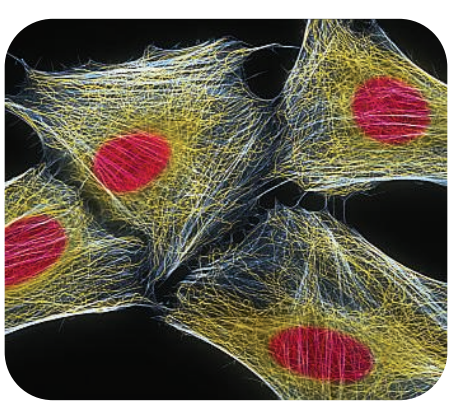
Cases of Mistaken Identity

For decades, biologists working with contaminated or misidentified cell lines have wasted time and money and produced spurious results; journals and funding agencies say it's not their job to solve this problem

IN THE 1980S, WHEN HE WAS A postdoctoral fellow at the Scripps Research Institute in San Diego, California, Reinhard Kofler received what was supposed to be a human cancer cell line from a collaborator. "We cultured it, we cloned genes into it," he recalls, then "[we] genotyped it and realized it was 100% mouse."

After scores of similar experiences with misidentified cells, Kofler and his colleagues at the Tyrolean Cancer Research Institute in Innsbruck, Austria, now authenticate every line as soon as it arrives at the institute. And periodically afterward, they use a simple, cheap, quick, and reliable DNA fingerprinting technique to verify that each cell line continues to be what it should be. "It's an absolute must now," says Kofler. His lab "repeatedly" encounters problems with cell line contamination, and without this constant vigilance, Kofler says, "I wouldn't be confident about our work."

Not every biologist is so wary. A 2004 survey of nearly 500 biologists by Gertrude Buehring of the University of California, Berkeley, and her colleagues, showed that less than 50% of researchers regularly verify the identities of their cell lines using any of the standard techniques such as DNA fingerprinting. "Everybody is in denial" about the widespread problem of cell line cross contamination, says Charles Patrick Reynolds of the University of Southern California and the Children's Hospital Los Angeles' Institute for Pediatric Clinical Research, who establishes new pediatric cancer cell lines and tests potential cancer drugs on existing lines.



Early warning. HeLa cells have contaminated scores of cell lines for more than 4 decades.

Indeed, many studies have shown that a surprisingly large number of cell lines have become contaminated, often by older, more well-established cancerous cells. For example, according to a 1999 paper by Roderick MacLeod and his colleagues at the German Cell Bank (DSMZ) in Braunschweig, 18% of 252 lines donated to the bank were misidentified or contaminated. The extent of the problem "always seems to come as a surprise for people," says John Masters of University College London, president of the European Tissue Culture Society.

And even though biologists read and hear about cross contamination, "people just think that this is not a problem in *my* lab," says Reynolds. If contaminated cell lines are used merely as "test tubes" to express proteins, a lab's work may not be affected. But, say Masters and others, research with contaminated lines continues to obscure potential drug leads and

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generate a large amount of artifacts in the scientific literature.

Troubled by this ongoing problem, Roland Nardone, a cell biologist and professor emeritus at the Catholic University of America in Washington, D.C., has taken it upon himself to become the Paul Revere of cell contamination. In a recent white paper chastising the scientific community, Nardone calls for stricter policing of cell identities. He argues that journals and funding agencies such as the U.S. National Institutes of Health (NIH) should mandate authentication of cell lines.

Several professional groups—including the Society for In Vitro Biology, the European Tissue Culture Society, and the American Society for Cell Biology—have endorsed the white paper, as have several cell repositories. But journals and NIH are wary of taking on the role of cell cop, and Reynolds is skeptical that Nardone will succeed where he and others have failed. "No amount of passioned discussion by myself or Dr. Nardone will fix what has been and continues to be a widespread problem," he says. Merely suggesting what needs to be done, Reynolds adds, "is a long way from people actually doing it." Kofler cites his own record as a cautionary tale: "We have started doing this [regularly fingerprinting lines] only 5, 6, or 7 years ago. Before that, even we were lazy."

