 5.	
SWEDEN	<p>Tissue Law: Lag (2008:286) om kvalitets- och säkerhetsnormer vid hantering av mänskliga vävnader och celler, som reglerar hanteringen av vävnader och celler som ska användas för transplantation, assisterad befruktning och tillverkning av läkemedel.</p> <p>Lagens bestämmelser konkretiseras ytterligare i de föreskrifter som tagits fram av Socialstyrelsen respektive Läke medelsverket. Socialstyrelsens föreskrifter om donation och tillvaratagande av vävnader och celler; beslutade den 18 november 2008.</p>
SWITZERLAND	<p>Federal Act of 19 December 2003 on Research Involving Embryonic Stem Cells (Stem Cell Research Act, StRA) (RS 810.3, Loi relative à la Recherche sur les Cellules Souches (LRCS)), http://www.admin.ch/ch/e/rs/c810_31.html</p> <p>Federal Act of 18 December 1998 on Medically Assisted Reproduction (Reproductive Medicine Act, RMA) (RS 810.1 Loi fédérale du 18 décembre 1998 sur la procréation médicalement assistée (LPMA) www.admin.ch/ch/fr/rs/c810_11.html</p> <p>Federal Act of 8 October 2004 on the Transplantation of Organs, Tissues and Cells (Transplantation Act)</p> <p>The Federal Act on Medicinal Products and Medical Devices (Therapeutic Products Act, TPA), in force since 1st January 2002 (www.admin.ch/ch/e/rs/c810_21.html)</p> <p>Federal Office of Public Health (www.bag.admin.ch/index.html?lang=en)</p> <p>Swissmedic (Swiss agency for the authorisation and supervision of therapeutic products): the responsible regulatory authority on behalf of the Federal Office of Public Health (www.swissmedic.ch/index.html?lang=en)</p>
JAPAN	<p>The Act of Pharmaceuticals and Medical Devices MHLW: Ministry of Health, Labor and Welfare (25/11/2014) Revision of former Pharmaceutical Affairs Act. Producing regenerative and cellular therapeutic products in firms</p> <p>The Act on Safety of Regenerative Medicine MHLW (25/11/2014) Providing regenerative medicines within hospitals and clinics. The previous guidelines "The Guideline on clinical research using human stem cells" and "Ethical Guidelines for Clinical Research" were abolished.</p> <p>Guidelines on Ensuring Quality and Safety of Products Derived from Processed Human Cell/Tissue Autologous: MHLW Notification No.0208003 (8/2/2008) Allogeneic: MHLW Notification No.0912006 (12/9/2008)</p> <p>Guidelines on Ensuring the Quality and Safety of Products Derived from Processed Human Stem cells Autologous Somatic Stem Cells: MHLW Notification No.0907-2 (7/9/2012) Allogenic Somatic Stem Cells: MHLW Notification No.0907-3 (7/9/2012) Autologous iPS(-Like) Cells: MHLW Notification No.0907-4 (7/9/2012) Allogenic iPS(-Like) Cells: MHLW Notification No.0907-5 (7/9/2012) Embryonic Stem Cells: MHLW Notification No.0907-6 (7/9/2012)</p> <p>Guidelines on the Derivation of Human Embryonic Stem Cells Guidelines on the Distribution and use of Human Embryonic Stem Cells MEXT : Ministry of Education, Culture, Sports, Science & Technology (25/11/2014) Revision of regulations for clinical use of hES cells</p>
THAILAND	<p>Thai Medical Council Regulation (November, 2009) Thai Food and Drug Administration Regulation (March, 2009) Medica I Council's Medical Practice Act BE2525 (AD 1982) Division of Medical Registration of the Department of Health Service Support's Sanatorium Act BE 2525 (AD 1982)</p>

