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TRANSLATIONAL STEM CELL STUDIES

Consensus Guidance for Banking and Supply of Human Embryonic Stem Cell Lines for Research Purposes

The International Stem Cell Banking Initiative

Keywords Human embryonic stem cells · Cell banking · Standardisation · Microbiological testing · Pluripotency · Quality control

Background and Scope

In just a few years hundreds of human embryonic stem cell (hESC) lines have been established in laboratories around the world and many programmes of research initiated to investigate their properties and broad ranging potential in therapy and for other research applications, such as developmental biology, toxicology and drug discovery. This work is being performed with a variety of cell lines using a variety of culture conditions; a situation that makes standardisation between projects and publications very difficult and could prevent the identification of cells that have undergone permanent deleterious changes. Clearly the consequence of using such cells would be wasted time and resources but, more seriously, the generation of erroneous data in the literature which could both confuse and delay scientific progress in this area. Thus ensuring that cell lines used in this dynamic field have the correct identity and characteristics is critical to the delivery of effective and efficient research of acceptable quality.

Many centres now distribute hESC lines around the world but the preparation and testing of cell stocks released to other researchers is generally based on local 'norms' and naturally varies from centre to centre. The challenge of preparing satisfactory cells for use in research work has been recognised and guidance has been developed by international groups on good cell culture practice [1] and cell banking [2, 3]. In response to the lack of formal coordination between the active distributing centres from different countries the International Stem Cell Forum, a group of national and international stem cell research funding bodies, has funded this initiative, the International Stem Cell Banking Initiative (ISCBI), to establish a dialogue between the distribution centres to develop a consensus on the principles of best practice for the banking, testing and distribution of hESC [4, 5] cells. Due to local conditions and procedures some distributors of hESCs may not address all items as described in this guidance. However, where this occurs the distributor in question must be able to justify their position. The first meeting of this group was held at the Jackson Laboratory (Maine, USA) in October 2007 and this guidance document represents the first output from the ISCBI. The document has been prepared from the perspective of hESC culture but, in many respects, is broadly applicable to all human stem cell lines including induced pluripotent stem cell lines.

Informed Consent, Traceability and Governance

General Principles

Banks must comply with their own domestic laws and regulations (see ISCF website) and should also verify compatibility with international ethical principles such as the guidance established by the International Society for Stem Cell Research (ISSCR) and other relevant bodies.

Banks should obtain documentation pertinent to the provenance of the cell line, which includes evidence – and

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scope- of the donor's free and voluntary informed consent, traceability measures and of the safeguards necessary to ensure the privacy of donors and donor information. Ideally, this should also enable traceability to the original donor but this should be coded, or otherwise anonymised, such that the bank cannot access details of donors directly, but through a formal mechanism via another independent party or organisation.

A review of international ethical safeguards and further information from the ISCF Ethics Working Party and others can be found at http://stemcellforum.org/.

Procedures to Assist Ethical Operation

Each bank should have an independent and transparent governance structure which reviews the ethical provenance of cell lines accepted by the bank consistent with 'General Principles' above. Depositors of cells should demonstrate that they have met all legal and ethical requirements associated with procurement of tissue and derivation of the cell lines. The depositor of the cell line should provide information that enables the banks governance structure to determine whether these conditions are broadly consistent with the bank's national regulation. Moreover, banks should have in place a mechanism to maintain awareness of changes in regulation.

Governance of Cell Supply

Only ethically approved projects should receive cells from the bank. This should be determined before the Materials Transfer Agreement (MTA) is signed in which this constraint is indicated (see Shipment of Cells and 'Information for Users'). To avoid distribution for unethical purposes, the users should be prohibited from third party distribution without prior permission from the bank.

Banks should also consider ethical responsibility in the case of discovery of infectious or genetic disease in a cell line according to domestic laws and ethical norms (see ISCF website for sources of information).

The Cell Banking Process

Procurement of Cell Lines

The conditions of consent for the use of donated cells and tissue for research purposes should be subjected to appropriate scrutiny. Accordingly, traceability should be established for each stem cell line as well as for the informed consent from the original donor of cells used to derive the cell line (see 'Informed Consent', 'Traceability and Governance').

The establishment of archive material is important for future reference and useful samples include cells from the original donor and fixed cells (e.g. "Guthrie cards", 'FTA' cards (Whatman)) or DNA from early passages of the cell line to enable confirmation of genetic identity of cell banks. In addition, vials of the cryopreserved cell line supplied by depositors can be held unopened in case the banked cells are later found to have altered characteristics or contamination.

Fundamental information from depositor of a cell line in the bank should include: cell identity, contamination status, viral testing, passage level, mycoplasma testing and key cell antigen and molecular marker tests. Details of the testing recommended and cell bank specifications and release criteria are given in 'Informed Consent', 'Traceability and Governance', 'Cell Characterisation' and 'Release Criteria for Cell Banks'.

Cell Banking Procedures and Documentation

Banks should be accredited, designated, authorised or licensed by an appropriate authority for the purpose of their activities. They should also comply with domestic laws, guidelines and international norms for the banking and use of hESC lines. Banks should be subject to formal mechanisms for legal, ethical and scientific oversight in order to maintain public trust.

Operation of a formal and documented Master Cell Bank and Working Cell Bank system [1] is strongly recommended to enable supply of reproducible cells at the same passage level over long time periods. Cells would normally be supplied from the 'Working Cell Bank' which may be called the 'Distribution Cell Bank'. In addition, the bank must establish a quarantine procedure for newly acquired cell lines in order to avoid spread of any contamination they may contain.

For all processes involving culture or manipulation of cells the risk of microbiological contamination from raw materials should be evaluated (see 'Microbiological Testing'). The use of antibiotics in culture media may be necessary for derivation of cell lines, but is not recommended for preparing cell banks and other experimental work, with the notable exception of cell clones expressing 'marker' genes that are maintained under antibiotic selection (this is important to avoid the longer term problems that can be associated with suppressed contamination).

Documentation of banking procedures is vital for the bank to be able to demonstrate that it has worked in compliance with appropriate regulatory standards and to address and 'troubleshoot' complaints from recipients and internal failures in quality control. Documentation should include all information provided with deposited cell lines, consistently recorded data from quality control and characterisation and standard operating procedures for all key processes and protocols. Materials and procedures used to prepare each cell bank should also be traceable. All data available on a particular cell

line should be collated or referenced in a cell line master file that will provide a central source of information on that cell line and the stocks established in the bank.

Frozen stocks of a particular cell line should not be stored in a single location. In order to avoid the risk of complete loss of cell lines, the bank should provide back-up storage facilities at a second site that would enable regeneration of the original stocks. Off-site stored material should be held under conditions equivalent to those at the bank site. The bank should operate an inventory management system and procedures should be in place to ensure the accuracy and reliability of labeling and location records. The loss of cell lines due to substandard maintenance of cold storage would have a significant impact on the credibility of the bank, and auditing the bank storage system is an important factor in assuring secure long-term storage of material and is strongly recommended. As a minimum, an internal audit process should be employed to carry out periodic checks on the reliability and robustness of maintenance procedures for stored materials. Where formal standards apply for medical use, testing or other purposes, a formal independent audit process may be required for all cell culture and testing procedures.

