Eradication of Cross-Contaminated Cell Lines: A Call for Action

By
Roland M. Nardone, Ph.D.

Abstract

This "white paper" was prepared and widely disseminated in an attempt to sound an alarm about the long-term existence of a grave, unresolved and growing problem that affects a significant portion of biomedical research, namely, the use of misidentified and cross-contaminated cell cultures. The "white paper" shows how bold action could bring about a profession-wide change in practice that will prevent further erosion. Misidentification and inter- and intra-specific cross-contamination of mammalian cell cultures used in research continues as a widespread problem despite an awareness that dates back more than 45 years. Awareness of the problem has led to a good understanding of the causes of cross-contamination and appropriate preventative measures. It has also led to the application of robust methods for the authentication of cell lines. Yet, the problem continues unabated. Estimates of the incidence of research papers flawed by the use of misidentified and cross-contaminated cell cultures approximate 15-20%. The gravity of the situation called for a strategy that would deliver a remedial message of authentication to virtually all cell culture researchers and also ensure compliance with the message. At the core of the strategy proposed herein is having cell line authentication as a condition for the award of research grants and for the publication of research findings.

Brief History

Cell line authentication by karyotyping and immunological approaches became objects of interest in the late 1950's and early 1960's (Rothfels et al, 1959, Defendi et al, 1960, Brand and Syverton, 1962). The reports indicated special concern for continuous cell lines, such as transformed cell lines and human tumor cell lines. Heightened attention followed in 1966 when Stanley Gartler reported at the Second Decennial Review Conference on Cell, Tissue, and Organ Culture (1967) that 18 human cell lines of independent origin were overrun by HeLa, the first human cancer cell to be established in culture (Gey et al, 1952). HeLa is a cervical adenocarcinoma cell derived from an African-American donor, Henrietta Lacks. Gartler based his conclusion on karyotypic markers, the presence of the Type A (fast mobility) isoenzyme glucose-6-phosphate dehydrogenase (which is found only in African Americans and at a frequency of 30%) and Type 1 phosphoglucomutase, antigenic markers, viral susceptibility, and nucleic acid hybridization profiles. We now attribute the extensive contamination to the following: HeLa, because of its celebrated status, was widely distributed and passed on from lab to lab, where practitioners did not always exercise stringent care and/or were oblivious to cross-contamination as a problem. Also, HeLa proved to be a very robust cell in culture capable of overgrowing many other cells in mixed culture (Masters, 2002). The reaction of the scientists at the Decennial

Review Conference ranged from disbelief to accepting (see paper pg 182-195 of NCI Monograph 26, 1967 for verbatim discussion).

The issue was sharply focused and brought to a broad audience by Walter Nelson-Rees and his associates who showed in a series of papers that extensive cross-contamination and misidentification characterized the cultures sent to him for inclusion in the repository he was maintaining under contract for the NCI. In his speech on the occasion of receiving a Lifetime Achievement Award from the Society for in Vitro Biology in 2004, Nelson-Rees recalled that from 1970 through 1974 he authored twenty-five research accounts "of greater or lesser importance, none of which caused a stir." The June 7, 1974 issue of *Science*, however, published a paper based on observations of twenty separate cell cultures, nine of which had HeLa banded markers as well as Type A G6PD. Two of these cell lines were purported to be breast carcinoma cells (HBT-3, HBT39B). A third, HEK (presumably derived from human embryonic kidney), was identical to HBT-3 and HBT39B, which were HeLa cells. Unfortunately, these cells were widely used for breast cancer research. Nelson-Rees recalled that this first major listing, particularly the wholesale use of the wrong cells in extensive programs of breast cancer research "caused quite a tremor." Barbara Culliton (1974), a columnist for *Science* wrote in the same issue, "If Nelson-Rees is right, a lot of people may have been spending a lot of time and money on misguided research."

Nelson-Rees pointed out that while HeLa cell contamination is widespread, other human and animal cells are contaminating one another "…techniques for maintaining cell purity must be applied to reduce it and the problems it presents to biologists through out the world."