Appendix 5.	
SOUTH KOREA	<p>Bioethics and Safety Act (Jun, 2008) http://www.moleg.go.kr/FileDownload.mo?flSeq=25769 (Article 15)</p> <p>Enforcement Decree of Bioethics and Safety Act (Nov, 2009) http://www.moleg.go.kr/FileDownload.mo?flSeq=31613</p> <p>Enforcement Rule of Bioethics and Safety Act (Dec, 2009) http://www.moleg.go.kr/FileDownload.mo?flSeq=31607</p> <p>Pharmaceutical Affairs Act (Apr, 2007)</p> <p>Enforcement Decree of Pharmaceutical Affairs Act (Jun, 2007)</p> <p>A draft of "Regulation of Review and Authorization of Biological Products" (Jul, 2009)</p> <p>law on human tissues (19th March 2010), Ministry of Human Welfare (MHW)</p> <p>Enforcement regulations (Oct 2004), MHW</p> <p>Guidelines for Management of cord blood bank (Act 2005), FDA.</p>
TAIWAN	<p>Regulation of Organ Banks</p> <p>Regulation of Human Biobanks</p> <p>The regulation of prevention of infectious diseases</p> <p>Guidelines of research usage of human biopsy, tissue and fluid</p> <p>Guidelines of research ethics for human embryo and embryonic stem cells.</p>
CHINA	<p>人体器官移植条例 Regulations on human organs transplantation (4–6–2007)</p> <p>http://wsj.sh.gov.cn/website/b/28586.shtml</p> <p>骨组织库管理 Standard for human musculoskeletal tissue bank(3–1–2011)</p> <p>眼库管理 Standard for human eye tissue bank(3–1–2011)</p> <p>http://www.moh.gov.cn/publicfiles/business/htmlfiles/mohzcfgs/s7850/201009/48944.htm</p> <p>脐带血造血干细胞治疗技术管理规范 Regulations on therapeutic technology of cord blood stem cells (11–13–2009)</p> <p>http://wsj.sh.gov.cn/website/b/48446.shtml</p> <p>医疗技术临床应用管理办法 Regulations on therapeutic technology for clinics (3–2–2009)</p> <p>http://wsj.sh.gov.cn/website/b/43522.shtml</p> <p>及人的生物医学研究伦理审查法 Ethical Guidelines on the use of human tissue in research (1–11–2007)</p> <p>http://wsj.sh.gov.cn/website/b/28676.shtml</p>
EU	<p>Commission Directive 2006/86/EC 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. (October, 2006)</p> <p>Commission Directive 2006/17/EC 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. (February, 2006)</p> <p>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. (April, 2004)</p> <p>European Parliament legislative resolution on the Council common position adopting a European Parliament and Council directive on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells (10133/3/2003 - C5–0416/2003 - 2002/0128(COD)) (December, 2003)</p>
ISBER	<p>Best Practice for Repositories: Collection, Storage, Retrieval and Distribution of Biological Materials for Research (2012)</p>
FACT	<p>Cellular Therapy Accreditation Manual (5th Edition, 2012)</p>
AHCTA	<p>Position Paper: Towards Global Standard for Donation, Collection, Testing, Processing, Storage and Distribution of Allogeneic HSC and Related Cellular Therapies (2008)</p>
ECVAM	<p>Guidance of Good Cell Culture Practice – A Report of the Second ECVAM Task Force on Good Cell Culture Practice (2005)</p>
NCI – NIH-USA	<p>NCI Best Practice for Biospecimen Resources (2011)</p>
ISSCR	<p>Guidelines for the Clinical Translation of Stem Cells (2008)</p> <p>Guidelines for the Conduct of Human Embryonic Stem Cell Research (2006)</p>
OECD	<p>Guidelines for Human Biobanks and Genetic Research Databases (HBGRDs) (2009)</p> <p>OECD Best Practice Guidelines for Biological Resource Centers (2007)</p>

Appendix 6. An example of release criteria*: characterization data for information** and specifications for seed stocks of undifferentiated hPSC lines.

