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Bertrand Russell (1872 - 1970)

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Success stories

In 2012, Germany's exports were clearly more successful than they should have been. "Calculated in euros, Germany's current account surplus was €169 billion," stated Ifo President Hans-Werner Sinn, commenting on the Institute's figures. This would constitute 6.4 percent of GDP for the year 2012. Ifo figures show that only China – the world's #1 exporter – recorded a figure larger than the German surplus. Taken as a dollar value, China's surplus increased from $202 to $234 billion last year, while Germany's rose from $204 to $218 billion.

With a certain amount of pride, then, German industry can also look ahead to a positive result in 2013. Quality and reliability do seem to be truly valued by countries around the world. On the other hand, it is also a result of the efforts made by German companies to raise the profile of their businesses and products. Participation in global trade fairs is steadily increasing, and our publishing house is also seeing a growing readership for our international journals.

As ever, success is the result of hard work, tenacity and the courage to innovate. One striking example of this appeared on the news tickers in the last few days. The European aerospace company Airbus has just won a record order from the Indonesian Lion Air airline. Lion Air stands to receive 174 aircraft from the new A320neo and A321neo series. Another 60 standard A320s complete the deal. Overall, the European aircraft maker will supply 234 aircraft for a sum of € 18.4 billion. French President François Hollande stated that the order is the "largest-ever for Airbus – both in terms of aircraft number and total sales volume."

"Chapeau" – as the French would say. In this issue, we wish our customers the best of luck in achieving similar kinds of success.

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R-Biopharm – RIDA®QUICK Norovirus available

The RIDA®QUICK Norovirus Test is a qualitative immunochromatographic lateral flow rapid test for determining Genogroup 1 (GG I) and Genogroup 2 (GG II) noroviruses in stool samples. It is used as an aid in the diagnosis of gastroenteritis and for the analysis of stool samples from children and adults with the symptoms of suspected gastroenteritis caused by the Norovirus. The test kit contains all utensils needed for successful assay performance and allows you to take 25 individual readings. It follows on consistently from the well established RIDA®QUICK Norovirus flow trough rapid test. The new test can be used without predilution of the stool specimen. Only four steps are needed to reach reliable results. In comparison with real-time RT-PCR, the new test format of RIDA®QUICK Norovirus offers an excellent standard of accuracy, with 92% sensitivity and 98% specificity.

→ **www.r-biopharm.com**

Merck – Study for TH-302 launched

Merck has just announced the start of a worldwide Phase III study by the name of MAESTRO (the acronym stands for MetastAtic or unrESectable pancreaTic adenocaRcinOma), to investigate the effectiveness and safety of TH-302 in the treatment of this form of cancer. The company is hoping to test the hypoxia-targeted drug, which is still the subject of research, in combination with Gemcitabin on patients with previously untreated, locally advanced, inoperable or metastatic adenocarcinomas of the pancreas. The study is being conducted under the auspices of the global licensing and development agreement on TH-302 between Merck and Threshold Pharmaceuticals Inc. The agreement provides an option for Threshold of joint marketing activities in the USA.

→ **www.merck.de**

Shimadzu – Gas chromatograph

The new Tracera GC System is now ready to meet trace analysis needs. This utilises innovative Barrier Discharge Ionisation Detector technology, coupled with a GC-2010 Plus capillary gas chromatograph, to create a GC system that can reveal trace components that escape other GC detectors. Notable features of the system are the high sensitivity (detection sensitivity 100x higher than TCD and 2x higher than FID), the Novel Universal Detector ('Single Detector Approach for your Complex Analyses') and long-term stability ('Long-Term Stability with New Discharge Design').

→ **www.shimadzu.eu**

Sartorius – PAT portfolio

Sartorius Stedim Biotech (SSB), a leading international supplier to the pharmaceutical and biotech industries, has entered into a global sales and development partnership with Aber Instruments, a Welsh company that makes bioprocessing and brewing equipment. Aber is a highly specialised supplier of the sensor technology used by the biopharmaceutical industry for in-line biomass measurement in cell cultivation and fermentation processes. Based on this partnership, SSB has secured exclusive global rights for the marketing and distribution of Aber's single-use sensor technology on the biopharmaceutical market.

→ **www.sartorius.com**

Hamamatsu – NTT's Communication Laser Technology

NTT Advance Technology Corp. and Hamamatsu Photonics K.K. have just entered into a partnership for the sale of a 1.3-µm-band wavelength swept light source for use in optical coherence tomography (OCT). OCT is a non-invasive in vivo imaging technique that is widely used in clinical applications. NTT-AT has developed this product using the same technology as that employed in the manufacture of an electro-optic crystal called KTN (potassium tantalate niobate: KTa1-xNbxO3). This was developed by Nippon Telegraph and Telephone Corporation for use in telecommunications. The new product is a marriage between this technology and high-speed variable wavelength laser technology making use of the crystal. Achieving a wavelength sweep speed of 200 kHz, which is a world record (double that of conventional products), this light source will speed up OCT-based examinations, significantly reducing the physical impact on patients. NTT-AT manufactures this product and will market it jointly with Hamamatsu Photonics K.K.

→ **www.hamamatsu.com**

Anton Paar – Multiwave PRO

After ten successful years, the Multiwave 3000 microwave reaction system has now been phased out, to be replaced by the new Multiwave PRO. This is based on the most up-to-date technology, as well as having a wide range of rotors and numerous accessories. The Multiwave PRO is a technological platform which combines sample preparation methods and microwave synthesis in just one piece of equipment.

→ **www.anton-paar.com**

Carl Zeiss – Second Prize at 2013 CLEAN!

In February 2013 the Microscopy business unit of Carl Zeiss received second prize in the 2013 CLEAN! Fraunhofer Cleanliness Technology Award for its Correlative Particle Analyzer (CAPA). The judges of the Fraunhofer Institute for Production Technology and Automation (IPA) paid tribute to the three award winners on day two of the Cleanroom Lounge trade fair in Karlsruhe. 'By bridging scanning electron and light microscopy, the Correlative Particle Analyzer is revolutionising cleanliness technology,' was the verdict of the Fraunhofer Institute judges. Carl Zeiss's brand of correlative particle analysis establishes a connection between scanning electron and light microscopy which did not exist in the past.

→ **www.zeiss.com**

AppliChem – a challenge accepted

From now on, unwanted germs have more difficult conditions to contend with. AppliChem's CellCultureGuard is the ideal solution for the sterilisation of bottles and petri dishes. Cell cultures can be contaminated in various ways by mycoplasm, bacteria or fungi (yeasts). The main measures to avoid contamination of cell cultures are sterile techniques, consistent observance of good laboratory practice and sterilised equipment and vessels. The addition of antibiotics to cell culture media further reduces the risk arising from microbial growth. CellCultureGuard is a combination of novel antibiotics to protect animal and human cell cultures from contamination by microorganisms. The antibiotics of CellCultureGuard replace conventional antibiotics such as penicillin, streptomycin, nystatin and amphotericin B.

→ **www.applichem.com**

Roche – Production at Penzberg plant in Germany

Swiss pharmaceuticals giant Roche has just launched the expansion of diagnostics production at its Penzberg plant. The company's investment of over 200 million euros in its 'Diagnostics Operations Complex II' (DOC II) project is a response to the continuing demand worldwide for working ingredients and reagents designed for immunological testing in laboratories and hospitals. The Penzberg Biotechnological Centre produces the greater part of the reagents and working ingredients that are used for Roche's immunological diagnostic tests all over the world.

→ **www.roche.de**

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Biofuels

Dynamic new connections

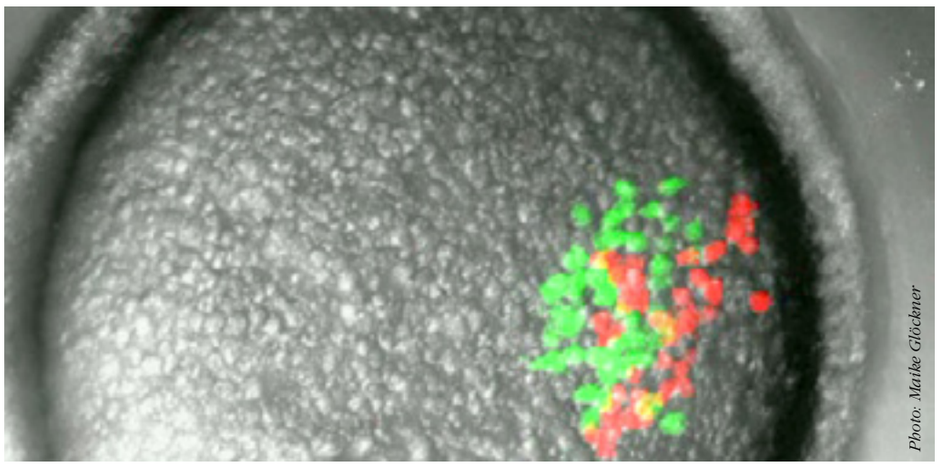


Photo: Maike Glöckner

A watering can sponge of the glass sponges genus. Thanks to the sophisticated structure, its glassy skeleton is practically unbreakable.

A Freiburg research team under Professor Wolfgang Driever has cast light on the molecular control mechanisms whereby the cells of the embryo of a zebra fish, which begin to come together following fertilisation, change in such a way as to start on the first major cell migration of their development. The Freiburg biologists were able to show that the stem cell factor Oct4 controls the formation of a signal (the Epidermal Growth Factor or EGF). The EGF governs the transport of the most important cell adhesion protein, E-cadherin, from the cell membrane to the interior of the cell. Carried out under the auspices of Special Research Department 850, their work gives rise to important results for investigation of the way in which cancerous metastases are formed.

Source: www.uni-freiburg.de
Original publication: Developmental Cell Vol. 24, p 486–501, DOI: 10.1016/j.devcel.2013.01.016

Geosciences

Extreme water

A group of German, Finnish and French researchers has shown what happens when you subject water to the conditions of pressure and temperature prevailing in the depths of the earth. At pressures over 22 MPa and temperatures in excess of 374° C, beyond the critical point, water becomes a highly aggressive solvent – a fact with crucial implications for the physical chemistry of the earth's mantle and crust. In the experiments carried out by researchers of the German Research Centre for Geosciences (GFZ) and the Technical University of Dortmund, the microscopic structure of water was investigated at different pressures and temperatures with the help of X-ray Raman scattering. It proved possible to demonstrate that the structure of water develops, under hypercritical conditions, from an orderly networked structure to a disorderly and dislocated one.

Source: www.gfz-potsdam.de
Original publication: PNAS, 2013, DOI: 10.1073/pnas.1220301110.

Biomaterials

First biotechnologically produced spider's silk fibre

Photo: AMSilk company, Planegg/Martinsried

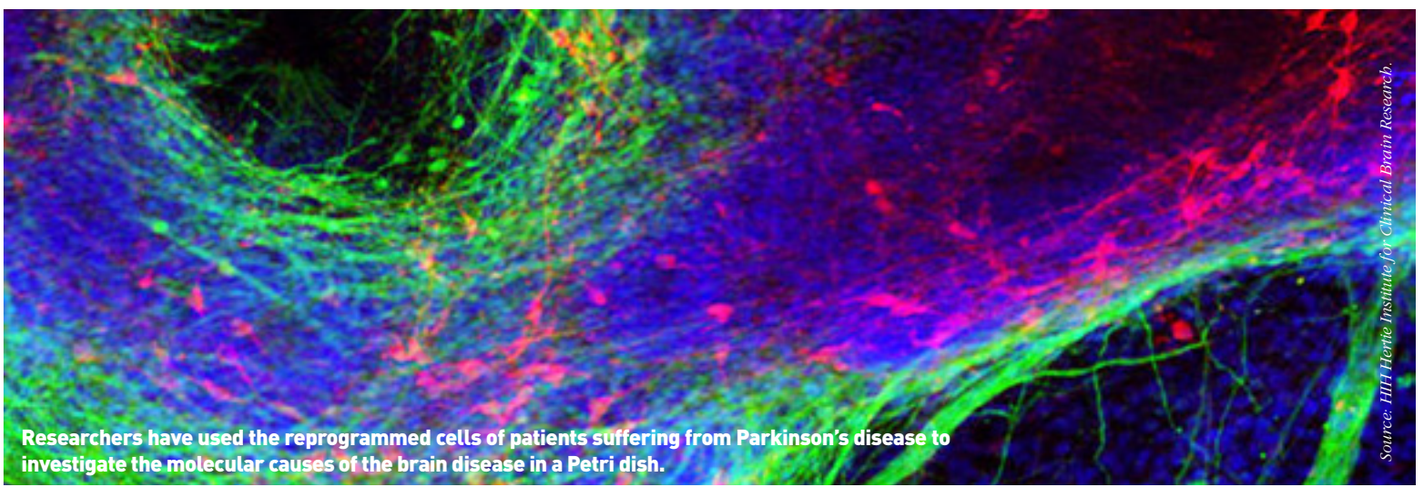
A high-performance product: spider's silk made from artificially produced fibres has just the same mechanical properties as are found in nature.

Researchers have been successful for the first time in using biotechnologically produced proteins to create spider's silk fibres which have exactly the same mechanical properties as those found to occur in nature. The artificial spider's web goes by the copyright-protected brand name of Biosteel, and is a product of the AMSilk company. Essentially it is the result of research and development work carried out by Professor Thomas Scheibel at the Chair of Biomaterials of the University of Bayreuth. The new spider's silk is characterised by its extreme toughness coupled with a high degree of stretch. It will find a use principally in medicinal and pharmaceutical products, cosmetics, composite materials and technical textiles. Spider's silk can be subjected to three times as much force as nylon before tearing. Moreover it is sustainably produced, can be recycled and presents no problem from a medical point of view, as it does not give rise to any allergic reactions.

Source: www.uni-bayreuth.de

Medicine

Parkinson's in the test tube



Researchers have used the reprogrammed cells of patients suffering from Parkinson's disease to investigate the molecular causes of the brain disease in a Petri dish.

Source: HIH Hertie Institute for Clinical Brain Research.

Brain researchers in Tübingen and Münster have succeeded in correcting the commonest genetic mutation in Parkinson's disease patients in the test tube. The test tube experiment went on to imitate what happens to the nerve cells of the brain, when the genetically modified cells showed no sign of neurological degeneration, but instead behaved like healthy nerve cells. The findings of the study give us a deeper understanding of the disease and of its possible triggers. At the same time, the study authors advise against premature euphoria. 'This method of genetic modification has only recently been developed, and so far has only been subjected to test tube trials. Only further studies can show whether this technique, or the modified cells, can actually be used to help patients.'

Source: www.hib-tuebingen.de
Original publication: Cell Stem Cell (2013), DOI: dx.doi.org/10.1016/j.stem.2013.01.008.

A Spring Without Birdsong

The theory behind biological trace analysis

Prof. Dr Bernd Herrmann, Historical Anthropology and Human Ecology, Göttingen University

Foto: CBS Photo Archive

In 1962, biologist Rachel Carson published her world-famous book "Silent Spring", in which she warned of the consequences of inconsiderate use of DDT. DDT accumulates in the food chain and, thanks to its hormone-mimicking effects, causes serious damage to the organisms at the end of such food chains. One such effect is that the shells of many birds' eggs become so thin that they break during incubation. The consequence: a silent spring without birdsong.

Natural awareness as a continuous process

The synthesis of organic compounds began more than 100 years ago: today, tens of thousands of these compounds can now be detected in environmental media (water, soil, air) all over the world. Such compounds have escaped into the environment through the production process itself, waste disposal or simple thoughtlessness. Via the food chain, some of these compounds induce severe clinical syndromes even in far-flung human communities. Compared to these, the runny nose now being experienced by some of our fellow humans due to pollen from spring blossomers like hazel and birch is fairly harmless. Those immune to hay fever may however end up being stung this summer for the first time by the Asian tiger mosquito – a recent European invader that can spread a whole host of dread diseases.

Beyond the philosophical aspect that life in general is lived exposed to the constant risk of hazards, these three examples do raise the question of whether – and how – such dangers can be forestalled. The first step is to monitor the environment. Many groups make such observations, from consumers and interested nature lovers to environmental researchers and the environmental monitoring institutions run by the federal and state governments.

