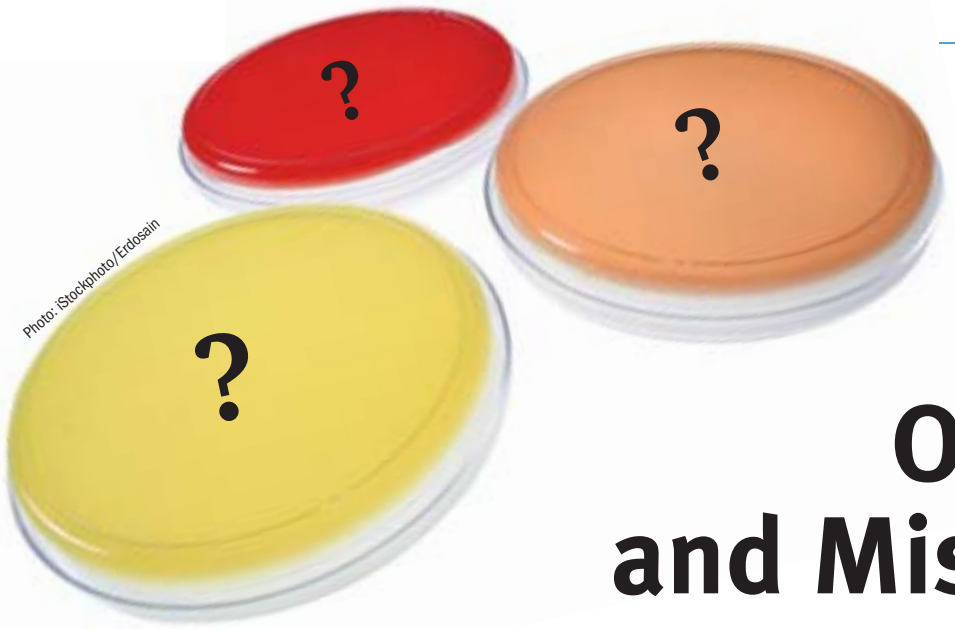

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Authenticity of lab organisms

Of Mix-ups and Mislabelling

Mistakes happen all the time but it gets bitter when your whole research depends on it. Is the cell line you are working with really the one it has been claimed to be?

It's in the nature of things that life science research requires the handling of living objects on a day-to-day basis – cell lines, bacterial strains, plant and animal models... Logically, therefore, the outcome of your experiments most critically depends on the nature, state and condition of these living "tools". Most probably you have received these tools from a culture collection, gene bank or a collaborating scientist and have also been taught how to best utilise them. And clearly, you have since devoted a fair amount of your time taking care of them and making sure that they propagate under the most optimum conditions.

Now, what would your reaction be if you found out that these bacteria, cell lines or mice were non-authentic? That they had been mislabelled or mixed up? That, all this time, you have been investing your time and effort in incorrect tools? Unfortunately, such muddle-ups and contamination have indeed become a serious reality in biomedical research and many an unsuspecting researcher has already fallen prey to this nuisance. Therefore, let's now review some famous "mix ups" from recent years – in order to understand how best to avoid them.

Lost and found: *Pseudomonas cissicola* and Adenovirus

In 1992, the paper "Taxonomic confusion of *Pseudomonas cissicola* originated from mislabeling by International Type Culture Collections" by the Japanese microbiologist Masao Goto appeared in the *International Journal of Systematic Bacteriology* (vol. 42: 503-5). It almost read like a mystery. In it, Goto explains how an internal mistake at the International Collection of Microorganisms from Plants in New Zealand led to the mislabelling of the type strain (one of the more fully characterised strains) and two reference strains of *P. cissicola*. And as if this wasn't enough, the mistake was repeated during a transfer to the National Collection of Plant Pathogenic Bacteria in England.

Needless to say, this led to some major confusion regarding the bacterium's taxonomic classification amongst the researchers who had received these cultures as well as the workers in charge of the culture collections. With his paper, Goto finally helped clear the air – and then went on to suggest that all researchers "should check the identity and purity of the bacterial strains that they use through a preliminary examination". In addition, he quoted a 1966 *Bacteriological Reviews* paper by Einar Leifson (vol. 30: 257-66) that had already warned about such a situation and recommended that all cell culture collections "should be urged to use a depositor approval check system to retain their reputations for the accurate identification and culture purity".