Cell Bank Quality Control

The past experience of other culture collections distributing cell lines has identified that an important principle of quality control (QC) for cell lines is that it enables the bank to state, subject to certain qualification, that the cells are what the bank 'says' they are and that microbial contamination was not detected. In order to achieve this for human stem cell lines and indeed any stored cells, it is vital to establish key criteria for the quality control and characterisation of the cells and these are addressed in detail in 'Microbiological Testing', 'Cell Characterisation' and 'Release Criteria for Cell Banks'. Clearly qualification of cell lines, through appropriate and rigorous quality control, is critical and should be a mandatory function for a bank. It should also be remembered that QC is just one part of overall quality assurance procedures for the bank, which should also cover aspects such as correct function of facilities and equipment, and staff training. In some applications of cell lines, where specific quality standards apply, the bank will need to understand and possibly comply with such standards, examples of which include standards from the international standards organisation (www.iso.org/):

- ISO9001:2000 a general quality management standard for provision of services and products
- ISO17025 for laboratory testing and monitoring including the use of cell lines for 'batch release' testing of medical products
- ISO13485 for diagnostic testing procedures including the use of cells or cell-derived reference materials
- ISO34 Guide for preparation of reference materials

Other international guidance is also available for general cell culture (e.g. reference 1), safety testing [6] and establishment of cell lines for the manufacture of medicinal products [7, 8].

The Process of Releasing Cell Banks

Historically researchers have often sourced lines from unqualified sources in their colleagues' laboratories and this practice unfortunately promoted the widespread use of cross-contaminated and mycoplasma infected cell lines [9, 10]. Banks should actively work towards establishing expedient procedures to promote prompt access to cell lines to minimise the need for 'colleague-supply' routes. As part of this process banks need to work towards low cost simple testing regimes that can also be used by recipients of stem cell lines.

For each cell bank there should be an associated 'specification' which describes the key characteristics of each line. The 'specification' should meet the requirements for the cell to be "suitable for use" i.e. for use in stem cell research work. The specification must be matched by the quality control and characterisation data for each bank of cells before they can be released (for details see 'Release Criteria for Cell Banks').

It is recommended that with current culture methods banks should avoid shipment of growing cultures. Pooling multiple straws or vials from the same frozen stock may be necessary for a recipient to successfully recover a culture. However, banks should aim to provide sufficient 'viable' cells in a single container to enable appropriately trained staff to recover a representative culture.

Culture manuals should be available from the bank, ideally 'on-line', along with key standard operating procedures. Release of cells to researchers should be accompanied with advice and training; recipients should either have evidence of past training or training should be provided as part of cell supply.

It is recommended that the bank should make stocks of especially critical reagents, such as feeder cells, available to recipients to get their work started efficiently. Banks should advise recipients to produce a cryopreserved stock of each stem cell line on supplied feeders before switching to local feeder cells.

A complaints procedure should be in place which should be responsive to avoid frustration for recipients and enhance reputation of the bank. The bank should also have a clear replacement policy for cultures which fail to thrive in the hands of recipients. All complaints should be reviewed to assess the effectiveness of the corrective actions taken and to look for opportunities to improve service.

Technical support is important to users of the bank and should be provided.

Microbiological Testing

Risk Evaluation

Microbial contamination can change the characteristics of cells without causing obvious cytopathic effects and the unwitting use of such cultures could not only affect the quality of research data (for example viral infection may be non-lethal to cells and alter their biological performance), but could also expose researchers to infectious agents. In principle, stem cell lines should be free of any microbial contamination as far as can be reasonably determined by the bank. Cells with established 'stable' infection with specific agents may have useful research applications but, should be handled separately from 'clean' cultures with no evidence of infection.

It is vital that the bank has a rationale for the tests performed. Sterility and mycoplasma testing are microbiological tests that should be performed on all cell cultures used in the bank; these are covered in technical aspects of release procedures. The bank's drivers for safety testing will normally be to protect bank staff and users and to deal with liability that may be associated with the supply of cells to research workers. In addition to microbiological risks there is a formal but low risk of tumor formation following subcutaneous inoculation of a tumorigenic cell line as a result of accidents in the use of 'sharps' such as hypodermic needles. Whilst this risk would appear to be very small, the bank should aim to minimize it and take special care with immuno-compromised bank staff. Furthermore, where human or animal cells have been subjected to transfer in animals, it should be remembered that there is potential for transfer of animal virus from the host.

The bank should be aware of donor testing results relating to individual cell lines, but these should not be used alone. Caution should be taken in the use of donor testing as the reliability of test data may vary and contamination can occur after cell isolation. In addition, 'positive' viral testing in donors does not necessarily exclude the use of cells for cell line derivation e.g. hCMV. Where reagents of biological origin are used there may be a risk of contamination (see Table 1 for examples). Banks should consider obtaining risk-evaluated products that have been appropriately tested for microbial contamination. The infectious risk represented by different cell growth media products can be difficult to assess, but can be evaluated in the light of a number of factors outlined below.

- Issues relating to source animals. Species and tissue of origin and the geographical location, husbandry and health/microbiological screening of source animals and raw product harvesting procedures. In particular it is important to evaluate the risks from potential microbial contamination of serum. This will require careful documentation of batches used and their origin.
- Product manufacture. Nature of the processing, purification and formulation methods, including assessment of any added materials and the capacity of different steps in this process for reducing the level of any potential contaminants.
- Nature, effectiveness and reliability of any sterilisation or disinfection steps. Many proteins, such as growth factors, will not survive methods of sterilisation such as autoclaving or destructive irradiation. However, processes such as 'Pasteurisation', filtration, chemical treatment and lower level irradiation can be quite effective at reducing the viable microbial load in cell culture products.
- Testing performed on the product. Viral testing of animal derived products will be most appropriate when no sterilisation process can be used. Care should be taken to assess viral testing to ensure that appropriate methods were employed that have been demonstrated to give defined and acceptable levels of sensitivity and specificity and were performed by a qualified or accredited laboratory with appropriate experience in the tests performed.

It should be remembered that no current testing regime can guarantee absolute absence of microbial contamination and, therefore, cells should be considered to remain potentially infectious even where comprehensive testing has been carried out.

Microbiological Test Procedures

Documentation of genetically modified components and pathogenic agents (e.g. recombinant cells, contaminated cell lines, control organisms) is important and may be a legal requirement (see relevant national rules).

Virus testing should be performed on early archive or master stocks that will be used for establishment of any future distribution stocks. It is recommended that banks should test human cell lines for serious and primarily blood born pathogens e.g. HepB, HepC, HIV, HTLV I/II, EBV and hCMV. This may be expanded to other viruses that may contaminate cells from the human reproductive tract (e.g. HPV, HSV, HHVs) depending on local policy, incidence of disease amongst donors and other risk factors identified for the individual cell line. The bank should have a documented procedure for dealing with positive microbiological results arising during testing.

Currently no recommendation can be made on tests for prions and agents of transmissible spongiform encephalopathies (TSEs), due to lack of validated sensitive methods and lack of information specific to contamination of stem cells. However, banks should carry out periodic reviews of developments in microbiological testing and, consider appropriate collaborative projects, to ensure that they maintain current best practice in testing regimes.