Research scientist activities play the predominant role in such environmental monitoring, since the continuously maturing process of natural awareness is based on the strategies for problem determination and solution developed by basic research. These were also the first to even recognise the potential dangers. Since the three examples cited trigger biological effects, they fall within the remit of biological scientists: biochemists, physiologists or developmental biologists research the effects of DDT, pollen is analysed by botanists skilled in plant ecology, the tiger mosquito is identified by zoologists, while microbiologists describe the parasites that use this mosquito as a host or vector. Where research pursues the goal of environmental observation and hazard prevention, it is ascribed to "environmental monitoring": alongside "forensic biology", this is the second field within "biological trace analysis".

Materials and effects in the spotlight

Biological trace analysis is an area of scientific research that observes and analyses biologically active substances, biological organisms and biogenic compounds or materials, so as to diagnose their mechanisms of action and use these to derive prognoses. Observation, analysis, diagnosis and prognosis are performed so as to observe, administrate and preserve the environment, and to avert dangers to human and other life or to materials. Unlike other fields of knowledge or scientific research, the establishment of biological trace analysis is a necessity backed by statutory law (German Basic Law (GG) ss. 2 and 20a, plus many other legal provisions and directives).

Biology and the environment

Environmental monitoring is a field within applied biology, and is the field with the greatest need for biologists drawn from the broadest spectrum of disciplines and the

Until his retirement in 2011, **Dr. Bernd Herrmann** was the Head of the Department of Historical Anthropology and Human Biology at Göttingen University, where he set up the Biological Trace Analysis degree course programme.

highest levels of specialisation. Such biologists test sugar beet pulp for residues of BSE prions from bone meal fertiliser, assess whether DNA from genetically modified organisms can escape into the environment, and verify that there's calf in the calf's liver pâté and that the Parma ham is from Parma. They investigate whether hormones from ovulation inhibitors can pass through waste water facilities into rivers, where they disturb sex ratios in fish, and they check bacterial loads in drinking water and hygiene in the hospital or catering sectors. They assess vermin affecting agriculture and forestry and how they can be ecologically controlled, whether the raccoon dog has crossed the River Main, how the salmon can be re-introduced and whether the cockchafer is still found around Darmstadt. They inspect goods from shipping or overseas freight to see if they carry pests, protected or invasive species, test returning holidaymakers for malaria, and establish which of our plant and animal species are under threat. And the list goes on and on. Lastly, they also recommend countermeasures, adjustments to tolerance ranges and the creation of nature reserves.

Biology and forensics

The epistemological principles for environmental monitoring are identical to those for the second area within biological trace analysis, namely forensic biology. Here, however, their implications are more direct. Forensic biology concerns itself with the examination of biological materials for the purpose of clarifying or reconstructing circumstances and events that form the subject of formal inquiries or investigations. The primary focus here is on investigating 'traces' in a narrower sense. Generally speaking, a 'trace' is material evidence of the former presence of an entity now absent. In the context of biological trace analysis, therefore, it is evidence that a living being was present. As a rule, this will be only parts of an organism – such as hair or saliva residues on cigarette butts – finger marks, etc. If a trace is to be first assessed on the basis of its material composition, this proves to be a highly problematic field of activity. Biological traces vary considerably in terms of their material composition (fluids, scales, hair, etc.), their species-specific origin (300,000 plant species, > 10 million animal species, ? species of microorganisms) and their state of preservation (fresh, degraded, fragmented, state dependent on media). Accordingly, their mere identification presents major challenges to the biologist's expertise in the field of biodiversity.

Photo: Centres for Disease Control and Prevention's Public Health Image Library, James Gathany

Asian tiger mosquito (*Stegomyia albopicta*, *formerly Aedes albopictus*)

No two traces are the same

In addition, three particular epistemological problems also arise. Firstly, a trace is the result of an event that is unique as to its historical and material nature – e.g. homicide with a knife. While there are many stabbings worldwide every single day, every crime is one of a kind: the time, place, motive, act and persons involved all differ. Since the event is unique, so too are the traces it produces. One-off events owe their allegiance to random chance and not the exigencies of regular processes. As one-time events, they themselves possess no recurrent structure. As a result, each trace is not just historically unique – but materially so. Furthermore, material qualities of traces also change local structures. Such changes follow empirical rules: by applying these rules, once can estimate the time a body spent lying on the grass from the degree of lightening shown by the grass leaves, for example, or examine a trail of blood in terms of drip patterns.

The second problem involves contextualising a trace – and this introduces an as-

sociated logical dilemma. At a crime scene, many traces will be present, yet not all are relevant to the crime, since some of them will stem from unrelated earlier events. From this overabundance of possible traces, only those with relevance must be recorded – i.e. placed in a crime-relevant context. This is possible only by advancing a hypothetical progression of events – yet it is precisely this that the trace analysis is supposed to establish. Initially, then, the possible contextualisation of a blood trail – whose drip pattern would fit the suggested progression of events – can only be provisional. Necessarily, one must perform subsequent molecular verification of the correlation of the blood trail to the victim, the perpetrator or uninvolved third parties.

This also broaches the third problem, centred on the "knowledge generating narrative" about the circumstances reconstructed by trace analysis. The term "drip pattern" already implicitly contains the description of a process. Such descriptions are founded on familiar, established scientific 'best practice' rules: "blood dripping from a blade will normally produce these kinds of drip patterns." Knowledge-generating narratives are deployed in many ways within research, in situations where the general level of knowledge permits a sound description of historical events although eyewitnesses are unavailable. The most famous examples of such narratives are the 'history of the universe' or the 'origin of species'. Each trace evaluation performed thus contributes to a knowledge-generating narrative about the progression of events. There is a danger, however, that such evaluations will embody persuasive tactics that hinge on the appraiser's own opinion and are not founded on generally accepted methods of reasoning – or engage in speculation beyond that which can be reliably stated. Both intellectually and methodologically, forensic biology is thus one of the most demanding areas within applied biology.

Trends in trace analysis

In German-speaking Europe, biologists qualified in (crime scene) forensics tend to work at institutes of forensic medicine or criminal investigation departments; a few also work as independent experts. In Germany itself, there is a wide choice of degree and training courses focused on the environment, although fewer that address biological trace analysis. For forensic science itself, the degree and training courses on offer in Germany are entirely inadequate. If we consider the fact that the impressive methodological advances in lab techniques in particular are producing greater volumes of trace material – since this increases the prospects of successful trace analysis – and that internal security needs to be addressed long into the future, then there is a lack of relevant funding instruments. The University of Göttingen is one of the few institutions in Germany where expertise in trace analysis can be obtained during an MSc in Biology. To date, demand for this course easily exceeds the number of places available.

→ **bherrma@gwdg.de**

Literature
Herrmann B, Saternus K-St (2007)(eds) Kriminalbiologie. Biologische Spurenkunde, Vol.1. Springer, Heidelberg

Mysterious mycoplasma – a question of culture?!

Firstly reported in 1956 (Robinson *et al.* 1956), the potential presence of mycoplasma in cell culture laboratories challenges scientists. The parasitic mycoplasmas represent a serious problem for all cell line-related fields in research as well as in industrial facilities for development or manufacture of cell-derived biological and pharmaceutical products, including vaccines, monoclonal antibodies, drugs, and products for gene and cell therapy. Still, there is no perceivable reduction of cell culture infection rates (Ryan 2008), even though risks and consequences caused by mycoplasma infections are known for decades, and strategies for their prevention, detection and elimination are well established. Why are so many cell lines – while commonly well fostered by their cell culturists – still insufficiently protected against the cell wall-free invader? Is this a cause of carelessness, or rather a lack of knowledge? Unfortunately we cannot provide any data regarding this question – but a lot of facts demonstrating the importance of the unpopular subject.

How do mycoplasmas commonly enter our labs and cultures?

Mycoplasmas are omnipresent; their broad range of hosts includes humans and other mammals, birds, reptiles, fish, insects and plants (Razin *et al.* 1998). However, in cell culture laboratories, 95% of all continuous cell line infections are caused by only six species originating from bovine (*M. arginini & Acholeplasma laidlawii*), swine (*M. hyorbinis*) – and human (*M. orale, M. fermentans, M. hominis*) (Drexler and Uphoff, 2002). The main source of mycoplasma contaminations today are mycoplasma-infected cell cultures used in the same laboratory (Rottem & Barile 1993, Drexler *et al.* 2002; Drexler & Uphoff 2002). The infection may be transferred by aerosols, particulates and inadequate cell culture technique directly – or indirectly via media, solutions and laboratory equipment contaminated by previous use in processing mycoplasma-infected cells. As a result, 15–35% of all continuous cell lines are positive for mycoplasma, but only 1% of the primary cell cultures (Drexler and Uphoff, 2002). The second leading source is the laboratory personnel, explaining the fact that mycoplasma species from human are the most common contaminates (responsible for 40–80% of the infections) with *M. orale*, commonly colonizing the oral cavity, representing the primary species isolated from contaminated cell cultures.

Mycoplasma species from bovine or swine can be traced back to contaminated sera and other animal-derived products, e.g. the prevalent presence of *A. laidlawii* and *M. arginini* implicates fetal or newborn bovine serum as the primary source of infection.

By now, sera and media are rarely the source of mycoplasma contamination (Lincoln and Lundin 1990; Armstrong *et al.* 2009) as long as they are purchased from reputable manufacturers that sterilize their products by several filtration steps using a 0.1 µm pore membrane filter and frequently control sterility.

What makes mycoplasma species worse than other bacterial contaminates – and why is it a must to banish them from cell cultures?

In contrast to "common" bacteria, these tiny prokaryotes do not possess a cell wall. Together with other cell wall-lacking bacteria – species of ureaplasma, acholeplasma, anaeroplasma, spiroplasma - they form the class of mollicutes. Nevertheless, the terms "mycoplasma" or formerly "pleuropneumonia-like organisms (PPLO)" and "mollicutes" are often used synonymously. Due to the absence of a cell wall, mycoplasmas are unaffected by antibiotics that interfere with peptidoglycan formation, namely beta-lactam antibiotics. These include penicillin-derivatives, cephalosporins, and carbapenemes. Furthermore they are very flexible in shape which in addition to their small size (ranging from 0.1 to 0.8µm in diameter, depending on the literature) makes them difficult to filter from solutions. Mycoplasma species easily penetrate the membrane of 0.2µm filters commonly used for sterilization of media, sera and other in-autoclavable reagents. Mycoplasma's general dependence on complex enriched media (including host cell nutrients) and defined environmental conditions – both perfectly realized in cell culture – and their very slow growth rates complicate identification of infected cells by common microbiological cultivation methods. Their small size and missing cell wall allows them to achieve high densities in cell cultures; often without being detectable by turbidity, cytopathogenicity or even microscopic examination. However, the consequences of mycoplasma contaminations should not be underestimated; neither in regard to research (and the researcher's career!), nor in terms of serious health risks for humans and animals. Please keep in mind that the mycoplasma family is composed of a number of pathogenic organisms!

By growing covertly and undisturbed within a cell culture, mycoplasma can easily take over the control of reagents, equipment, and other cell lines within weeks (McGarrity 1976). Be aware that the lack of

visible effects provides a false sense of security: While often behaving inconspicuous at first glance, the fastidious organisms are able to influence nearly every single cellular function, ranging from a decelerated growth rate to metabolic (including protein, RNA, DNA synthesis) and morphologic changes. All these effects are mainly based on a competition for essential nutrients (nucleosides, nucleotides, nucleobases, arginine and other amino acids, fatty acids, sugars, etc.) and the release of toxic, cytolytic or acidic metabolites. By up- and down-regulation of cytokines and growth factors, stress-response genes, transport proteins, receptors, ion channels, oxidases, tumor supressors and oncogenes, mycoplasmas significantly alter the gene expression profiles of cultured cells (Miller *et al.* 2003). Therefore they make any experiment carried out with infected cells questionable! Furthermore they are known to cause chromosomal aberrations in vitro, with chromosomal breakage, translocation events, and reduction or augmentation in chromosome number being the most frequent outcomes. Virus propagation might also be influenced in both directions, positively (by inhibiting interferon induction and activity) as well as negatively (by competing for essential nutrients). Even though there are a large number of potential effects described in literature, it is unpredictable which effect will occur. Possible effects depend on mycoplasma species and strain, the infected cell type, and certainly on environmental conditions (Rottem & Barile 1993).

Finally, besides biosafety concerns, the consequences of mycoplasma contamination on laboratory work are loss of time, efforts, money (regarding cells, media, materials, but also valuable biopharmaceuticals if cultures were used for production of vaccines, antibodies or drugs) and good reputation. Research based on mycoplasma-contaminated cell lines will produce inaccurate or erroneous results yielding misleading publications. Consider the personal embarrassment and maybe the loss of good reputation if the published results are proven to be faulty due to a contamination problem. And how awkward will it be to get informed by a colleague that the cell line you provided him is contaminated? All these factors should be rethought when calculating to take the risk of covert mycoplasma infections by NOT testing cell cultures and NOT actively preventing them by good laboratory practise.

To sum up, a mycoplasma-free cell culture is PRECONDITION for safety and purity of cell-derived products and reliable results in scientific experiments.

The good message: it is possible to minimize the risk of general mycoplasma contaminations – and to exclude serious outbreaks.

How can I avoid contaminations?!

Probably, there will never be a point in time when mycoplasma contaminations are completely banished from our labs – as long as humans are working there. But carrying out some general principles will surely minimize the risk of contaminations and prevent costly or embarrassing situations.

- Strictly follow aseptic techniques and practices, including no unnecessary talking, no mouth pipetting, no media supply by pouring, regular hand washing and disinfection! Do not use the laminary flow for storage of solutions and equipment! Only work with ONE cell line at a time and use separate materials for each cell line to avoid cross-contaminations! Make sure all media, solutions and materials are properly sterilized – the same is true for any kind of occurring waste of course!
- Frequently clean and disinfect surfaces, laminar flows, incubators, water baths and all other equipment – before AND after the working procedure. Make sure the laboratory is cleaned up regularly and only authorized persons have access to the working area.
- Use antibiotics responsibly. For routine culture work antibiotic-free media should be employed. General usage of antibiotics to mask low hygiene levels, a lack of good aseptic techniques, or improper cell culture facilities is not a solution to the problem! Quite the contrary, non-responsible use of antibiotics will make the situation even worse.
- Isolate incoming cell cultures (use a separate incubator or at least sealed flasks as well as separate culture media and materials) until the mycoplasma test results are proven to be negative.
- Test frequently for contamination – regardless of whether the cell culture contains any antibiotics or not! Routine testings for the presence of mycoplasma species are an absolute must for the responsible scientist! Only by identification and treatment or elimination of the infected cell line the risk of further (cross-) contaminations is banished and experiments yield stable and reliable results.

Furthermore, it is highly recommended to freeze a cell stock as a backup for damaged or lost cell cultures. When dealing with cells of limited life span, cryopreservation is invaluable anyway. But also a stock of continuous cell lines should be stored properly below -130°C to prevent in vitro cellular alteration (Hughes *et al.* 2007; Stacey & Masters 2008) and maintain reliable cultures of consistent quality for research and biopharmaceutical production. The advantages of a cryopreserved cell bank are a reduced risk of (cross-) contamination with microorganisms or other cell lines, prevention of phenotypic or genotypic drifts, and damages due to cell aging. But caution: Please be aware that mycoplasmas are able to survive freezing in liquid nitrogen – even without cryopreservation. For that reason, a contaminated liquid nitrogen container (e.g. due to an inadequatly closed or contaminated sample of cells) might be a source of mycoplasma. But how do they enter the cell culture-containing cryo tube? Storage in the liquid phase together with a non-sufficient tube

AppliChem offers a series of products preventing microbial contaminations – in hoods, incubators, water baths and cell cultures. More information about this product line is available in the product table at the end of this article.

filling level might be the way in. Liquid nitrogen has the tendency to permeate the cryo tube, especially if it is filled insufficiently. To avoid any contamination risk, cryogenic vials should be properly stored in the vapour phase of the liquid nitrogen container.

