A Proposal for An Institutional Research Policy Requiring Authentication of Cell Lines and Tissue Samples

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The biomedical research community has a major problem with the authenticity of established cell lines and tissue samples and the use of misidentified cell lines occurs frequently (20, 21, 24, 26, 30, 31). Studies worldwide have found the incidence of misidentified cell lines ranges from 18 to 85%, with an approximately 22% average (26). Furthermore, 10% or more of human genomic data may be sequences derived from mycoplasma contamination (27). Retractions of research articles are costly and detrimental for the reputations of researchers and their institutions (38).

In response to this alarming problem, the NIH issued three notices that "key biological and/or chemical resources" need to be regularly authenticated (NOT-OD-15-103, NOT-OD-16-011, NOT-OD-16-012, effective January 25, 2016). Several granting agencies now require the authentication of key biologic reagents, including cell lines and tissue samples. Furthermore, a growing number of journals require that authors provide evidence that cell lines were authenticated prior to manuscript acceptance.

Therefore, members of the research community must alter research practices in a manner that recognizes these policy changes and addresses this important problem. Several publications describe "best practices" for the culturing, handling, and authentication of cell lines and tissue samples (3, 4, 9, 22, 23, 28, 34-37). Building on the Cell Line Authentication Policy of the MD Anderson Cancer Center (40), we propose the following recommendations for a cell line and tissue authentication policy for biomedical research institutions.

1. All laboratories should practice the Best Laboratory Practices and safe tissue culturing techniques, as referenced above, to prevent contaminating the established cell lines with cells from foreign cell lines or microbes including mycoplasma. These precautions are described on the ICLAC website (iclac.org) and by Freshney (22), Geraghty et al. (23) and others (9, 28, 34-36).

   Each laboratory should develop a set of documents detailing these standard best laboratory practices for the consistent and uniform training of all laboratory personnel. The goal is to minimize inconsistent and irreproducible results arising through variable verbal transmission of laboratory practices.

2. Before obtaining cell lines for a research project, the names of these cell lines should be checked for what is known about them on the ICLAC (ICLAC.org) (13), Cellosaurus (7) (https://web.expasy.org/cellosaurus/), and the Cell Line Project at the COSMIC Catalogue Of Somatic Mutations In Cancer websites. Are any of the cell lines known to be misidentified? Is there a reference STR or SNP genotype available for comparison?

3. Established cell lines should only be obtained from sources that can demonstrate the authenticity of the material. Commercial sources, such as the ATCC, RIKEN, JCRB, or DSMZ, do this routinely. Most colleagues are not likely or able to provide authenticated cell lines.

4. A large cell line batch should be generated and multiple aliquots preserved in liquid nitrogen. Test an aliquot of this batch to determine its STR or SNP genotype and for mycoplasma before the cells are used for research.

5. Cell lines should also be considered potential sources of infectious agents such as pathogenic viruses. This could be due to the original patient sample being infected or could have occurred sometime between the time that the cells were first established and when received by the researcher's laboratory. Therefore, these cultures should be handled with appropriate caution. The CDC and WHO have published online protocols for the safe handling of human cell cultures (14, 41).

6. All cell cultures should be incubated in a separate Quarantine Incubator until the cultures are shown to be both free of microbial contamination and not to be cross-contaminated with other cell lines (e.g., HeLa).

7. Parson et al. (33) and Korch et al. (25, 26) have described how MSI-Unstable cell lines can change with passaging. Cell lines should also be checked for microsatellite instability (MSI) using tests such as described by Bacher et al. (6) or the Promega kit (MD1641, see https://www.promega.com/products/molecular-diagnostics/amplification/microsatellite-instability-msi-analysis/?catNum=MD1641).

8. Cell lines routinely used by a laboratory should be authenticated at regular intervals to confirm their identity; a minimum of semi-annual testing is recommended. Cell lines should be authenticated at the beginning and conclusion of a study so as not to jeopardize the submission of manuscripts and grants. The best testing regime would be at the following time points:

    a. The start of the project,
    b. During a project,
    c. Whenever a novel phenotypic behavior is noticed (see item 12 below),

d. After phenotypic selection (e.g., drug resistance, growth as xenografts; see item 13 below),
e. At the end of a project,
f. Before submission of grant applications, and
g. Before manuscript submissions.

