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.

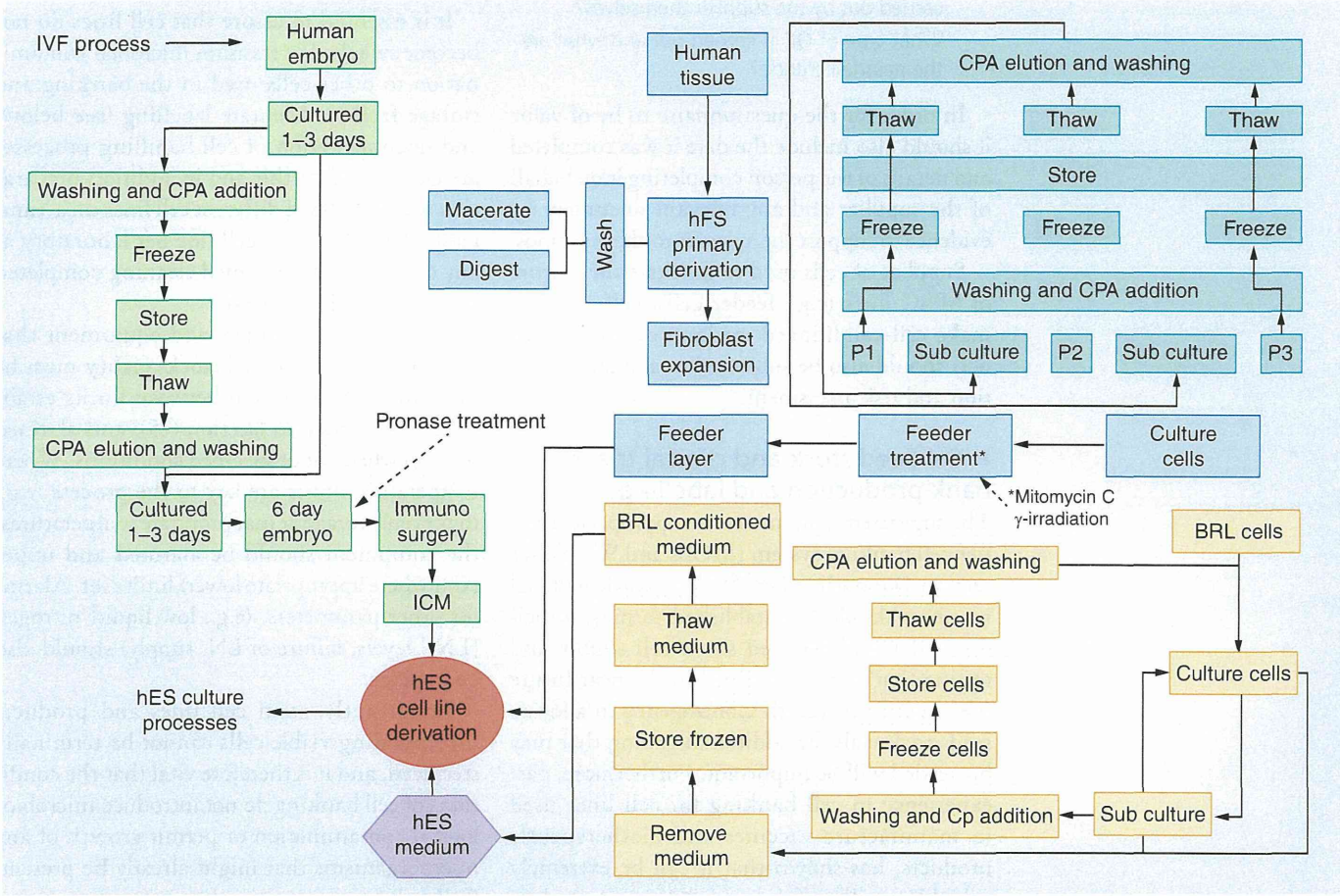

Figure 1. Example of a process map for derivation of a human embryonic stem cell line.
Courtesy of C Hunt, UK Stem Cell Bank, NIBSC, 2013.

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.

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, LN_2 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 Co-operation 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 over view 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

**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.*

***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].*

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 put 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_organs/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 ISCBI 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