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Improving rigor and reproducibility through implementation of the ISSCR standards for human stem cell use in research

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The International Society for Stem Cell Research (ISSCR) document "Standards For Human Stem Cell Use in Research" put forward a checklist for scientific journals to use in the assessment of compliance with its reporting standards. A trial implementation of this checklist at *Stem Cell Reports* revealed consistent gaps in the reporting of critical data relating to the cells and experimental methodologies employed in published manuscripts.

In 2023, the International Society for Stem Cell Research (ISSCR) published its document "Standards for Human Stem Cell Use in Research" (https://www.isscr.org/basic-research-standards), a guide to best practice in laboratory use of human pluripotent stem cells (PSCs) and tissue stem cells (TSCs). The document was the result of nearly 2 years of work by an international committee of experts, ISSCR staff, and outside reviewers (Lefkopoulos, 2023; Ludwig et al., 2023). It was recognized early in the development of the standards document that if it were to have a real impact on the field, it would be important not only to promulgate its recommendations but also to endeavor to ensure that they were taken up into practice as widely as possible. The objective of the Standards Committee and the ISSCR was not to police the field but to raise awareness of standards and to ensure transparent and comprehensive reporting of details of experimental methodology relevant to quality control, rigor, and reproducibility.

Journal editors were among the stakeholders consulted at the outset of the process, because it was felt that scientific journals could play an important role in standards implementation. Many editors recommended that the Standards Task Force develop a simple checklist that would enable editors, reviewers, and readers to assess the degree of compliance of research manuscripts with the key recommendations in the document. Accordingly, the Task Force produced a two-page checklist for authors to complete that asked them to indicate whether their manuscript provided information on compliance with a particular set of recommendations for the use of PSCs or TSCs and where in their article the relevant information could be found. The concept was based on existing author checklists already in use in journals in the biological and biomedical sciences.

The official journal of the ISSCR, *Stem Cell Reports*, introduced the use of the checklist on an experimental basis in October 2023. In this initial trial, authors were required to complete the checklist with the understanding that it would be collected for information only and that it would neither be made available to reviewers nor considered by the Editorial Team in reaching decisions on their manuscripts. The goal of the trial was to introduce authors to the checklist without prejudice to the review process and to assess their responses to it. This trial continues to date, but during the period from October 2023 to April 2025, we compiled a survey of author responses to all queries in the checklist from a randomly selected pool of manuscripts using PSCs and TSCs that were eventually accepted for publication. Manuscripts were chosen prior to review without reference to the accompanying checklists; for manuscripts studying TSCs, we made some effort to ensure representation of diverse cell types.

The results of the survey are shown in Table 1. All authors submitted a checklist, though some were more complete than others. Table 1 contains information on responses to each checklist query for 49 studies using PSC and similar data for 22 studies based on TSCs. It is apparent that response rates for many checklist queries were quite low. Considering PSCs first, information on cell line identification was varied; almost all authors provided the names of cell lines, but a much lower proportion provided registry identifiers, and a very low fraction reported authentication of cell identity. Details of cell culture practice were provided in about two-thirds of manuscripts, but less than half reported details of cryopreservation methodology or the passage level of the cell line used in experiments. Only a little over one-third of authors using PSCs reported genomic

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Table 1. Percentage of manuscripts using PSC (top) or TSC (bottom) in this survey that reported data requested in the Standards Checklist