It is also recommended to frequently save aliquots of the residual cell suspensions after passaging cells, and store these frozen at -20°C (viability is not necessary). If future analysis detects cross-contamination or sample mix-ups at one of the above steps 8a through 8g, the frozen samples can be analyzed to ascertain when cross-contaminations or mix-ups may have occurred.

9. Generally, MSI-Stable cell lines should not be passaged more than 10-20 times and MSI-Unstable cell lines should not be passaged more than 5-10 times to minimize the effects of genetic drift of these cultures.

10. The level of confluence of cells can affect the expression of many genes in cell lines. Therefore, one should harvest cells at consistent levels of confluence to maintain reproducibility.

11. Media components are variable between suppliers and between batches from the same supplier. Therefore, each batch of culture media and their components should be recorded and checked, if necessary, for suitability before being used so that results are reproducible.

12. Sudden changes in cell line behavior (e.g., grows faster, change of morphology or other phenotype) often indicates contamination, an outgrowth of a variant subline, or even the presence of another species. Authenticate such cell lines to verify their identity.

13. The identity of cell lines subjected to selection after modification (e.g., stable transfection and drug selection) should be verified as this process can lead to outgrowth of variants or cross-contaminating ("imposter") cells.

14. Newly established cell lines and preclinical models (xenografts, 3D cultures, stem cells, etc.) generated by a laboratory should be subjected to short tandem repeat (STR) genotype analysis and any other molecular techniques, including species confirmation (5, 15) and functionality, to establish baselines for future authentication efforts.

15. When establishing new cell lines or xenografts, it is critical to determine the STR genotype of the original patient tissue sample to serve as a reference genotype for all future work. Preserve original tissue samples and, if possible, a normal sample of tissue or blood for any other future molecular characterization.

16. This policy should apply also for the use of cell lines from other organisms and to check for interspecies contamination when working with cells from different species (e.g., xenografts in mice). When possible, authenticate cell lines and tissues from mouse, rat, dog, and other species using STR or SNP genotyping (2, 5, 7, 10, 15, 16, 29, 32, 39). New databases and technologies are under continuous development and should be incorporated for best practices in research.

17. Langdon (27) showed that about 10% of DNA sequences from the Human Genome 1000 project are from mycoplasma, a common human parasite or commensal and a common contaminant of cell cultures. Mycoplasma is known to alter metabolic responses and impact DNA and RNA analysis of contaminated cell lines (1, 8, 11, 17-19). Therefore, all cell lines should be routinely checked for mycoplasma infection and treated when necessary.

18. When publishing, the frequency of STR genotyping and percent match to the reference sample should be reported using the "Tanabe" algorithm (12).

19. Any investigator sharing cell lines with fellow researchers, has the ethical responsibility to provide only authenticated cell lines. Results of STR genomic profiling and mycoplasma testing should accompany the cell line transfer to the new investigator.

20. Investigators should understand that cell lines or tissue samples having identical or nearly identical STR- or SNP-based genotypes does not prove that the samples are genetically identical. It only shows that they were derived from the same donor.

References

1. **Aldecoa-Otalora E, Langdon W, Cunningham P, Arno MJ.** 2009. Unexpected presence of mycoplasma probes on human microarrays. Biotechniques **47:**1013-1015. PMID: 20047202
2. **Almeida JL, Hill CR, Cole KD.** 2014. Mouse cell line authentication. Cytotechnology **66:**133-147. PMID: 23430347
3. **American Type Culture Collection Standards Development Organization Workgroup ASN-0002.** 2010. Cell line misidentification: the beginning of the end. Nat Rev Cancer **10:**441-448. PMID: 20448633
4. **ATCC SDO.** 2011. ASN-0002. Authentication of Human Cell Lines: Standardization of STR Profiling. ANSI eStandards Store, *on* ATCC-Standards Development Organization (SDO). https://webstore.ansi.org/RecordDetail.aspx?sku=ANSI%2FATCC+ASN-0002-2011. Accessed May 2019.
5. **ATCC SDO.** 2015. Species-level identification of animal cells through mitochondrial cytochrome c oxidase subunit 1 (CO1) DNA barcodes, *on* ATCC-Standards Development