The litany continued throughout the seventies with additional revelations of inter-and intraspecies cross-contamination and more vehement accusations being exchanged including concealment of knowledge and manipulation through editing. This is well illustrated in response to a paper published in *Nature* in 1981. A team of seven scientists, including Nelson-Rees, analyzed four "unknown" cultures purported to be of Hodgkin's disease origin. All four, including three identical cultures, were not Hodgkin's nor were they HeLa. Three were of unidentified human origin and the fourth was non-human, with a karyotype identical to that of the Northern Colombian brown foot owl monkey, a cell line carried by the contributor of the four cell cultures (Harris et al, 1981). Such a large-scale mix-up invoked the verbal wrath of Washington reporter David Dickson, whose diatribe included, "corruption of scientific literature…misleading colleagues… forgery…falsifying data…lying…false claims…fraud against the federal government…a criminal offense"(Dickson, 1981).

A more quantitative and broader picture follows. Nelson-Rees encountered 279 contaminated cell cultures submitted from 45 different laboratories. Recent submissions to the German DSMZ cell bank include cohorts of human hematopoietic cell lines, 14% of which are cross-contaminated. In another survey, they found that 45 of 326 submissions were contaminated. Forty-two were intraspecific contaminants. These were submitted by 27 of 93 scientists (29%) who made submissions. vanHelden (1988) reported that the human esophageal squamous carcinoma cell lines HCu 10, 18, 22, 27 and 39 are genetically identical, while Ogura et al (1997) reported that lines JTC-3, OF and OE isolated in 1959, 1969, and 1971, respectively, were HeLa cells. More recently Melcher (2005) reported the putative normal colon epithelial cell line NCOL-1 probably was derived from the colon carcinoma LoVo. Furthermore, spectral karyotyping and DNA fingerprinting revealed that a subline of NCOL 1 and LoVo are identical while another putative subline of NCOL 1 had additional markers. See Kniss et al (2002) and Drexler et al (2001) for other examples.

It is hard to estimate how much misguided research is attributable to cross-contamination. But, again, we do have data that provide a conservative but shocking estimate. Masters (2005) reported at the Annual Meeting of the Society for in Vitro Biology of a Medline search for the years 2000-2004 regarding the continued use of contaminated cell lines known to be HeLa. The outcome was as follows: There were 19 citations for the putative intestinal cell, Int 407, 45 citations for the putative amnion cell, WISH, 59 citations for Chang liver, 470 citations for the putative human nasal carcinoma cell, Hep-2, and 556 citations for the putative oral carcinoma, KB. A PubMed search by Buehring et al (2004) uncovered 220 publications which involved cross-contaminated cultures. A survey distributed and analyzed by Buehring et al (2004) in order to obtain a profile of active cell culture workers revealed that of the 483 respondents 32% use HeLa cells, 9% unwittingly were using HeLa contaminants, 33% of the investigators tested for authenticity, 35% obtained their cell lines from other labs rather than from a major repository. Their paper also includes the outcome of a PubMed database analysis which uncovered 220 research papers based on the use of cross-contaminated cell lines. Buehring et al (2004) also revealed a disturbing trend. While the number of publications in the PubMed database increased steadily from 1969 through 2004, the number of papers involving HeLa contaminants increased far more rapidly. An analysis of one of their figures shows use of contaminated cultures increasing about 10-fold and the number of cell culture papers in the database increasing slightly more than 2-2.5-fold during the same time period. An identical search covering 2005 and some of 2006 revealed that the pattern of misuse continues (Buehring, personal communication, 2006).