How can I identify a mycoplasma contamination?

The most sensitive method to detect mycoplasma is the direct culture method in suitable broth and solid media to obtain visible colonies. Theoretically, a single CFU (colony forming unit) per sample volume is detectable. Unfortunately this method is also the most time consuming (up to 28 days; due to the slow growth of mycoplasma species), and it requires experienced personnel conducting the experiments under controlled environmental conditions. Even if the difficult procedure (to start with the complex medium composition often requiring non-standard adjustments for individual species up to analysis of the results) is properly conducted, the method is not 100% effective since some fastidious strains may not grow in pure culture. Therefore, an indirect detection method should be performed in addition. The most sensitive indirect mycoplasma tests are based on DNA fluorochrome staining (e.g. using DAPI) and PCR. Even if the detection limit of these methods is lower than for the direct culture method, they are absolutely sufficient for routine testings. Commonly, mycoplasma-contaminated cell cultures show high densities of mycoplasma (up to 10^7–10^8 CFU/ml) that are well suitable for the detection limits of these methods. In contrast to the PCR alternative, the traditional fluorescence staining method requires more time and experience. In addition, the DNA-binding fluorescent stain is cancerous and needs to be handled carefully. Hence, for routine mycoplasma screenings, PCR analysis is recommended (Drexler & Uphoff 2002). This method is sensitive (depending on the kit almost as sensitive as the direct culture method), very fast (results are obtained within hours) and detects cultivable as well as non-cultivable mycoplasma species. Furthermore, at least with commercially available kits, this method is very easy to perform and does not require a specific expertise.

What can I do to eliminate mycoplasma from an infected cell culture?

This answer is easy: Autoclave the contaminated cells, at best, together with any bottle of medium and solution used with this relevant culture. Don't forget subsequent cleaning and disinfection of surfaces, hoods, incubators, pipettors etc. – or better, the whole lab! Make sure that other cell lines are not infected as well! Cleaning up a contaminated culture with an anti-mycoplasma treatment is recommended only for very valuable or irreplaceable cultures, and if the potential source of mycoplasma was previously banished from the laboratory. Efforts for the attempted rescue are high and, until now, no universal mycoplasma-eliminating reagent is available. Antibiotic resistance, cytotoxicity, and a reduced viability of chronically or multiply infected cells may be reasons to prevent curing (Fleckenstein & Drexler 1996).

Despite assured resistances, the most reliable and efficient treatment of mycoplasma contaminations is the addition of suitable antibiotics, such as quinolones, tetracyclines and macrolides (Drexler & Uphoff 2002). In an experiment with 251 chronically mycoplasma-positive cell lines, treatment with ciprofloxacin provided healing-levels of 78%, with 15% of the cell cultures remaining contaminated due to resistance and 7% loss by cell death during the elimination procedure. The combination of tiamulin and minocycline even reached curing of 82% of all treated cell cultures, showing a lower resistance level (7%) but higher cytotoxicity (11% of the cell cultures died during the treatment) (data taken from Drexler & Uphoff 2002). Besides the traditional mycoplasma-eliminating agents Myco-1 & 2 (tiamulin and minocyclin) and Myco-3 (ciprofloxacin), AppliChem now offers a new solution for effective and permanent removal of mycoplasma species from cell culture: Myco-4 provides a broad spectrum of activity (including any type of mycoplasma, acholeplasma, spiroplasma and entomoplasma) combined with very low cytotoxicity and a low resistance risk due to an initial biophysical mode of action.

The "mycoplasma problem" is known for decades – why does it still exist?!

There are two main reasons why mycoplasma contaminations are not banished from cell culture laboratories yet: First, half of the researchers still do not test their cell cultures for mycoplasma (Ryan 2008) and second, there is a tendency to rely on antibiotics instead on good aseptic practices.

Even though cell culture experts agree that general use of antibiotics can increase the severity of contamination problems, the routine use of antibiotics in cell culture laboratories is still prevalent. Particularly mycoplasma contamination rates are much higher in cell lines grown in antibiotic-containing medium than in antibiotic-free cultures (Barile 1973). If microorganisms, bacteria or fungi, are accidentally brought

AppliChem's PCR Mycoplasma Test Kits enable identification of mycoplasma-contaminated cell cultures - fast and effective! The PCR technique allows highly sensitive detection of both cultivable and non-cultivable mycoplasma species. Reproducible results are provided within hours, making PCR the method of choice for frequent routine testings.

AppliChem's Myco-4 effectively eliminates mycoplasma from infected cell cultures. This new product combines a mycoplasma-specific biophysical reagent with a highly efficient combination of standard antibiotics.

Prod. No	Description	Features
DETECTION		
A3744	PCR Mycoplasma Test Kit	Ready-to-use (*Taq* Polymerase included!); contains a positive control DNA.
A8994	PCR Mycoplasma Test Kit II	Highest sensitivity of <10 CFU/ml; according to Ph. Eur. (section 2.6.7). Besides a positive control DNA (non-infectious!) this kit provides an internal control DNA to visualize potential PCR inhibitions. The DNA Polymerase is not included, we recommend a Hotstart *Taq* Polymerase, e.g. SuperHot *Taq* DNA Polymerase (A5231)
A9019	qPCR Mycoplasma Test Kit	Designed for qPCR applications. Provides a non-infectious positive control (ROX) and an internal control (FAM) to visualize potential PCR inhibitions. The DNA Polymerase is not included, we recommend a Hotstart *Taq* Polymerase, e.g. SuperHot *Taq* DNA Polymerase (A5231).
A1001	DAPI	Excellent fluorescent dye for mycoplasma detection via DNA-staining followed by microscopy.
ELIMINATION		
A8360	Myco-1 & 2 Set	2-step treatment with Tiamulin and Minocycline.
A5240	Myco-3	Single-phase treatment with Ciprofloxacin.
A8366	Myco-4	2-step treatment with a mycoplasma-specific biophysical reagent followed by an appropriate antibiotic combination.
PREVENTION		
A5230	Incubator-Clean™	Non-toxic and biodegradable disinfectant for incubators and sterile benches; prevents contamination with and growth of fungi, bacteria (including mycoplasma) and viruses (including HIV and Hepatits B). Fully compatible with common work surfaces (non corrosive!).
A5219	Incuwater-Clean™	Non-toxic, non-volatile, and extremely effective disinfectant for CO_2-incubator water baths.
A9390	Aquabator-Clean (100X)	Disinfectant for prevention of microbial growth in common water baths.
A8906	CellCultureGuard	Combination of especially selected antibiotics to prevent microbial growth (extra- and intracellular bacteria, mycoplasma, protozoa and fungi) in cell cultures; provides high compatibility with resistance markers and low risk of resistance development.

Please note that AppliChem also provides, •transfection reagents •growth factors and cytokines, •vitamins, •amino acids, •antibiotics, •antimycotics and other •reagents for cell culture applications.

into antibiotic-free culture medium, they will replicate non-inhibited, soon leading to visible indicators of contamination: turbidity, filamentary structures, color changes due to pH alteration. In contrast, the presence of antibiotics will prevent the microbial growth – maybe. Unfortunately there is no absolute guarantee that the added antibiotics act against the introduced microorganisms (probably a mixture of different species), and sooner or later the user will face some kind of resistance phenomenon. If the introduced germ is fully resistant to the antibiotic, it will hopefully rapidly overgrow the culture and become visible within a short period of time. If the introduced microorganism only shows a partial resistance the situation is worse. Due to the latent static level of partly resistant contaminations, the risk of cross contaminations and usage of the affected culture in experiments or bio-production should not be underestimated. This worst case is very likely if the invader belongs to the species of mycoplasma (e.g. brought into the culture through aerosol droplets from the mouth of the cell culturist), since most common antibiotics used in cell culture do not act on mycoplasma! Besides the beta-lactams being ineffective anyway, high resistance levels of mycoplasma against streptomycin (88 %), kanamycin (73 %), gentamicin (80 %) and neomycin (86 %) (Lundin & Lincoln 1994) were determined.

Apart from Barile's observation of strongly increased rates of mycoplasma contamination, morphological and functional changes are other disadvantages one has to take into account (Kuhlmann 1996) when using antibiotics on a routine basis. Anyhow, there exist useful applications of antibiotics in cell culture, e.g. within the first two weeks of primary culture. In order not to create new resistances due to inactivation of the antibiotic, the antibiotic-containing medium should be refreshed frequently.

As an alternative to classical cell culture antibiotics like penicillin-streptomycin, AppliChem provides a new product to prevent microbial growth in cell cultures: CellCultureGuard. This combination of selected antibiotics (one being a fluoroquinolone) offers a wide range of anti-microbial activity, making it our first choice cell culture reagent: CellCultureGuard is active against extra- and intracellular bacteria, mycoplasma, protozoa and fungi (yeast). Additionally, it is highly compatible with resistance markers and bears a low risk of resistance development.

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Pharmaceuticals in wastewater

Instrumental and effect-directed analysis in the oxidative degradation of micropollutants

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Occurance and fate of micropollutants, metabolites and transformation products in the water cycle is placing increasingly higher demands on instrumental analysis in terms of resolution and sensitivity. As yet, effect-directed analysis methods play a subordinate role in water analysis work. Yet for evaluating unknown samples or ingredients, these methods form a perfect complement to instrument-based identification. Indeed, in the detection of estrogenically active substances, bioanalysis is considerably more sensitive than mass spectrometry detection methods.

Jochen Türk studied Chemistry at the University of Dortmund. He has worked as a research assistant at the Institute of Energy and Environmental Technology e.V. (IUTA) since 2001. His PhD thesis at the University of Duisburg has focused on LC-MS/MS analysis techniques in the field of occupational safety and environmental protection. From 2003 to 2008 he worked as project and lab manager in the field of environmental medicine. From 2009 to June 2012, he was the head of the department for Research Analysis. He has been managing the Environmental Hygiene & Trace Substances unit since July 2012. In the services sector, Dr Turk is involved in biomonitoring and environmental monitoring work on cytostatics plus cleaning validation. Key scientific research topics include trace analysis using LC-MS, as well as the development of oxidative procedures for municipal and industrial wastewater treatment. The chemical and biological characterisation of transformation products plays a major part in this research work.

Rising concentrations of micropollutants in treatment plant effluent are placing increasing demands on wastewater treatment. Conventional facilities using standard technologies remove only a small portion – if any – of such substances from the wastewater. Elimination of such substances can be achieved by making use of advanced oxidation processes (ozonation, UV oxidation). The removal of hormonally active substances, pharmaceutically pharmaceuticals and personal care products (PPCP) and industrial chemicals from wastewater goes hand in hand with reducing the chemical oxygen demand (COD) and dissolved organic carbon (DOC) compounds. Key questions about the formation of toxicologically relevant transformation products (TPs) still remain unanswered, however. For the evaluation of oxidation products, cost-effective and sensitive detection methods are essential. Despite the availability of increasingly powerful GC-MS and LC-MS equipment, instrumental analysis as it currently stands is incapable of quantifying the environmental quality standard recommended by the European Union of 0.035 ng/l for the synthetic hormone 17α-ethinylestradiol [1]. Following enrichment, in vitro assays can be used to measure estrogenic activity as a sum parameter. As a reference point, a 17-β estradiol equivalent quotient (EEQ) is selected. Biological analysis is unable to furnish statements about individual substances, however. This being so, instrumental and effect-directed forms of analysis supplement each other ideally as complementary methods.

Determining endocrine effects

Figure 1 shows the basic principles of the in vitro assay A-YES®_aqua 1.0 (new_diagnostics GmbH, Freising), an assay based on the transgenic yeast *Arxula adeninivorans*. The sum parameterisation of estrogenic activity is based on the binding of estrogenically active substances to the corresponding human receptor that is integrated into the yeast cell genome. The binding of estrogenically active substances to the receptor induces the expression of the phytase reporter enzyme, which is released into the medium (Figure 1a). The phytase is then detected photometrically, using an enzyme-substrate reaction (figures 1b and 1c). In this context, it was possible to demonstrate that the test system validated for mineral water is also suitable for testing treatment plant effluent. Without further concentration, the limits of detection achieved lay between 13 and 19 ng/l EEQ. Following enrichment on an Oasis HLB solid phase (Waters, Eschborn), a limit of determination of 0.02 ng/l was achieved [2]. Accordingly, the method is suitable for determining whether or not treatment plant effluent and surface waters comply with the recommended environmental quality standard.

Online SPE-LC-MS/MS determination of fluoroquinolones

In the course of the projects presented here, an investigation was made of the chemical and toxicological evaluation of oxidative wastewater treatment techniques. In the context of drinking water treatment, estrogenic effects join cytotoxicity, genotoxicity and the mutagenic properties of unknown substances in the water cycle as the most worrying factors to consider.

As one example, the following discussion concentrates solely on the fluoroquinolones ciprofloxacin and ofloxacin, present at concentrations of between 30 and 200 ng/l in WWTP effluent. Investigations of the toxicity of fluoroquinolones before and after oxidative treatment are of particular interest, since previous research has provided evidence that ciprofloxacin produces toxic effects [3, 4]. An online SPE-HPLC-MS/MS method has been developed for the rapid and sensitive analysis of ciprofloxacin and ofloxacin. Following the enrichment of 10 ml of the water sample on an HySphere C18HD SPE cartridge (Spark Holland), elution proceeds in HPD focusing mode with 200 µl acetonitrile:water (40:60) injected within one minute into the HPLC flow of 300 µl/min 5% acetonitrile in water with 0.1% formic acid. After one minute the solvent gradient starts with a flow of 500 µl/min. To keep analysis times as rapid as possible and achieve a

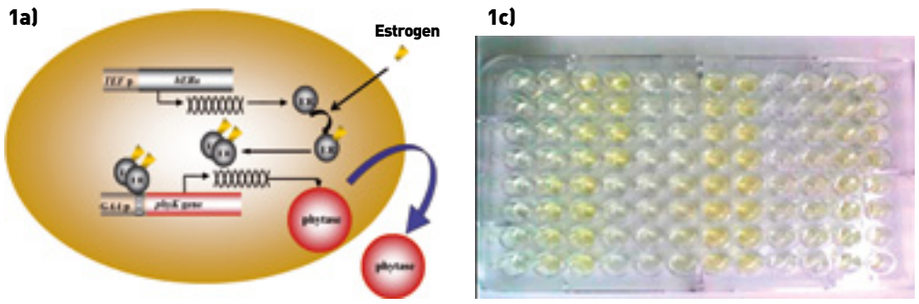

Fig. 1 Schematic representation of the basic A-YES mechanism. a) Binding of estrogenically active substances to the continuously expressed human estrogen receptor (ER) and subsequent formation of the phytase reporter enzyme (photo 1a: IPK Gatersleben). This reaction takes place during incubation for 24 h at 30 °C and 700 – 750 RPM. b) Enzyme-substrate reaction for detecting the phytase formed. The p-nitrophenolate formed produces a yellow colour on addition of the developer solution (NaOH). c) 96-well microtitre plate with yellow-coloured samples, measured in photometer at 405 nm.

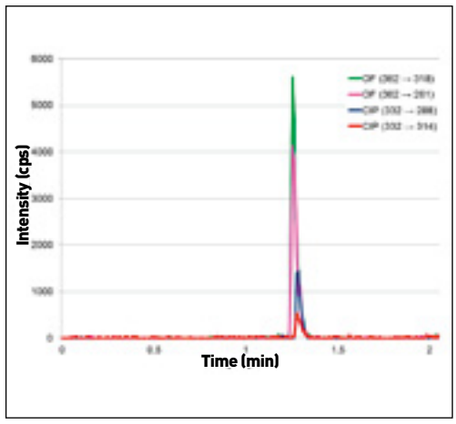

Fig. 2 Online SPE-LC-MS/MS method for the determination of ciprofloxacin (CIP) and ofloxacin (OF) using Spark Holland Symbiosis Pico and AB Sciex Q Trap 3200. (c = 1 ng/l, enrichment volume: 10 ml, SPE cartridge: Spark HySphere C18HD, HPLC column: Phenomenex 50 x 2.1 mm Kinetex 2.6 µm C18 100 Å).