Organization (SDO). https://webstore.ansi.org/Standards/ATCC/ANSIATCCASN00032015. Accessed
6. **Bacher JW, Sievers CK, Albrecht DM, Grimes IC, Weiss JM, Matkowskyj KA, Agni RM, Vyazunova I, Clipson L, Storts DR, Thliveris AT, Halberg RB.** 2015. Improved Detection of Microsatellite Instability in Early Colorectal Lesions. PLoS One **10:**e0132727. PMID: 26252492
7. **Bairoch A.** 2018. The Cellosaurus, a cell-line knowledge resource. J Biomol Tech **29:**25-38. PMID: 29805321
8. **Barile MF.** 1968. Mycoplasma and cell cultures. Natl Cancer Inst Monogr **29:**201-204. PMID: 4976863
9. **Baust JM, Buehring GC, Campbell L, Elmore E, Harbell JW, Nims RW, Price P, Reid YA, Simione F.** 2017. Best practices in cell culture: an overview. In Vitro Cell Dev Biol Anim **53:**669-672. PMID: 28808859
10. **Callari M, Batra AS, Batra RN, Sammut SJ, Greenwood W, Clifford H, Hercus C, Chin SF, Bruna A, Rueda OM, Caldas C.** 2018. Computational approach to discriminate human and mouse sequences in patient-derived tumour xenografts. BMC Genomics **19:**19. PMID: 29304755
11. **Callaway E.** 2014. Contamination hits cell work. Nature **511:**518. PMID: 25079533
12. **Capes-Davis A, Reid YA, Kline MC, Storts DR, Strauss E, Dirks WG, Drexler HG, MacLeod RA, Sykes G, Kohara A, Nakamura Y, Elmore E, Nims RW, Alston-Roberts C, Barallon R, Los GV, Nardone RM, Price PJ, Steuer A, Thomson J, Masters JR, Kerrigan L.** 2013. Match criteria for human cell line authentication: where do we draw the line? Int J Cancer **132:**2510-2519. PMID: 23136038
13. **Capes-Davis A, Theodosopoulos G, Atkin I, Drexler HG, Kohara A, MacLeod RA, Masters JR, Nakamura Y, Reid YA, Reddel RR, Freshney RI.** 2010. Check your cultures! A list of cross-contaminated or misidentified cell lines. Int J Cancer **127:**1-8. PMID: 20143388
14. **Center for Disease Control and Prevention C.** 2009. CDC Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th edition *on* USA Department of Human Health and Services. https://www.cdc.gov/labs/BMBL.html). Accessed May 2019.
15. **Cooper JK, Sykes G, King S, Cottrill K, Ivanova NV, Hanner R, Ikonomi P.** 2007. Species identification in cell culture: a two-pronged molecular approach. In Vitro Cell Dev Biol Anim **43:**344-351. PMID: 17934781
16. **Didion JP, Buus RJ, Naghashfar Z, Threadgill DW, Morse HC, 3rd, de Villena FP.** 2014. SNP array profiling of mouse cell lines identifies their strains of origin and reveals cross-contamination and widespread aneuploidy. BMC Genomics **15:**847. PMID: 25277546
17. **Drexler HG, Dirks WG, MacLeod RA, Uphoff CC.** 2017. False and mycoplasma-contaminated leukemia-lymphoma cell lines: time for a reappraisal. Int J Cancer **140:**1209-1214. PMID: 27870004
18. **Drexler HG, Uphoff CC.** 2002. Mycoplasma contamination of cell cultures: Incidence, sources, effects, detection, elimination, prevention. Cytotechnology **39:**75-90. PMID: 19003295
19. **Drexler HG, Uphoff CC, Dirks WG, MacLeod RA.** 2002. Mix-ups and mycoplasma: the enemies within. Leuk Res **26:**329-333. PMID: 11839374