Murphy's law

How do cell lines assume secret identities, and why does it happen so often? "It's like Murphy's law," says Kofler. "Everything that can go wrong will go wrong. It's just a matter of time." Although most researchers are aware of the possibility of contamination and cautious when handling cells, accidents happen. Cell lines get mislabeled or contaminated with fast-growing cells that can in no time take over the original lines.

The only way to prevent cross contamination is to spot it before it spreads. In 2001, Masters, who has been advocating for increased awareness of the problem for decades, published a description of a DNA fingerprinting technique that has become the standard tool for authenticating cell lines. When a line is established, it is crucial to record the donor's genetic profile and then do the same for the new line, says Masters. If this is done and the fingerprints made available publicly, it would provide other scientists with an authentic signature to verify the identity of the lines. Reynolds and his colleagues recently estimated the cost for a single DNA fingerprinting experiment to be $30. "It's so cheap, so obvious, so trivial, and yet it's not being done," says Masters.

Ignoring history

The roots of the contamination problem go back to the beginning of studies with cell lines. Between the mid-1960s and the early 1980s, Walter Nelson-Rees of the Cell Culture Laboratory of the University of California, Berkeley, at Oakland found more than 40 different cell lines—both human and animal—cross-contaminated by the HeLa line, the first human cell line to be grown successfully in a laboratory. By the time he published his findings, there were already hundreds of papers describing research using the contaminated lines.

Nelson-Rees made it his personal mission to warn others about the dangers of

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WHEN 60 LINES DON'T ADD UP

Even the bedrock of present-day cancer research, the NCI-60 panel—a group of 60 cancer cell lines maintained by the U.S. National Cancer Institute (NCI) and used widely for both basic research and drug discovery—has not escaped the scourge of cross contamination. In the late 1990s, Mordechai Liscovitch of the Weizmann Institute of Science in Rehovot, Israel, had obtained from the institute the breast cancer line MCF-7 and its drug-resistant daughter line, once known as MCF-7/AdrR (for Adriamycin resistance)—both part of the NCI-60 panel. A few years ago, a comparison of the lines in his lab revealed certain biochemical differences that illustrated how cancer cells become resistant to drugs. Three years of work with these lines had unfolded "a nice story," says Liscovitch.

Early in 2001, he submitted a manuscript on the work to *Oncogene* and was awaiting its publication. Then, one of his students stumbled upon a 2000 letter in the *Journal of the National Cancer Institute*, saying that DNA fingerprinting had revealed that MCF-7 and MCF-7/AdrR were in fact unrelated; Liscovitch and his team immediately realized that their interpretations in the upcoming paper were no longer valid. Disappointed at the years of wasted time and effort, they withdrew the paper before it went to print. "It was a big blow for us," Liscovitch says.

Not only was MCF-7/AdrR unrelated to MCF-7, but it also turned out to be identical to an ovarian cancer cell line also in the NCI-60 panel. That's not the only case of mistaken identity within the NCI-60 panel. The SNB19 and U251 lines, once thought to be distinct central nervous system lines, are identical to each other and came from the same individual. And MDA-MB-435, a prevalent model for metastatic breast cancer, is identical to the panel's melanoma line, M14. NCI has tried to trace the history of MDA-MB-435, which was originally established in 1976 at M. D. Anderson Cancer Center in Houston, Texas. NCI found that the NCI-60 panel's version is the same as a sample of the line originally deposited at a cell bank by M. D. Anderson and as a sample given to an NCI researcher by the cancer center. "The mix-up with melanoma cell line M14 likely happened early in the history of the cell line," NCI says on its Web site.