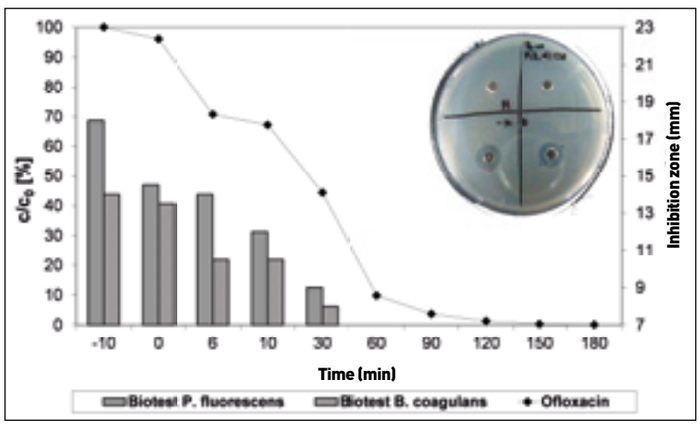

Fig. 3 Degradation of ofloxacin and reduction of microbiological activity by UV/H_2O_2 oxidation in spiked WWTP effluent. The photo shows the disk-diffusion test with B. coagulans (c_0 = 50 µmol/l, B = blank array, -10 = sample before switching on UV lamp, 0 = sample after UV lamp warm-up phase, before addition of H_2O_2).

high level of sensitivity, the decision was made to skip baseline separation of the two fluoroquinolones; the system was re-equilibrated again after just 2.5 minutes. If we look at the 4 MRM transitions shown in Figure 2, we can clearly see that mass spectrometric separation for the purposes of quantifying and verifying the result is eminently possible. Without requiring further process steps, this method permits the determination of starting concentration, elimination and reaction kinetics for oxidative wastewater treatment. With an acetonitrile portion of 25% and a slower acetonitrile-water gradient, baseline separation is also possible with a total analysis time of about 10 minutes. The weaker elution from the SPE cartridge results in peak broadening, however, which has the result of reducing process sensitivity (data not shown).

Thank you!
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Investigation and evaluation of transformation products

Both in ultrapure water and in treatment plant effluent, ozonation with 10 mg O_3/l achieved substance degradation of at least 80% for ciprofloxacin and 88% for ofloxacin. By using UV oxidation in combination with H_2O_2, complete substance degradation was achieved in treatment plant effluent. Follow-up investigations utilising QTRAP and TOF-MS were able to detect a total of 22 transformation

products from ciprofloxacin and 15 transformation products from ofloxacin [5]. Effect-directed analysis was extended to antibacterial activity with a disk-diffusion test utilising the bacterial strains Pseudomonas fluorescens and Bacillus coagulans. Figure 3 shows the degradation of ofloxacin plus the simultaneous decline in antibacterial activity. The formation of inhibition zones around the applied sample can be clearly seen in both pre-treatment samples (photo below left and right). No inhibitory effects versus the two bacterial strains were observed in the blank array, the control samples and the samples after 30 minutes of oxidative treatment.

Summary

The results from instrumental and effect-directed analysis prove that substance degradation goes hand in hand with the loss of antibacterial activity. Considered alongside the results from cytotoxicity, genotoxicity and mutagenicity testing, an indirect conclusion may be drawn that the transformation products formed have no toxicological relevance in contaminated and uncontaminated real-world wastewater.

By combining instrumental and effect-directed analysis, it has been possible to confirm the working hypothesis that the types of procedures investigated (O_3, UV and UV/H_2O_2) can be safely applied for the degradation of trace substances in real-world treatment plant effluents [5]. Since wastewater composition is not identical across multiple wastewater facilities, installation of large-scale ozonation plant should be preceded by guideline investigation work on ozonation and the formation of potentially relevant transformation products, conducted using instrumental and effect-directed analysis. This is of key significance for bromide-rich waters, for example, as these can result in bromate formation.

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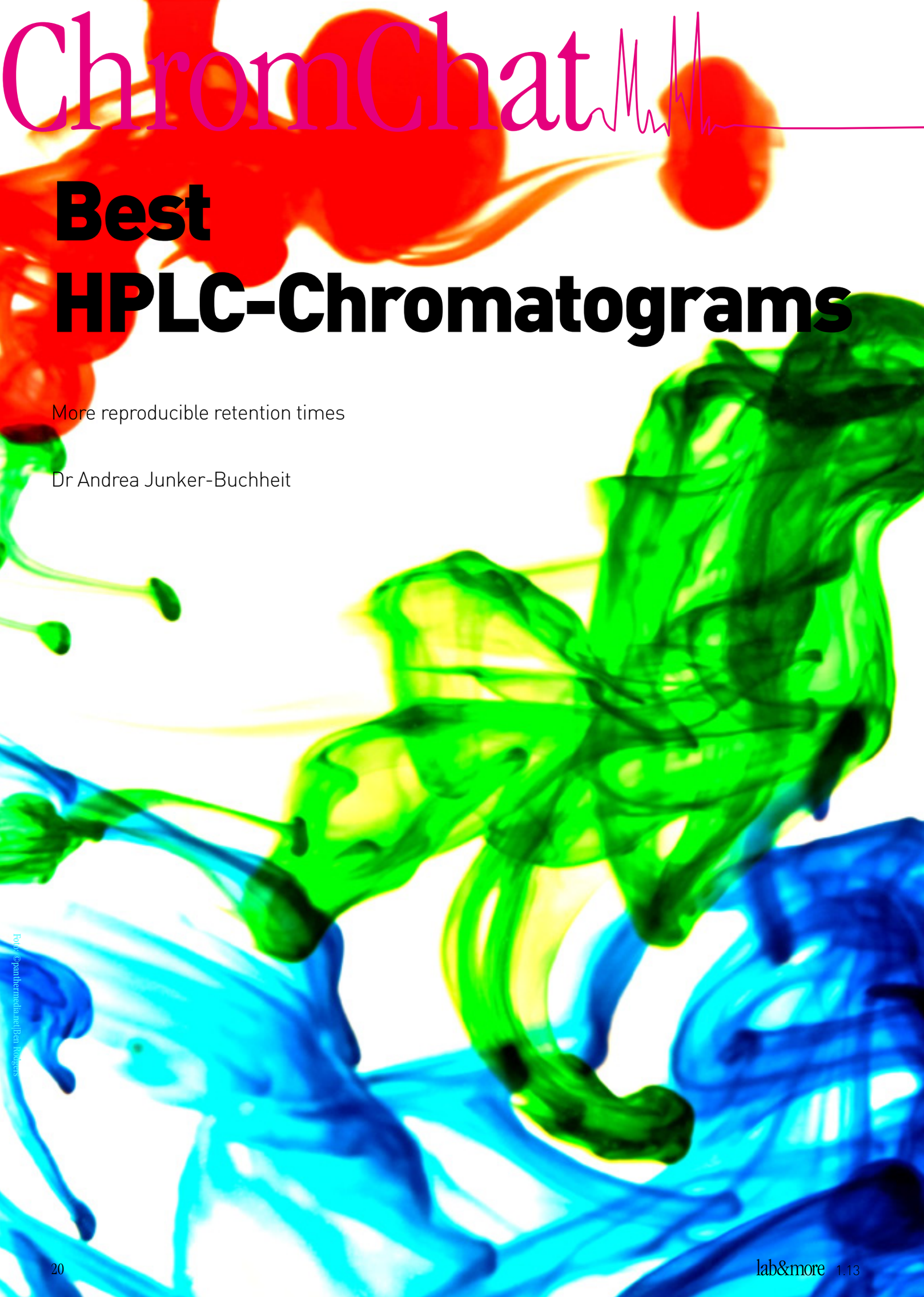
Best HPLC-Chromatograms

More reproducible retention times

Dr Andrea Junker-Buchheit



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The high health hazard potential of many organic solvents is well known to anyone working in a laboratory. Solvent vapours can not only endanger people's health, but also increase the fire and explosion risks for laboratories and buildings, in addition to causing air pollution. This is also the case for analytical and preparative HPLC laboratories.

Permanent air contamination by solvent vapours is in most cases caused by faulty sealing of the supply and waste containers. This is not only a permanent health risk, but has also a very practical impact: Contamination of the eluent can occur as well as the mixing ratio due to different vapour pressures of the single solvent components can be continuously changed. This necessarily leads to separation results that can hardly be reproduced, like e.g. retention times, and might finally cause incorrect analytical data. This is why safety measures must be taken and be assessed again and again and adapted to the actual working place conditions.

In the following contribution, the impact of eluent evaporation on the long-time reproducibility of retention times of a selected HPLC test mixture will be demonstrated and correct solvent disposal will be addressed as well.

Influences on the HPLC selectivity

Selectivity changes of the HPLC column or the mobile phase are often the reason for a slow, but constant change of HPLC retention times. Sometimes, a non-adequate sample preparation of genuine samples like lipids, polymers, carbohydrates, cellulose, may cause this phenomenon. Matrix compounds from these samples can be adsorbed onto the column's reversed-phase surface, thereby changing the chemistry of the column. Consequently, it's properties will change and the retention times of the analytes as well. The same fact is observed, if the composition of the eluent changes. This can be caused by unnoticed mistakes when mixing a mobile phase, e.g. wrong volumes of each solvent in the mixture, change in the sequence of mixing, or mixing techniques. Sometimes, decomposition effects can occur in certain mobile phases or HPLC pump defects can lead to a change in the eluent composition and thereby to a retention time shift [1]

Here, a simple testing procedure for the reproducibility of retention times is described. The purpose was to check what happens if a HPLC user is using solvent bottles with "open caps", instead of using Safety Caps (S.C.A.T. Europe). When solvent is evaporated, the composition of premixed mobile phases can change. To demonstrate this, chromatograms of Polycyclic Aromatic Hydrocarbon (PAH) compounds were regularly checked over a period of 20 days. An acetonitrile-water

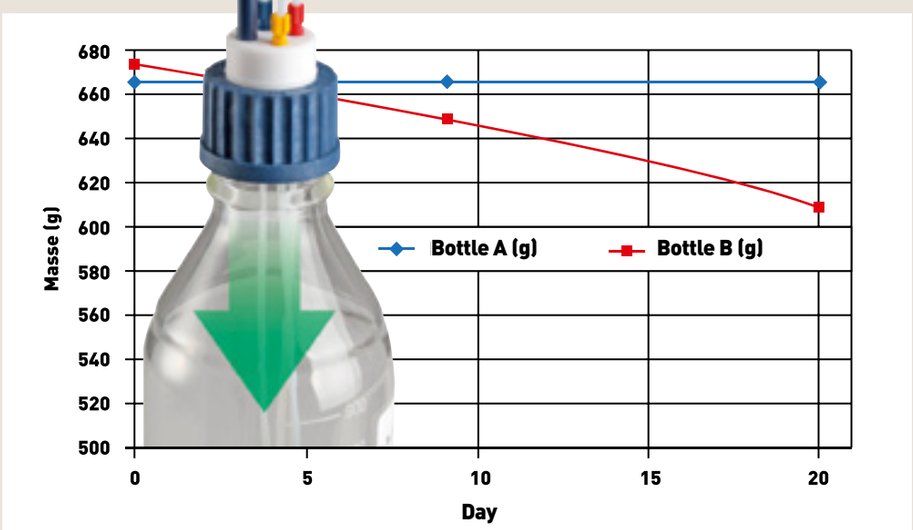

Fig.1 Comparison of the weight-losses of solvent bottle A and B [2]

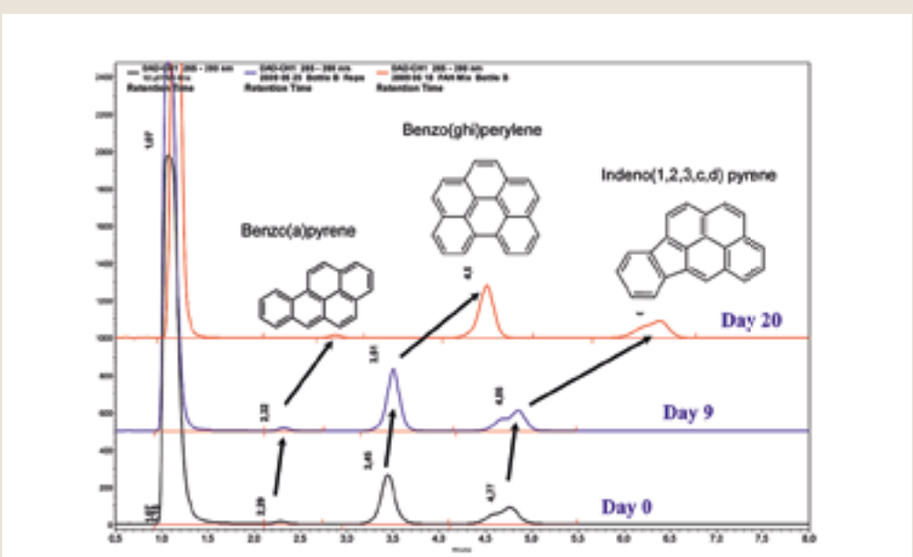

Fig. 2 Chromatogram of 3 PAH compounds at day 0, 9, and 20 (Bottle B has been stored without Safety Cap)

Tab. Weight-loss of bottles over a period of 20 days.

Day	Bottle A (g)	Bottle B (g)
0	666,44	673,59
9	666,22	650,85
20	665,26	609,12

Fiig. 3 SafetyCaps

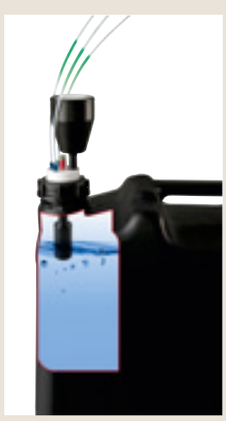

Fig. 4 SafetyWaste Cap

mixture has been used as eluent in the isocratic mode. As stationary phase, an end-capped RP column with small-diameter has been applied. As the flow rate was low, the eluent consumption was also reduced [2].

The following testing conditions have been selected:

Bottle A: This bottle was closed with a S.C.A.T. Safety Cap, precisely fitting the used standard GL45 glass bottle thread. No additional adapters were necessary.

Bottle B: This bottle was closed with a cap having a 10-mm hole in the plastic material, giving an open exit of an area of about 0,785 cm^2.

Experimental set-up:

HPLC system: HITACHI LaChrom® Elite system with diode array detector, controlled by the EZChrom® Elite software. Isocratic pump conditions with pre-mixed mobile phase. HPLC-Column: Purospher® RP-18e (5 µ), 125 x 2 mm. Flow rate: 500 µL/min

Performance of the test

At the beginning of the test, both bottles have been filled with the identical mixture of water and acetonitrile (20:80, w/w). The weight change within the period of 20 days has been regularly checked by differential weighing.

Using bottle A as a reference, a chromatogram of a PAH mixture (Benzo(a)pyrene, Benzo(g,h,i)perylene, and Indeno(1,2,3-c,d) pyrene) at day 0 was acquired for comparison. After having measured the reference chromatogram, both bottles were stored under room temperature in a fume hood, which guaranteed a gentle air flow over the top of the bottles. HPLC analyses have been repeated on day 9 and 20.

Results

This experiment cleary reveals that without using tightly sealed solvent bottles as containers for pre-mixed HPLC eluents, there is a risk of uncontrolled vaporization, resulting in non-reproducible analytes' retention times. As expected, bottle A didn't show any significant weight change, thus no solvent vapours did escape from this bottle, see Tab. 1 and Fig. 1. In contrast to bottle A, bottle B showed a significant and

uncontrolled solvent loss by evaporization. This results in a change of the acetonitrile-water mixing ratio and thereby in a change of the selectivity of the mobile phase, leading to a retention time shift of the eluted compounds. They are all prolonged because in bottle B more acetonitrile has been evaporated. Especially for real sample analysis, this retention time shift can have an impact on the resolution of separated peaks.