Test	Examples of test method(s)	Criteria/specification	Test results prior to release
Identity*	Typically Short Tandem Repeat (STR) Testing (other techniques may be used such as, Human Leukocyte Antigen (HLA) Testing)	All alleles match parent cell line	Meets Specification
Bacteria/Fungi (sterility) 1*	Inoculation of microbiological media to detect growth of bacteria and fungi	No detectable contamination	Meets Specification
Mycoplasma1*	Pharmacopeia tests include direct culture, direct stain (DAPI or Hoechst 33258) and Vero culture followed by direct stain. Alternative PCR tests are now becoming acceptable	No detectable contamination (sensitivity and specificity to be validated with service provider)	Meets Specification
Karyotype2*	Chromosome count of 20 metaphases and G-banded analysis of a further 10 metaphases (see [1] and Section 4)	Diploid chromosomes predominant in cells analysed (for specifications see section 4).	Meets Specification
Viability1*	Viability must be quantified using a validated method. A lower limit for acceptability should be indicated	Viability should typically be $\geq 50\%$ of thawed cells (N.B. this does not necessarily equate with functional performance of the culture and is merely an indicator of the ability to expand cells from production purposes)	Meets Specification
Growth characteristics*	Determine doubling time	Typically 20 to 40 h	Meets Specification
Characterization and stability (N.B. stability testing will need to be established by each repository, but may include culture to passages or population doublings to limits anticipated for cell therapy products)**			
Antigen expression	Flow cytometry of hPSC markers of self renewal and hPSC state (these are to be selected and qualified the repository but possible examples include Oct-4, TRA 1-60, TRA 1-81, SSEA-3, SSEA-4, Alkaline Phosphatase, Rex-1, SSEA-1 negative)	Typically $\geq 70\%$ of hPSCs expressing hPSC markers and $\leq 10\%$ of hPSCs expressing SSEA-1 (N.B actual values should be based on local experience with each cell line)	Meets Specification
Pluripotency**	Tests indicating potential pluripotency (e.g., teratoma production, embryoid body formation, directed differentiation - see section 5.4)	Criteria should be set by repository depending on method used, but embryoid bodies and teratomas should express markers of ectoderm, mesoderm and endoderm.	Meets Specification
Viral contamination3*	<i>In vitro</i> and <i>in vivo</i> non-specific and specific (virus screening should be directed by risk assessment and where there is risk of blood born virus contamination may include viruses such as HIV 1 and 2, HBV, HCMV, HCV, HHV 6-8, EBV, HTLV I&II, B19 etc.)	No detectable contamination (N.B. levels of sensitivity will need to be validated by the repository or service provider)	Meets Specification
Reprogramming factors*	Test to assure silencing of reprogramming vectors or elimination of episomal non-integrating vectors	RTPCR/qPCR, antibody based detection	Reprogramming vectors and/or exogenous expression of reprogramming factors not detectable

*Release criteria should include test sensitivity and test specificity where appropriate.

**Characterization for information, but not release.

***Testing should be performed on at least 1% of vials, but no less than 2 of the Bank from which cells are to be released.

1. Some suitable tests are described in the European Pharmacopoeia methods and 21 CFR 610.30.

2. For further information, refer to the Consensus Guidance for Banking and Supply Of Human Embryonic Stem Cell Lines For Research Purposes (reference ISCBi (2009) in main text) (1)

3. Examples of suitable tests are described in the ICH guidelines Q5A [105].

4. Cell banks should be free of extraneous material apart from that which is unavoidable in the manufacture process. For further information, refer to ICH Q3 on 'impurities' [106]

5. It is important to note that the tests indicated here are examples of tests applied typically to pharmaceutical products, and whilst they may add value by detecting contamination that may not be detected in a standard pharmacopoeial 'sterility' test, they may also miss certain bacterial contaminants lacking the cell wall components detected in pyrogenicity and limulus lysate assays. These tests may also give false positive results where contamination is not present but bacterial components persist in cell culture reagents. Cell banks should keep a watching brief for alternative qualified tests, which may become available and give broader capacity for detecting both bacterial and fungal contamination such as PCR for microbial ribosomal RNA.

