



Published in final edited form as:

Radiat Res. 2018 June ; 189(6): 551–552. doi:10.1667/RR15086.1.

Identity Crisis – rigor and reproducibility in human cell lines

Jaimee C. Eckers, Adam D. Swick, and Randall J. Kimple

Department of Human Oncology, University of Wisconsin Carbone Comprehensive Cancer Center, University of Wisconsin School of Medicine and Public Health, University of Wisconsin, Madison, WI, USA

Cell line identity (more precisely, misidentification) has become a major source of concern for funding agencies, conscientious investigators, publishers, and pharmaceutical companies. Worldwide, up to half of commonly used cell lines are believed to be misidentified (1–4). Incorrect, or misidentification, of cell lines is a concern for both cancer and non-cancer research and should be a concern for the readers of *Radiation Research* due to concerns about data replicability, and the not-insignificant impacts upon the pyramidal nature of scientific research. Cross-contamination appears to often be an event occurring shortly after cell line establishment leading to hundreds of lines with no known authentic stock (5, 6). It has been estimated that publications using contaminated or misidentified cells affects as much as 10% of the literature (7) and could cost the US as much as \$20 billion/yr through publication of irreproducible results(8).

Mistaken identities of cell lines can arise as a result of cross-contamination during routine culture, accidental mistakes in labeling of cell lines, or through malicious intent. Over the last 10 years, improved genomic techniques have been established that enable high-confidence identification of cell lines for relatively low cost. Short-tandem repeat (STR) testing (9) has become the de-facto and recommended standard used for human cell line identification (10, 11). At least eight core STR loci plus amelogenin should be used, although many assay kits now assess more loci (12). Large cell line repositories (e.g. German Collection of Microorganisms and Cell Cultures, American Type Culture Collection) provide STR profiles on all cell lines they distribute which can be used to manually cross reference test results with published standards. In addition, online resources such as Cellosaurus (<http://web.expasy.org/cellosaurus/>) enable users to match their cell line's STR profiles to published databases. A minimum of 80% match has been recommended to discriminate between related and unrelated samples (12, 13). An increasing fraction of journals now require cell line identity confirmation although their requirements and the rigor with which they are imposed vary greatly (14–17).

For our lab, the effort to ensure cell line identity starts prior to obtaining the cells. Whenever possible, all cell lines are obtained from verified commercial sources with published STR profiles. When this is impossible, we request shipment of cell lines directly from the originating laboratory. Cells are handled by a single technician in a dedicated incubator and

*CORRESPONDING AUTHOR: Randall J. Kimple, M.D., Ph. D., 3107 WIMR, 1111 Highland Ave., Madison, WI 53705, USA, Phone: (608) 265-9156, Fax: (608) 262-7224, rkimple@humonc.wisc.edu.

undergo initial STR testing at the time of large-scale cryopreservation to confirm the identity of the cells. This initial preservation forms the reference stock for our laboratory that will be used in our ongoing and future work. Each individual lab member who wishes to work with a given cell line can obtain a single vial of this cell line and is responsible for establishing their own cryopreserved repository to avoid depleting the central resource. Details regarding STR testing are available on a local network and researchers are encouraged to routinely confirm the STR profile of their cells. All cell lines are cultured for less than 3 months before returning to an identity-confirmed early-passage frozen stock or having STR profile performed. For projects that require longer duration passaging of a given cell line, such as developing drug resistant lines, we confirm the identity by STR at the end of project and at regular intervals during culture. While the cost of these steps is not insignificant in a lab that routinely has 10–12 cell lines in use, it is a fraction of the potential cost associated with use of incorrect or misidentified cell lines and is critical for the comparison of results between research groups and the translation of research findings to clinical practice.

We would encourage the use of the following best practices:

1. Perform STR profiles of all cell lines upon receipt from both commercial or academic labs.
2. Perform (and publish) STR profiles of any newly established cell lines. Share STR profiles simultaneous with sharing cell lines and publish to publicly accessible database such as Cellosaurus.
3. Provide (in a supplemental table) STR profiles of cell lines used in manuscripts. Include the loci tested, reads, date of testing, and where the test was performed.
4. Provide source of cells (commercial, collaborator, author generated) culture conditions, and date (yr) of receipt in all manuscripts. Our preference is that this data be included in a supplemental table so that it does not require printed space.
5. Encourage journals to include supplemental data in the online pdf that most readers download.
6. As a reviewer, ask for details of STR testing on cell lines to be included as described above.

