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 pharmacopeia. 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 pharmacopeia [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 Tumorigencicity 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* tumorigenicty 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/IL2Rγnull) [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 *in situ* 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 *in situ* 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 tumorigenicitiy 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 fiunction. 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-flourochrome 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 regprograming 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.

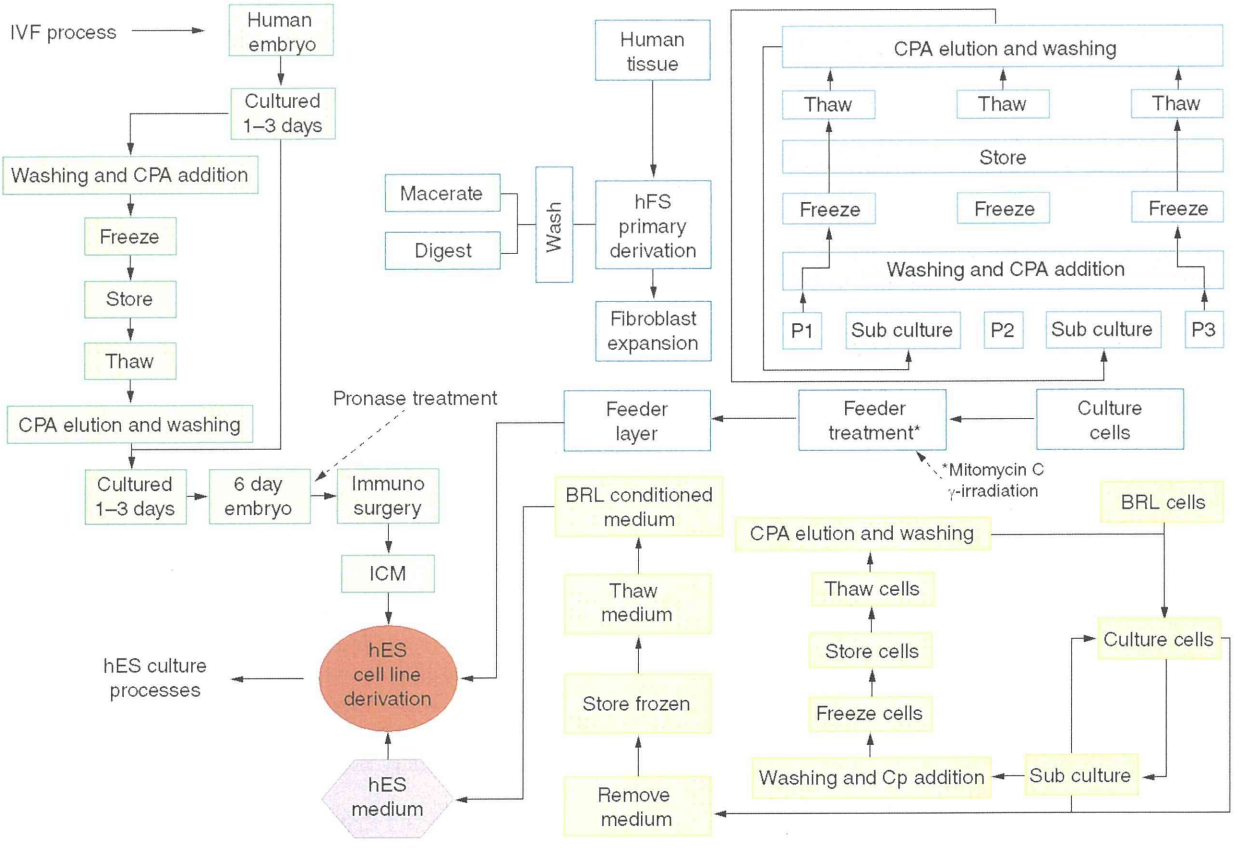

Figure 1. Example of a process map for derivation of a human embryonic stem cell line.
Courtesy of C Hunt, UK Stem Cell Bank, NIBSC, 2013.

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 [LN_2] levels, failure of LN_2 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.