Without using tightly sealed solvent bottles as containers for pre-mixed HPLC eluents, there is clear risk of uncontrolled vaporization of solvents, thus leading to a higher health risk for the employees. In summary, it is evident that the use of S.C.A.T. Safety Caps definitely prevents such uncontrolled emissions, and in addition leads to a clear reduction of the exposure of solvents in the atmosphere in a laboratory.

How to put things right

SafetyCaps (Fig. 3) ensure safe withdrawal of solvents without release of harmful vapours. The emission of organic solvent vapours is verifiably being reduced by 73%, thus contributing to health protection of the laboratory staff. The integrated air valve allows safe solvent removal; it provides as usual for venting during removal, and at the same time, the valve membrane absorbs dust and contaminant particles from the incoming air. The air filter integrated in the air valve ensures that the solvent remains uncontaminated, provided the filters are being replaced every 6 months.

The sophisticated system has freely rotatable closure caps so even when several connectors are used, containers can be quickly changed without twisting the tubes. SafetyCaps with shut-off valve have proven to be very practical; they prevent air pockets in the tube, make changing supply containers easier and are ideal for repairs or maintenance work on the HPLC system. When restarting the HPLC system, SafetyCaps allow for fast and easy purging. Also available are SafetyCaps with level indicator, showing the solvent reservoirs running short.

SafetyCaps specifically designed for preparative HPLC are equipped with a special valve and easily deliver supply volumes of up to 400 mL/min.

SafetyWasteCaps for solvent waste disposal

It is well-known that particular caution is needed when collecting combustible fluids. Static charges may build up during outflow, which may cause sparks and increase the hazard of fire. Therefore, a sophisticated system has also been developed for safe disposal of HPLC solvents. The well-proven SafetyWasteCaps (Fig. 4) provide a direct and gas-tight connection of the drainage tubes or capillaries of the HPLC system to the solvent disposal container.

The SafetyWasteCaps consist of the required fittings for the tubes, as well as an exhaust filter with special granulates of activated charcoal. The lifetime of the filter depends on the load of solvent vapour absorbed. The safety funnels are only opened at the moment filling starts, otherwise the container remains closed. S.C.A.T. Europe installation solutions, made from electrically conductive PE-HD, prevent static discharge and can be secured via an additional ground cable.

Take Home

The use of SafetyCaps for solvent withdrawal and waste disposal considerably reduces solvent emissions and consequently helps to decrease health risks for the employees. Moreover, the S.C.A.T. technology significantly minimises solvent blank values – the solvent bottles remain tightly sealed. Retention times remain constant – in particular when using pre-mixed solvents and isocratic conditions – as the solvent composition cannot change due to solvent evaporation.

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Literature

[1] For a detailed description of the potential causes of changes in selectivity, please refer: https://www.novia.de/hplc-tipps/details/artikel/verschiebung-der-retentionszeit/ and www.kromidas.de.

[2] Publication in VWR ChromJournal Edition 8, Spring 2010, pp. 7, 14

[3] The topic „Protect your health" is dealt with in detail in S.C.A.T. Europe Catalogue 2012, pp. XIV-XVI.

Sure, it tastes good

Food safety: new approaches to analysis

Prof. Dr. Dr. Alfonso Lampen, Dr Thorsten Buhrke and Hermann Broll,
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Photo: © istockphoto.com|Oliver Hellbig

Food safety is a requirement stipulated by Section 14 of EU Basic Regulation (EC) No. 178/2004. Food safety monitoring activities conducted by state inspection agencies ensure nationwide compliance with the high levels of food safety in Germany. As well as monitoring microbial loads in foodstuffs, the agencies also focus on a wide range of chemical contaminants, such as dioxins. Whether the foodstuffs have been contaminated with these kinds of potentially toxic substances deliberately or inadvertently as a result of an accident is initially irrelevant to the agencies' work: the job of the enforcement laboratories is to track down contaminated food and make sure it is withdrawn from sale.

The number of chemical substances officially monitored as potential contaminants of foodstuffs is continuously increasing: as a result, food safety monitoring is a field that is facing a growing set of challenges. According to estimates, the European Union has approved a total of approximately 80,000 substances, which also have the potential to enter the human body via the food we eat. The presence of substances such as e.g. various pesticides is established using validated chemical analysis methods. These methods generally consume a lot of time, expenditure and resources. A further disadvantage is that a chemical analysis method of this kind will identify and/or quantify only a single, predefined analyte: such a method cannot establish the presence of related substances with comparable toxicological potential. Examples of such substances include the multifarious members of the PAH and PCB families or dioxins – which are so numerous that it is impossible to analyse every last one of them. Although potentially toxic, if a dedicated search is not made for these substances, conventional chemical analysis methods will not detect them. Ideally, therefore, one needs to develop innovative procedures that are not only faster, less costly and less resource-intensive to complete but which are also capable of detecting both individual substances as well as substance groups with similar toxicological properties – and thus permitting a 'screening' of the substance in question.

Enter 'effect-directed analysis' (EDA): in contrast to conventional chemical analysis, which investigates substances on the basis of their physical and chemical properties, EDA is based on the concept of detecting the effects of individual substances or groups of substances. The foundations of such an analysis are provided by existing biological systems, which trigger a biological signal when they interact with a substance contained in the foodstuff. The substance therefore achieves a biological effect via a kind of biosensor. One example of such a substance would be the aryl hydrocarbon receptor (AhR), a transcription factor that initiates the expression of multiple xenobiotic-metabolising enzymes by direct interaction with dioxin and dioxin derivatives. This occurs by the binding of the activated AhR to 'dioxin responsive elements' (DREs) – i.e. specific promoter sequences that are located above the AhR-dependent target gene. Genetic manipulation now offers the possibility of coupling the DRE with an entity known as a 'reporter': a typical reporter is the gene for a protein named 'luciferase', which is responsible for giving glow-worms their glow since it excites a substance named 'luciferin' to create light. If one now sets up a test system where the reporter gene is controlled by a DRE, then the concentration of dioxin or dioxin-like substances in a sample will correlate to the AhR-induced expression of luciferase. The quantity of luciferase protein formed is proportional to the conversion of luciferin and the associated emission of light, which is relatively simple to quantify with the aid of a luminometer (Figure 1; [1, 2]). Since only a few molecules are needed to activate the DRE, a test of this kind is highly sensitive and capable of detecting all AhR-activating substances in a specified extract for analysis.

Alongside the well-characterised AhR, other biological systems are also suitable for use as starting-points for designing reporter gene test systems. Members of the

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Hermann Broll is a biologist and has worked as a research assistant at the BfR since 1997. His past work has focused above all on questions of traceability when applied to methods within molecular biology. He has developed and validated both national and international standards for the detection of GMOs. He has also played a leading role in several European projects.

Thorsten Buhrke, a research chemist, worked from 1996 to 2007 at the Humboldt University of Berlin (doctorate 2002). He has worked as a research assistant in the Food Safety Department at the Federal Institute for Risk Assessment (BfR) since 2008. Key areas of research: molecular mechanisms in the toxicity of food ingredients and contaminants, plus the development of methods for effect-directed analysis.

family of nuclear receptors, for example, are transcription factors that are activated by the binding of specific ligands. These natural systems are superbly qualified for detecting a wide range of substances or substance groups that are of interest for food safety monitoring. Similarly to AhR, for example, the constitutive androstane receptor (CAR) and the pregnane X receptor (PXR) also interact with a large number of foreign substances with an aromatic base structure – such as are contained in many pesticides, for example. Then again, other food contaminants – such as a number of mycotoxins (aflatoxin B1) or ergot alkaloids, for example – are also capable of activating CAR and/or PXR. The glucocorticoid receptor (GR) is also a suitable model for developing a reporter gene system: in this case, for the detection of a range of steroid hormones – such as might be deployed illegally within the meat production industry, for example. Finally, a test system based on the oestrogen receptor (ERα) could be used for detecting hormonally active substances. Receptor-induced effects present just one option for approaching solutions within effect-directed analysis. Other endpoints include cytotoxicity, for example, genotoxicity/mutagenicity, as well as reproductive toxicity.

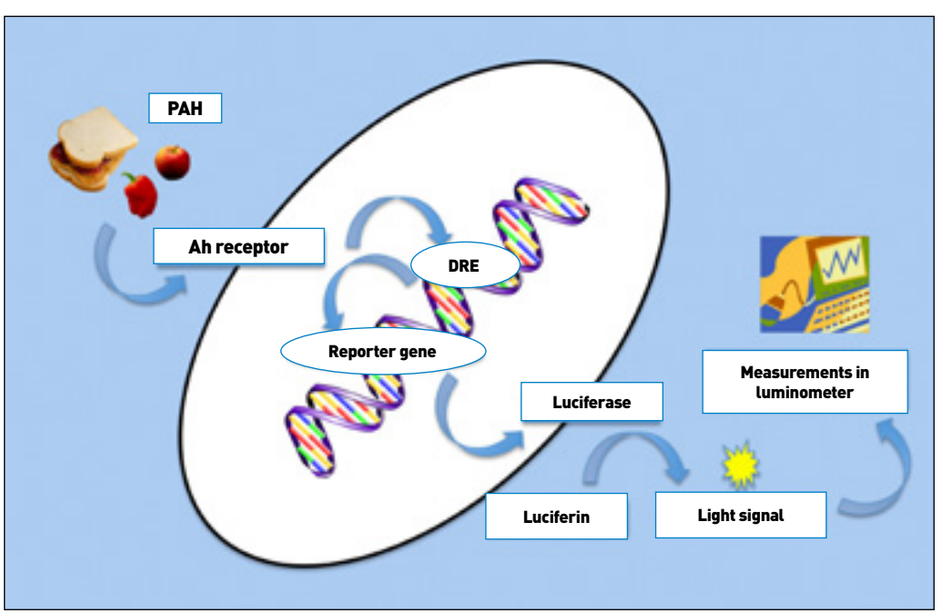

Fig. 1 Diagram showing mechanism of a luciferase-based reporter gene system, using the Ah receptor as an example

Reporter gene test systems based on the above-mentioned nuclear receptors have existed for some time and have been utilised extensively within research for several years now. To make these systems capable of deployment in day-to-day food safety monitoring work, they must be developed further, – especially in terms of simplification and handling, and as regards the standardisation and automation of the test systems in question. One possible strategy for establishing standardised procedures is the principle of reverse transfection. This involves the prefabrication ("coating") of microtitre plates with the plasmids needed for the respective test system – a process that can also be automated by using pipetting robots. This approach would not only permit the standardised production of larger batches of plates prepared in this way, but would also mean that these plates could be stored stably for prolonged periods without efficiency losses. For reverse transfection, the plates coated with plasmids need then only be populated with the cells and an appropriate transfection reagent, plus – as a second step – furnished with the extracts from the foodstuffs that are to be in-

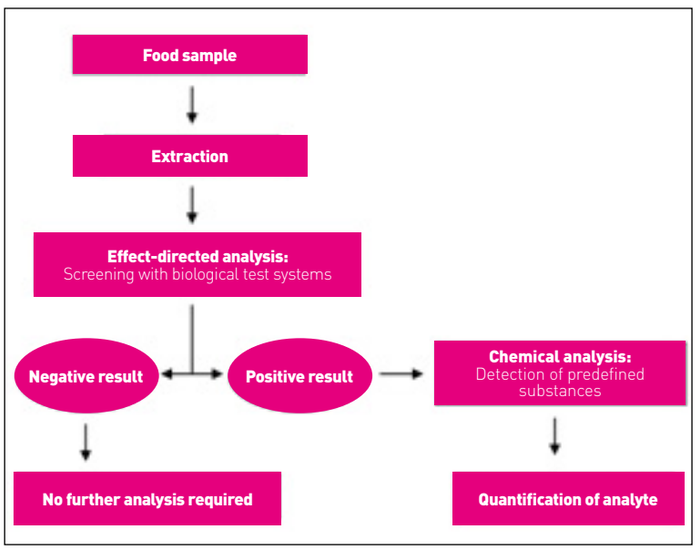

Fig. 2 A model for food safety monitoring that applies the innovative methods from effect-directed analysis

vestigated. An analysis of the results is typically complete within 24 hours, depending on the test system deployed. At first, this period of time seems very long in contrast to conventional chemical analysis. Two points must be borne in mind, however: first, biological test systems can investigate many samples in parallel; second, the actual luminometer measurements taken at the end of the incubation period need mere minutes to complete.

The BfR is particularly interested in developing these kinds of effect-directed analytical procedures to a point where they are resilient and accurate enough to produce reliable test results in food safety monitoring. Ultimately, the goal is for biological test systems to supplement – not replace – conventional chemical analysis. The goal should be to establish biological procedures as screening methods that are applied as an initial step – such as when sample volume is high, for example, a scenario that makes the methods of instrumental chemical analysis impractical to apply because of time and/or cost considerations. Only those samples returning a positive result from biological screening would then need to have these results verified in a second step by applying conventional chemical analysis techniques (Figure 2). Overall, then, a combination of biological screening and chemical analysis would facilitate high sample throughput while keeping time and costs within justifiable limits. If we consider the steadily expanding remit and the growing challenges facing food safety monitoring, this is a goal well worthy of pursuing.

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[2] Kuhn, K. et al. (2008) J. Food Prot. 71,993-999.

glycopeptides

Photo: © panthermedia | Sebastian Kaulitzki

An anti-cancer jab?

Chemical synthesis of vaccines

Prof. Dr Horst Kunz, Sebastian Hartmann, Björn Palitzsch,
Institute for Organic Chemistry, Johannes Gutenberg University Mainz

Vaccinations against disease: a blessing for humanity

The discovery made by Emil von Behring in 1890 – namely that bacteria in humans and animals trigger a powerful immune response in the form of antibodies (immunoglobulins) targeting the invaders – represents a phenomenal advance in the history of medicine. His work, which paved the way for diphtheria and tetanus immunisation, was honoured with the first Nobel Prize for Physiology and Medicine in 1901. The basic principle of immunotherapy against infectious diseases is that molecules (saccharides, polysaccharides, proteins) in the bacterial cell wall are identified as foreign by the mammal organism and are combated by the formation of antibodies targeting these foreign structures. The immune response against viral infections such as flu proved much harder to trigger, since the viruses utilise the biosynthetical apparatus in mammalian cells for synthesising their membrane molecules. Accordingly, the organism does not directly identify these membrane molecules as foreign. Diseased cells such as tumour cells carry endogenic molecules produced by the organism itself (proteins, glycoproteins and glycolipids) on their membranes. These molecule structures are only weakly immunogenic: they are therefore shown natural tolerance by the organism's immune system.

Over 30 years ago, however, G. F. Springer and co-workers [1] established that membrane glycoproteins in normal epithelial cells differ markedly from those found in epithelial tumour cells. The differences are not so much to do with protein sequences, but are to be found in the carbohydrate side chains. Biochemical research over the last 20 years has discovered that these structural differences are particularly applicable to the MUC1 mucin (episialin) and its tumour-associated form.

The MUC1 mucin as a target structure for an immune differentiation of normal and tumour cells

MUC1 is a glycoprotein anchored in the external cell membrane; it is highly glycosylated and extends far into the extracellular space (>100 nm). It is expressed in almost all epithelial tissue (breast, prostate, colon, pancreas); it is generally strongly overexpressed in the equivalent tumour tissue. MUC1's large extracellular portion contains an extended domain consisting of numerous repeat sequences – known as 'tandem repeats' – which vary in number according to the individual (Figure 1). Each of these repeat units incorporates five potential glycosylation sites – three threonines and two serines.

In MUC1 as present on normal epithelial cells, the carbohydrate side chains are very long: they cover the protein backbone in this portion completely. The MUC1 molecule thus assumes an elongated form that protrudes far into its environment: it looks like a test tube brush thickly set with bristles. On account of the characteristically altered activity of glycosyltransferases (enzymes that transfer carbohydrates), the carbohydrate side chains in tumour cells are often severely shortened and prematurely sialylated, i.e. they terminate in the C-9 carbohydrate N-acetylneuraminic acid frequently found in mucus glycoproteins.

