*Cell cultures*

# Trouble in the Dishes

Cell lines play a key role in biomedical research but surprisingly, many scientists pay little attention to them. Lack of rigour and knowledge often leads to contamination in the culture dish. For years, a handful of dedicated cell biologists has been issuing warnings to the scientific community, but more often than not their warnings fall on deaf ears.

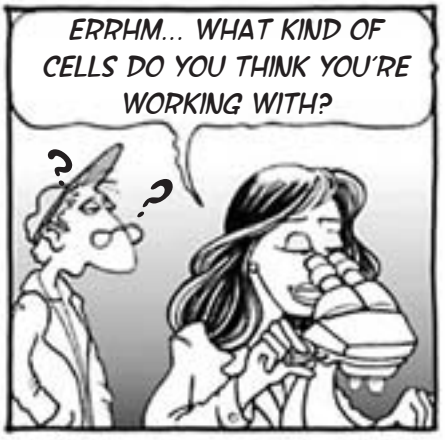
When Jurjen Boonstra xenografted cells of the TE-7 cell line on nude mice to analyse the histology of the tumour cells, he got a nasty surprise. The cells that ought to behave like oesophageal adenocarcinoma cells grew like squamous cell carcinoma cells. Repeating the experiment gave the same results.: "We thought that something must be wrong with the cell line", says Winand Dinjens, from the Department of Pathology at the Erasmus Medical Center (Rotterdam, Netherlands). So they asked the Japanese Collection of Research Bioresources (JCRB), who had sent them the original TE-7 cells, if they could send them earlier passages of the line. But when the experiments were repeated the results were the same; TE-7 cells showed squamous, instead of adenocarcinomal, characteristics.

By now, thoroughly wary of these cells, the scientists went into a detailed analysis, checking the genotypes of their lines together with TE-lines from other laboratories. "We are now sure that the TE-7 line is indeed a squamous cell carcinoma cell line", says Dinjens. "And we discovered that TE-2, TE-3, TE-7, TE-12 and TE-13 have a single origin! They are the same single squamous cell carcinoma cell line!"

## Which cancer?

"What's the problem?" some of you might say. After all, these researchers spotted the problems with TE-7 at the start of their experiments – they didn't waste months or years doing futile experiments. Well, unfortunately, other labs have. TE-lines are used in biomedical research throughout the world. Established in Japan in the late 1970s and early 1980s, fourteen TE-lines were derived from human oesophageal squamous cell carcinomas and just one, TE-7, from putative primary oesophageal adenocarcinoma. The work by the Dutch scientists, published last September in *Cancer Research* (67, pp.7996-8001), means that all the previous work on TE-7 cells, from molecular data to pharmacological tests, does not in fact have anything whatsoever to do with adenocarcinoma.

Reports about cell lines being classified wrongly, contaminated with cells of other tissue or even of other organisms (*e.g.* mouse cells in human cell lines) or mycoplasma, are not published that often. Although rare, these reports certainly underline the paramount importance of controlling cell lines. However, the "quality control of cell lines is widely neglected by the scientific community", says John Masters from University College, London, President of the European Tissue Culture Society. Hans Drexler from the German Collection of Microorganisms and Cell Cultures (DSMZ) in Braunschweig further laments that "people don't look upon control of their cells as being important. And if experiments lead to inexplicable results they will be pigeonholed instead of published."




## Rude awakenings

While scientists routinely control new plant and mouse mutants, or cloned genes and plasmids obtained from other laboratories or commercial stocks, their control of cell lines is remarkably rare. Not surprisingly, such casual handling of cell lines in defiance of basic scientific principles can sometimes lead to a bad end. For example, bad experience led to a rude awakening for one Croation scientist. He ordered MRC5-cells from the National Institute for Biological Standards and Controls (UK) and cheerfully started developing a new vaccine against measles. Some time later he sent his MRC5-cells to the DSMZ for routine testing. The results led to some raised eyebrows as there was no Y-chromosome in the cells. Yet, MRC5-cells, used for years in vaccine development, were originally derived from a male foetus! "The Croatian scientist had to discard half a year's work", reports Roderick MacLeod from the DSMZ.