Information reported in manuscript (%) Pluripotent stem cells						
Checklist category						
	Y	**N**	**P**	**X**	**NA/X**	**NA**
Metadata						
Name	94	6	0	0	0	0
ID Registry #	47	31	12	0	4	6
Source/catalog number	65	13	12	0	8	2
Atl info	33	43	12	0	8	4
Culture details						
Passaging	69	14	13	0	2	2
Cryopreservation	39	43	4	0	12	2
Culture reagents	68	14	16	0	0	2
Passage number	40	39	8	0	11	2
Basic characterization						
Authentication	14	70	0	2	14	0
Mycoplasma	41	36	6	4	14	0
Sterility	22	51	6	4	17	0
Genomic characterization						
Method	37	36	4	2	21	0
Timing	20	49	4	4	19	4
Pluripotency						
Method	49	27	8	2	14	0
Statistics	35	37	4	2	20	0
Timing	27	49	4	2	18	0
Disease model validation						
Disease mutation	14	8	2	4	0	72
Genetic modification	35	6	2	4	0	53
Experimental details						
Samples/replicates	90	0	6	0	4	0
Number and type of replicates	90	2	4	0	2	0
Data practices						
Statistical methodology	98	0	0	0	2	0
Code	33	4	2	4	16	41
FAIR/CARE	5	16	3	3	60	13

Information reported in manuscript (%)
Tissue stem cells

Checklist category	Y	N	P	X	NA/X	NA
Metadata						
Name	63	14	0	0	0	23
ID Registry #	13	18	0	0	5	64
Source/catalog number	54	14	5	0	0	27
Atl info	36	32	5	0	0	27
Culture details						
Passaging	59	23	4	0	0	14
Cryopreservation	32	22	5	0	0	41
Culture reagents	73	9	18	0	0	0
Passage number	41	18	9	0	5	27
Basic characterization						
Authentication	14	50	0	5	0	32
Mycoplasma	9	22	14	5	45	5
Sterility	10	38	5	5	38	5
Genomic characterization						
Method	0	18	0	9	9	64
Timing	0	13	0	14	9	64
Confirmation of cell type						
Starting material	41	9	5	9	36	0
Post expansion	41	13	5	5	36	0
Lineage potential	41	5	5	9	40	0
Disease model validation						
Disease mutation	9	0	0	0	0	82
Genetic modification	4	0	0	9	0	87
Experimental details						
Samples/replicates	86	5	0	5	4	0
Number and type of replicates	86	9	0	5	0	0
Data practices						
Statistical methodology	86	0	0	5	5	4
Code	14	4	0	9	9	64
FAIR/CARE	21	5	0	16	53	5

Y, information reported; N, information not reported; P, information partially reported; X, field blank; NA/X, information reported not applicable but should have been reported; NA, correctly reported as not applicable.

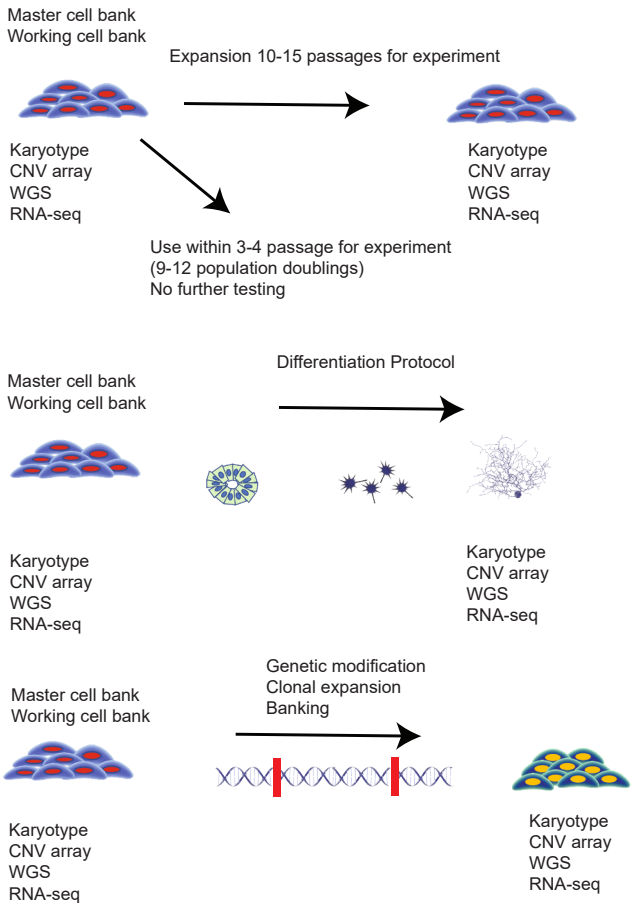


Figure 1. Process validation for stem cell culture quality assurance with emphasis on genetic integrity

