

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

by
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Summary

The use of cross-contaminated cell lines in cancer and other biomedical research is at a high level and increasing. Consequently, a significant proportion of the literature using cell lines is misleading or false, tainting up to 20% of publications. What are the consequences?

1. The wastage of funds on biomedical research that is flawed and often misleading. Millions of dollars are spent on research using false cell lines every year worldwide.
2. Many scientists believe or claim that they are working with cells derived from one tissue, when they either know or could easily find out that the cells are derived from a different tissue. This situation is unnecessary, unacceptable and the antithesis of fundamental scientific principles.
3. Loss of public support for biomedical science. The longer the current situation continues, the more likely it is to damage the credibility of biomedical science and risk the loss of public funding.

In the past, there were excuses for the use of cross-contaminated cell lines. Now, standardized simple inexpensive methods are available to detect and eradicate the problem. We must take action now.

Preamble

Extensive cross-contamination of human and animal cell cultures with a variety of human and animal cell lines is a long-standing problem. Although extensive awareness dates back to the 1950's, the problem is not diminishing. Estimates based on submission of cell lines to major repositories indicate that up to 20% of the cultures may be cross-contaminated. As continuous cell lines increase in use and as immortalization of cell lines becomes more common the problem will become exacerbated unless strong, realistic measures are taken to correct the situation.

The Consequences and Why Cross-Contamination Needs to be Eliminated

This "white paper" outlines major initiatives which, when applied in concert, could rapidly diminish the problem. These initiatives call for cell line authentication as a condition for receipt of grant funds from major agencies (NIH, NSF, HHMI, ACS, etc.), authentication as a condition for publication of cell culture-based research in leading journals, and focused education opportunities for technicians and scientists regarding prevention and detection of cross-contamination.

The importance, the breadth, and the complexity of the subject require broad participation in the evaluation and possible modification of the strategies outlined herein, as well as due consideration of alternative strategies. Hence, a suitable conference, to evaluate these strategies is recommended.

This document shows that the basic understanding of causes, prevention and detection of cross-contamination are in place. It also advances the view that a broad base of outstanding leadership can rectify the situation.

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) isozyme 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. 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, 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, in a review in *Science Year*, admonished 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 (vol. 289: 211-212). A team of seven authors, 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). 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.”

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 (17.9%) were contaminated. Forty-two were intraspecific contaminants. These were submitted by 27 of 93 scientists (29%) who made submissions. Van Helden (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 reported 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.

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.

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, organized by relevant professional societies and funded by an NIH R13 Conference Grant, should be convened to analyze the major features of the proposed strategy, as well as alternative ones. (The document, NIH Support for Conferences and Scientific Meetings, specifies that support is highly contingent on the scientific interests and priorities of the individual Institutes and Centers. The problem of cross-contamination of cell lines should be of great interest to several Institutes. Hence, joint support by several Institutes is recommended).

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 put 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 and isozyme profiling 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.

STR analysis has been adopted for forensic work and by major repositories for intraspecies authentication. 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.

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.

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