THE CATHOLIC UNIVERSITY OF AMERICA

The Discovery Center for Cell and Molecular Biology

McCort-Ward Building, Cardinal Station

Washington, DC 20064

An Open Letter Regarding the Misidentification and Cross-Contamination of Cell Lines: Significance and Recommendations for Correction

July 11, 2007

Secretary Michael O. Leavitt
U.S. Department of Health and Human Services
200 Independence Avenue, S.W.
Washington, DC 20201

Dear Secretary Leavitt:

This letter has been drafted by a group of leading cell culture scientists and other experts involved in the use of DNA methods for the identification of biological material. This group is seriously concerned about the continued use of misidentified and cross-contaminated cell lines in current biomedical research. We urge you to read this letter, share our concerns and take appropriate action consistent with the responsibility of your office.

Cell cultures are important tools for biomedical research and have been used successfully in diverse fields such as medicine, genetics, drug discovery, vaccine development, reconstructive medicine, basic science, HIV testing and treatment and cell biology. Without doubt, all these, and many more, have progressed using cell culture models. The fact is that cell culture technology, which is not trivial science, is of major importance in biomedical research and technology and, therefore, to all of us. However, its usefulness continues to engage investigators at a pace and frequency that often leads to significant gaps regarding fundamental principles of quality control such as cell line authentication.

Irrefutable evidence has demonstrated that cross-contamination and misidentification of mammalian cell cultures is widespread.[1] Major cell line repositories such as the ATCC (American Type Culture Collection), the Coriell Institute for Medical Research, the European Collection of Cell Cultures (ECACC), Deutsche Sammlung von Mikrorganismen und Zellkulturen (DMSZ, the German Cell Repository) and the Japanese Collection of Research Resources (JCRR) have experienced cell line submissions that, upon authentication testing, were determined to have been misidentified by the depositor. Similar experiences have been shared by countless scientists worldwide, after receipt of cell cultures donated by their colleagues. Although we recognize that the incidence of cross-contamination may vary among scientific specialties and individual groups, the problem of misidentification and cross-contamination is prevalent and continues unabated, at a rate that has increased in the half-century following its uncovering. Its consequence, the use of cultures that are not what they are purported to be, has spawned a segment of research and subsequent scientific literature that is blemished – that is false. Estimates vary, but as many as 20% of scientific publications using cultured cells may be involved. Using a perspective from another scientific discipline – a paper would not be published in the chemical literature without some indication of chemical purity. In direct analogy, if a scientific investigator introduces a cell line, there should be the expectation that it is authenticated. A cell line is a reagent in an experiment just like a chemical. If something is wrong with the cell line, the validity of the experiment is compromised. Therefore, publication of a research paper based upon faulty cell line identification inevitably leads to a cascade of other misleading misinformation, with a potential for dire consequences.

The resulting corruption of biomedical research, no matter the frequency of occurrence, has the potential for grave public health consequences. Drugs, vaccines, and other biomedical products may be developed using misleading or false data, causing delays in availability of needed medical treatments. Additionally, huge amounts of resources, both public and private, are wasted. Critical time and vast sums of money are wasted in support of research using misidentified or cross-contaminated cell lines and in the development, testing and manufacture of biomedical products based upon erroneous data. Two real-life examples include:

- An NCI-60, multiple drug-resistant breast cancer cell line has been distributed by the National Cancer Institute (NCI) and widely used to compare other breast cancer cells. The drug-resistant breast cancer cells were ultimately determined to be ovarian cancer cells.[2,3] The discovery authors, Liscovitch and Rabin, estimate that there are probably 300 published papers with the erroneous identification.

- In human metastases studies, human tumor cells placed in animals frequently turn out to be (nonhuman) mouse cells or recruited host cells.[4,5]

The irony of the situation is that techniques used for authentication today are inexpensive and fast,[6,7,8,9] while the errors caused by lack of cell line authentication are in many cases too costly for society!