Microbiological testing should be performed using qualified methods and banks should hold information on sensitivity, specificity, robustness and other validation work that indicates fitness for the purpose of testing cell lines specifically. Viral screening may be mandatory under certain regulatory environments.

Endogenous retroviruses could arise in any cell culture and are known to be expressed at the RNA level in numerous cancer-derived cell lines and in murine cells. Retrovirus-like sequences provide normal functional elements in some key processes in human biology including the development of the placenta. However, the potential hazards arising from interaction of these sequences with other retroviruses (e.g. contaminants, deliberate infection of cells) to form novel viruses, should be considered.

Microbiological Issues for Supply of Cells

The bank must assure the capability of cell line recipients to handle potentially contaminated cells safely. The bank should also provide advice to recipients on safe handling that can be incorporated in local rules.

Banks should provide recipients with a statement listing and qualifying the testing performed on cells supplied including indication of the 'analyte' (i.e. test material) used.

The bank should have a policy on dealing with new microbiological data that may arise after cell banks have been released. This may include notification of all recipients of potentially affected cells (see ISCF website for information on national and local rules applicable to national banks).

Cell Characterisation

General Principles

Banks are considered to have a primary role to play in meticulous characterization of stem cell lines available for research. They must also confirm consistency for each cell line between deposited cells, master stocks and cells for distribution ('The Cell Banking Process', 'Banking Procedures').

A critical feature regarding the pluripotency of hESC cell lines is that they should at least form 'teratomas' in immune-compromised mice. The definition for nomenclature of these tumors has been a matter of recent debate but the conclusion recently adopted by *Nature Biotechnology* [11], following expert consultation, is as follows:

'.... Nature Biotechnology will adopt the term 'teratocarcinoma' to describe malignant tumors comprising both somatic tissues and undifferentiated malignant stem cells, identifiable as EC cells. .... We will apply the term 'teratoma' only to tumors composed of normal, 'benign' somatic tissue and their immature (fetal) precursors derived from more than one of the three embryonic germ layers (ectoderm, mesoderm and endoderm). Teratomas comprising nonproliferating somatic tissue may be further labeled as 'benign', 'mature' or 'fully differentiated'. Teratomas composed of immature, proliferating fetal-like tissues may be labeled 'immature'?.

It is strongly recommended that distributors of stem cell lines should adopt this terminology.

Morphology continues to be an important criterion in stem cell research and banks should provide representative images of undifferentiated and differentiated cells (NB under defined culture conditions and post seed/subculture time) for users to compare with their own cultures. Ideally the bank should also make images available of typical immuno-cytochemistry and examples of undesirable features such as differentiation.

Table 1 List of biological reagents used in stem cell derivation and culture and associated microbiological hazards

Reagent	Source	Potential Contaminants
Fetal calf serum	Bovine fetuses	Bovine viruses e.g. bovine viral diarrohea virus, bovine polymoma virus (numerous serum free media available but may still contain materials of animal origin)
Trypsin	Porcine pancreas	Porcine viruses (risk of contamination reduced by using recombinant trypsin produced in microorganisms or plants[a])
Bacterial enzymes such as Collagenase	Bacterial cultures of *Clostridium spp.*	Spores and organisms from the original culture[a]
Growth factors	Animal/human tissues and cells	Viral contamination depending on the species of origin[a]

[a] **NB** Altering the source of a biological reagent using recombinant DNA approaches may eliminate certain hazards but if the new source of material is still of biological in origin (e.g. recombinant organisms) then there may still be materials used in its preparation that represent a risk of contamination, however, this will generally not be a significant issue for materials used for research purposes.

There are certain key cell markers for the characterisation of stem cell lines that should be used by the bank, and these are described in Phenotype below.

Methods and Measurements

Phenotype

A standardised international study [12] of hES cell phenotypic markers confirmed for a large group of hESC cell lines that a typical surface marker profile for these cells is SSEA-1 (negative or very low), SSEA-3 'positive', SSEA-4 'positive', TRA-1-60 'positive' and TRA- 1-81 'positive' (NB, SSEA-3 & -4 have the potential to reveal different patterns for a very small number of individual donors i.e. 1%). In the same study microfluidic arrays for Q-RT PCR revealed that expression of six genes could be recommended for confirmation of the hESC phenotype: Nanog, Oct 4, DNMT 3B, TDGF, GABRB3, GDF3. These markers were found to be strongly correlated in stem cells. It is important to note that other cell types may express these markers but it is the overall profile of expression, not expression of individual markers, that is key. Flow cytometry (FC) is the central methodology for canonical markers of stem cells identified above. Immunocytochemistry is also valuable to provide additional data on morphology and localization of antigen. Multiple fluorochromes are useful for flow cytometry to evaluate coexpression, etc. Measurement of expression by FC should be recorded and available to bank users along with the method of FC data collection and the FC instrument used. Care should be taken in how data is collected and expressed and this should be clear to bank users. In particular, the bank should carefully and unambiguously report the meaning of data referring to "% positive cells". Banks should use appropriate controls in FC analysis (fixed cell preparations are under development in the International Stem Cell initiative www.stemcellforum.org.uk). Bank SOPs for such phenotypic analyses should be made available to users.

RNA expression profiling gives desirable additional supporting data on associated gene groups but for critical interpretation such data should be confirmed at the protein level.

In all analytical work on feeder cell dependent stem cells, care should be taken to exclude the feeder cells from cell samples to avoid interference in data on stem cell lines. In addition, it is useful to use a preparation of pure feeder cells as a control.

Genotype

Each genotyping technique gives a different type of data and it is important that the bank understands and reports the advantages and limitations of the methods it uses.

Karyology by Giemsa-banding (G-banding) should be performed as a routine genotyping technique. However, it should be recognized that some diploid cells may carry undetected genetic alterations; and techniques such as "spectral karyotyping" (SKY), comparative genome hybridization (CGH) microarray and multiple single nucleotide polymorphism (SNP) analysis can provide useful additional information. However, comparative data from different methods will need to be collated from different centres over time. It is important to remember that the number of cells analysed can be critical for sensitivity of detecting abnormal clones and the bank should make such details available to users.

G-banding analysis can detect the appearance of chromosomally abnormal clones, however, the number of metaphase spreads analysed is critical to the sensitivity of their detection. Where karyologically abnormal cells are found, repeat testing is recommended to confirm the findings (see below). Abnormal sub-clones of stem cell lines should be renamed and information and cells made available as they may be useful in genetic research or for high throughput screening methods.

The following guidance indicates the minimum work necessary for the cytogenetic analysis of hESC cell cultures. Standard G-band analysis of prepared cell metaphase 'spreads' should include a chromosome count for 20 metaphases and banding patterns analysed in a minimum of 8 metaphases. It is to be expected that occasional abnormal karyotypes will be observed in hESC cell line analyses and these may appear to be present in all cells (i.e. clonal) or in a minority of cells (non-clonal). Chromosomal abnormalities that appear to be of clonal origin should be confirmed by repeat analysis. Abnormalities seen in single cells (i.e. presumed non-clonal) may result from a technical artifact, but may be due to a developing clonal abnormality or low level mosaicism [13, 14]. Chromosomal analysis of a repeat sample from the culture may be helpful in determining the basis of apparent anomalies. Standard terms and methodology for G-banding analysis of hESC cells adopted in this guidance are given in Table 2. These were developed as a consensus between the standards used in the USA and the Association of Clinical Cytogeneticists General Best Practice Guidelines (2007) [15] available on that organisation's website (see website list). New international guidance on definitions and standards for cytogenetic analysis are given in ref [16].