Nevertheless, incidents like this have since continued to happen. For example, Ijad Madisch and Albert Heim from the Medical University Hannover only recently reported a similar "mislabelling incident" regarding certain viral serotypes *(J. Clin. Microbiol.*, 2007, 45(6): 2092). In this case, two different serotypes of adenovirus were reciprocally mislabelled and, in August 2000, submitted to the American Type Culture Collection (ATCC) and the Centers for Disease Control and Prevention (CDC) in Atlanta. The CDC discovered this mistake almost immediately in September 2000, and reported it to the ATCC. However, the story did not end there. In 2002, the authors ordered these strains from the ATCC for a phylogeny study and discovered that the samples still bore incorrect labels. When seeking answers from the ATCC, they first received no response; it took until February 2004 for the ATCC to finally confirm the suspicion.

Cross-contaminations in cell lines

Cell lines are another issue in this respect. In 2000, for example, Glyn Stacey from the National Institute for Biological Standards and Control in South Mimms, UK, together with five colleagues, wrote a correspondence letter to *Nature* titled "Cell contamination leads to inaccurate data: we must take action now" (vol. 403: 356). The letter started with citing the famous 1981 re-

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Joining its brethren's colony from an unidentified stock

port by Walter Nelson-Rees *et al.* that brought to light the cross-contamination of numerous cell lines with HeLa cells, a human cervical cancer cell line (*Science* 212: 446-52). Nevertheless, according to Stacey *et al.,* reference culture collections had still been finding cases of HeLa contamination in various cell stocks. The ATCC discovered 15 non-authentic cases among newly acquired cell lines, while a survey performed by the German Collection of Microorganisms and Cell Cultures (DSMZ) in Braunschweig (D) found that up to 18% of these stocks had been cross-contaminated by their originators. The authors' lamentable statement was, "In such cases, the only remaining characteristic of the original cultures is their name!"

In another guest editorial, titled "Mix-ups and mycoplasma: the enemies within" (*Leukemia Res.*, 2002, 26(4): 329-33), Hans G. Drexler from the DSMZ in Braunschweig and his co-authors concentrate on cross-contamination of human leukaemia-lymphoma (LL) cell lines. According to them, this can happen in three ways:

- A cross-contamination occurs during the time that a cell line is being established – this is termed as "early cross-contamination".
- An established cell line A is contaminated during culture with another cell line B – this is termed as "late cross-contamination". In this case, cell line A may be completely lost if cell line B has a growth advantage, for example, via shorter doubling times.
- The last group, "misclassification", simply refers to instances that arise due to incorrect identification of a cell line.

Interestingly, Drexler & co. also mentioned what happened when, after they had identified a cross-contamination, they informed the scientist who had submitted the sample. Many scientists responded with a "false-cell-line-denial-syndrome", either arguing or completely ignoring the finding of the DSMZ.

In both those reports, the authors recommend authentication and archiving of cell stocks by resource centres and scientific organisations. They also advise using DNA fingerprinting and cytogenetics as a means of detecting cross-contamination. Moreover, they emphasise that cell lines should only be disseminated if they are from authenticated sources and if they carry complete documentation regarding origin, provenance and biohazard information. And, last but not least, they certainly try to pinpoint the reasons for contamination: "faulty cell culture technique, inappropriate handling of cell lines, and lack of knowledge and information about the consequences and effects of contaminations" (Drexler *et al.*).

Mice with questionable genetic backgrounds

And mouse models? Everybody knows that, in recent decades, they have proved enormously valuable in addressing questions on basic genetics, physiology, drug discovery and human disease modelling. For this reason, in 2000, more than 25 million mice were raised worldwide for research. However, as early as 1974, Michael Festing from the Medical Research Council Laboratory Animals Centre in Carshalton, UK, published a report in *Laboratory Animals* (vol. 8: 265-70), in which he stated that several of the "common albino outbred stocks" of laboratory mice in the United Kingdom were not "genetically authentic" and that "it is questionable whether the published background information on such stocks is valid".