The major repositories, because of their diligence in monitoring cross-contamination are now able to fulfill their mission of storing and distributing authenticated cell lines. However, this does not diminish the need for periodic authentication of cultures received directly from other investigators, from commercial sources, and from major repositories. Liscovitch and Ravid (2006) reported that DNA fingerprinting analysis revealed that NCI/ADR-RES cell line is actually an ovarian tumor cell line, OVCAR-8, rather than a breast cancer cell line. NCI/ADR-RES is included in the NCI panel of 60 cell lines distributed for evaluation of potential anti-cancer drugs. Liscovitch and Ravid estimate that about 300 papers have been published with the incorrect identification. Mistakes can happen even in the finest laboratories. Only a universal practice of cell line authentication can provide us with the security our research and commitment warrants!

A Call for Remedial Action

The cross-contamination and misidentification disclosures of the last four decades tainted the reputations of many respected laboratories. This led to denial, paranoia, and mockery. The climate so engendered was not conducive to the generation of policies and practices that would be embraced profession-wide. Contributing to the lack of action was the mistaken belief by some that disclosure would automatically be followed by individual heightened awareness and remedial action. Periodic reports, conferences, and symposia came and went and had insufficient impact (as is evidenced by the high frequency of misidentification). No encompassing, remedial plan with reasonable expectations and with measured inducement was developed to challenge the profession. The compelling need for changed practice was not matched by compelling solutions.

Clearly, the current situation is intolerable and requires a broad, coordinated effort involving those who do research, fund research, publish findings of research and educate researchers. The strategy described below derives its merit from the compelling need, its reliance on compliance methods used in selected situations by government agencies and scientific

journals, and the role of professional societies as guardians in advancing the search for truth and maintenance of high standards.

Generation of a plan and its implementation should have as its hallmark the spirit of collegiality and mutual concern. However, it must also be unyielding regarding the need for decisive action that leads to the elimination of this scandalous situation.

A conference should be convened to analyze the major features of the proposed strategy, as well as alternative ones.

1. It is proposed that government and private funding agencies be prevailed upon to require cell line authentication as a condition for the award of grant and contract funds.
2. It is also proposed that key scientific journals be prevailed upon to require cell line authentication as a condition for publication.
3. Furthermore, it is proposed that relevant professional societies a) endorse the policies pertaining to grants and publications and b) sponsor conferences, workshops and/or training activities to facilitate the adoption of cell line authentication standards.
4. It is further recommended that laboratory directors and chiefs as well as academic department heads be encouraged to ensure that staff members are cognizant of the problem of cross-contamination and the quality control measures that should become standard operating procedure.

By focusing on these select groups we would be dealing with entities that have a large constituency and a clear, related mission. Grants and publications are at the heart of the scientific enterprise. As precedence, it should be noted that the FDA has a requirement for cell line authentication as a condition for approval. The Human Genome Office requires grantees to report the sequences of DNA fragments on the internet as soon as they are deciphered. Also, several journals require the author to specify the two methods used for mycoplasma detection. Requiring cell line authentication as a condition for grants and publication would not be unreasonable.

The Methods for Authentication

Good, reproducible methods for interspecies and intraspecies cross-contamination detection exist. Most frequently, karyotyping (Nelson-Rees et al, 1974; Lavappa, 1978; Lee et al, 2002) and isoenzyme profiling (O'Brien et al, 1977) are used for interspecies cross-contamination detection while DNA analyses are used for intraspecies investigations. The latter applications have evolved over time as new methods for DNA analysis became available. DNA/RNA hybridization gave way to RFLP analysis which has been supplanted by short tandem repeats (STR) analysis (Bar et al,1997; Coble et al, 2004; Dirks and Drexler, 2004; Gilbert et al 1990; Milanesi et al, 2003; Muller et al, 2004; Satoli and Takeuchi, 1993; Schneider, 1997; Stacey et al, 1992; Steube et al, 2003; vanHelden et al, 1988).

STR analysis has been adopted for forensic work and by major repositories for intraspecies authentication (Gill et al 1997; Masters et al, 2001; Parson et al, 2005). Its attributes are that after PCR multiplex amplification of polymorphic loci and separation on a gel, a profile unique for that DNA sample source is obtained. It can be distinguished from the DNA of any other source. Furthermore, when the sizes of the products (accurate to one base pair) are determined, a series of numbers are generated which can be used as a bar code for that DNA source. A registry of bar codes would make it easy to compare DNA samples. The STR method is easy, reliable, and can be done "in house" or analyzed by a commercial laboratory for a few hundred dollars per sample (Masters, et al 2001).