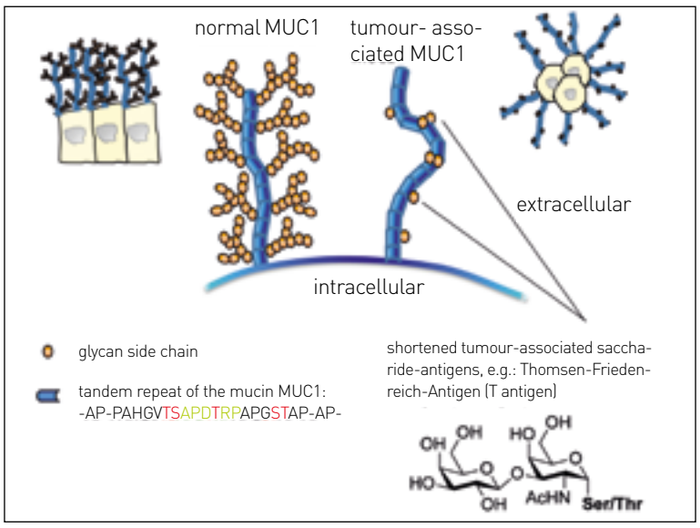


Fig. 1 MUC1 membrane glycoproteins on normal epithelial cells and epithelial tumour cells. Amino acids in single-letter coding.

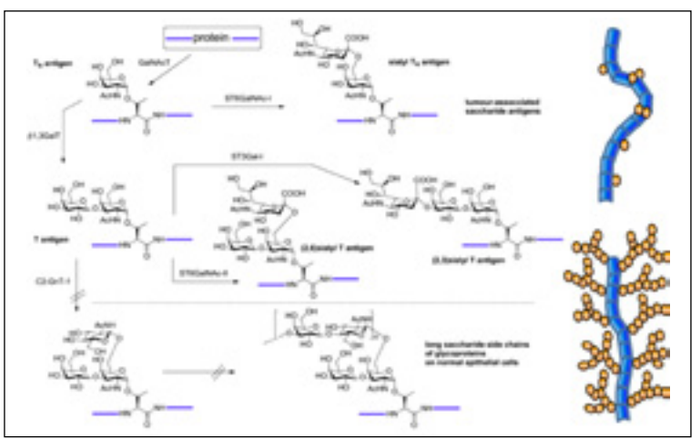
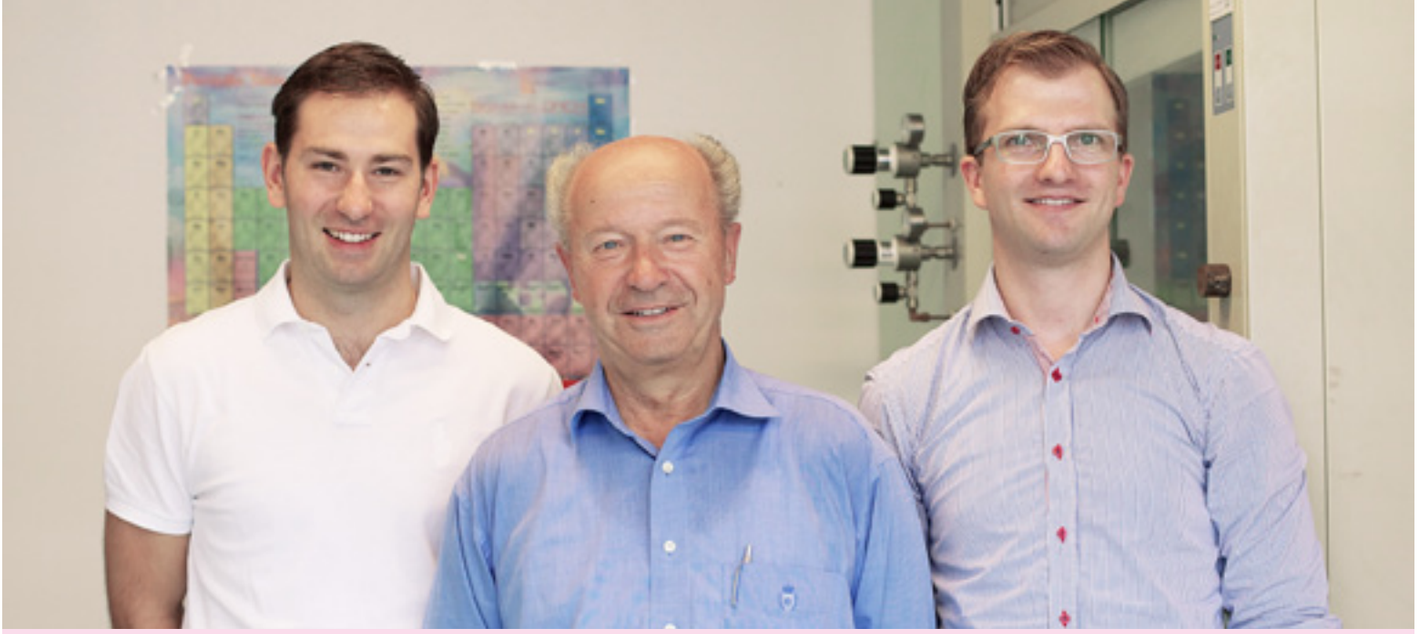


Fig. 2 Schematic display of the biosynthesis of mucin carbohydrate side chains. The five saccharide structures shown in the top part of the image are – bound to threonine or serine – tumour-associated carbohydrate antigens on epithelial cells. The corresponding fluorenylmethoxycarbonyl (Fmoc)-protected glycosyl amino acids (serine, threonine derivatives) are required for the synthesis of the tumour-associated glycopeptide antigens.

These short saccharide side chains, α-glycosidically linked to threonine or serine, are held to be tumour-associated carbohydrate antigens [1–3]. We believe these short saccharide side chains make peptide sequences in the tandem repeat region of the protein backbone of tumour-associated MUC1 accessible to the immune system; in normal, healthy cells, these sequences are completely covered by the long saccharide side chains. Alongside the tumour-associated carbohydrate antigens (Figure 2), these sequences also then embody peptide structural information typical to tumours.

The shortened saccharide side chains in tumour-associated MUC1 clearly also have the effect of changing the conformations of the peptide chain. The elongated form can now develop knobbed folds (turn conformations) [4] that represent further structures identified as typical for tumours. From the structural differences discussed so far, one could conclude that the immunisation of patients with MUC1 mucin isolated from tumour cell membranes is able to induce a tumour-specific immune response. While antibodies of this kind are indeed found in cancer patient serum, they do not cause an immune response against tumour tissue because of the tolerance already mentioned. Furthermore, attempts to deploy tumour cell MUC1 for immunisation have failed largely due to the fact that every protein strand of tumour-associated MUC1 is populated both by short, tumour-associated saccharide antigens and by



(left to right) Dipl.-Chem. Sebastian Hartmann, Prof. Dr. Horst Kunz, Dipl.-Chem. Björn Palitzsch

Sebastian Hartmann was born in 1984 in Dillingen a.d. Donau and studied Biomedicinal Chemistry at Johannes Gutenberg University Mainz. In 2010, he completed his Diplom thesis in the Institute of Organic Chemistry, entitled "Synthesis and Multivalent Presentation of MUC1 Antigen Structures on Particulate Carriers". Since January 2011, he has been working in Professor Kunz's study group on experiments for his doctoral thesis in the field of synthetic glycopeptide vaccines.

Horst Kunz was born in 1940 in Frankenhausen (Saxony), and studied chemistry at the Humboldt University of Berlin and Johannes Gutenberg University Mainz. He completed his doctorate under Leopold Horner in 1969 while working cyclic organophosphorus compounds. Habilitation followed in 1977, the appointment as professor (C2 scale) in 1979 and, after declining an outside offer, appointment as C4-scale Professor of Organic and Bioorganic Chemistry at Mainz University. His key areas of research are stereoselective reactions and the chemistry of alkaloids, peptides, carbohydrates and glycopeptides. He was awarded the Max Bergmann Medal in 1992, the Emil Fischer Medal in 2000 and the Adolf Windaus Medal from the University of Göttingen in 2001. In 1988 he was elected a Correspondent Member of the Saxonian Academy of Sciences and Humanities in Leipzig.

Björn Palitzsch was born in 1981 in Erlabrunn (Erzgebirge) and studied Biomedicinal Chemistry at Johannes Gutenberg University Mainz. In 2010, he completed his Diplom thesis at the University Dermatologic Clinic under the supervision of Dr H. Jonuleit, entitled "Investigation of Human CD8+T cells in Immunodeficient Mice with Transgenic HLA Expression". Since March 2011, he has been researching for his doctoral thesis under Professor Kunz in the field of synthetic glycopeptide vaccines.

the long carbohydrate side chains typical for normal MUC1, in alternating combinations. The pure glycoprotein structures typical for tumours cannot be isolated. Modern chemical synthesis techniques can produce pure partial glycopeptide structures typical for tumours from MUC1, however [5]: since these are too weakly immunogenic, they must be linked to immune stimulating factors to produce effective vaccines.

MUC1 glycopeptide vaccines via linking with T helper cell epitopes

Considering the differences in molecular structure for the MUC1 mucin in normal epithelial cells on the one hand and tumour cells on the other, as outlined above, target structures for the immune system are set up in the form of glycopeptide antigens, in which the tumour-associated saccharide antigens – such as the TN antigen, the Thomsen-Friedenreich (T) antigen or their N-acetylneuraminic (sialic acid)-substituted forms, such as the sialyl-TN antigen – are linked with peptide sequences from the MUC1 tandem repeat region. Multistage chemical and stereoselective synthesis is required to obtain the chemically pure forms of the fluorenylmethoxycarbonyl (Fmoc)-protected glycosyl amino acid components. Compared to normal Fmoc amino acids, the glycosidic bonds present in these compounds (and in the products obtained from them) have the effect of producing an elevated sensitivity to bases and acids. They can be successfully deployed in the automated solid-phase synthesis of MUC1 glycopeptide antigens, however, if conditions are maintained as established by recent work in this field. The example shown in Figure 3 summarises the use of a 2-(trimethylsilyl) ethyl ester anchor (1) and a sialyl-TN threonine component [6].

Cleavage of the completely protected glycopeptide was achieved by cleaving the anchor – in this case via fluoride-induced βelimination under neutral conditions. Following hydrogenolysis of the benzyl ester, all acid-labile protective groups were removed from the amino acid side chains by using trifluoroacetic acid (TFA), triisopropylsilane (TIPS – serves to scavenge tert-butyl cations) and water. Particular attention is due to the cleavage of the O-acetyl groups, since the O-glycosidic bonds to serine/threonine are characteristically base-labile. A pH value of 11.4 should not be exceeded. As a rule, the glycopeptides (such as 2) were isolated in their pure form by subsequent preparative reversed-phase HPLC in quantities >10mg. Their purity and structure are not only verified by means of retention times in analytical HPLC and mass spectra but also via high-field NMR spectra [6].

Following a similar procedure in chemical synthesis, it is also possible to construct fully synthetic vaccines, in which the MUC1 glycopeptide is linked as a B cell epitope via an immunologically inactive oligoethylene glycol spacer with a T cell epitope peptide immune system stimulant, taken in this case from ovalbumin (Structure 3, Figure 4). Following purification via HPLC, this pure, wholly chemically synthesised vaccine was obtained in a quantity of 15mg – an adequate volume for immunising a veritable host of mice.

The subsequent presentation of the T cell peptide by the major histocompatibility complex (MHC) II on the B cell then effects – due to identification by the aforementioned CD4 receptor – the activation of the T helper cells, which ensure, via cytokines and co-stimulating factors, that this selfsame B cell transforms itself into a plasma cell and proliferates. This in turn triggers secretion of large quantities of antibodies, which are quasi equivalent to being copies of the immunoglobulin receptor on the B cell (Figure 4).

Corroborating this, strong immune responses were triggered in the transgenic

mice with synthetic vaccine 3. For their characterisation in an enzyme-linked immunosorbent assay (ELISA, Figure 5), one needs microtitre plates with immobilisable forms of the glycopeptide antigens against which the immune response is targeted. These can be obtained by conjugating these glycopeptides (such as 2) to carrier proteins. For linking to carrier proteins such as BSA, the glycopeptides were N-terminally extended in solid-phase synthesis by a triethylene glycol spacer amino acid. The fully unblocked form was transformed with bi-functional linker molecules – in the present scenario via squaric acid diesters at pH 8 – into the monoamide. The monofunctional form reacts with the βamino functions of the lysines in the protein in water (here, the squaric acid monoamide ester at pH 9.5) to yield the BSA conjugate. MALDI mass spectrometry shows it carries an average of six tumour-associated glycopeptide antigens in an unaffected structure [6]. This glycopeptide BSA conjugate is applied in an aqueous solution to the microtitre plate wells, where it remains adhered to the hydrophobic surface. Following rinsing with water one obtains a coating that one can understand as the simulation of a cell surface (Figure 5).

If one now applies the antiserum induced in the transgenic mouse by vaccine 3 to the microtitre plate, subsequent washing will leave only those mouse antibodies adhered to this conjugate that specifically target the glycopeptide (contained in the vaccine). These induced mouse antibodies are detected using a biotinylated sheep anti-mouse antibody, whose adhesion is, in turn, visualised using a streptavidin-horseradish peroxidase (HPO) conjugate, which catalyses the oxidation of a colourless heterocycle via hydrogen peroxide to a green radical cation. In the ELISA diagram (Figure 6), its absorption is plotted against the increasing dilution of the mouse antiserum (green line).

We found that such a strongly elevated immune response occurred in one of the three transgenic mice after the second booster dose with the fully synthetic vaccine 3. This is a marker for the formation of immunological memory and indicates a changeover from IgM to IgG type antibodies. Neutralisation of the antibodies induced in the mouse with the synthetic tumour-associated glycopeptide antigen 2 (red line) impressively demonstrates the high structural specificity of the immune response triggered. While the sialyl-TN glycopeptide 2 present in the vaccine completely neutralises the antibodies (Figure 6, red line), neither the same-sequence unglycosylated MUC1 peptide nor the sialyl-TN glycopeptide with a peptide sequence from the MUC4 mucin effect a neutralisation of these antibodies. One may therefore state as fact that synthetic vaccine 3 has triggered an eminently structurally selective immune response, which is targeted specifically against the MUC1 glycopeptide structure typical for the epithelial tumour cells.

The selectivity of the immune response induced by the synthetic vaccine is astonishing and highly promising for the ultimate goal of being able to achieve active immunisation in the patient against the patient's own cancer cells. On average, however, the vaccine produces this effect only in every third mouse. Moreover, the transgenic mouse model is a model that cannot be extrapolated to humans.

Anti-tumour vaccines from MUC1 glycopeptide antigens and tetanus toxoid

To obtain glycopeptide vaccines that are not only reliably highly immunogenic but which could also be deployed in humans, we proceeded to link the synthetic tumour-associated glycopeptides via the linkage elements of an amino acid spacer and squaric acid linkers with tetanus toxoid as a carrier protein [7]. Tetanus toxoid is a protein with a molecular weight of >150,000 and which contains numerous T cell epitopes. It is a strong immunostimulant and has already been deployed successfully in many vaccines – such as flu vaccine – for human immunisation.

Synthesised using the described method, a MUC1 tetanus toxoid (TTox) vaccine, described in ref. [7] was used to vaccinate ten wild-type BALB/c mice. All ten animals exhibited a very strong immune response. Following the second booster dose, the ELISA test detected an antibody titre of >500,000, i.e. following dilution of the antiserum of the vaccinated mouse by a factor of 500,000, 50% of the absorption of the green radical cation was still found using ELISA (see above). This immune response is so powerful that it would break down the natural tolerance mentioned earlier against endogenic structures in each and every case. Moreover, it is also a structurally selective immune response targeting the tumour-associated MUC1 glyco-

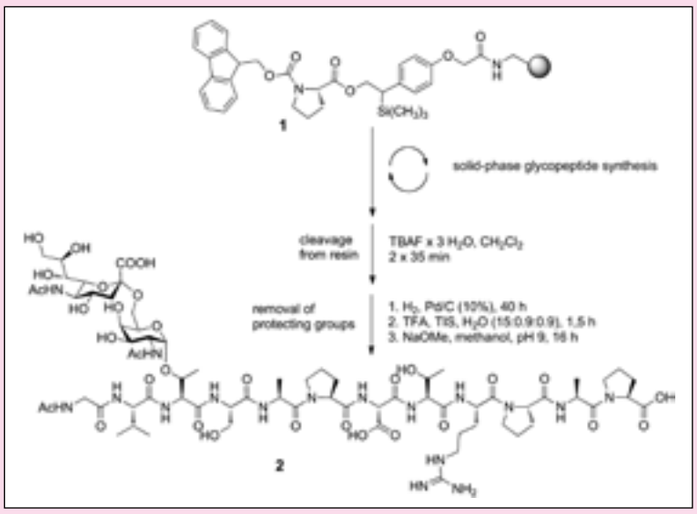


Fig. 3 Solid-phase synthesis of a glycopeptide 2 from the tandem repeat region of the tumour-associated MUC1 mucin.