Pluripotency

The bank should provide some form of evidence for the potential of each stem cell line to produce cells representative of the three germ layers that ultimately give rise to all cells of the body. The 'gold standard' for this pluripotency is considered to be the ability of stem cells should at least form teratomas in SCID mice. This should be performed on

each cell line at least once in its history and the bank should make all efforts to ensure that this characteristic is not lost by *in vitro* manipulation in the bank.

Assays of cell features deemed to be subject to variation in culture should be carried out by the bank. If certain hESC lines are found that do not form teratomas, they may also have value for research into pluripotency. It is considered important that teratoma formation tests should be applied to all cells that may be used for clinical purposes.

Alternative methods are available for the determination of potential pluripotency including formation and characterisation of embryoid bodies and *in vitro* induced differentiation. Standardisation of any pluripotency assay will prove challenging. This is an area which is under development and banks should keep up to date on current technical progress. In the future it will be extremely helpful if the methods used are sufficiently accessible that they can be reproduced in all user laboratories.

Cell Line Stability and Epigenetics

In order to avoid any subtle changes in cell characteristics, it is recommended that for each cell line banks use the depositor's methodology, at least up to the first cryopreserved stock of cells or Master Cell Bank. Banks should monitor cultures for adaptation to *in vitro* conditions (e.g. changes in growth rate, cloning efficiency) and attempt to minimise the risk of changes in stem cell cultures by:

- Minimising culture passages (a Master- and Working cell bank system is key to this; see 'The Cell Banking Process') and being aware of culture methods that could induce change
- Publishing subculturing methods for recipients to use
- Passaging cells beyond typical levels used by recipients of cell lines and requalify cells at these time points
- Making recommendations to recipients not to use cells beyond a specified passage level from a characterised cell bank

It is important to record actual passage number and to notify recipients of the passage level of cells provided. Population doublings may be difficult to estimate with current protocols, however, this is considered a more accurate and useful measure of replicative 'age' of a cell culture and it would be highly desirable to have such data available from the bank. Banks should keep up to date with current technical developments in this area to ensure that bank procedures reflect current best practice.

Investigation of epigenetic variation occurring in stem cell lines is at an early stage and more data is needed before routine tests can be recommended for stem cell banks. However, banks should keep up to date on current scientific and technical developments in this area.

Release Criteria for Cell Banks

General Principles

Bank cultures should be representative of the originally deposited material i.e. quality control and characterisation data should show no evidence that the banking process has altered the cell line in comparison to the cells provided by the depositor.

The bank should provide assurance that cell lines released have no detected microbial contamination and, that as far as can be determined, they are 'monoseptic' i.e. a single uncontaminated viable cell line. This assurance can be provided through a combination of cell-identity testing and microbiological isolation methods but should be qualified based on the types of organisms that might be detected by the testing regime used.

Identity testing is critical to demonstrating that the cell line is unique (i.e. not switched with, or cross-contaminated by, another cell line). Banks should share identity data to avoid spread of cross-contaminated cultures, but should take care in general with dissemination of genetic information that may be donor-specific i.e. could have a significant influence on the healthcare of the donors or lead to donors being identified.

'Sterility' tests from antibiotic-free cultures should be performed on cell banks to provide evidence for absence of bacterial and fungal contamination. Banks should seek to extend these tests to cover fastidious organisms, including mycoplasma and certain bacteria that may contaminate cell cultures but are not detected by standard sterility test methods for bacteria and fungi.

Mycoplasma species are a frequent cause of contamination in cell culture and can be introduced from a variety of sources including bovine serum, feeder cells, animal-derived raw materials (e.g., trypsin) or humans. Mycoplasma species are one of the greatest risks for potential contamination of mammalian cell lines with several studies estimating rates of contamination as high as 15-30% in cell lines that are shared between research labs [17–19]. Although over 20 different mycoplasmas have been isolated from cell lines, the vast majority of contaminations are typically due to eight species: *Mycoplasma hyorhinis* (porcine trypsin), *M. arginini* (bovine serum), *M. fermentans* (human), *Acholeplasma laidlawii*, *M. hominus* (human), *M. orale* (human), *M. bovis* (bovine) and *M. pulmonis* (murine). Due to their small size, filtration is not always effective at removing mycoplasma from cell culture

Table 2 The karyological analysis of hESC lines: recommended terms, standard method and procedures for investigation of abnormal results and reporting of data

Standard G-band Analysis	A minimum of 8 metaphases analysed	
	20 metaphases counted	
Procedure for investigation of clonal abnormal findings	Clonal chromosome abnormalities should be confirmed in a second later passage culture, to allow further interpretation of their significance (see Genotype)	
Procedure for investigation of abnormalities observed in single cells	Single cell abnormalities (e.g. aneuploidy, structural rearrangements) will require further investigation in some cases to exclude mosaicism, depending on the chromosome involved.	
	Aeuploidy of chromosomes 1, 8, 12, 14, 17, 20 and X (incl. unbalanced rearrangements)	A minimum of 30 G-banded cells counted from initial culture. Examine 100 interphases using FISH with appropriate probe of a follow up later passage culture and 30 G-banded cells.
	Other aneuploidy and structural abnormalities	A minimum of 30 G-banded cells counted from initial culture.
Minimum quality score	ISCN 400 band level is the minimum level of G-banding analysis necessary, although effort should be made to analyse cells of ISCN 500 band level and above.	
	The method used to score the banding is at the laboratory's discretion. An example of a banding scoring system can be found in the UK's Association of Clinical Cytogenetics Best Practice Guidelines (2007) (ref. 15).	
	www.cytogenetics.org.uk/prof_standards/acc_general_bp_mar2007_1.01.pdf	
Substandard analysis	If analysis at the ISCN 400 band level cannot be achieved, the analysis can proceed as normal but should be reported with the caveat that it is a "substandard analysis" and may need to be repeated.	
Report	The result reported should include:	
	–The karyotype designation using current correct ISCN nomenclature 2009 (ref. 16), where practicable.	
	–The types of analysis used (e.g. karyotype, FISH, CGH, special types of banding etc).	
	–The average banding level achieved. Single cells with aneuploidy or structural abnormalities involving chromosomes 1, 8, 12, 14, 17, 20 or X (this list is evolving) should be reported, even after extended analysis, as it is necessary to analyse a second, later passage culture to fully interpret the abnormality	
Definitions (Adapted from ACC General Best Practice Guidelines, 2007) (ref. 15)	**Analyse** – 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.	
	Count – To enumerate the total number of chromosomes in any given metaphase in such a way that gross structural abnormalities would be identified, or in FISH analysis to enumerate the number of signals in an interphase nucleus.	
	Score – To check for the presence or absence of abnormalities in a cell or metaphase without full analysis.	
	Clone – A cell population originally derived from a single cell.	
	Such cells will have an identical chromosome constitution. A clone is said to exist if three cells have lost the same chromosome, or two cells contain the same extra or structurally rearranged chromosome.	

media and reagents. In addition, mycoplasma contamination of cell cultures is often difficult to detect since gross changes in culture (e.g., turbidity) are not commonly observed. Mycoplasma contamination, however, can result in significant changes in cell metabolism and growth characteristics and can have a dramatic effect on cell phenotype and chromosome structure. Due to the high frequency of mycoplasma contamination, good cell banking practices should include measures for early detection and preventing the spread of contamination.