Karyotyping of G-banded chromosomes can be used alone or to complement isozyme analysis in order to distinguish among cell lines with characteristic karyotypes, such as man,

mouse, rat, and hamster. More specialized karyotypic analysis is required to distinguish among cells from closely related organisms such as different genera of the Order Primates. Fluorescent in situ by hybridization (FISH) with species-specific probes, can resolve the differences. The methods are relatively simple but do require some experiences for reliable interpretation. Hence, using the services of a commercial cytogenetics lab (or a skilled colleague) may be required.

Other methods such as HLA typing and spectral karyotyping may be used for further resolution (Melcher et al, 2005).

Change the Status Quo

This problem of cross-contamination and misidentification of cell lines continues to cast a shadow over published research with cell cultures. The problem is not disappearing; it is growing. It can be eradicated by bold yet reasonable approaches.

One can think of many excuses and/or reasons why the problem was not suitably addressed in the past. These excuses no longer apply. Our psychic, social and monetary investment in research demands that this deplorable situation be changed.

What is needed is firm resolution to end the travesty through the implementation of the strategic approaches, such as those described above or others. A conference would facilitate democratization of the decision-making, ensure careful scientific evaluation, and encourage acceptance of standards appropriate for the burden of trust bestowed on us.

Addendum to Original White Paper

An earlier version of this white paper, identical except for the identification of a specific source for conference funds and 2006 references, has been endorsed by the governing boards of the American Society for Cell Biology, the European Society for Tissue Culture, and the Society for in Vitro Biology. At its 2006 meeting, the Board of the American Cancer Research Society endorsed the call for a conference. They also indicated their willingness to play a leadership role in the cancer research community with regard to promoting awareness of the problem. Endorsement for a Call for Action has also been received from the following major cell culture repositories: The American Type Culture Collection, the Coriell Institute, the European Cell Culture Collection, the Japanese Cell Repository Bank, and the German cell bank (DMZB).

1. Roland M. Nardone is Professor Emeritus at the Catholic University of America (Nardone@cua.edu). He has used cell cultures as a research tool for more than forty years. He has served as Chairman of the Third Decennial Review of Cell, Tissue, and Organ Culture and as an officer of the Tissue Culture Association. Each year he teaches a cell culture course for the Foundation for Advanced Education in the Sciences at the NIH (www.biotrac.com). Revised September 2006 to include response of scientific community to original white paper (October 2005) as well as new reports.
2. Science Magazine featured misidentification and cross-contamination in the February 16, 2007 issue (Science, vol. 315, 928-931). The article includes other examples as well as snippets of interviews with scientists, editors, and administrators. The interviews provide some insight regarding indifference to the status quo and the absence of leadership.

References

Bar, W., Brinkmann, B., Budowle, B., Carracedo, A., Gill, P., Lincoln, P., Mayr, W., and Olaisen, B. 1997. DNA recommendations. Further report of the DNA Commission of the ISFH regarding the use of short tandem repeat systems. International Society for Forensic Haemogenetics. *Int. J. Legal Med.* 110, 175-176

Brand K. and Syverton, J.T. 1962. Results of species-specific hemogglutination tests on transformed, non-transformed, and primary cell cultures. *J. Nat. Cancer Inst.* 25; 147-157.

Buehring G.C., Eby E.A., Eby M.J. 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(7):211-5.

Buehring, G.C. 2006. Personal communication.

Coble, M.D., Just, R.S., O'Callaghan, J.E., Letmanyi, I.H., Peterson, C.T., Irwin, J.A., and Parsons, T.J. 2004. Single nucleotide polymorphisms over the entire mtDNA genome that increase the power of forensic testing in Caucasians. *Int. J.Legal Med.* 118, 137-146.

