[Letter to the Editor]

The culture of cell culture practices and authentication—Results from a 2015 Survey

Concerns continue to grow regarding irreproducible basic biological and preclinical research (1,2). Excluding scientific misconduct (3), lack of preclinical reproducibility typically results from errors or flaws in one or more of the following areas: (i) biological reagents and reference materials, (ii) study design, (iii) laboratory protocols, and (iv) data analysis and reporting (4). There are no simple solutions to this problem. However, remediating misidentified and contaminated cultured cell lines, which have been workhorses in biomedical research labs for decades (5), provides one path toward improving preclinical reproducibility rates.

Accurate documentation of cell line tissue of origin (i.e., identity), sex, and species are critical to ensure the credibility, reproducibility, and translation of data and results from cell culture-based experiments (6). The accurate identification of the origin of a human cell line (authentication) is a key quality

control (QC) test that can be achieved through the determination of a genetic signature using DNA profiling and then comparing that signature with established databases (e.g., ATCC, RIKEN) (7). Yet cell lines are cultured, passaged, and processed using widely varying QC procedures, and sharing cell lines remains endemic. Consequently, misidentified cells—including inter- and intraspecies cross-contamination and simple cell line naming and mislabeling errors—continue to be reported in the scientific literature (8). Microbial contamination, particularly by mycoplasma or other bacteria (9), along with genotypic or phenotypic changes (i.e., drift) over time (10), are yet additional problems.

Evidence of this cell line authentication problem has been available for decades. In a 2004 survey, Buehring et al. (11) reported that just one-third of laboratories tested their cell lines for identity. In 2007, researchers reported

that the usage of contaminated or misidentified cancer cell lines ranged from 18% to 36% over a 20-year period (12). Surprisingly, cross-contamination rates in China were recently reported to range from 25% to more than 85% for cell lines established both outside and inside that country, respectively (13). A more recent review of almost 60 articles using cell lines published across several *Nature* journals during 2013 and 2014 concluded that “Only 10% of authors said that they had authenticated the cell line. This is especially problematic given that almost one-third admitted that they had obtained the cell lines as a gift from another laboratory” (14). The International Cell Line Authentication Committee (ICLAC), which formed following the 2012 publication of the STR (short-tandem repeat) DNA profiling standard (15), promotes awareness of cell line misidentification and use of authentication testing. They also host and update the Database of Cross-contaminated or Misidentified Cell Lines (<http://iclac.org/databases/cross-contaminations/>). However, even with these educational developments, Sigma-Aldrich’s “Second Annual State of Translational Research: 2014 Survey Report” (<http://go.sigmaaldrich.com/Translational-Survey>) found that less than half of survey respondents were familiar with the database, and only 11% had searched it during the preceding year.

The financial implications of misidentified or contaminated cell lines can be profound. A search of the National Institutes of Health (NIH) RePORTER database revealed 9000 projects and sub-projects using cell lines at a total estimated taxpayer expenditure of \$3.7 billion. If we were to estimate, conservatively, that 20% of the cell lines used in these projects were misidentified or contaminated, this would equate to over \$700 million dollars in research that could be at risk.

Figure 1. Survey responses: Quality control of cultured cells/cell lines. (A) Frequency of use of authentication and other species-related quality controls among respondents. Note, “at all” includes weekly, monthly, biannually, annually, and only prior to publication use of a particular quality control. (B) Percentage of respondents who never perform short-tandem repeat (STR) profiling by work setting.

Please indicate which of the following specific trainings you received when you were taught cell culture. Select all that apply. (N = 366)

Figure 2. Survey responses: Frequency of targeted trainings received as part of overall cell culture training.

The Global Biological Standards Institute (GBSI) Cancer Cell Authentication and Standards Task Force was formed in 2014 to (i) identify and overcome existing barriers to the use of currently available cell authentication tools, and (ii) support the development, evaluation, and application of policies, novel technologies, and standards for expanded cell authentication. In order to understand the extent of cell line authentication efforts within the biomedical community at the moment, we designed and conducted an online survey focusing on current cell culture and authentication practices. A summary of the responses to all survey questions is provided in the Supplementary Material.