Acknowledgments

Acknowledgements: RJK received support from the NIH/NCI P30 CA014520-UW Comprehensive Cancer Center Grant.

REFERENCES

1. Huang Y, Liu Y, Zheng C, Shen C. Investigation of Cross-Contamination and Misidentification of 278 Widely Used Tumor Cell Lines. *PLoS one*. 2017;12(1):e0170384. [PubMed: 28107433]
2. Lacroix M Persistent use of “false” cell lines. *Int J Cancer*. 2008;122(1):1–4. [PubMed: 17960586]
3. Kniss DA, Summerfield TL. Discovery of HeLa Cell Contamination in HES Cells: Call for Cell Line Authentication in Reproductive Biology Research. *Reprod Sci*. 2014;21(8):1015–9. [PubMed: 24520087]

4. Bian X, Yang Z, Feng H, Sun H, Liu Y. A Combination of Species Identification and STR Profiling Identifies Cross-contaminated Cells from 482 Human Tumor Cell Lines. *Sci Rep.* 2017;7(1):9774. [PubMed: 28851942]
5. MacLeod RA, Dirks WG, Matsuo Y, Kaufmann M, Milch H, Drexler HG. Widespread intraspecies cross-contamination of human tumor cell lines arising at source. *Int J Cancer.* 1999;83(4):555–63. [PubMed: 10508494]
6. Capes-Davis A, Theodosopoulos G, Atkin I, Drexler HG, Kohara A, MacLeod RA, et al. Check your cultures! A list of cross-contaminated or misidentified cell lines. *Int J Cancer.* 2010;127(1):1–8. [PubMed: 20143388]
7. Horbach S, Halfman W. The ghosts of HeLa: How cell line misidentification contaminates the scientific literature. *PloS one.* 2017;12(10):e0186281. [PubMed: 29023500]
8. Freedman LP, Cockburn IM, Simcoe TS. The Economics of Reproducibility in Preclinical Research. *PLoS Biol.* 2015;13(6):e1002165. [PubMed: 26057340]
9. Masters JR, Thomson JA, Daly-Burns B, Reid YA, Dirks WG, Packer P, et al. Short tandem repeat profiling provides an international reference standard for human cell lines. *Proc Natl Acad Sci U S A.* 2001;98(14):8012–7. [PubMed: 11416159]
10. Barallon R, Bauer SR, Butler J, Capes-Davis A, Dirks WG, Elmore E, et al. Recommendation of short tandem repeat profiling for authenticating human cell lines, stem cells, and tissues. *In Vitro Cell Dev Biol Anim.* 2010;46(9):727–32. [PubMed: 20614197]
11. American Type Culture Collection Standards Development Organization Workgroup ASN. Cell line misidentification: the beginning of the end. *Nat Rev Cancer.* 2010;10(6):441–8. [PubMed: 20448633]
12. Capes-Davis A, Reid YA, Kline MC, Storts DR, Strauss E, Dirks WG, et al. Match criteria for human cell line authentication: where do we draw the line? *Int J Cancer.* 2013;132(11):2510–9. [PubMed: 23136038]
13. Bady P, Diserens AC, Castella V, Kalt S, Heinemann K, Hamou MF, et al. DNA fingerprinting of glioma cell lines and considerations on similarity measurements. *Neuro Oncol.* 2012;14(6):701–11. [PubMed: 22570425]
14. Blow NS. Right cell, wrong cell. *Biotechniques.* 2011;51(2):75. [PubMed: 21806546]
15. Potash J, Anderson KC. What's Your Line? Clinical cancer research : an official journal of the American Association for Cancer Research. 2009;15(13):4251. [PubMed: 19549765]
16. Identity crisis. *Nature.* 2009;457(7232):935–6.
17. Lichter P, Allgayer H, Bartsch H, Fusenig N, Hemminki K, von Knebel Doeberitz M, et al. Obligation for cell line authentication: appeal for concerted action. *Int J Cancer.* 2010;126(1):1.7. [PubMed: 19882693]