Test	Examples of test method(s)	Criteria/specification	Test results prior to release
Purity [5]			
Differentiated cells**	Flow cytometry using hPSC and non-hESC markers	Contamination with non-hPSC markers should be below levels	Meets Specification
Cell debris**	Flow cytometry (of note, markers and acceptable limit may vary with cell line and local culture procedures)	Maximum levels of cell debris specified based on local data on each cell line.	Meets Specification
Non-specific tests for bacterial contamination	Examples include: a) Endotoxin [5]**: limulus amoebocyte lysate (LAL) test b) Pyrogenicity [5] **:	Acceptable levels will need to be defined and validated locally (international standards to qualify)	Meets Specification
	Rabbit pyrogen test method c) PCR for microbial rRNA genes:		
Vial labelling**	A water-resistant written, printed or graphic indication must be affixed to each container/ package of hPSCs describing critical information about the cells/product	See section 6.4	Meets Specification
<p>*Release criteria should include test sensitivity and test specificity where appropriate. **Characterization for information, but not release. ***Testing should be performed on at least 1% of vials, but no less than 2 of the Bank from which cells are to be released. 1. Some suitable tests are described in the European Pharmacopoeia methods and 21 CFR 610.30. 2. For further information, refer to the Consensus Guidance for Banking and Supply Of Human Embryonic Stem Cell Lines For Research Purposes (reference ISCB (2009) in main text) (1) 3. Examples of suitable tests are described in the ICH guidelines Q5A [105]. 4. Cell banks should be free of extraneous material apart from that which is unavoidable in the manufacture process. For further information, refer to ICH Q3 on 'impurities' [106] 5. It is important to note that the tests indicated here are examples of tests applied typically to pharmaceutical products, and whilst they may add value by detecting contamination that may not be detected in a standard pharmacopoeial 'sterility' test, they may also miss certain bacterial contaminants lacking the cell wall components detected in pyrogenicity and limulus lysate assays. These tests may also give false positive results where contamination is not present but bacterial components persist in cell culture reagents. Cell banks should keep a watching brief for alternative qualified tests, which may become available and give broader capacity for detecting both bacterial and fungal contamination such as PCR for microbial ribosomal RNA.</p>			

Appendix 7. Examples of QA definitions used in GMP manufacture

The terminologies given here are purely examples drawn primarily from the FDA tissue banking regulation [108]. There are no wholly agreed terminologies for this area and it is therefore important to use the definitions of QA terms recommended in national guidelines. In some cases there are significant difference in the scope of a definition under different jurisdictions such as the definitions for serious adverse events in the EU and the USA. ICH definitions [109] is very similar to FDA Medwatch and is probably one of the best harmonized terminologies. The PAS terminology [2] provides the UK and EU definitions and the regulatory reference for QA terms in Europe is the European Tissues and Cells Directive.

QA DEFINITIONS

ACCEPTANCE CRITERIA: The specifications and acceptance/rejection criteria, such as acceptable quality level and unacceptable quality level, with an associated sampling plan, that are necessary for making a decision to accept or reject a lot or batch of raw material, intermediate, packaging material, or product. This term can also be applied to validation.

ADVERSE EVENT: Any untoward medical occurrence in a patient or clinical investigation subject administered with a pharmaceutical product and that does not necessarily have to have a causal relationship with this treatment.

ADVERSE REACTION: A noxious and unintended response to any human cells, tissues, and cellular and tissue-based products for which there is a reasonable possibility that the HCT/P caused the response.

ASEPTIC PROCESSING: The processing of cells/product by methods that avoid or minimize contamination with microorganisms from the environment, processing personnel and/or equipment.

AUDIT: A review of procedures, records, personnel activities, reagents, materials, equipment and facilities to determine adherence to standards and regulations.

BATCH: A batch, sometimes called lot, is defined as an entity, by either time or quantity or both, of a product that is intended to have a uniform character and quality. A batch must be produced within predefined and specified conditions following a defined manufacturing process.

BATCH MANUFACTURING RECORD (BMR): The necessary quality documentation for tracing the complete cycle of manufacture of a batch or lot.

BATCH RECORD REVIEW: The process of reviewing and approving all Product Manufacturing and control records is called the batch record review. This includes, but is not limited to, packaging and labeling. The batch record review is performed by the quality unit to determine compliance with all established approved written procedures before a batch is released.