"Scientists are simply not interested in controlling their cell lines", states Masters. This is reflected in the Materials and Meth-

ods sections of publications where restriction enzymes are described in far more detail than the cell line used. And it's the exception rather than the rule when the authors deign to disclose the origin of their cells, whether it be from a friend in the next lab or from a cell bank.

Cell biologists assume the reason for this culpable negligence is a lack of knowledge about the hidden life of cells in culture. "Cells aren't simple reagents, they are living organisms. Yet, many scientists ignore this fact and use cells like other lab tools", complains Petra Boukamp from the German Cancer Research Centre (Heidelberg, Germany). This cell biologist knows exactly what she's talking about. She has established several cell lines. "Many people working with our cells do not know what they should look like, how they should be cultivated, or how often they can be passaged in culture."

## Of mice and men

Falsely typified cell lines are not to be sneezed at. As far back as 1968, Stanley Gartler from the American Type Culture Collection (ATCC) published, that 18 out of 20 cell lines he had analyzed were misclassified. In the dishes grew something else – alive and kicking HeLa cells! Walter Nelson-Rees and Robert Flandermeyer, in the 1970s, both working in the cell culture laboratory of the University of California, Oakland, published HeLa pollution in over 40 different lines. Many HeLa-cultures were also identified by scientists at the Welcome Trust Sanger Institute (Hinxton, UK) working for the Cancer Genome Project. Indeed, these robust HeLa cells thrive and prosper so well that they overgrow any other cell line if they get into the culture. Moreover, contamination of mouse cells in human cell lines are not uncommon.

## Warnings ignored

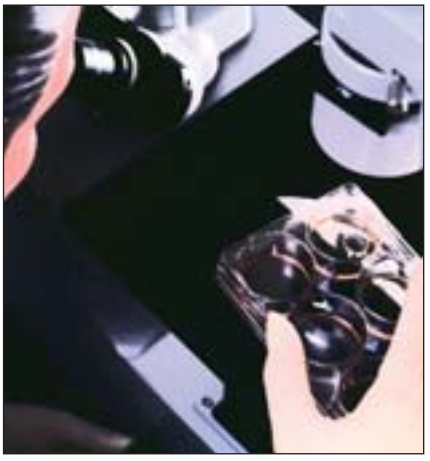
People searching systematically for wrong lines, frequently discover them. The team around Drexler and MacLeod investigated more than 500 leukemia and lymphoma cell lines. The results were staggering: 14 percent of the lines were falsely classified, 26 percent were infected with mycoplasma. Michael Stratton, leader of the Cancer Genome Project, reported in an email to *Lab Times*, "As part of this project we genotyped all the lines we ever used and found lots of cross contamination, *i.e.* lines with different names, which are clearly derived from each other."

Especially alarming is the case of the NCI-60 panel. These 60 cell lines from the National Cancer Institute (USA) are among the best-characterised cell lines in the world. In 1990, the NCI began offering these cells for screening potential anti-cancer substances and pharmaceutical products. But even this distinguished panel isn't immune against contamination. Various research groups proved that the NCI/ADR-RES cells are not derived from breast cancer tissue but from ovarian cancer cells. In addition, cells lines SNB-19, U251 and MDA-MB-435 are not authentic. Expression analysis of the panel cell lines (*BMC Genomics* 7, pp. 166) performed by scientists of pharma giant Eli Lilly showed that SF-295 and SF-539 cannot be derived from central nervous tissue, that

SN-12C doesn't contain kidney tumour cells, and that NCI-H23 and NCI-H522 are not derived from lung tumours, nor PC-3 and DU-145 from prostate carcinomas.

The ATCC collection also contains incorrectly characterised lines. The cell bank's website sheepishly lists six lines of putative female origin that, in fact, contain Y chromosomes (OV-1063, CHP-234, NCI-H738, NCI-1514, NCI-H1622, HBL-100). Furthermore, the identities of SNB-19, U-373MG, U-118MG, U-138MG and ECV-304 are "in question".

The ECV-304 case is clear. The line, established in the early 1990s, was thought to be the first endothelial cell line. It became a popular model line in its heyday. Unfortunately in 1999, it turned out that these cells are in fact bladder cancer cells, derived from the T-24 cell line. Although this finding has been circulated by cell banks and published in the International Journal of Cancer, many scientists – mainly Chinese – happily continue to use this line as an endothelial model! If you don't believe us, take a look in PubMed! It seems that despite the efforts of some robust scientists, like Masters and Drexler, who untiringly advise scientists of the widely neglected problems concerning contaminated or falsely classified cell lines, their warnings are often ignored by the scientific community, as was Cassandra of Troy.