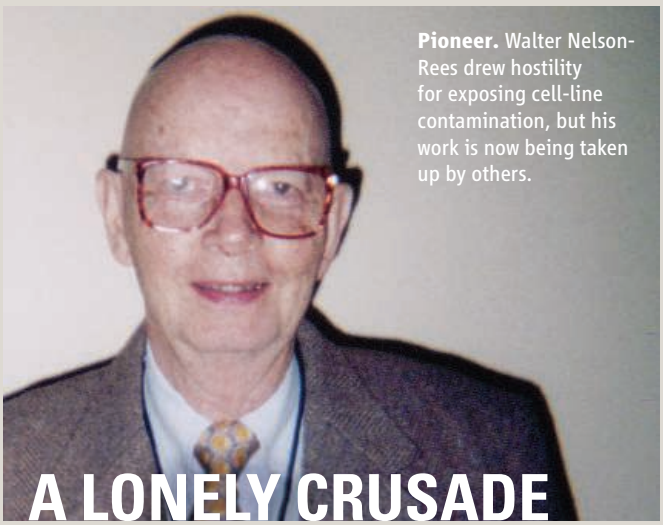
Although the NCI-60 panel's Web site now details the history behind its "mischaracterized" cell lines—Daniel Zaharevitz, chief of the information technology branch at NCI's Developmental Therapeutics Program, considers that description more accurate than contaminated or misidentified—the institute hasn't gone out of its way to inform researchers who obtained these lines in the past that the lines are now suspect. Zaharevitz says the agency is wary of creating undue concern, because much of the work with such lines, such as drug testing, is unlikely to have been compromised.

Liscovitch feels that greater exposure of the problem is needed. He publicized the story of MCF-7/AdrR, now known as NCI-ADR/RES, in the 8 January *Cancer Letters*. There may be more such stories in the future. There is some evidence that the NCI-60 panel's version of the colon cancer cell line HCT-15 is not the same as the original line. **–R.C.**

HeLa contamination. But the scientific community mostly reacted with hostility, and Nelson-Rees eventually gave up (see sidebar, below). No one was willing to withdraw their papers or lose their credibility—and most researchers continued using the contaminated lines. Nardone, Reynolds, Kofler, and other researchers are worried that history is being repeated, especially because the number of new cell lines has proliferated dramatically.

In 2003, MacLeod and Hans Drexler of DSMZ and their colleague, Yoshinobu Matsuo, then at Fujisaki Cell Center in Okayama, Japan, checked the identity of 550 lymphoma-leukemia lines collected from researchers around the world and found 15% of them to be contaminated, mostly with faster-growing, well-established cell lines. In a letter in the 23 February 2006 issue of *Nature*, they estimated that 29% of all human-tumor cell line submissions to the DSMZ include cross contaminations. Because of the small sample sizes, these figures are, at best, "a significant underestimate," says MacLeod.

Estimating the real extent of the problem is difficult; there are far too many cell lines being established every year, and very few of them ever get their identities profiled. Repositories such as the German Cell Bank and the American Type Culture Collection (ATCC) profile every line in their labs. But most new lines are established in individual labs and from thereon are freely exchanged between labs, rarely having their identities checked. "These cell lines never pass through our doors, so they are never subject to accurate authentication," says MacLeod. He and his colleagues have found that about 90% of scientists ignore or refuse a cell bank's request to send in new lines, and MacLeod argues that depositing lines should be required so that DNA fingerprints can be established and stored for



Pioneer. Walter Nelson-Rees drew hostility for exposing cell-line contamination, but his work is now being taken up by others.

A LONELY CRUSADE

In 1951, a 31-year-old African-American woman was admitted to Johns Hopkins Hospital in Baltimore, Maryland, for treatment for cervical cancer. The hospital sent a sample of her cancerous tissue to Hopkins tissue culture expert George Gey, who successfully cultured it in his lab. Henrietta Lacks's ferocious cancer cells spread throughout her body and eventually killed her. And her immortalized cells, named HeLa cells after her, quickly spread through labs across the world—and not always because researchers had requested a sample for study.

In 1966, Stanley Gartler of the American Type Culture Collection found that 18 of the first 20 human cell lines established were chromosomally and biochemically identical to HeLa cells. All 18 lines were known to have come from Caucasian individuals. Yet Gartler found that each had a genetic variant of an enzyme found only in the small percentage of African-American population that Lacks had belonged to. Gartler published his findings in *Nature* in 1968, marking the first reported case of HeLa contamination. It was only the beginning.