20. **Freedman LP, Gibson MC, Ethier SP, Soule HR, Neve RM, Reid YA.** 2015. Reproducibility: changing the policies and culture of cell line authentication. Nat Methods **12:**493-497. PMID: 26020501
21. **Freedman LP, Gibson MC, Wisman R, Ethier SP, Soule HR, Reid YA, Neve RM.** 2015. The culture of cell culture practices and authentication--Results from a 2015 Survey. Biotechniques **59:**189-190, 192. PMID: 26458546
22. **Freshney RI.** 2016. Culture of animal cells: a manual of basic technique and specialized applications, 7th ed. Wiley-Blackwell, Hoboken, New Jersey, U. S. A.
23. **Geraghty RJ, Capes-Davis A, Davis JM, Downward J, Freshney RI, Knezevic I, Lovell-Badge R, Masters JR, Meredith J, Stacey GN, Thraves P, Vias M.** 2014. Guidelines for the use of cell lines in biomedical research. Br J Cancer **111:**1021-1046. PMID: 25117809
24. **Horbach S, Halffman W.** 2017. The ghosts of HeLa: How cell line misidentification contaminates the scientific literature. PLoS One **12:**e0186281. PMID: 29023500
25. **Korch C, Spillman MA, Jackson TA, Jacobsen BM, Murphy SK, Lessey BA, Jordan VC, Bradford AP.** 2012. DNA profiling analysis of endometrial and ovarian cell lines reveals misidentification, redundancy and contamination. Gynecol Oncol **127:**241-248. PMID: 22710073
26. **Korch C, Varella-Garcia M.** 2018. Tackling the Human Cell Line and Tissue Misidentification Problem Is Needed for Reproducible Biomedical Research. Adv Mol Pathol **1:**209-228.e236. PMID:
27. **Langdon WB.** 2014. Mycoplasma contamination in the 1000 Genomes Project. BioData Min **7:**3. PMID: 24872843
28. **McLaren RS, Reid Y, Storts DR.** 2013. Human cell line authentication: the critical first step in any project using human cell lines. Methods Mol Biol **963:**341-353. PMID: 23296621
29. **Morgan AP, Fu CP, Kao CY, Welsh CE, Didion JP, Yadgary L, Hyacinth L, Ferris MT, Bell TA, Miller DR, Giusti-Rodriguez P, Nonneman RJ, Cook KD, Whitmire JK, Gralinski LE, Keller M, Attie AD, Churchill GA, Petkov P, Sullivan PF, Brennan JR, McMillan L, Pardo-Manuel de Villena F.** 2015. The Mouse Universal Genotyping Array: From Substrains to Subspecies. G3 (Bethesda) **6:**263-279. PMID: 26684931
30. **Neimark J.** 2014. Trial and Error - The Dirty Little Secret of Cancer Research. Discover Magazine**:**44-51. PMID:
31. **Neimark J.** 2015. Line of attack. Science **347:**938-940. PMID: 25722392
32. **Ono K, Satoh M, Yoshida T, Ozawa Y, Kohara A, Takeuchi M, Mizusawa H, Sawada H.** 2007. Species identification of animal cells by nested PCR targeted to mitochondrial DNA. In Vitro Cell Dev Biol Anim **43:**168-175. PMID: 17516125
33. **Parson W, Kirchebner R, Muhlmann R, Renner K, Kofler A, Schmidt S, Kofler R.** 2005. Cancer cell line identification by short tandem repeat profiling: power and limitations. FASEB J **19:**434-436. PMID: 15637111
34. **Price PJ.** 2017. Best practices for media selection for mammalian cells. In Vitro Cell Dev Biol Anim **53:**673-681. PMID: 28726187
35. **Reid Y, Storts D, Riss T, Minor L.** 2013. Authentication of Human Cell Lines by STR DNA Profiling Analysis. https://www.ncbi.nlm.nih.gov/books/NBK144066/. Accessed

36. **Reid YA.** 2017. Best practices for naming, receiving, and managing cells in culture. In Vitro Cell Dev Biol Anim **53:**761-774. PMID: 28986713
37. **Schweppe RE, Korch C.** 2018. Challenges and advances in the development of cell lines and xenografts. Adv Mol Pathol **1:**239-251. PMID:
38. **Stern AM, Casadevall A, Steen RG, Fang FC.** 2014. Financial costs and personal consequences of research misconduct resulting in retracted publications. Elife **3:**e02956. PMID: 25124673
39. **Steube KG, Koelz AL, Drexler HG.** 2008. Identification and verification of rodent cell lines by polymerase chain reaction. Cytotechnology **56:**49-56. PMID: 19002841
40. **University of Texas MACC.** 2013. Cell Line Authentication Policy UTMDACC Institutional Policy # ACA1040. https://www.mdanderson.org/documents/core-facilities/Characterized%20Cell%20Line%20Core%20Facility/CCLC_%20Policy_ACA1044.pdf. Accessed
41. **World Health Organization WHO.** 2004. Laboratory Biosafety Manual, 3rd edition, *on* World Health Organization (WHO). https://www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/. Accessed May 2019.