This potentially grave situation warrants firm commitments and expeditious action by organizations, institutions and individuals for the purpose of achieving without further delay a profession-wide correction/mandate. This panel recognizes that the task is large, but not unduly so. It shares the common sense belief that there is no place in 21st century biomedical research for the use of cell lines that have not been authenticated. The factors needed to rectify the situation exist; however, they are awaiting bold leadership. These factors include an understanding of the causes and prevention of cross-contamination and misidentification, robust methods for the authentication of cell lines and bioinformatic tools to achieve profession-wide access to globally acquired authentication data. The task can be reduced to initiatives directed at education and compliance.

Education

Education initiatives can and should be launched and developed through cooperative efforts of relevant professional societies. Also, participation by interested sponsors of research and by representatives of scientific journals should be solicited. Among the diverse initiatives should be those directed at causes and prevention of cross-contamination and misidentification and methods for the detection of intra- and inter-species cross-contamination. A variety of tailored programs and formats (newsletters, courses, workshops, symposia) should be developed to serve bench scientists, technicians, laboratory directors, journal editors and peer reviewers, grant peer reviewers, and high school and college biology teachers. The keys to success of education initiatives are quality, delivery of information via appropriate venues, and sustained saturation maintained for as long as needed.

The education committees of professional societies are ideally positioned to undertake this task, perhaps in conjunction with a "trans-society" authentication education committee. A "trans-society" authentication education committee, suitably funded by grants, could serve as a valuable resource to coordinate and update new developments and to assist those in need of help.

Compliance

Education alone cannot accomplish the goal of bringing about a profession-wide change in cell culture practices. Education programs, although very significant, will not reach all workers nor will they have the force to ensure compliance. Any plan to permanently rectify the current situation must be based on rules or guidelines which apply broadly and encourage or guarantee compliance. The panel endorses the implementation of two approaches that are, in part, complementary and could reinforce one another.

We advocate that all researchers using cell cultures incorporate a specific cell line authentication protocol into their experimental framework and the first approach virtually ensures compliance:

- Cell line authentication would be required by granting agencies as a condition for the award of funds; *no authentication/no grant.*
- All grant funding bodies would be asked to include in funding contracts the requirement to operate to GCCP,[10] Good Cell Culture Practice, analogous to Good Laboratory Practice (GLP), initiated at the 3rd World Congress on Alternatives and Animal Use in the Life Sciences, Bologna, 29 August – 2 September 1999.
- Authentication would be required by journals as a condition for acceptance of research articles. If exceptions are made, it should be disclosed.

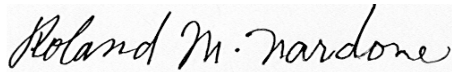
The panel recommends that major granting agencies and journals adopt these policies and begin implementation using a timetable reflective of the urgency. The panel further recommends that those agencies and journals with disproportionate leverage and a high vested interest be called on to invoke authentication requirements thereby leading the profession-wide reformation that is so urgently needed. The extramural grant program of the NIH and the journals Cell *and* Cancer Research *are among the most logical candidates.*

A parallel approach would lead to the development of a set of voluntary consensus standards to address the needs of the cell biology community for standardization of authentication and characterization of cell lines for use in research and manufacturing. We further suggest that an additional consensus standard (or standards) be developed that outlines proper procedures for all funding organizations and journals to require the demonstration of authenticity before funding and/or publication is granted.

It is important to note that implementation of the approaches delineated above have been successful. For example, the U.S. FDA (1993 Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals) mandates that the cell substrates used in biopharmaceutical production be identified. This forced compliance has resulted in very low levels of interspecies cross-contamination within the biopharmaceutical industry (in one report only 2 cases of misidentified cells were discovered in an evaluation of over 900 samples from 1996 to 2006[8]). The biopharma industry is very highly regulated and the contamination rate very low due to that regulation.

We urge you to take action! Contact us to let us know you want to participate and will support these efforts.