Cells should not be released if they are known to carry a viral hazard to laboratory workers or a virus that may influence research data from the culture in question. It is possible that persistently infected cells would have significant value in research but these would have to be handled separately in appropriate facilities and the infection notified to recipients.

In addition to tests for certain viral pathogens (see 'Microbiological Testing'), banks should also consider inclusion of testing for other contaminants prevalent in the local environment, media supply and donor groups.

Technical aspects of release procedures

Each bank should establish a clear set of release criteria that should apply to each bank of cells. a recommended minimum set of criteria for release of hESC cell banks is given in Table 3.

A similar test regime should be established for feeder cell banks based on a minimum requirement for sterility testing, mycoplasma testing and viability as described for hESCs (Table 3). In addition, identity testing and appropriate virological testing (see 'Microbiological Testing') should be carried out for human feeder cells. Mouse embryonic feeder

Table 3 Proposed testing for hESC master cell banks[a]

Test	Specification	Examples of test methods	Typical test result pass specification	Release criteria
Identity	Matches parent cell line	Short Tandem Repeat (STR) Testing Human Leukocyte Antigen (HLA) Testing	Shares all alleles of parent cell line	Passes test result specification
Bacteria/fungi	No contamination detected	Inoculation of microbiological culture media to detect growth of bacteria and fungi[b]	No culturable bacterial or fungal organisms detected[d]	Passes test result specification
Mycoplasma	No contamination detected	Direct culture in broth and agar and indirect test using indicator culture/ DNA stain[b]	No culturable mycoplasma detected[d]	Passes test result specification
Karyotype	Report karyotype from a specified number of metaphase analyses (see Methods and measurements)	Perform G-band analysis of 20 metaphase spreads (see Table 2). Further analysis may be performed using FISH.	Single karyotype in all cells analysed. No alternative karyotypes at or above 5% of metaphase 'spreads' in the cell preparation (see 'Methods and Measurements')	Passes test result specification
Post-Thaw Recovery	Viable colonies recovered (quantified efficiency of recovery of each bank/lot should be given)	Test for the ability to recover viable hESC colonies	Colonies recovered that are representative of the original cell line as demonstrated in other quality control and characterization data[e]	Passes test result specification
Pluripotency	Report data available	Formation of teratomas in immune-deficient (SCID) mice (see 'Pluripotency' of this guidance). Also formation of embryoid bodies and *in vitro* 'directed' differentiation.	Data presented	(see footnote [f])
Growth Characteristics	Report value	Growth characteristics under standard cell culture conditions. Determine doubling time.	Growth rate estimate presented	–
Cell Antigen Expression	High proportion of cells (typically >70%) positive for each marker	Flow cytyometry for a arrange of hESC markers (e.g.SSEA-3, SSEA-4, TRA-1-60, TRA-1-81, Oct-4) and SSEA-1 negative..	Data presented	–
Cell Gene Expression	Report data available	Gene expression profiling using DNA microarray or Q-PCR analysis. Analyze for expression of core hESC genes as well as markers of differentiated cell types.	Data presented if available	–
Genetic stability	Report data available	Analysis of multiple Single Nucleotide Polymorphisms. Comparative Genome Hybridization by DNA microarray methodology.	Data presented if available	–

[a] Testing on stocks other than the Master cell bank may not need to be so extensive but should be defined by the bank

[b] Tests consistent with pharmacopoeial methods are recommended

[c] Cell differentiation capacity can be determined during characterization studies performed on distribution lots/banks. These studies should be performed on cells passaged beyond the distribution lot passage level e.g. 20 passages

[d] Use appropriate controls to assist in validation of result, sensitivity determination and false negatives due to inhibition of assays. Sensitivity should be known and validated for hESC samples in the hands of the testing laboratory

[e] The bank should establish a justified specification that may be dependent on the needs of customers. This is a developing area in which banks should maintain knowledge of current developments and best practice. Coordination between banks is vital to continue to improve viability of preserved cells supplied to bank users

[f] 'Pluripotency' should be a release criterion for stem cell lines which the bank asserts to be potentially pluripotent. Where this is included in release criteria the cell bank report should be based on a defined protocol for the methodology and data evaluation

cells should be obtained from animal colonies with an appropriate standard of husbandry compliant with national and/or international standards, including colony screening for common murine pathogens. Mouse antibody production tests can be used for virus detection in mouse feeder cells and it is also recommended to prepare large homogenous stocks of these cells to promote consistency and economy. The significance of potential murine virus contamination of human stem cells should also be born in mind when introducing them into animals especially SCID mouse colonies.

Table 4 Mycoplasma detection methods

Technique/Test Method	Comments
Selective broth and agar subculture/industry standard methods are published in national pharmacopoeia	Generally considered the most sensitive method for detecting culturable *Mycoplasma* and *Acholeplasma* species.
	Long incubation periods are required with final results not available for several weeks.
DNA stain (Hoechst or DAPI) of inoculated Vero cells (industry standard methods are published in national pharmacopoeia).	Can isolate mycoplasma strains that do not grow in broth/agar method.
	Simple to carry out providing a high magnification uv-epiflourescent microscope is available.
	Results available within a few days give good sensitivity but generally not considered to provide the level of sensitivity obtained using culture methods.
	Rapid direct staining can be performed, but may suffer from cell and bacterial artefacts leading to ambiguous results.
PCR/ variety of methods based on amplification of mycoplasma DNA e.g., 16S rRNA, 23S rRNA, rpoB, ITS. Representative references include [20–24].	Detects non-culturable organisms
	Useful for screening cultures due to easy sample preparation and rapid production of test results (within hours)
	May be very sensitive but some methods can lead to false positives (important to validate the specific method used for sensitivity and specificity)
	PCR assays are also subject to limited sensitivity due to small sample volume and inhibition of the PCR reaction by sample components. Sensitivity can be addressed by use of diluted DNA controls and sample spiking with positive controls ([23]).
Detection of mycoplasma specific enzymes/examples of commercially available kits are given	MycoTect (Invitrogen): detects a wide range of mycyoplasma species based on levels of the mycoplasma-specific enzyme adenosine phosphorylase.
	MycoAlert (Cambrex): reported to be capable of detecting down to 20 cfu/mL of several mycoplasma species.

Mycoplasma tests should be performed on newly deposited cells under quarantine and all cell banks established. Several different assay methods are currently available for mycoplasma testing, including culture methods, PCR-based assays and detection methods based on the presence of mycoplasma-specific metabolic enzymes. Mycoplasma testing of distribution cells is typically conducted using either culture or PCR-based assays since these methods can provide broad coverage of *Mycoplasma* and *Acholeplasma* species. A brief overview of these methods is provided in Table 4. The chosen test methods should be validated for their specificity and sensitivity for testing cell lines. For the testing cell banks, a combination of methods used to give high sensitivity and detection of non-culturable strains is recommended.