Defendi, V., Billingham, R.E., Silvers, W.K. and Moorhead, P. 1960. Immunological and karyological criteria for identification of cell lines. *Journal National Cancer Institute*. 25: 359-385.

Dirks, W.G., and Drexler, H.G. 2004. Authentication of cancer cell lines by DNA fingerprinting. *Methods Mol. Med.* 88, 43-55

Dickson, D. Contaminated cell cultures. *Nature* 289: 227.

Drexler, H.G., Dirks, W.G., Matsuo, Y., and MacLeod, R.A. 2003. False leukemia-lymphoma cell lines: an update on over 500 cell lines. *Leukemia* **17,** 416-426

Drexler H.G., Dirks W.G., MacLeod R.A. 1999. False human hematopoietic cell lines: cross-contaminations and misinterpretations. *Leukemia.* 13(10): 1601-7.

Gartler, S.M. 1968. Apparent HeLa cell contamination of human heteroploid cell lines. *Nature*. 217:750-751.

Gilbert, D.A.; Reid, Y.A.; Gail, M.H., et al. 1990. Application of DNA fingerprints for cell-line individualization. *Am. J. Hum. Genet*. 47:499-514.

Gill, P., Brinkmann, B., d'Aloja, E., Andersen, J., Bar, W., Carracedo, A., Dupuy, B., Eriksen, B., Jangblad, M., Johnsson, V., et al. 1997. Considerations from the European DNA profiling group (EDNAP) concerning STR nomenclature. *Forensic Sci. Int.* 87, 185-192.

Harris, N., Gang, D., Quay, S., Poppema,S.,Zamecnik,P.,Nelson-Rees, W., and O'Brien, S.J. 1981. Contamination of Hodgkin's disease cell cultures. *Nature* 289,228-230.

Kniss D.A., Xie Y., Li Y., Kumar S., Linton E.A., Cohon P., Fan-Harvard P., Redman C.W., Sargent, IL. 2002. ED(27) trophoblast-like cells isolated from first-trimester chorionic villi are genetically identical to HeLa cells yet exhibit a distinct phenotype.
Placenta. 23(1):32-43.

Lavappa K.S. 1978. Survey of ATCC stocks of human cell lines for HeLa contamination. *In Vitro*. 14(5):469-75.

Lee, J.Y., Lee, C.H., Shim, S.H., Seo, H.K., Kyhm, J.H., Cho, S., and Cho, Y.H. 2002.
Molecular cytogenetic analysis of the monoblastic cell line U937. karyotype clarification by G-banding, whole chromosome painting, microdissection and reverse painting, and comparative genomic hybridization. *Cancer Genet. Cytogenet.* 137, 124-132

Liscovitch, M. and Ravid, D. 2006. A case study in misidentification of cancer cell lines: MCF-7/AdrR cells (re-designated NCI/ADR/RES) are derived from OVCAR-8 human ovarian carcinoma cells. *Cancer Lett.* Feb. 24.

MacLeod R.A., Dirks W.G., Matsuo Y., Kaufmann M., Milch H., Drexler, H.G. 1999.
Widespread intraspecies cross-contamination of human tumor cell lines arising at source.
International Journal of Cancer. 83(4):555-63.

Masters, J.R. 2002. HeLa cells 50 years on: the good, the bad and the ugly. *Nat. Rev. Cancer*. 2:315-319.

Masters, J.R. 2005. DNA profiling and the authentication of cell lines. *In Vitro Cell. Develop. Biol. 41 (abstract) 6A.*

Masters, J.R.; Thomson, J.A.; Daly-Burns, B., et al. 2001. Short tandem repeat profiling provides an international reference standard for human cell lines. *Proc. Natl. Acad. Sci.* USA. 98:8012-8017.

Masters, J.R.W.; Bedford, P.; Kearney, A.; Povey, S. and Franks, L.M. 1988. Bladder cancer cell-line cross-contamination: Identification using a specific locus-specific minisatellite probe. *Brit. J. Cancer*. 57, 284-286.