A few years later, Walter Nelson-Rees began discovering contaminations in lines from laboratories across the world. At the time, he was at the Cell Culture Laboratory of the University of California, Berkeley, at Oakland, characterizing, storing, and distributing cell lines for the U.S. National Cancer Institute (NCI). Over more than 10 years, he counted 279 contaminated lines from 45 different laboratories. Many were contaminated with cells from other species, but the bulk—more than 40 individual lines—had been overcome by HeLa cells. "This sort of scenario happened many, many times; people who thought they were working with one type of cells [were later found to be] working with HeLa cells," he says.

Eponymous. HeLa cells came from Henrietta Lacks's cervical cancer.

Nelson-Rees published his results in a series of papers in *Science* in the 1970s, urging scientists to stop using contaminated cell lines, re-evaluate their previous research, and employ simple quality-control practices such as regularly verifying their lines' authenticity.

Nelson-Rees's revelations threw the community into a frenzy. Many studies were called into question, and Nelson-Rees was naming names. Some biologists reacted with hostility, and *Nature* in an editorial called Nelson-Rees a "self-appointed vigilante." In a 2001 commentary on cell line authentication, Stephen O'Brien of NCI in Bethesda, Maryland, who had worked with Nelson-Rees, recalled the tension: "Human emotions were on edge, red faces were appearing in the most prestigious laboratories, and discussions of the problem lost any semblance of civility." Nelson-Rees even remembers an anonymous telegram offering to send him a one-way ticket to South Africa. "My aim was to clear up a morass of contamination, and it wasn't easy," he says.

The attacks ultimately took their toll. In 1981, Nelson-Rees quit science and opened an art gallery in San Francisco.

HeLa continues to spread today. In 2004, Gertrude Buehring of the University of California, Berkeley, and her colleagues surveyed 485 researchers from 48 countries who were working with specific cell lines and found that 49 were using seven lines that others had shown to be contaminated by HeLa. When Buehring conducted a PubMed search to identify the number of publications from researchers wrongly using HeLa-contaminated lines as though they still had cells of the original line, she found a total of 220 papers between 1969 and April 2004. And the number of publications on research using cell lines shown to have become contaminated by HeLa had increased by a factor of 10 between 1969 and 2004, whereas the total number of publications had increased by only a factor of 2.7.

But perhaps Nelson-Rees will finally get his due. Other scientists are now taking up his fight against cell line contamination (see main text). And in 2004, the Society for In Vitro Biology publicly recognized his contribution to science with a lifetime achievement award.

–R.C.

future verification attempts.

Researchers sometimes publish papers on individual mix-ups, hoping to warn the rest of the community about a particular cell line. But these warnings are typically restricted to specialized journals and fail to grab the attention of the larger scientific community. For example, Mordechai Liscovitch, a cancer researcher at the Weizmann Institute of Science in Rehovot, Israel, says he and his lab wasted 3 years because they hadn't noticed a publication revealing that the two breast cancer lines they were studying were not actually related—a fact the U.S. National Cancer Institute knew and attempted to publicize, although it continues to use and distribute the contaminated lines for drug testing (see sidebar, p. 929).

A birthday resolution

Nelson-Rees may have failed to stop the spread of HeLa cells, but Nardone is taking up his battle. The retired director of R/M Nardone Associates, a biotechnology training company, Nardone has for more than 2 decades educated graduate students and postdocs at NIH about cell culture techniques. "Each year, I give a lecture on cross contamination," he says. "And each year, I get the same blank stares that tell me they aren't adopting the techniques."

In 2005, he happened to give this lecture on his 77th birthday. After the class, when his son asked him whether he had a birthday resolution, Nardone realized that he was "so damn mad" about the reluctance of scientists to acknowledge the seriousness of the problem that he decided to do more than give an annual talk to a few biologists.

Several weeks later, Nardone put together a white paper titled *Eradication of Cross-Contaminated Cell Lines: A Call for Action*. "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," he writes.

Nardone's "call for action" seeks two broad changes: more regulations and increased education efforts. Nardone argues that journals and funding agencies should impose strict rules on researchers, forcing them to submit proof of cell line identity along with their manuscripts and grant proposals, respectively. This, he says, has to be supplemented by renewed education efforts to increase awareness of the cross-contamination problem, especially among younger researchers who are unfamiliar with its history.