Identity testing should be performed on samples of early stage material (early passage cell DNA preserved either frozen or on storage cards e.g. 'Guthrie cards', 'FTA' cards (Whatman)) and all cell banks established (see 'The Cell Banking Process'). It is recommended to use systems with core common alleles represented in forensic work and examples from commercially available STR kits are given in Table 5. Publication of STR profiles of recently isolated human stem cell lines may present ethical issues in some countries. However, it is recommended that banks should share such data for the purpose of identifying cross-contaminated cell lines and preventing their release. Failure to do this could have a serious long term effect on the validity of research data performed with such lines.

"Viability" is a difficult parameter to define - frozen vials or straws should enable recovery and expansion of a 'representative' culture within a certain time (see Table 3). The bank should formally qualify the viability method it uses to test thawed vials from frozen stocks intended for distribution. Viability test data can be used as a release test but should not be used as an absolute indicator of the 'quality' of a stem cell bank. This can only be demonstrated through successful completion of a range of quality control and characterisation tests.

The composition of cell types should be evaluated for test samples from each bank and should include percentage of cells positive for markers and the level of apparently differentiated cells (see 'Cell Characterisation'). The functional properties of cells should also be addressed and the key property of pluripotency is dealt with in 'Cell Characterisation'.

Table 5 Examples of STR genetic alleles represented in commercially available kits

Gene locus name	Applied biosystems				Promega
	Cofiler (USA)	ProfilerPlus (USA)	Identifiler (UK)	SGM + (UK)	Power Plex 16 (UK, USA)
Amelogenin	+	+	+	+	+
D3S1358	+	+	+	+	+
D16S539	+	–	+	+	+
TH01	+	–	+	+	+
CSF1PO	–	–	+	–	+
TPOX	+	–	+	–	+
D7S820	+	+	+	–	+
D13S317	–	+	+	–	+
D21S11	–	+	+	+	+
D18S51	–	+	+	+	+
D8S1179	–	+	+	+	+
FGA	–	+	+	+	+
vWA	–	+	+	+	+
D5S818	–	+	+	–	+
D2S1338	–	–	+	+	–
D19S433	–	–	+	+	–
Penta D	–	–	–	–	+
Penta E	–	–	–	–	+

Key: + , indicates alleles for which each kit has primer sets

Supply Issues

The bank should remind recipients of lines that the testing performed on each cell bank may not detect microbial contamination below the level of detection or organisms not covered in the testing regime and thus recipients should be advised to handle the cells as if potentially infectious.

Shipment of Cells and 'Information for Users'

General Principles

"Instructions For Use"(IFU) and/or Standard Operational Procedures for culture and preservation should be provided to bank users. The IFU should typically contain information prescribing general culture and preservation methods and what procedures the cells have been qualified or consented for (e.g. "*in vitro* research only", "not for generation of gametes", "not for reproductive cloning").

Lot numbers for the cells shipped should be provided to users that are traceable to the 'lot' or cell bank. It is also recommended that data on the Master Cell Bank is made available by the bank. Banks should supply, with the cells, test results obtained from that specific stock of cells (i.e. a Certificate of Analysis for each bank listing data including quality control and characterisation). It is also desirable for these to be available from the bank website. A statement or materials safety data sheet on hazards associated with the cells should be supplied with cell shipments. Terms and Conditions or a warranty should be provided which qualifies cell potential and characteristics based on testing performed by the bank.

Information Available to Users of the Bank

Such information should include: standard operating procedures (culture, preservation etc.) and characterisation data from the depositor for each cell line in the bank. Each bank should provide a statement on policy for the quality and sourcing of certain raw materials subject to national or international restrictions (e.g. serum). It would also be desirable for data generated on cell lines supplied by each bank to be made available to assist future users of the bank.

Ethical issues may be particularly important for bank users and each bank should provide information to facilitate efficient selection of suitable lines. Relevant information would include date of preservation of tissue, date of attempted 'derivation' (for hESCs, this is usually considered to be the date the inner cell mass was isolated or plated *in vitro*); whether fresh or frozen embryos used;

whether payment was made for the donation of embryo/tissue; whether the embryo was created for research; the existence of fully informed consent obtained from the donor for use of the original tissue for research; and any associated constraints on the use of the derived line. Information should also be available on the derivation procedure, as well as seminal and key publications for each cell line.

Materials Transfer Agreements (MTAs)

Banks should work within national guidelines and laws on ownership and patenting of biological materials (see the World intellectual property organisation website www.wipo.int/ for general information and for information on patent office contacts in the 184 WIPO member states see www.wipo.int/members/en/).

A common MTA would not be practicable for all banks; however, biological resource organisations have identified key generic elements that should be included (e.g. European Culture Collection Organisation www.eccosite.org/) and there are other national examples that could be considered as templates (e.g. National Cancer Institute – see also links to national banks on the ISCF web site to obtain MTAs from suppliers of stem cell lines).

Transportation

Technical Issues

Vials and straws shipped should be from a homogenous distribution bank of cells (see 'The Cell Banking Process', 'Banking Procedures') and contain sufficient cells to readily recover a culture (see 'Release Criteria for Cell Banks'). The method of transport should be appropriate for the method of preservation and validated by the bank. Typically, cryopreserved vials (i.e. preserved by a slow cooling method) can be shipped on "dry-ice" (solid carbon dioxide) and 'vitrified' materials should be shipped in the vapour phase of liquid nitrogen (i.e. in 'dry-shippers'). In the international distribution of mouse embryos, test vials (or 'dummy' straws) have proven helpful for recipients to test their thawing process.

Preservation methods are developing and improved methods are needed to assist in stable storage and shipment and it is important that banks maintain awareness of current developments in preservation science and technology.

Administrative Procedures

Banks should have a planned and documented shipment process to identify recipient contacts (i.e. a detailed local contact name/tel. no. to receive cells as well as the shipment address), shipment, delivery dates and shipment tracking information from the courier.

For each shipment the bank should check all local import regulations with the recipient. Helpful information is available from the World Federation of Culture Collections on import regulations http://www.wfcc.org - see also the ISCF stem cell banking webpages for links to different national import/export regulations. Before dispatching cells the bank should also check the necessary international packing and labeling requirements for national and international shipment (see www.iata.com).

Preference should be given to use of shipping agents that can refresh 'dry-ice' or liquid nitrogen and the chosen method of shipment (insulated box of 'dry-ice' or nitrogen 'dry-shipper') and required storage temperature on receipt should be communicated to the recipient laboratory.

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ORIGINAL PAPER PS17

Transfusion errors and their prevention

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Although the current risks of acquiring viral transmission through blood components and products are very small, mistransfusion, in which a blood unit is incorrectly administered to a non-designated recipient, remains the most common type of error in transfusion practice. ABO-incompatible blood transfusion attributable to the incorrect identification (ID) of the patient or the blood unit is one of the most serious transfusion hazards. Many studies revealed that transfusion errors occur frequently in clinical areas, with the most common error being failure to perform the final patient ID check at the bedside. Thus, the pretransfusion bedside check is the most critical step for the prevention of mistransfusion. Machine-readable ID technology, especially a bar code-based ID system, is ideally suited to bedside check requirements. Our experience at the Juntendo University Hospital shows that the bar code-based ID system has worked well over a 7-year period on a hospital-wide basis, and approximately 70 000 blood components have been transfused without a single mistransfusion. The overall rate of compliance with 'second' electronic bedside checking for blood components was 99%. Human error was the most frequent cause of errors leading to the failure of the bedside bar code ID check. The bar code-based ID system has become applicable to the bedside pretransfusion check for paediatric transfusion, i.e. blood dispensed in syringes, and preoperative autologous blood donation, as well as regular allogeneic blood transfusion. If we want to reduce the risk of mistransfusion to improve transfusion safety, we have to address the issue at the hospital level, with a system-based approach.