DISPOSITION: The destination of cells/product for research, transplantation or discard.

DISTRIBUTION: A process including the receipt of a request for, selection of, and inspection of cells/product, and their/its shipment for delivery to recipient.

DOCUMENTATION: Any procedures, instructions, logbooks, records, raw data, manuals, and policies associated with the development, manufacture, testing, marketing and distribution of a product required demonstrating compliance with applicable worldwide regulatory requirements.

EQUIPMENT QUALIFICATION: Protocols to evaluate equipment performance following installation and before use, to ensure normal function within required tolerance limits.

FACILITIES: The facilities are used for the manufacturing of cell therapy products with predefined environmental control following the applicable standards of e.g., particulate and microbial contamination. The facilities should be constructed and used reducing the introduction, generation and retention of contaminants within the area.

IN-PROCESS CONTROL (IPC): Testing and activities performed during production to monitor and, if necessary, adjust the process to assure that the product conforms to its specifications.

INTERMEDIATE: An intermediate product e.g., cell line that must undergo further processing before it becomes a final product.

LABEL: A written, printed or graphic indication affixed to a container/ package describing critical information about the cells/product.

LOT: Cells/product derived from one donor, banked at one time using the same reagents and materials, and identified by a unique identification number.

PROCEDURE: A series of ordered steps designed to achieve a specific outcome when followed.

PROCESS VALIDATION: Establishing documented evidence that provides a high degree of assurance that a specific process will consistently produce a product meeting its pre-determined specifications and quality attributes.

QUALITY: The term quality is used as the totality of features and characteristics of a product that bears on its ability to satisfy stated or implied needs including the conformance to requirements to specifications.

QUALITY ASSURANCE: A formal methodology designed to provide adequate confidence that the entire production of a product will fulfil requirements for quality under a wide conditions of operation. Quality assurance includes formal review of care, problem identification, corrective actions to remedy any deficiencies and evaluation of actions taken.

QUALITY ASSURANCE UNIT: Sets policies, procedures and specifications, audits, reviews, assesses and training including continuous evaluation of the adequacy and effectiveness of the overall quality program.

QUALITY CONTROL: A procedure or set of procedures intended to ensure that a manufactured product adheres to a defined set of quality criteria.

QUALITY CONTROL UNIT: The function in the quality unit that is responsible for the ongoing control of product and environment quality. Therefore the quality control unit (QC) has the overall responsibility for acceptance or rejection of e.g., raw materials, cell lines/intermediate products/final products, packaging components.

IN-PROCESS CONTROLS (IPC), LABELLING AND INSPECTION: Assurance that supporting systems are being controlled and monitored.

QUALITY SYSTEM: Organizational structure, responsibilities, procedures, processes, and resources for implementing quality management.

QUARANTINE: The storage of materials/cells/ product in an isolated area until deemed safe (cleared/approved) for use.

SERIOUS ADVERSE EVENT/REACTION (ICH DEFINITION: Topic E2A1): Is an untoward medical occurrence which is: fatal, life-threatening (risk of death at the time of the event), disabling, or incapacitating resulting in hospitalization, or medically significant congenital abnormalities.

SERIOUS ADVERSE EVENT (EU TCD): Any untoward occurrence associated with the procurement, testing, processing, storage and distribution of tissues and cells that might lead to the transmission of a communicable disease, to death or life-threatening, disabling or incapacitating conditions for patients or which might result in, or prolong, hospitalization or morbidity

SERIOUS ADVERSE REACTION (EU TCD): Unintended response, including a communicable disease, in the donor or in the recipient associated with the procurement or human application of tissues and cells that is fatal, life-threatening, disabling, incapacitating or which results in, or prolongs, hospitalization or morbidity.

SPECIFICATIONS: Used for the predefined written, chemical, physical, biological and environmental characteristics for testing a product or system. This includes, but is not limited to, starting materials, packaging materials, intermediate, bulk, or product.

STANDARD: An accepted or authoritatively established principle or practice for quality assurance (e.g., SOP).