The journals and agencies targeted by Nardone seem to embrace his warnings but not his solutions. In an e-mail, Sally Rockey, deputy director of NIH's Office of Extramural Research, told *Science* that "NIH is aware that contamination of cell lines is a serious issue that can result in loss of biological products and render research worthless. … The career and reputation of scientists can be affected if research is conducted using contaminated cell lines." Yet, Rockey argues: "It would be impractical to require authentication as a condition of award as cell lines are used routinely in thousands of basic science studies that NIH funds. … NIH believes that professional societies and scientists themselves should be driving the profession toward best practices in avoiding cell culture contamination instead of placing the responsibility on the funding agency."

Journals also hesitate to assert authority. "This is a requirement that would be imposed by the field, not by the journal," says *Science* Deputy Editor for Biology Katrina Kelner. "We do not have an explicit policy but will certainly keep our eye on this if it is something that becomes a standard." *Nature* did recently mandate DNA fingerprint data for papers reporting new human embryonic stem cell lines, but this policy doesn't extend to all cells. "I think we would agree with the sentiment" in the white paper, says Natalie DeWitt, a biology editor at *Nature*. But "you can't just suddenly say we need to verify cell lines; we don't have labs in our offices, and we can't check the lines ourselves and say it's from hamster and not from mouse."

Rebecca Chasan, executive editor of the *Journal of the National Cancer Institute* (*JNCI*), says reviewers sometimes raise questions about cell line identity, but after reviewing Nardone's white paper, *JNCI* may take a firmer line. The journal is planning to begin asking authors to confirm that they have authenticated their lines. Some issues need to be worked out, however. For example, should that request come before or after a paper undergoes review? "If a paper has gone through the peer-review process and the authors aren't able to confirm the identity of the cell line, it's not yet clear what we would do," says Chasan. If the genetic signatures of all established cell lines were available in a public database, then it would be easier for journals to step in, notes DeWitt.

As journals wrestle with the problems posed by cell line mix-ups—Reynolds goes so far as to estimate that journals would have to retract 35% to 40% of their previously published cell biology papers to weed out invalid data—some organizations are trying to help in different ways. The Society for In Vitro Biology will hold a symposium at its 2007 annual meeting in which Yvonne Reid of ATCC will talk about how contamination can be prevented. Nardone, Masters, and Joseph Perrone of ATCC are also organizing a conference to discuss standards and guidelines that could lead to profession-wide compliance for authentication. And ATCC, which has for decades sold lines overtaken by HeLa, recently decided to stop routinely distributing the lines, except for special requests from researchers. But these efforts will have limited effect, says Nardone, if journals and grant-awarding agencies won't mandate cell line authentication. What biologists need, he concludes, is a "stick saying that if you don't do this, there will be a consequence."

–RHITU CHATTERJEE

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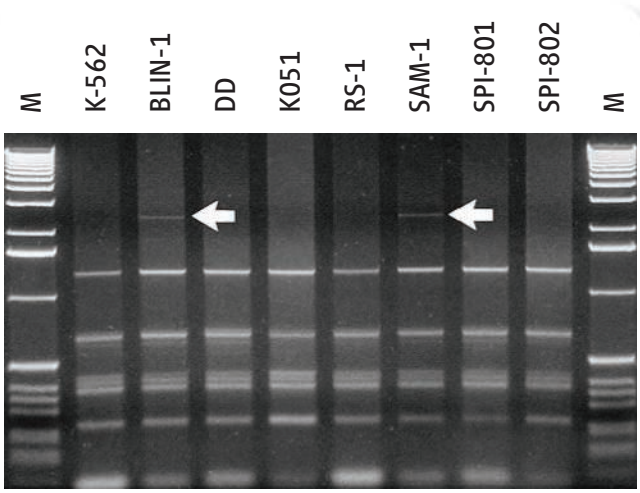

Identity theft. DNA fingerprinting of these cancer cell lines shows that most, if not all, are identical to the chronic myelogenous leukemia line K-562. RS-1, for example, had been thought to be an acute myelogenous leukemia line.

CREDIT: DREXLER *ET AL.*, *LEUKEMIA* 17 417 (2003)