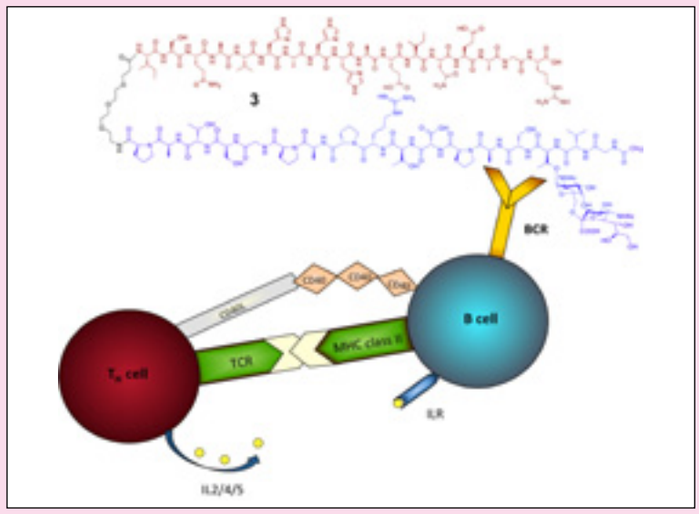


Fig. 4 Humoral immune response to a MUC1 glycopeptide-OVA T cell peptide conjugate 3, stimulated by TH cells.

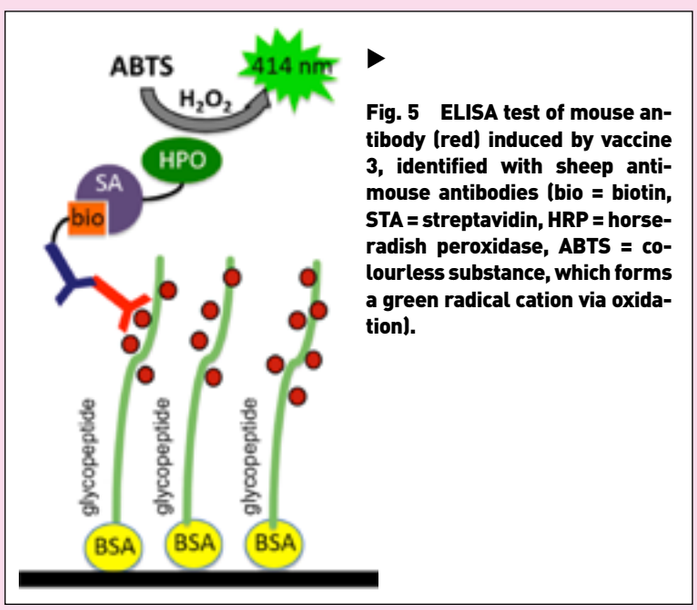


Fig. 5 ELISA test of mouse antibody (red) induced by vaccine 3, identified with sheep anti-mouse antibodies (bio = biotin, STA = streptavidin, HRP = horseradish peroxidase, ABTS = colourless substance, which forms a green radical cation via oxidation).

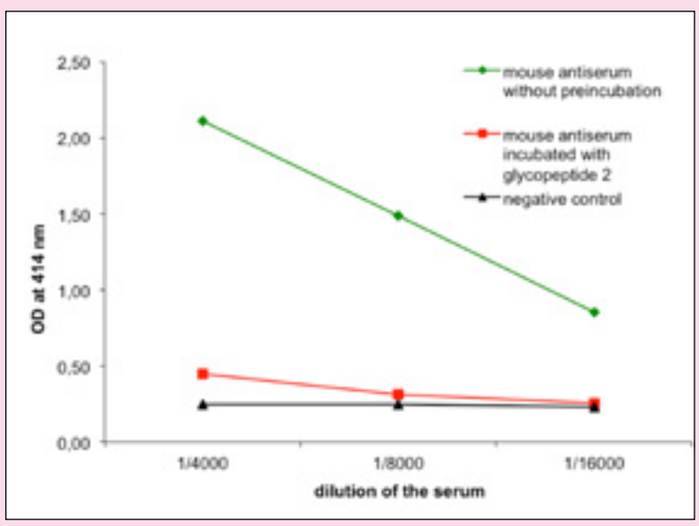


Fig. 6 ELISA identification of the immune response induced in transgenic mice by the synthetic vaccine 3 Black is negative control; Neutralisation of the mouse antiserum by glycopeptide 2

peptide. If one adds this glycopeptide without TTox and squaric acid portion to the antiserum, then the induced antibodies are neutralised and binding to the MUC1 glycopeptide-BSA conjugate on the microtitre plate can no longer be detected using the ELISA test. This selectivity of the strong immune response is absolutely essential: if the induced antibodies would also bind to glycopeptide structures on normal epithelial cells, this could have undesirable consequences.

Regrettably, the antibodies triggered by this first synthetic TTox vaccine exhibited only weak binding to cells from the MCF-7 breast cancer cell line in a flow cytometer. In this kind of flow cytometer analysis, the tumour cells are first incubated with 1:1000 diluted serum from a vaccine-immunised mouse before then being washed and the bound mouse antibodies labelled with a fluorescent labelled goat anti-mouse antibody. During the fluorescent-activated cell sorting (FACS) analysis in the flow cytometer, all cells passing through are counted by Laser beam scattering. For the cells identified by the antibodies – and therefore fluorescing – a separate count is maintained.

Since the antibodies triggered by the vaccine with the sialyl-TN side chain excited a strong immune response in the mice, but the induced antibodies demonstrated only weak binding to the MCF-7 breast cancer cells in the flow cytometer analysis, we then used the technique as described to synthesise a MUC1 glycopeptide TTox vaccine 4, in which the glycosylation position is located at a different site (here: serine 17; see Figure 7). This vaccine also triggered a very strong, tolerance-breaking immune response in all mice [8]. In the FACS analysis (Figure 8a, negative control), the antibodies thereby formed now exhibit complete binding to the breast cancer cells (Figure 8b).

Neutralisation of the antiserum with the glycopeptide contained in the vaccine (equivalent to 2 in vaccine 3) cancels out this tumour cell labelling by the antibodies present in the antiserum (Figure 8c) [8].

The binding of the vaccine 4 induced antibodies to the membrane glycoproteins of the MCF-7 breast cancer cells is reflected by the labelling of tumour cells in tissue sections. Figure 9 presents tissue sections of mammary carcinomas from three female patients incubated with antiserum taken from a mouse immunised with synthetic vaccine 4 (Figures 9a–c) [8].

The mouse antibodies binding to the tumour cells were in turn rendered detectable using a dye-coupled secondary antibody (here: a goat antibody) [8]. One observes that the antibodies induced by vaccine 4 barely bind at all to early-stage tumour tissue (Figures 9a,). They bind clearly to tumour tissue at the median G2 stage (Figure 9b), however, while the tumour cells taken from the advanced-stage tumour (G3, Figure 9c) are fully labelled by the antibodies triggered by vaccine 4. This variable degree of antibody labelling is undoubtedly attributable to the progression

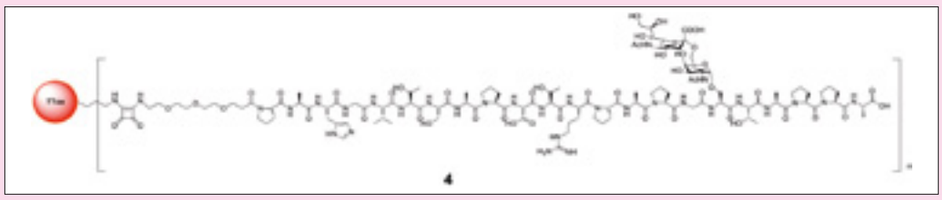


Fig. 7 Anti-tumour vaccine 4 from a MUC1 glycopeptide glycosylated on Ser17 and tetanus toxoid.

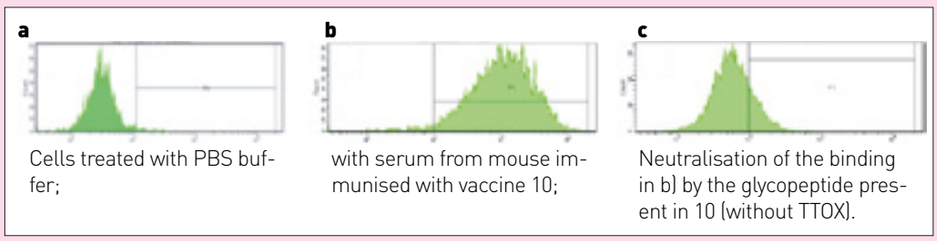


Fig. 8 Flow cytometer FACS analysis of the binding of antibodies induced by vaccine 4 to breast cancer cells of the MCF-7 cell line

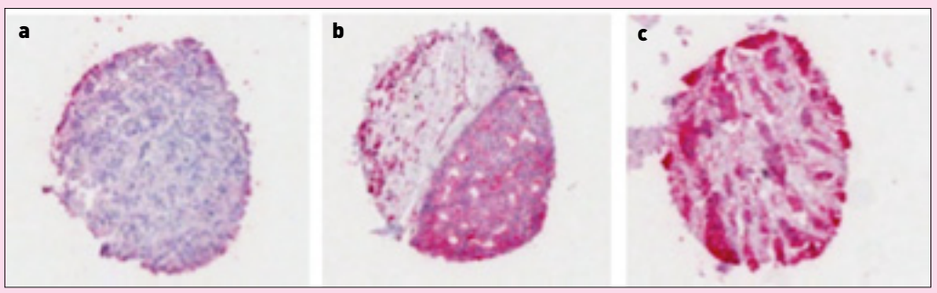


Fig. 9 Tissue sections from mammary carcinomas at different stages of progression;
a)–c) following treatment with antiserum from a mouse immunised with vaccine 4 (colouration results from enzyme-linked secondary goat antibody, colour from oxidation of 3-amino-9-ethyl-carbazol).

of the tumour and the concomitant rise in the proportion of tumour-typical MUC1 glycoprotein structures on the breast cancer cell membranes.

Investigations of the subtypes of the antibodies triggered by the synthetic glycopeptide TTox vaccine have shown [7, 8] that the vast majority of antibodies formed are of the IgG subtype (IgG1). In accordance with immunological mechanisms, the labelling of the tumour cells by these specific antibodies should initiate the degradation of tumour cells by the immune system. These results entitle us to express the hope that, one day, immunisation with chemically synthesised vaccines using the procedure described here will achieve active immunisation of patients against their own tumour tissue, thus opening up vast new horizons in the field of cancer therapy.

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Literature at the authors

A Higher Level of Live Cell Research

New highly expandable platform for live cell imaging

Designed with the scientist's workflow in mind the innovative new IX3 series of inverted research microscope systems from Olympus are developed for effortless, intuitive live cell imaging and clinical analysis. Built using worldwide customer feedback and designed to meet the needs of a wide range of users, the new systems offer exceptional ease-of-use and unprecedented optical flexibility via a new, customisable light path. New components can be easily slid into the light path using a series of swappable decks. This opens up many new avenues for exploration, allowing researchers to follow their imaginations.

The new IX3 inverted microscope systems (Fig. 1) are optimised for live cell imaging. The series also utilise the latest Olympus innovations in frame design, optics and software, providing exceptional stability, optical quality, accuracy and reliability.

This includes the fully automated IX83 for high-end research applications, the flexible IX73, which can be configured in manual, semi-motorised or motorised modes, and the easy-to-use IX53 with fluorescent capabilities, which is optimised for the routine examination of tissue samples.

Truly 'Open Access'

In particular, the IX73 and IX83 are built using a new swappable deck design, which allows optical modules to be easily and rapidly slipped in and out as needed, including magnification changer, filter turrets, a right side port and more (Fig. 2). This means that the systems are truly 'open access' and can be fully customised to meet the requirements of a wide range of applications, even allowing the utilisation of user-designed custom modules to provide the ultimate level of flexibility. This design also allows the systems to grow alongside the evolving demands of the users life science research projects. This is especially true of the IX73, which is highly configurable and capable of integrating with a range of computer-coded or motorised components.

Intuitive as Standard

The IX73 has been designed with an emphasis on ease-of-use and working efficiency. This makes it ideal for a range of routine and advanced research tasks. As it can be manual or semi-motorised and contains an ‚open access' to the infinite light path, the IX73 can be rapidly modified. The microscope comes as a one-deck system with an ergonomic low stage height, or a two-deck system with additional expansion capabilities. Users can build a uniquely flexible system that enables them to produce expert results with minimal training. This extends from brightfield and phase contrast methods, through to fluorescent imaging.

In addition, every image produced is of the highest quality, even during long or complex time lapse experiments, thanks to an improved focus mechanism, which reduces any drift phenomenon to a minimum.

Ideal Capturing of Time-Lapse Images

With new frame architecture and focus drive design, the IX3 system offers enhanced rigidity that reduces the impact of vibration and heat. It maintains the desired positions along the X, Y and Z axes to allow reliable time-lapse imaging. The real-time Z-drift compensation system capabilities of the IX3-ZDC combined with the Olympus ultrasonic stage

Fig. 1 IX3 Family

Fig. 2 A diverse range of units is available for the Olympus IX3 microscope system

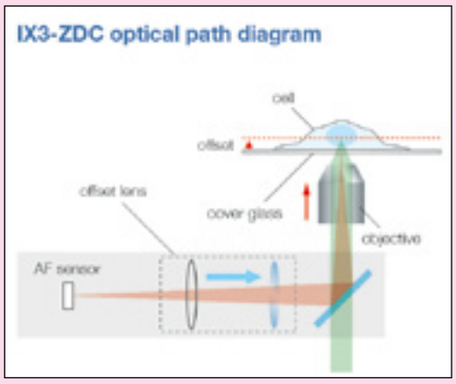


Fig. 3 X3-ZDC optical path diagram

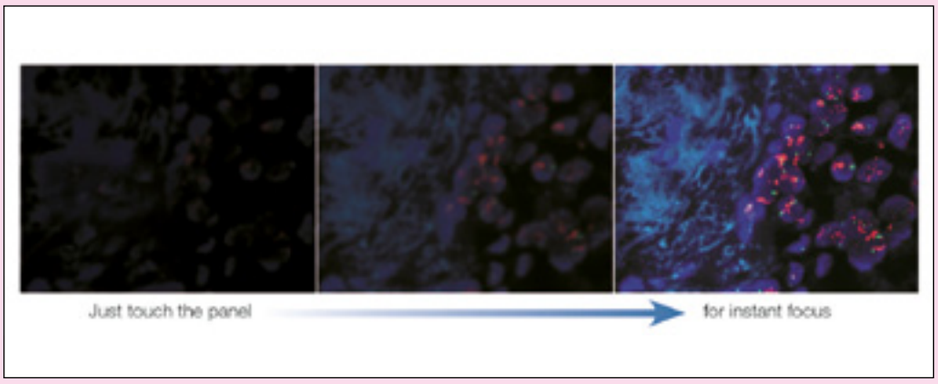


Fig. 4 ZDC one-shot function detects focus fast, even in high-magnification observation

is capable of multipoint imaging to capture high-precision multipoint time-lapse images that are never out of focus or misaligned.