Best regards,



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We, the following, are in agreement with the content of this Open Letter:

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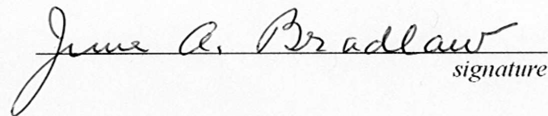

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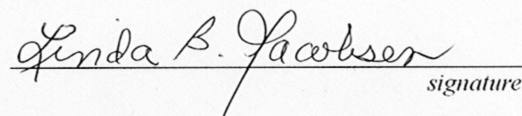

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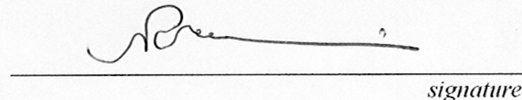

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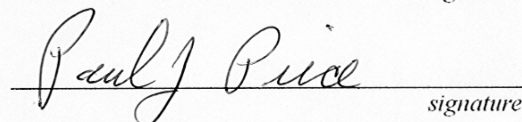

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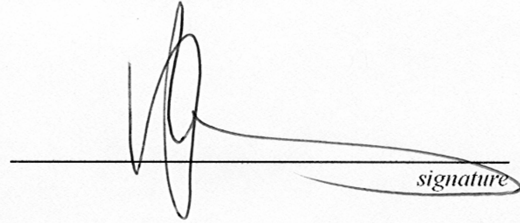
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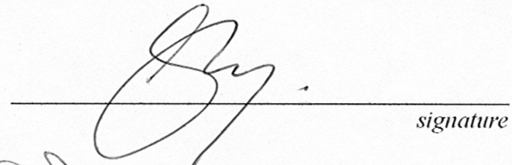
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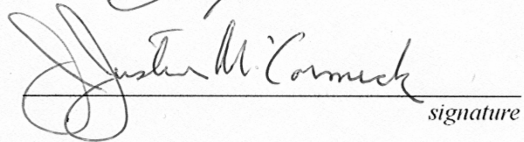
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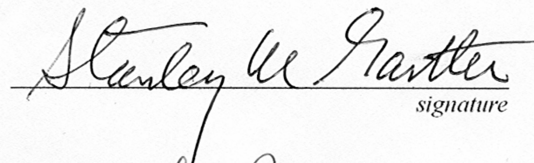
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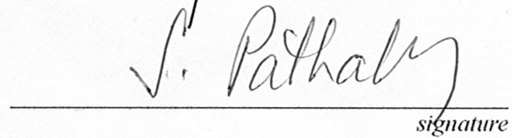
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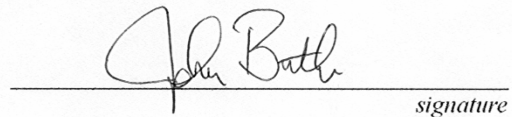
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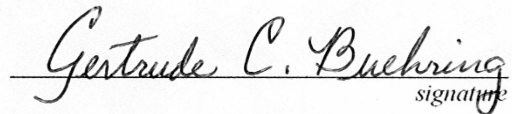
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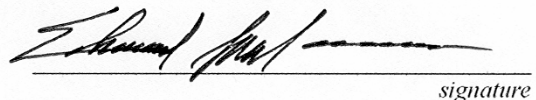
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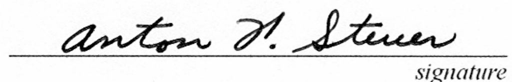
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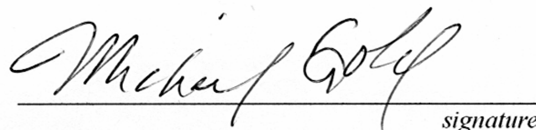

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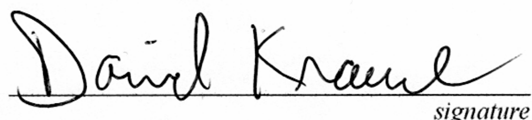

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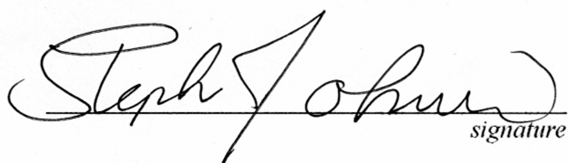

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[10] Coecke et al. "Guidance on Good Cell Culture Practice: A Report of the Second ECVAM Task Force on Good Cell Culture Practice", ATLA 33, 261-287, 2005, PMID: 11248855. An initiative for standardization and quality control in *in vitro* studies and the establishment of an ECVAM Task Force on GCCP.

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