STANDARD OPERATING PROCEDURE: A detailed description of a procedure or process for quality assurance.

TRACEABILITY: The ability to locate cells/product at any point/step during production, processing, testing, storage, distribution or disposition.

VALIDATION: The procedure for establishing documented evidence that a specific system is constructed and operates according to a predetermined set of specifications and guidelines. Validation includes but is not limited to: equipment, computer systems, production processes, cleaning procedures, facilities, utilities as well as analytical methods.

Appendix 8: Preservation technologies and methods

■ Mode of cryopreservation

Two approaches to cryopreservation have been applied to stem cells: vitrification and slow cooling [101]. Both of these are capable of ensuring high survival if appropriately applied.

■ Vitrification

The vitrification method currently applied is a non-equilibrium approach relying on ultra-rapid cooling with low concentrations of CPA to achieve the ice-free vitreous state. This is a meta-stable state which is prone to de-vitrification (with the potential for subsequent damaging ice formation) if those conditions necessary to maintain the vitreous state (notably a stable low temperature) are not maintained (see storage and transportation).

The choice of container and the unit volume of material being cooled should be considered when vitrification methods are employed, since both these will affect the maximum attainable cooling and warming rate. The ultra-rapid cooling rates necessary to effect vitrification require both high surface to volume ratios (with regard to container geometry) and small volumes (of the order of microliters per unit sample). In preparing large banks of cells, consideration should be given to the practicality of this method for scale-up due to the need to preserve relatively small numbers of cells at one time.

Consideration should also be given to the use of open straws and Dewars containing non-sterile liquid nitrogen (LN₂) into which the straws are plunged. Neither of these can be considered to be best practice both from a microbiological or regulatory perspective. Alternatives to the open straw method, such as closed straws and straw-in-straw methods, should be considered, but there may also be important logistical constraints (e.g., size of the bank, mode of transportation) which must be reconciled with the requirements of the preservation method.

Other alternatives for ice-free preservation, such as equilibrium approaches utilizing high concentrations of CPA [99] and/or the addition of natural or synthetic ice blocking molecules coupled with slow cooling [110] have not as yet been applied to stem cells.

■ Conventional slow cooling

During slow cooling, ice formation will occur within the system resulting in the concentration of solutes in the remaining liquid phase in which the cells reside. Damage results mainly from exposure to these high solute concentrations (so-called solution effects), but may also occur as a result of intracellular ice formation. Ice formation within cells is generally a consequence of rapid cooling, but may occur in tissues at slow cooling rates as a random event leading to ice propagation to surrounding cells. It should be noted that CPAs militate against damaging solution effects of slow cooling but not against damage caused by intracellular ice formation.

Conventional slow cooling methods are generally more amenable to the production of large banks of cells produced as 'single' cell suspensions. For stem cells cryopreserved as colony fragments, if slow cooling methods are to be applied, consideration should be given to methods to control ice nucleation such as the inclusion of ice nucleating agents or seeding samples at high sub-zero temperature [111].

■ Methods of cooling

The high cooling rates required for vitrification are generally obtained by direct immersion of the sample into a cryogen, usually liquid nitrogen. Slow cooling can be effected by either controlled rate cooling or the use of passive cooling devices. In both cases, consideration must be given to issues of sample contamination and contamination of the cleanroom as well as those of reproducibility and validation (see validation).

Controlled rate freezers (CRFs) in which the chamber containing the product is cooled by the injection of LN₂ will generally be located outside the cleanroom environment unless the resulting nitrogen vapour can be ported to the outside of the cleanroom and the chamber can be effectively sterilized between cooling cycles. If such devices are used, outside the production area, consideration should be given to the method by which cells are moved to the CRF, to ensure that CPA exposure time/temperature does not compromise cell survival or lead to contamination.

Liquid nitrogen-free, CRFs, such as those employing the Stirling Cycle principle, may provide an alternative [112,113]. While such equipment provides a clean room-compatible solution for controlled rate freezing, they should be assessed for their ability to provide the required cooling rates, unit volumes and bank sizes appropriate to the cell lines being banked.