Routine testing of cells need not be carried out in every experiment. Following establishment of master and working cell banks, cells are subject to quality control testing. If the experiments are carried out within 3–4 passages (9–12 population doublings at a 1:8 split to yield ~ a net 1,000-fold expansion) of establishment of banks, some reviewers may conclude no further testing is required. If experiments require extended cultivation, cultures should be tested again at the end of the culture span encompassed by the study (for example 10–15 passages). Similarly, studies reporting new cell culture or reprogramming methodologies should subject cells to testing after at least 10–15 passages (top). A similar approach can be used for novel differentiation protocols (middle) or following introduction of genetic modifications (bottom). It is known that recurring genetic variants may emerge during differentiation protocols, and in particular, extended differentiation protocols involving expansion of progenitor cells and/or selective pressure (significant cell death or loss, for example) should undergo scrutiny. In any case, authors should report the number of passages and split ratios from banked stocks that cover the span of the experimental period.

characterization, and only around half provided any confirmation of pluripotency. For disease models, a majority but not all authors reported confirmation of disease-related mutations in patient-derived cell lines; most authors using targeted genetic modification reported confirmation of the gene edit. Details of statistical analysis were almost uniformly reported by all authors, but this is due to the fact that the *Stem Cell Reports* editorial team routinely sends back manuscripts lacking such information prior to review. Very few authors reported compliance with FAIR/CARE data standards; written responses suggested that many were unfamiliar with either standard. Authorities in the data science field agree that these standards are crucial, particularly given the explosion of omics and phenotyping data, including imaging and high-content screening, now accompanying papers centered on cell-based models (Hu et al., 2025).

Similar patterns of response for manuscripts based on TSCs were observed for those queries that overlapped between these studies and those using PSCs. With respect to tissue stem cell studies, only around 40% reported confirmation of the correct cell type in the starting material or following expansion *in vitro*, and a similar minority documented the lineage potential of the cells under study.

It is apparent from this exercise that certain key features of cell line characterization, cell culture practice, and data analysis are underreported in manuscripts submitted to this journal. Some factors we did not explore might mitigate some of the deficiencies noted here. For example, since the initial trial specifically excluded incorporation of the checklist into the editorial or review process, we did not return the manuscripts to authors for clarification on any of the checklist queries. It is possible that some authors had the data requested but simply failed to report it (this has been the experience of our editorial team with regard to statistics and statistical analysis for example). Additionally, it is possible that some concerns could be addressed simply by reference to the original literature describing a cell line or the cell line provider (hIPSCREG for example requires some documentation of pluripotency prior to registration). We recommend that the completion of the checklist be made mandatory and that it should

be made available to reviewers and editors during the review process. If replies to the following checklist queries are unclear, the document should be returned to authors for clarification: name, registry number, and source of the cell line; passaging methodology and passage number used in experiments; mycoplasma testing; confirmation of disease mutation or genetic modification, if applicable; and information on experimental replicates and statistical methods employed. Furthermore, in future, revision of the checklist might expand coverage to other key quality control issues, such as the epigenetic status of cell lines or issues relating to rigor and reproducibility of novel alternative methodologies based on cell models. It is not clear whether this survey is representative of the field as a whole, but lack of adherence to some of the basic standards covered in the checklist, including cell line identification (Souren et al., 2022), experimental methodology (Anderson et al., 2021), and genetic integrity (Halliwell et al., 2020; Ludwig et al., 2023), could compromise rigor and reproducibility of studies with human stem cells. Currently, the use of the checklist is recommended by the Company of Biologists journals Development and Disease Models and Mechanisms and by Nature Methods.