Key words: bar code identification, bedside pretransfusion check, mistransfusion, transfusion safety.

Introduction

The risks of blood transfusion fall into several broad categories: immune and serologic transfusion reactions; blood-transmitted infections; lesser known complications, including transfusion-related acute lung injury, transfusion-associated circulatory overload and transfusion-associated graft-*vs*-host disease; and those associated with human error, especially mistransfusion. Traditionally, attention to increasing transfusion safety has focused mainly on the prevention of known viral transmission. The current risks of acquiring viral transmission through blood components and products are very small [1]. Therefore, non-infectious serious hazards of transfusion have emerged as the most common complications [2]. The risk of non-infectious complications, including risks related to hospital-based steps in transfusion care, is at least 100 times greater than the risk of acquiring human immunodeficiency virus or hepatitis C virus infection through blood components [3].

Mistransfusion, where a patient receives a blood component that did not meet the required specification or was intended for a different patient, remains the most common type of error in transfusion practice. In particular, ABO-incompatible transfusions attributable to incorrect identification (ID) of the patient or the blood unit are among the most serious of transfusion hazards [3–5]. Mistransfusion is the final outcome of one or more procedural errors or technical failures in the transfusion process, starting with the decision to transfuse a patient and ending with the actual administration of blood components [3]. The prevention of

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ID check errors, regarding either the patient or blood unit, is of major importance in current transfusion practices, and reducing the rate of human errors increases transfusion safety.

Frequency of transfusion errors

Sazama [4] described the issue of transfusion-associated deaths reported to the Food and Drug Administration from 1976 through 1985, in which 51% of 256 reported deaths resulted from acute haemolysis following the transfusion of ABO-incompatible blood products. Linden *et al.* [5] calculated the risk of ABO-incompatible transfusion to be 1 in 38 000 units, and erroneous administration was observed for 1 of 19 000 RBC units administered in New York State. The Serious Hazards of Transfusion (SHOT) scheme in England showed that, of 2630 reported adverse events between 1996 and 2004, 1832 (70%) were incorrect blood component transfused (IBCT) and the frequency of IBCT events was calculated as 7 per 100 000 components [6]. Similar findings have been reported from the haemovigilance program in Quebec, in which ABO-incompatible transfusion was the most common major adverse event occurring at a rate of 1 in 13 000 transfusions [7]. However, the true incidence of mistransfusion seems to be even higher due to a failure to recognize many errors and because of a lack of complete denominator data on transfusion episodes.

Pretransfusion check at the bedside

The SHOT scheme showed that approximately 70% of IBCT event errors take place in clinical areas, the most frequent error being failure of the final patient ID check at the bedside [6]. Mistransfusion typically results from an error made during the bedside check just prior to transfusion, through which the blood unit is confirmed to be correct for the intended recipient. The pretransfusion check at the bedside is the most critical step for the prevention of mistransfusion, and represents the final opportunity to interrupt a mis-guided blood component. However, a large observational audit revealed a failure to perform the final bedside check [8]. Machine-readable ID technology, especially a bar code-based ID system, is ideally suited to bedside check requirements and has been recently reported to significantly improve transfusion practice [9–12]. When a bar code-based ID system was implemented in a hospital, the checking procedure at the bedside involved only one nurse [13]. The bedside pretransfusion check, where one nurse carries out the verification procedure using a bar code-based ID system, seems to be plausible. We recently showed that the overall compliance rate with the bedside bar code ID check was 97·8% on a hospital-wide basis [14]. If electronic bedside checking fails because of human error, one-person bedside checking without new technology may present a higher risk of mistransfusion than a standard two-person double-check, although the number of people required to check the identity of the patient and blood unit at the bedside is controversial [15].

The Juntendo experience

Pretransfusion check at the bedside

The SHOT scheme reported that approximately 30% of errors pertaining to IBCT events occur in the hospital transfusion laboratory [6]. These may involve the selection of the wrong sample for testing, transposition of labels, technical or transcription errors in manual pretransfusion testing, or knowledge-based errors such as the selection of components not of the appropriate specification. The transfusion policy for blood administration in our hospital includes a standard two-person (doctor/nurse or two nurses) visual and verbal double-check first, followed by bar code ID check using a hand-held device just prior to blood administration [12]. We carry out verification procedures separately at the transfusion service (issuing verification) and bedside (bedside verification). The transfusion service carries out issuing verification to ensure that the staff members of the transfusion service have attached the correct compatibility label to the correct blood after the completion of compatibility testing. In the issuing verification procedure, the staff member sequentially scans bar codes of his/her own ID badge, blood unit, and compatibility label using a hand-held device. If the blood components are issued by dispensing in syringes for paediatric transfusion, the bar code of the primary bag of blood is copied on compatibility labels using a bar code printer to perform the electronic verification of blood dispensed in syringes. Then, the compatibility labels are attached to syringes. All blood components are delivered from the transfusion service after the completion of issuing verification. Although the issuing verification does not play an essential role in the electronic pretransfusion check, it may contribute to enhancing the overall safety of transfusion.

The bedside verification procedure is somewhat different between inpatient wards and operating rooms. In inpatient wards, where the wired method is employed, the transfusionist sequentially scans bar codes of his/her own ID badge, the patient's wristband, and blood unit using a hand-held device. In operating rooms, where the wireless technology is employed, electronic verification procedures are composed of two parts, i.e. 'entrance' and 'bedside' verification. In the entrance verification procedure, a nurse scans the bar code of the patient's wristband using a hand-held device when the patient enters the operating room. In the bedside verification procedure, after completing the

two-person double-check, the transfusionist scans only the bar code of the blood with one scanning for each unit just prior to blood administration. Reciprocal scanning of bar codes of the wristband and blood is unnecessary for each unit. This procedure made it possible to sequentially scan bar codes of multiple blood components in an emergent situation in operating rooms. If the bar codes of the wristband and blood are identical, the screen of the hand-held device displays 'OK' [12]. Non-matching data result in a warning of 'NG' with an alarm sound. After the completion of bedside verification, the transfusionist initiates the administration of blood components. Since initiating this bar code-based ID system, approximately 70 000 blood components have been transfused over a 7-year period without a single mistransfusion, even with the failure of the 'second' bar code ID check at the bedside. If electronic bedside checking is combined into a standard two-person double-check, it may reduce the risk of mistransfusion.