The end point temperature at which cells are transferred from the CRF to low-temperature storage should be set at a sufficiently low temperature to ensure that during handling and transfer to permanent storage any rise in temperature of the samples does not expose the cells to damaging sub-zero temperatures (above approximately -70°C for frozen cells and above the glass transition temperature for vitrified material).

Passive cooling devices are generally placed inside a mechanical freezer to equilibrate. A uniform and reproducible cooling rate can be obtained if there is careful control of the sub-zero environment. A sub-zero temperature of at least -70°C should be employed in order to limit the cells exposure to the most damaging sub-zero temperatures (between the equilibrium freezing point and ~ -40 to 60°C) and assist in providing a relatively linear cooling rate over this temperature range. Consideration should be given to temperature logging of a control sample for QC purposes, the use of a designated freezer and procedures to control access to this freezer during cryopreservation.

Appendix 9. Review of teratoma methodologies used in different laboratories

18 laboratories from 16 countries responded to a set of questions (see table) on the teratoma assays they used for evaluation of hESC lines. Methodologies used for teratoma assays showed little concordance for strain of mouse, site of injection, number of cells/volume, end point, inspection for metastasis and processing and analysis of tumors (data obtained by S Oh and L Healy of ISCBI).

Method component	Details requested	Range of methods reported
Preparation of hPSCs	Culture method used prior to harvesting	Feeder and feeder-free cultures used (some labs used both methods).
	Harvest method used	4 methods used (TrypLE, trypsin, collagenase, mechanical)
	Post-harvest processing before inoculation	3 methods used (cell centrifuged and resuspended, cells washed in growth medium or PBS and resuspended in the same, cells resuspended in medium with Matrigel™)
Cells inoculated	Number and viability of cells inoculated	Range of methods based on cell number (1000–10,000,000), cell viability (range 80–95%), injection volume 20–150µl, and no determination of cell number or viability.
Site of inoculation	Anatomical site and means of administration (e.g. syringe, surgical incision with cells on a substrate)?	4 different sites used (legs intramuscular (both sides), Kidney capsule, intra-testicular, subcutaneously head and neck and flank)
Test animal	Strain of mouse used	Seven different strains of mouse used (Nude Balb/c, SCID undefined, Nude/nude, SCID/Beige, SCID c gamma c -/-, SCID undefined, NOD/SCID, NOD/MrkBomTac-Prkdc SCID).
	Frequency and natural onset of spontaneous tumors in the mouse strain	None identified or 'low'
	Age of animals used	2 age ranges used (7–8 weeks (majority) or 5–8 months)
Replicates of test	No. of animals used for each test	Ranged from 1–4 per cell line (3/cell line (majority), 1/cell line with 2–4 injection sites, 4/cell line)
Observation of animals	Typical number of weeks post inoculation at which mice are sacrificed	4–12 weeks
	Is there a maximum end point for incubation or are mice kept until natural death?	3 different limits used (2–4 months, tumor growth allowed to reach 1–1.5 cm or tumor growth allowed to reach 2 g)
Tumor incidence	Frequency of mice developing tumors per experiment	Ranged from none–100% with an equal number of participants reporting incidence of tumors in mice at 30–50% and 80–100%.
	Method of tumor location	Palpation and observation
	Numbers of tumors expected per mouse	1–2 tumors per site
	Frequency of metastasis	Majority of participants reported metastases
Tumor preparation	Point at which palpable tumors are recovered	5–12 weeks or maximum size of 0.5–1.5 cm or maximum weight of 2 g
	Processing of tumor	Tumors fixed by alternative methods (paraformaldehyde or formalin, paraffin, cryosections) depending on post-fixation testing including 3 different techniques (histology [H&E, PAS etc], immune-staining or PCR).
Evaluation and reporting of tumors	Minimum criteria (in terms of histological data) to establish a cell line is 'pluripotent'	Evidence of 3 germ layers
	Are results from more than one mouse used in combination?	50% responded 'no', 50% responded 'yes' if using the same cell line
	Variation in results observed between cell lines	Of those responding 50% reported no variation and 50% did see a significant variation