The survey was conducted April 16–June 5, 2015, following extensive outreach to prominent researchers and notifying the memberships of over 20 biomedical and related research societies and organizations. Of the 446 total respondents, nearly half described themselves as a senior (15+ years since completing training) or mid-career (6 to 15 years since completing training) researcher. The majority of survey respondents conducted basic research (91%), worked in an academic setting (79%), and used cultured cells on a daily basis (59%). Seventy-one percent of respondents used cancer cell lines, whereas 70% used primary cells or other immortalized cell lines in their research; the species of origin of these

cell lines were predominantly human (89%) or rodent (74%). Notably, 73% of respondents rated themselves as either expert or above average cell-culturists.

The majority (52%) of respondents never perform authentication or other species-related QC tests on cell lines used in their experiments. Moreover, 74% never conduct STR profiling, a percentage that varied little between academia, industry, government, and non-profit researchers (Figure 1A and 1B). In contrast, respondents were more likely to perform some sterility (microbial contamination) quality controls, particularly visual inspections, even though such inspections will not typically detect mycoplasma-infected cells (9). The top 3

reported barriers to performing cell line authentication include cost (61%), time (53%), and delays in research (35%). More troubling is the apparent complacency (24% of respondents indicated “don’t see the necessity; I am careful”) and ignorance (22% reported that Principal Investigators were “unaware of or ignore the issues”) surrounding the need to authenticate cell lines. Nonetheless, the majority of respondents (73%) do support development and use of additional standardized techniques and procedures for authenticating cell lines, such as single-nucleotide polymorphism (SNP) genotyping (8).

When it comes to training, only 62% of respondents (Figure 2) had received specific training on the problems of cell line misidentification (cross-contamination) and mislabeling, and less than one-third (30%) were trained on the importance of cell line authentication as a quality control measure for species confirmation. Furthermore, only 65% of respondents indicated that their laboratory follows specific written procedures for the handling and storage of cultured cells and cell lines.

Figure 3 summarizes respondents’ levels of support for several statements regarding cell culture practices and authentication use, training, and their impact on the credibility and reproducibility of biomedical research. Respondents overwhelmingly agreed that not performing cell line authentication can adversely impact research quality. They also supported a variety of activities that

Please indicate your level of agreement with the following statements: (N = 333)

Figure 3. Survey responses: Support for the importance of activities to improve cell culture and authentication-related practices in biomedical research.

would expand training, funding, cell line authentication, and the use of cell culture best practices.

Our survey results show little has changed in the past decade. Despite increasing scientific and even mainstream media attention (e.g., National Public Radio; www.npr.org/sections/health-shots/2014/12/10/368776420/scientists-often-skip-a-simple-test-that-could-verify-their-work) on the extent and cost of using misidentified and contaminated cell lines, the majority of biomedical researchers still do not authenticate their cells. Even among survey respondents who have authenticated cells in their labs, only 26% authenticate cell lines received from a non-reputable source, and only 16% authenticate their cell lines annually.

It should be noted that biomedical journals are increasingly recommending cell line authentication for submitted manuscripts, as well as comparisons of cell lines against a database of misidentified cell lines (e.g., ICLAC's database) as part of their author guidelines (for a list, see www.scoop.international/t/cell-line-contamination/p/4040895974/2015/04/08/which-journals-ask-for-cell-line-authentication). Still, almost no journals require documentation of authentication as a condition of publication. Not surprisingly, establishing but not enforcing the use of reporting guidelines does not improve study reproducibility (16), nor does it make use of authenticated cells more likely (17). While recent NIH initiatives (e.g., <http://grants.nih.gov/grants/guide/notice-files/NOT-OD-15-103.html>) to improve the reproducibility of funded research have recognized the importance of establishing best practice guidelines and validating key biological and chemical resources such as cell lines (18), the NIH does not require authentication as a condition for funding. We believe that checking cell lines against a database of misidentified or cross-contaminated cell lines or simply attesting that cell lines were authenticated (i.e., "box-checking") is not enough—documentation of authentication is needed.

Clearly, the failure to authenticate cell lines is still not a major impediment to publication or to obtaining research funding. A systematic approach by the entire biomedical research community

that requires and specifically funds routine and documented cell authentication is needed to resolve this global problem (6). The scientific community must also commit sufficient time, resources, and expertise to adequately train and educate young scientists in best practices for cell culture experiments.

Author contributions

M.G., Y.R., and R.N. designed the survey. M.G. wrote initial draft, L.F., R.W., S.E., H.S., Y.R., and R.N. revised the paper.

Acknowledgments

We would like to thank the staff of McKinley Advisors for their invaluable assistance in hosting, launching, and analyzing the results of our survey.

Competing interests

The authors declare no competing interests.