Information about misclassified cell lines only seems to seep very slowly through the scientific community. Probably the best way to gather the latest information is by looking through the cell banks' websites. The ATCC describes incorrect lines in a nutshell, without references. After an investigative search of the NCI-website "DTP Human Tumor Cell Line Screen", we found "Please note the links for more information on the SNB-19, U251, NCI/ADR-RES, and MDA-MB345 cell lines" which provided extensive documentation. However, the fishy expression analysis results from the Eli Lilly scientists are not yet listed.

ATCC and NCI comply with the need to inform their customers. But to be honest, who actually screens all the websites of cell banks regularly for the latest data updates? The DSMZ is more active. If Drexler or his colleagues pinpoint a bad egg they inform all the people that previously received this cell line from them.

## Weeding out duplicate cell lines

Data on all the cancer lines genotyped by the Cancer Genome Project scientists is listed on the COSMIC database (Catalogue Of Somatic Mutations In Cancer). According to Stratton, this is "the standard for academic and industrial scientists interested in mutations in human cancer." He thinks that "in this era, having an updatable web release is probably the most useful way of making the data available." In the next couple of months a large analysis of copy numbers in the set of roughly 800 cancer cell lines will be published, mentioning which lines have been weeded out as duplicates.

So, what can the scientific community do to get to grips with this issue? In an open letter to the US Department of Health and Human Services, Roland Nardone from the Catholic University of America (Washington DC, USA) and 18 other scientists called for specific education of scientists through tailored programs and formats. "However, education alone cannot accomplish the goal of bringing about a profession-wide change in cell culture practices", wrote Nardone. He and his comrades-in-arms want all major grant agencies and journals to ask for cell line authentification if a scientist applies for a grant or submits a paper. The authors of this letter clearly think that only active constraints will cure scientists of the kind of lazy sloppiness that regularly produces such misinterpretable data. Although the letter appeared last summer, Masters, who also signed it, hasn't noticed any results. "This issue has been around for 40 years and I'm not very optimistic. I'm not holding my breath."

## Like lemmings

The really puzzling thing is why scientists are prepared to risk losing months, or even years, of their work instead of just taking a few days to check their cells. With readily available modern methods for genotyping DNA, checking the cells' integrity is surely a matter of clearly defined routine. Those who still consider this too difficult can buy a complete genotype analysis from the DSMZ for a mere 200 Euros. Diligent cell banks and commercial cell culture distributors routinely check DNA profiles and karyotypes of their lines – or at least they're meant to. If you order a cell line you should insist on details of the quality control.

Unfortunately it's not always possible to verify the origin and authenticity of a cell line by comparing it to the original tissue from which it was derived. Either the tissue is no longer available – in the case of the TE-7 line, the stored tissue was destroyed by an earthquake (no kidding) – and/or the original tissue was simply never stored. This is why Drexler not only tries to get his hands on new cell lines for his DSMZ bank but also on the original sources of the new lines as a control reference. However, his efforts are not always crowned with success. He reports that after launching a worldwide request for cell lines, almost all UK-based scientists sent them to him, as did two-thirds of scientists in France. However, virtually none of the requested cell culture probes were sent from Germany, Japan or the United States. Why not? Drexler thinks there are two main reasons: firstly, scientists worry that the cell banks will merchandise their cells, which they don't; and secondly, scientists want to keep their hands on their cells to ensure co-authorship on publications each time the cells are used. But Drexler refuses to laugh off such behaviour, "If the production of a new cell line is paid for by tax-payers then it must be deposited in the national cell bank!"

## Unpopular campaigns

Naturally, with such a determined stance, he isn't necessarily making friends for himself. But at least he hasn't met the fate of Nelson-Rees, who discovered the HeLa contaminations. His campaign made him famous but also so unpopular that he had to give up science.

Winand Dinjens, however, says he has experienced good cooperation when asking other labs for additional oesophageal carcinoma cell lines. "We are checking all lines we can get hold of, and that's quite an effort", he said. "But I'm convinced that this important work needs to be done."

If only more scientists would follow his example!

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