A key objective of the Standards Task Force was to raise awareness of many of these basic quality control issues across the field. As the checklist is incorporated into our review process after this introductory period, we are confident that the levels of compliance will rise. To assist authors in achieving better compliance, we provide several aids. First, Figure 1, based loosely on the concept of process validation used in drug manufacture, illustrates that it is not absolutely necessary to carry out the full panoply of quality control tests in every experimental series. If a cell line is obtained from a reputable source, when master and working cell banks are established, quality control tests can be performed on the master and working cell banks. Then, the cells are taken through the degree of expansion required for experiments, and quality control checks are repeated. If the properties of the cell line are stable during this interval, this gives some degree of assurance that the culture process is not markedly altering the properties of the cell during the period encompassed by the experiments, because, for example, recurrent genetic variants generally take over cultures rapidly (Avery et al., 2013; Price et al., 2021); if they do not, this suggests that the culture system is not selecting for them. The same approach could be applied to differentiation protocols, to determine for example their effect on genetic stability. Thus, if the investigator operates within the culture period covered by the testing, while it is not certain that quality is maintained in every experiment, the overall likelihood is that it will be.

Second, Table 2 provides some information on key quality control testing methodologies, including references to the literature for open source protocols and links to commercial suppliers of test kits or reagents. In general, while there will be some expense associated with implementing these checks, if they are built into the study design, they will ultimately save considerable time and costs associated with the use of cell lines that are contaminated with adventitious agents, bear recurrent genetic abnormalities, or are used in unsound experimental protocols.

Third, the ISSCR has worked with STEMCELL Technologies to develop a free, on-demand learning course on the Standards for Human Stem Cell Use in Research. This educational initiative will provide practical guidance on how to apply the ISSCR Standards to their research through interactive video modules focused on key considerations and reporting criteria. The learning course can be accessed through this link register: Applying the ISSCR Standards for Research On-Demand Course.

Finally, we note the important role that cell line repositories and stem cell core laboratories can play in supporting high research standards, through provision of high-quality reagents including well-characterized cell lines and, importantly, through training in proper stem cell culture methodologies and quality control testing.

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AUTHOR CONTRIBUTIONS

J.M.: conceptualization, data curation, formal analysis, funding acquisition, methodology, and writing – review and editing; Y.F.: data curation, formal analysis, methodology, writing – review and editing; I.B.: data curation and writing – review and editing; C.S.: data curation and writing – review and editing; M.F.P.: conceptualization, formal analysis, methodology, visualization, writing – original draft, and writing – review and editing.

DECLARATION OF INTERESTS

J.M. and Y.F. are employees of the International Society for Stem Cell Research. Y.F. is the Managing Editor of *Stem Cell Reports*. M.F.P. is an Associate Editor of *Stem Cell Reports*. I.B. is a member of the Scientific Advisory Board of WiCell and a scientific advisor for Broken String Biosciences.

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Table 2. Exemplary methodologies for characterization of human pluripotent stem cell lines