While meticulously monitoring 20 different mouse colonies sourced from ten separate breeders for more than a year, Festing stumbled upon three major cases of "lack of authenticity". In

the first case, mice that had been deemed a "pure strain" were in fact born from an unidentified cross. In the second case, two to three different stocks had been put together in a single colony and then sold with three different names, none of which were authentic. And in the last case, differences were identified between stocks that carried identical names but came from two separate sources.

At that time, Festing recommended strict protocols for mouse breeders that wished to supply such strains. Moreover, he suggested enforcing regular checks and multiple sampling during the year, to monitor the breeders and also advised users to perform independent checks to ensure the "genetic consistency" of their mice colonies. Finally, the paper ends on an optimistic note, "In this way, the genetic authenticity of experimental mice available to research workers in the U.K. should be substantially improved".

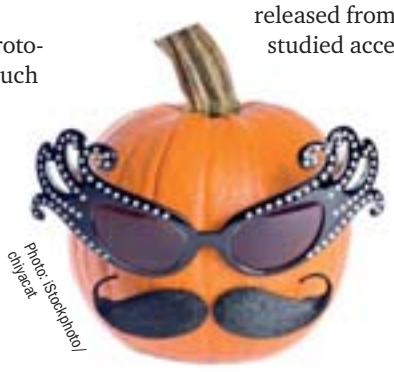

Photo: iStockphoto/chivacat

Even in disguise, this Cucurbita clearly can't be mistaken for *Lactuca sativa*, lettuce

Unfortunately, this matter did not close in 1974. In fact, in recent years the need for a more reliable method to detect mice with incorrect genetic make-ups has become even more urgent. In an attempt to answer these calls, Frauke Nitzki and colleagues from German universities in Göttingen and Mainz published a report in 2007 suggesting the application of SNP genotyping to mice colonies, in order to "monitor mouse genetic background" (*Laboratory Animals* 41: 218-28). Utilising a panel of 100 single-nucleotide polymorphism (SNP) markers, the researchers could confirm a suspected contamination of their commercially available inbred C57BL/6N mouse strain with alleles from a different strain.

Mixed-up cultivars in gene banks

However, mix-ups are not restricted to the animal world. Many plant gene banks (seed banks), especially older collections, contain accessions bearing the incorrect cultivar labels (any cultivable plant specifically selected for its desirable qualities). To add to that confusion, it seems that many older cultivars with identical or synonymous names are maintained in multiple collections, and quite often more than once within the same collection.

Mark van de Wouw and colleagues at the Centre for Genetic Resources (CGN) in Wageningen, the Netherlands, wanted to assess the extent of this non-authenticity. They analysed the DNA marker profiles of different accessions of the lettuce *Lactuca sativa* cultivar collection at the CGN. Their results published in the January issue of *Crop Science* (vol. 51, 736-746), "show that the non-authenticity of the investigated cultivars, particularly the oldest ones, is high. Even in more recent cultivars, such as those released from the 1960s to1990s, a substantial 10% of the studied accessions were not authentic".

But how did this situation occur in the first place? The paper explains that when modern gene banks were established, many older cultivars were no longer in use. Thus, they had to be brought in from all possible sources like botanical gardens, research institutes and seed companies. Quite often, however, the accompanying documentation was ambiguous or incomplete, as these institutions did not follow strict protocols. And due to the unavoidable manual handling of cultivars, mislabelling could occur at any stage.

This is an alarming finding, given that such gene banks constitute the principal sources in providing plant samples to research institutes and hobby growers for further breeding. Moreover, they are indispensable for the conservation and preservation of cultivar diversity. And not least, the people maintaining these collections also bear the responsibility of maintaining our bio-cultural heritage.

Take-home message

Although it has already been stated many times, it still seems necessary to stress: everybody ought to realise that cell culture collections or gene banks may not be blindly trusted. One must independently ascertain the identity of all received material to prevent any scientific mishaps. Additionally, the culture collections must maintain the highest levels of stringency in their day-to-day proceedings and invest extra effort in regular authenticity checks.

In the light of the notion that good research demands strict quality control, it is, furthermore, important that one is well informed about such events. For when the origin or purity of an experimental tool is questioned, it regrettably also casts a shadow of doubt on the researchers involved.

Latika Bhonsle

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