References

1. Begley, C.G. and J.P. Ioannidis. 2015. Reproducibility in science: improving the standard for basic and preclinical research. *Circ. Res.* 116:116-126.
2. Collins, F.S. and L.A. Tabak. 2014. NIH plans to enhance reproducibility. *Nature* 505:612-613.
3. Gunn, W. 2014. Reproducibility: fraud is not the big problem. *Nature* 505:483.
4. Freedman, L.P., I.M. Cockburn, and T.S. Simcoe. 2015. The economics of reproducibility in preclinical research. *PLoS Biol.* 13:e1002165.
5. Wrigley, J.D., E.J. McCall, C.L. Bannaghan, L. Liggins, C. Kendrick, A. Griffen, R. Hicks, and L. Froderberg-Roth. 2014. Cell banking for pharmaceutical research. *Drug Discov. Today* 19:1518-1529.
6. Freedman, L.P., M.C. Gibson, S.P. Ethier, H.R. Soule, R.M. Neve, and Y.A. Reid. 2015. Reproducibility: changing the policies and culture of cell line authentication. *Nat. Methods* 12:493-497.
7. Marx, V. 2014. Cell-line authentication demystified. *Nat. Methods* 11:483-488.
8. Yu, M., S.K. Selvaraj, M.M.Y. Liang-Chu, S. Aghajani, M. Busse, J. Yuan, G. Lee, F. Peale, et al. 2015. A resource for cell line authentication, annotation and quality control. *Nature* 520:307-311.
9. Rottem, S., J.D. Kornspan, and N.S. Kosower. 2012. Contamination of tissue cultures by mycoplasmas, p. 35-58. In L. Ceccherini-Nelli (Ed.), *Biomedical Tissue Culture*. INTECH, Rijeka, Croatia.
10. Masters, J.R. and G.N. Stacey. 2007. Changing medium and passaging cell lines. *Nat. Protoc.* 2:2276-2284.
11. Buehring, G.C., E.A. Eby, and M.J. Eby. 2004. Cell line cross-contamination: how aware are Mammalian cell culturists of the problem and how to monitor it? *In Vitro Cell. Dev. Biol. Anim.* 40:211-215.
12. Hughes, P., D. Marshall, Y. Reid, H. Parkes, and C. Gelber. 2007. The costs of using unauthenticated, over-passaged cell lines: how much more data do we need? *Biotechniques* 43:575-582.
13. Ye, F., C. Chen, J. Qin, J. Liu, and C. Zheng. Genetic profiling reveals an alarming rate of cross-contamination among human cell lines used in China. *FASEB J.* (In press.)
14. Announcement: time to tackle cells' mistaken identity. 2015. *Nature* 520:264.
15. ATCC/SDO. 2012. ANSI/ATCC ASN-0002-2011: Authentication of Human Cell Lines: Standardization of STR Profiling. ATCC-Standards Development Organization (SDO), Manassas, VA.
16. Baker, D., K. Lidster, A. Sottomayor, and S. Amor. 2014. Two years later: journals are not yet enforcing the ARRIVE guidelines on reporting standards for pre-clinical animal studies. *PLoS Biol.* 12:e1001756.
17. An update on data reporting standards. 2014. *Nat. Cell Biol.* 16:385.
18. Lorsch, J.R., F.S. Collins, and J. Lippincott-Schwartz. 2014. Fixing problems with cell lines. *Science* 346:1452-1453.

Received 02 September 2015; accepted 09 September 2015.

BioTechniques 59:189-192 (October 2015)
doi 10.2144/000114344

Keywords: authentication; preclinical; reproducibility; survey

Supplementary material for this article is available at www.BioTechniques.com/article/114344.

Leonard P. Freedman¹, Mark C. Gibson¹, Rosann Wisman¹, Stephen P. Ethier², Howard R. Soule³, Yvonne A. Reid⁴, and Richard M. Neve⁵

¹Global Biological Standards Institute, Washington, DC, ²Hollings Cancer Center, Medical University of South Carolina, Charleston, SC, ³Prostate Cancer Foundation, Milken Institute, Santa Monica, CA, ⁴American Type Culture Collection (ATCC), Manassas, VA, and ⁵Gilead, Inc., Foster City, CA

Address correspondence to Leonard P. Freedman, Global Biological Standards Institute, 1020 19th Street, NW, Suite 550, Washington, DC, 20036. E-mail: lfreedman@gbsi.org

To purchase reprints of this article, contact: biotechniques@fosterprinting.com