Basic cell line characterization

Aim	Method	Publications (examples)*	Kit (examples)*	Services (examples)*
Authentication of cell lines	microsatellite PCR	https://www.ncbi.nlm.nih.gov/books/NBK144066/ (Almeida and Korch, 2004)	https://www.qiagen.com/us/products/human-id-and-forensics/str-technology/investigator-24plex-kits https://www.promega.com/products/cell-authentication-sample-identification/cell-line-authentication/geneprint-10-system/?catNum=B9510 https://www.thermofisher.com/order/catalog/product/A47624	https://www.wicell.org/home/characterization/identity/short-tandem-repeat-str/short-tandem-repeat-str.cmsx https://www.northgene.co.uk/cell-line-authentication/ https://eurofinsgenomics.eu/en/genotyping-gene-expression/applied-genomics-services/cell-line-authentication/
Mycoplasma testing	PCR, immunofluorescence microscopy	https://pmc.ncbi.nlm.nih.gov/articles/PMC10192841/ https://doi.org/10.1007/s00253-005-0062-2 (Siegl et al., 2023) https://osf.io/jcen3/files/osfstorage	https://www.thermofisher.com/order/catalog/product/4460623?SID=srch-srp-4460623 https://www.invivogen.com/mycostrip https://www.atcc.org/products/30-1012k	https://www.wicell.org/home/characterization/mycoplasma/mycoplasma-detection-by-pcr/mycoplasma-detection-by-pcr.cmsx https://www.atcc.org/services/cell-authentication/mycoplasma-testing
Sterility	PCR, microbiological assay, staining	https://doi.org/10.1002/9780470167526.ch1 https://doi.org/10.1007/s00253-005-0062-2 (Cobo et al., 2005)	https://www.thermofisher.com/order/catalog/product/C7028? https://shop.sartorius.com/us/p/microsart-atmp-sterile-release-detection-kit/ATMP-Sterile-Release-Kit	https://www.idexxbioanalytics.com/biologics-cell-health/sterility-testing
Pluripotency				
Demonstrate pluripotency	germ layer trilineage differentiation, embryoid body or 2D culture neutral or directed differentiation Immunofluorescence, PCR, or Scorecard	https://www.sciencedirect.com/science/article/pii/S2666166722004488 (Lu et al., 2022) https://pubmed.ncbi.nlm.nih.gov/35507155/ (Keller et al., 2022) https://doi.org/10.1038/s41467-018-04011-3 (International Stem Cell Initiative, 2018)	https://www.stemcell.com/products/stemdiff-trilineage-differentiation-kit.html https://www.miltenyibiotec.com/US-en/products/stemmacs-trilineage-differentiation-kit-human.html https://qkine.com/product/tri-lineage-differentiation-kit-qk516/	https://www.wicell.org/home/characterization/flow-cytometry/pluripotency-assessment/pluripotency-assessment.cmsx

Genomic characterization

Method	Types of aberrations detected	Publications (examples)*	Kit (examples)*	Services (examples)*
Karyotyping	aneuploidy duplications/deletions balanced and unbalanced translocations	https://doi.org/10.1007/978-1-0716-1908-7_17 (Baker and Barbaric, 2022)	–	https://www.stemcell.com/forms/hpsc-services-quote.html https://www.wicell.org/home/characterization/cytogenetics/karyotyping/g-banded-karyotyping.cmsx https://www.stemgenomics.com/range-of-assays/genomic-stability-karyotyping-assays/
SNP/CGH arrays	aneuploidy duplications/deletions unbalanced translocations	https://doi.org/10.1002/cpz1.606 (Steventon-Jones et al., 2022)	–	https://www.wicell.org/home/characterization/cytogenetics/snp-microarray/single-nucleotide-polymorphism-snp-mircroarray-.cmsx
eSNP karyotyping	aneuploidy duplications/deletions	https://doi.org/10.1007/978-1-4939-9224-9_18 (Weissbein, 2019)	–	–
qPCR/ddPCR	single-nucleotide variants copy number variants genomic gains/losses	https://doi.org/10.1038/s41419-024-07236-x (Krivec et al., 2024); https://doi.org/10.1016/j.stemcr.2016.10.003 (Baker et al., 2016)	https://www.stemcell.com/products/hpsc-genetic-analysis-kit.html	https://www.stemgenomics.com/range-of-assays/genomic-stability-karyotyping-assays/fast-ipsc-qc-test/
FISH	aneuploidy genomic gains/losses gene fusions	https://doi.org/10.1007/978-1-0716-1908-7_17 (Baker and Barbaric, 2022)	–	https://www.wicell.org/home/characterization/cytogenetics/fluorescence-in-situ-hybridization-fish/flourescence-in-situ-hybridization-fish.cmsx https://www.stemcell.com/forms/hpsc-services-quote.html
Next-generation sequencing (whole genome [WGS] or whole exome [WES])	sequence changes indels	https://doi.org/10.1038/s41467-020-15271-3 (Thompson et al., 2020)	–	https://web.genewiz.com/ngs-inquiry https://www.novogene.com/eu-en/

To learn more about the ISSCR Standards please see https://engage.isscr.org/ISSCR/ISSCR/Events/Event_Display.aspx?EventKey=WEBD_SCR. *Listing of publications and commercial reagents is exemplary only; listing of commercial reagents does not imply endorsement.

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