Melcher, R., Maisch, K., Koehler, S., Bauer, M., Steinlein, C., Schmid, M., Kudlich, T., Schauber, J., Luehrs, H., Menzel, T., and Sceppach, W. 2005. SKY and genetic fingerprinting reveal a cross-contamination of the putative normal colon epithelial cell line NCOL-1. *Cancer Genetics and Cytogenetics.* 151(1): 84-87.

Milanesi, E., Ajmone-Marsan, Bignotti Losio, M.N., Bernardi, Jo, Chegdani, F, Sorcini, M. and Ferrari, M. 2003. Molecular detection of cell line cross-contaminations using amplified fragment

length polymorphism DNA fingerprinting technology. *In Vitro Cell Div. Biol. – Animal*. 39: 124-130.

Muller, S., Eder, V., and Wienberg, J. 2004. A nonredundant multicolor bar code as a screening tool for rearrangements in neoplasia. *Genes Chromosomes Cancer* 39, 59-70

Nelson-Rees, W., Flandermeyer, R., and Hawthorne, P. 1974. Banded marker chromosomes as indicators of intraspecies cellular contamination. *Science.* 184, 1093-1096

Nelson-Rees, W., and Flandermeyer, R. 1977. Inter-and Intra-species contamination of human breast tumor cell lines HBC and Br Ca5 and other cell cultures. *Science.* 195, 1343-1344.

Nelson-Rees W.A., Daniels D.W., Flandermeyer R.R. 1981. Cross-contamination of cells in culture. *Science.* 212(4493):446-52. Review.

Nelson-Rees, W. 2004. Acceptance speech, Lifetime Achievement Award, Annual Meeting, Society for in Vitro Biology (personal communication).

O'Brien SJ (2001) Cell culture forensics. *Proc Natl Acad Sci USA*, 98, 7656-7658.

O'Brien SJ, Kleiner G, Olson R & Shannon J.E. 1977. Enzyme polymorphisms as genetic signatures in human cell cultures. *Science*, 195, 1345-1348.

Ogura H., Fujii R., Hamano M., Kuzuya M., Nakajima H., Ohata R., Mori T. 1997.
Detection of HeLa cell contamination-presence of human papillomavirus 18 DNA as HeLa marker in JTC-3, OG and OE cell lines. *Jpn J Med Sci Biol*. 50(4-5):161-7.

Parson, Walther, Romana Kirchebner, Roswitha Muhlmann, Kathrin Renner, Anita Kofler, Stefan Schmidt, and Reinhard Kofler. 2005. Cancer cell line identification by short tandem repeat profiling: power and limitations. *The FASEB Journal* express article 10.1096/fj.04-3062fje.

Rothfels, K.K. Axelrod, A., Siminovitch, L., McCullogh, E., and Parker, R.C. 1958. The origin of altered cell lines from mouse, monkey, and man, as *indicated* by chromosome and transplantation studies. *Proc. 3rd Canadian Cancer Research Conference*, 1988. Berg, R.W. ed, New York Academic Press, Inc. 189-214.

Satoh M., Takeuchi M. 1993.Cross-contamination of cell lines as revealed by DNA fingerprinting in the IFO animal cell bank. *Res Commun Inst Ferment*. 16 18-23.

Stacey, G.N.; Bolton, B.J.; Doyle, A. 1992. DNA fingerprinting transforms the art of cell authentication. *Nature* (Lond.). 357:261-262.

Steube K.G., Meyer C., Uphoff C.C., Drexler H.G. 2003. A simple method using beta-globin polymerase chain reaction for the species identification of animal cell lines—a progress report. *InVitro Cell Dev Biol Anim*. 39(10):468-75.

van Helden P.D., Wiid I.J., Abrecht C.F., Theron E., Thornley A.L., Hoal-van Helden E.G. 1988. Cross-contamination of human esophageal squamous carcinoma cell lines detected by DNA fingerprint analysis. *Cancer Res.* 48(20):5660-2.