Monitoring of pretransfusion check at the bedside

Intercommunication between the transfusion service and inpatient wards or operating rooms via a network permits us to monitor compliance with the bedside bar code ID check [12]. Manipulations of a hand-held device alter the information contained in the host computer of the transfusion service. The issued blood components are listed on the screen of the host computer. When a nurse downloads the transfusion data into a hand-held device via a network, an 'open circle' symbol appears on the screen. After the transfusionist performs the bedside verification just before the initiation of transfusion, the symbol on the screen changes from an 'open circle' to a 'closed circle'. Thus, the transfusion service can monitor the electronic bedside verification, confirming that the correct units are for the correct patient. If the mark on the screen of the host computer remains as an 'open circle' 1 h after the issuing of blood components, the transfusion service judges that the bedside bar code ID check was not completed. Thereafter, the transfusion service contacts the clinic by telephone, clarifies the cause of failure, and finally indicates that the issued blood should be administered immediately if it has not yet been used.

Paediatric transfusion

Because of differences in the age, size and maturity of physiological systems, children undergoing transfusion may incur different risks compared with adults. A multi-institutional analysis of blood transfusion has been reported in a large cohort of paediatric patients not more than 18 years of age, in which the overall complication rate in the study was 10·7 per 1000 transfusions [16]. The complication rate in adults has been reported to be 2·5 per 1000 transfusions in a national incident reporting system of France [17]. In addition, the SHOT scheme [18] showed that 264 of 321 reports (82·2%) related to transfusion in patients < 18 years of age were instances of IBCT event errors, and that the proportions of IBCT event errors in paediatric patients were higher than those in adults. These findings suggest that paediatric patients are more vulnerable to transfusion-associated complications than adults.

Paediatric transfusion has special requirements regarding the transfusion of blood components, including small-volume transfusions, dispensing blood in plastic syringes, and infusion through small-gauge needles with or without a mechanical infusion pump. Many transfusion services use only RBC components for infants < 4 months, because they are unlikely to develop acute haemolytic transfusion reactions. However, a safety strategy for preventing mistransfusion is needed in paediatric patients being transfused blood components of platelets or fresh-frozen plasma. We applied the bar code-based ID system to the pretransfusion check at the bedside in the setting of paediatric transfusion [19]. Blood dispensed in syringes was attached to compatibility labels, on which the bar code of the primary bag of blood was printed, making it possible to perform electronic bedside checking using hand-held devices. Wristbands were occasionally attached to the patient's ankle or nameplate at the bedside in cases of neonates and preterm infants. The bar code-based ID system for paediatric transfusion has worked well over a 4-year period, and a total of 3957 blood components (10% of all transfusions) have been administered to paediatric patients without a single mistransfusion. The compliance rate with the electronic bar code ID check at the bedside for blood dispensed in syringes was 99%. This was slightly superior to the overall compliance rate with electronic bedside verification of our previous study on a hospital-wide basis [14], suggesting the acceptance of the bar code-based ID system for paediatric transfusion by nursing staff.

Preoperative autologous blood donation

Preoperative autologous blood donation (PABD) involves collecting and storing the patient's own blood prior to surgery to administer it, if necessary, postoperatively. It has been advocated both to reduce the risk related to allogeneic blood transfusion and to augment the blood supply. However, both autologous blood donation and transfusion are associated with risks. One (0·006%) in 16 783 autologous donations has been reported to be associated with an adverse reaction severe enough to require hospitalization [20], which is nearly 12 times the risk associated with allogeneic donation. This difference is probably attributable to the fact that autologous blood donors have underlying pathological conditions such as cardiac, vascular and

respiratory disease. The transfusion of autologous blood involves many of the same complications as that of allogeneic units, such as the risk of administrative errors. Patients may receive another patient's autologous blood as a result of clerical errors or may receive allogeneic blood when autologous blood is available. There have been few studies examining the frequency of errors in PABD transfusion. Ohto *et al.* [21] reported a nationwide survey in Japan regarding autologous blood donation and transfusion, and identified 117 errors or problems (0·47%) in 24 929 PABD transfusions, of which one unit (0·004%) was administered to the wrong patient. Domen [22] reported a total of 20 (0·072%) adverse reactions in 27 859 autologous blood transfusions at the Cleveland Clinic, of which one (0·0054%) in 18 506 intra-operatively salvaged blood products was transfused to the wrong patient. Because the error rate of autologous and allogeneic blood transfusions appears to be similar, autologous transfusion should be approached with the same level of care and consideration as allogeneic transfusion.

We applied the bar code-based ID system for the pre-transfusion check at the bedside in the setting of PABD transfusion [23]. Compatibility labels were affixed to PABD units, on which an in-house bar code identifying the product type and number was printed, making it possible to perform electronic bedside checking using hand-held devices. The bar code-based ID system for PABD transfusion has worked well over a 5·5-year period, and a total of 5627 PABD units have been administered to patients without a single mistransfusion. The overall compliance rate with the bedside bar code ID check for PABD transfusion was 99%. This high compliance rate seems to be derived from the simple checking procedure employing the system in operating rooms. Of 5627 PABD units transfused, 75% were administered in operating rooms.

Causes of the failure of electronic bedside checking

We recently reported the causes of the failure of 'second' electronic bedside checking in the administration of blood components [14]. All errors leading to the failure of bedside bar code ID check were categorized into four broad groups: human, hand-held device, system and wristband errors. Human errors were further divided into manipulation and neglect-based errors. The categorization and definition of errors leading to the failure of the bedside bar code ID check are described in Table 1. From April 2004 to December 2007, a total of 43 068 blood components were transfused without a single mistransfusion, and 958 transfusions (2·2%) were performed without 'second' electronic bedside checking. The overall compliance rate with bedside bar code ID check at the time of this study was 97·8%, being 99% over the last one year. The cause of the failure of electronic bedside checking was human error in 811 cases (84·7%), hand-held device error in 74 (7·7%), system error in 50 (5·2%) and wristband error in 23 (2·4%). Of the human errors, 698 (86·1%) were manipulation errors, and the remaining 113 (13·9%) were neglect-based errors. In May 2006, the transfusion service initiated notification at 1 h after the issuing of blood components to clarify the cause of failure and indicate the immediate use of the issued blood, as described above. The total numbers of blood components transfused before and after the initiation of notification were 24 727 and 18 341, respectively. Of the human errors, manipulation errors mainly decreased after the initiation of notification, and neglect-based errors were still reported, although the number was small. The mean number of human, manipulation and neglect-based errors significantly decreased from 26, 22 and 4 to 9, 7 and 2, respectively, after the initiation of notification. These errors may be decreased by the further education and training of medical staff and continued support from the transfusion service.

Table 1 Categorization and definition of errors leading to failure of the bedside bar code ID check

Category	Definition
Human errors	
Manipulation errors	The transfusionist did not manipulate the hand-held device correctly
Neglect-based errors	The transfusionist did not act in accordance with the transfusion policy[a]
Hand-held device errors	Failures of the device itself, including battery failure or it being dropped
System errors	Failures of the bar code-based ID system or network communication, including down- or up-loading errors using hand-held devices
Wristband errors	Failures of the patient's wristband, where patients were not wearing them at the time of transfusion because they were intentionally removed

[a]The transfusion policy in our hospital includes a standard two-person visual and verbal double-check that should be performed first, followed by a bar code ID check using a hand-held device just prior to blood administration. In this case, the 'second' bar code ID check might be omitted due to urgency or time constraints.

Bedside ID technology

Bar code technology is a widely used, reliable and inexpensive machine-readable ID system. Although our electronic ID system presently employs the linear bar code that has been adopted by the Japanese Red Cross Blood Center, more advanced systems such as radiofrequency ID (RFID) will be