The IX3-ZDC uses low-phototoxicity IR light to detect the correct focus position as set by the user. One-shot AF mode allows several focus positions to be set, enabling efficient Z-stack acquisition in multi-position experiments. Continuous AF mode keeps the desired plane of observation precisely in focus, avoiding focus drift due to temperature changes or the addition of reagents, making it ideal for measurements such as TIRF that require more stringent focusing (Fig. 3).

IX3-ZDC focus detection can be performed via the innovative touch panel independent of software (Fig. 4).

Clear and Bright Images

Olympus UIS2 infinity-corrected optics provide bright, high-resolution images from ultraviolet to near-infrared. The system ensures high optical transmittance with a broad range of objectives providing wide chromatic correction and high resolution, as well as high S/N primary images regardless of the observation method. The wide field of view and fly-eye lens system provide uniform fluorescence images and enable the use of sCMOS cameras with large chips.

For a Myriad of Research Needs

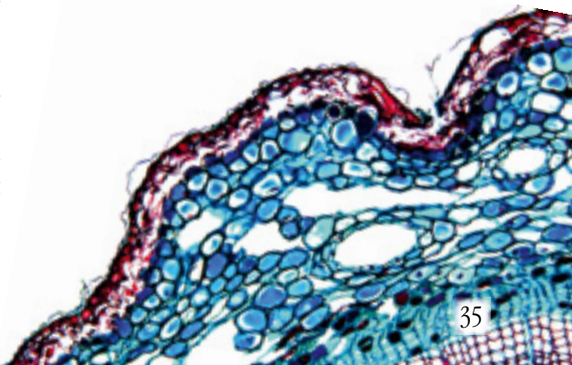
In particular, the highly-stable and robust IX53 is ideal for continual use in research and clinical laboratory environments, as it is exceptionally easy-to-use and has an ergonomic, low stage.

The fully motorised and automated IX83 along with the semi-motorised IX73 are designed to satisfy a myriad of research needs. With additional modules providing expanded functionality, both microscopes provide the ability to enable a multitude of imaging techniques, ranging from long-term time-lapse imaging and other demanding cutting-edge techniques to casual documentation. The systems also integrate seamlessly with the powerful and intuitive Olympus cellSens software*, providing hassle-free imaging and documentation.

No matter what the task, the IX3 series delivers the performance and expandability needed to accommodate the demands of tomorrow.

→ microscopy@olympus-europa.com

** cellSens software is not for clinical diagnostic use.*



Advertisement

High Precision and Speed

JULABO Temperature Control Solutions

JULABO presents an extensive program of temperature control solutions ranging from -95 to +400 °C. Since JULABO's foundation in 1967 the company has pioneered the development of technology for liquid temperature control. JULABO equipment is used successfully around the world providing application solutions for science, laboratories, technology centers and process industries. During every step of development and production JULABO equipment has to meet high quality criteria and its „made in Germany" technology reliably fulfills customers' requirements.

Sophisticated design, user-friendly operation

The current program features heating and refrigerated circulators, highly dynamic temperature control systems, recirculating coolers, water baths and additional special equipment. All JULABO products impress with sophisticated design and user-friendly operating concept. Their integrated highly precise control technology is unique. It guarantees highly accurate temperatures and rapid reaction to temperature changes. Another special feature of JULABO units: no side vents. Operation of JULABO instruments is intuitive. All important information is displayed intelligibly and easy to read. The parameters for any application are set quickly using only a few keys.

The EasyTEMP and WirelessTEMP by JULABO products facilitate innovative control and software solutions for remote operation, control, monitoring and documentation of applications – powerful tools for simplifying workflow automation.

From routine to high performance temperature applications

JULABO's product range includes temperature control systems for a variety of applications. Heating and refrigerated circulators accomplish almost every temperature control application with temperatures ranging from -95 to +400 °C and are available in many variants. JULABO water baths are high-quality and durable products, with a working temperature range from 20 to 99.9 °C, equipped with the latest microprocessor technology. In laboratories they are ideal for temperature control of samples, incubations, material testing, corrosion tests, cell cultivation or testing of food and beverages. State-of-the-art heating and refrigerated circulators by JULABO provide rapid and precise temperature control for a wide range of applications. Devices such as jacketed reactor vessels can be controlled within a temperature range from -92 to +250 °C. JULABO recirculating coolers are used for a variety of cooling applications in laboratories and industry. Users may choose from a large range of air or water cooled models with cooling capacities from 0.3 to 20 kW. JULABO also offers specialized equipment for individual applications and an extensive list of accessories for all products.

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JULABO takes pride in offering customers expert advice for pairing the proper JULABO temperature control solution to their specific application. JULABO service and support options include installation and calibration, equipment qualification documentation and application training. These invaluable services ensure customer confidence in the operation and maintenance of their JULABO unit.

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JULABO units function reliably providing years of operation with quality at your fingertips. Every unit meets strict internal requirements and conforms to the highest national, European and international standards. Each JULABO instrument must pass numerous tests in every production phase. A sophisticated quality management program in accordance with DIN EN ISO 9001 guidelines guarantees that only technologically superior products are shipped from our facility. Find comprehensive information on the JULABO program in the current catalog.

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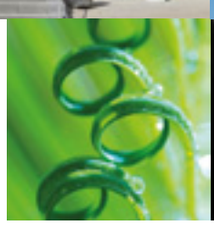

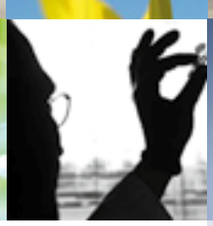

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status: march 13

New versatile ORCA-Flash4.0 V2 sCMOS camera

Hamamatsu Photonics has released the ORCA-Flash4.0 V2, a 4-megapixel scientific CMOS camera that offers unrivalled flexibility across a wide range of microscopy applications. The ORCA-Flash4.0 V2 has many new features such as two scan speeds, a readout mode for light sheet microscopy, and USB 3.0 and Camera Link interfaces.

Camera users have the option of running the ORCA-Flash4.0 V2 in a blazing fast scan mode or a virtually noiseless slow scan mode. The fast scan (standard) produces a low read noise of only 1.9 electrons rms (1.3 electrons median), which is suitable for most experiments. The slow scan with read noise of 1.5 electrons rms (0.9 electrons median) is designed for experiments where lower noise is more important than raw speed.

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A preparative HPLC system should be as versatile as possible, since purification tasks can change frequently. An AZURA Preparative HPLC system, fitted with a feed pump and fractionation valve, makes working with large sample volumes easier than ever.

The systems feature a pulsation compensated pump drive with selectable constant flow or constant pressure solvent delivery. Supported flow rates range from 0.1 to 1000 ml/min. Pump heads are available in different materials. AZURA purification solutions are scalable and offer gradient support, flexible fraction collection, solvent and peak recycling, leak detection and practical GMP features. KNAUER will be at ARABLAB, German Pavilion, booth #414.

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Phenomenex Inc., a global leader in the research and manufacture of advanced technologies for the separation sciences, introduces roQ QuEChERS kits, for the cleanup of multiple pesticide residues in food prior to HPLC or GC analysis. The roQ kits offer a number of usability features specifically designed to facilitate and improve the largely manual QuEChERS technique. Rigorously tested tubes and superior tube caps ensure leak-free performance. The low-extractable tubes ensure the cleanest possible extracts. In addition, roQ flat-bottom centrifuge tubes are designed to stand upright so they can be easily placed on a scale for sample weighing. The roQ salt packets have a unique, easy-pour design that minimizes spills.

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Novel Plasma Technology

Tracera is equipped with the newly developed barrier discharge ionization detector (BID). This system will make its debut at PITTCON 2013, an international analytical instrument exhibition convened in the United States (March 17 to 21 at the Pennsylvania Convention Center in Philadelphia, Booth#1434). With the new BID, the plasma is generated inside a quartz tube, so it makes no contact with the discharge electrode used for plasma generation. As a result, the detector electrode is not degraded, achieving longterm analytical stability.

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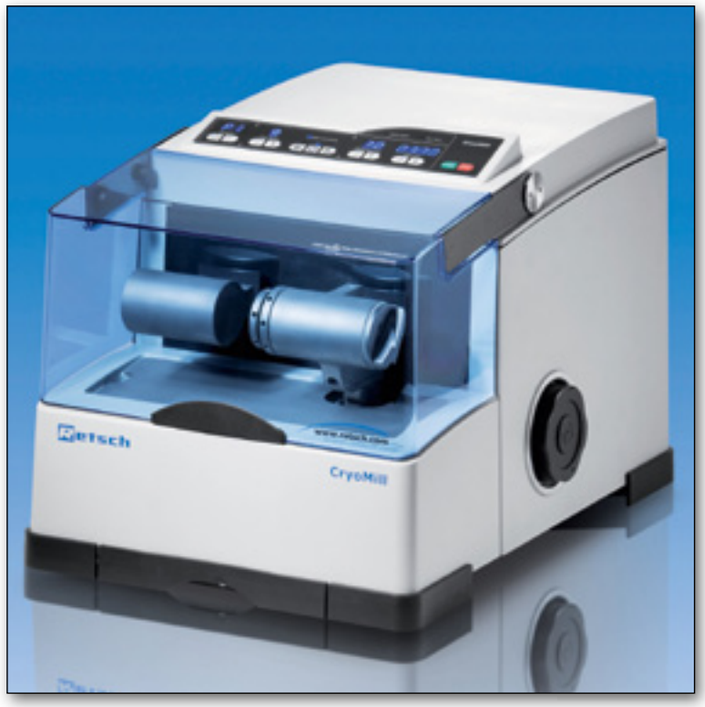

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RETSCH's CryoMill is the ideal choice for cryogenic grinding of samples which cannot be processed at room temperature. Thanks to an integrated cooling system the grinding jar is cooled with liquid nitrogen before and during grinding. Thus the sample is embrittled and volatiles are preserved. An Autofill System provides liquid nitrogen in the required amount so that the user never gets into direct contact with the LN_2 which makes operating the mill particularly safe. The new generation of the CryoMill provides substantially improved grind sizes thanks to the increased oscillation frequency of 30 Hz.

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Begin shipping samples of the next-generation Micro PMT

The µPMT is a next-generation, ultra-compact photomultiplier tube that is fabricated through the use of in-house semiconductor processes for MEMS (micro-electro-mechanical systems) devices.
It can be mass produced, is only 1/7th of the volume and 1/9th of the weight of the smallest conventional photomultiplier tube. The characteristics of this new µPMT will enable the development of high-precision measurement instruments for individual users, such as for point-of-care medical testing and environmental monitoring. The new µPMT assembly features a bialkali photocathode that is sensitive in the visible wavelength range and a divider circuit.

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Low Retention Pipette Tips – superior sample recovery

Thanks to optimised surface properties, the new Low Retention Pipette Tips from Sarstedt are ideal for superior sample recovery. Pipetting viscous material or samples containing detergents quite frequently leaves residues in the pipette tip. Using Sarstedt's new Low Retention Pipette Tips excludes this deficiency. Apart from providing enhanced pipetting accuracy, the new tip option therefore also minimises the loss of valuable reagents like restriction enzymes or polymerases.
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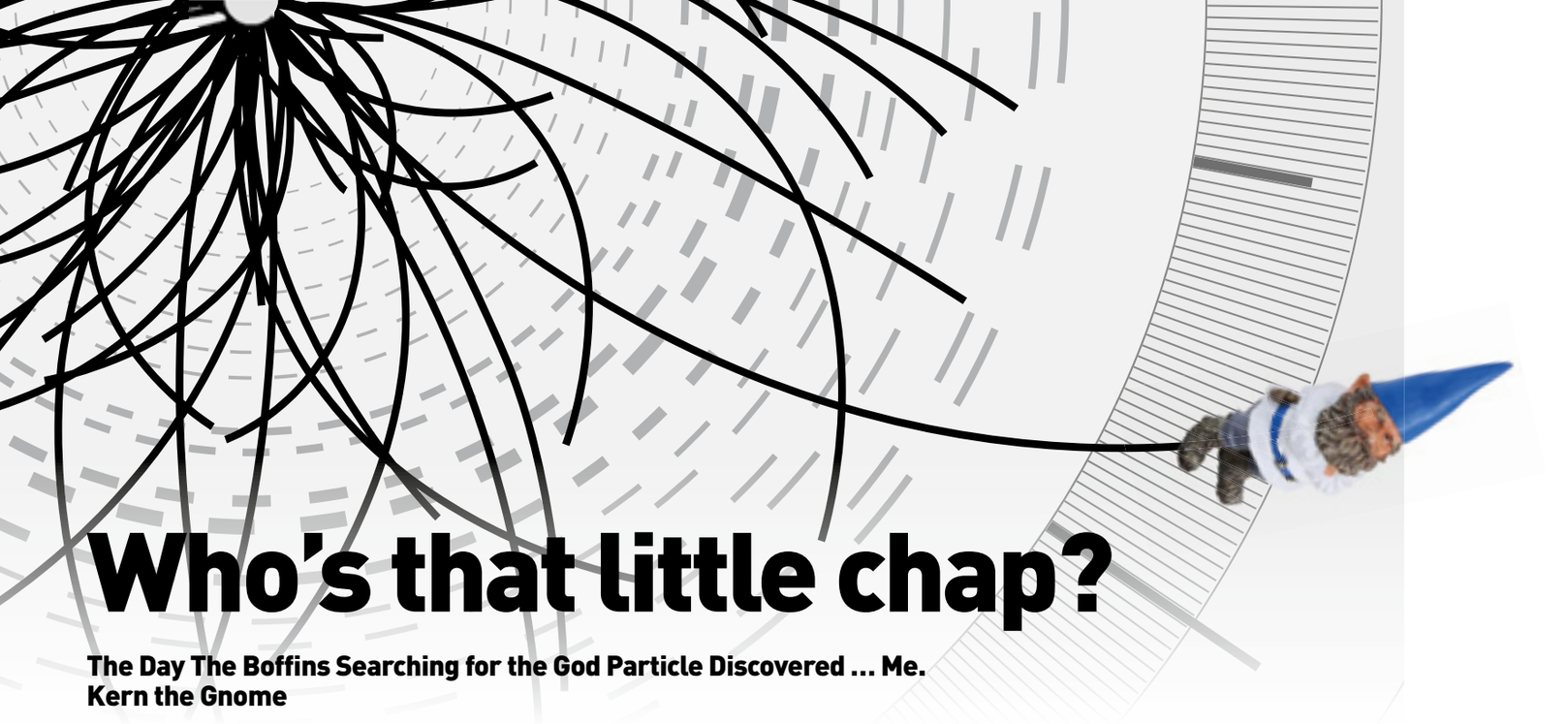

Who's that little chap?

The Day The Boffins Searching for the God Particle Discovered … Me.
Kern the Gnome

It took more than 10,000 scientists, ten years and cost £ 2.6 billion to build the Large Hadron Collider at CERN, near Geneva. It was designed to answer some of the most profound questions about the universe. But the question they find themselves addressing today is, 'Who's that little chap in the pointy hat?'

Er… yes, sorry, that would be me. Kern the Gnome. To be honest I was hoping to drop in without the press turning up. CERN is just one of a number of stops on my epic round-the-world scientific tour. My purpose here is to investigate a rather remarkable fact

about our own planet, namely that it is slightly potato-shaped. (See fig. 1). It's not something you would notice on a walk to the shops. But the irregularity does mean the strength of gravity varies by infinitesimal amounts at different points on Earth's surface.

We're talking differences of up to 0.5% here, not much more than the weight of a gnat's top hat. Still, if you have a set of some exquisitely sensitive laboratory scales, such as the ones made by Kern & Sohn, you can detect it. Which is why those extremely nice people at Kern sent me on the world's first scientific gnome expedition along with a set of their wonderful scales.

So far I've been to the Amundsen-Scott research station at the South Pole, where I weighed my heaviest (309.82g). From there I went to Mount Evans, Colorado (307.52g). The Snolab underground Cosmic Ray detector in Ontario (307.63g) and even NASA's celebrated Zero Gravity Training Plane – the so-called 'Vomit Comet' (where unsurprisingly, I didn't weigh anything at all).

After all that, CERN should be a breeze. But just as I was boarding the plane for Geneva, the Press got hold of the story.

The next thing I know my face is all over the front pages. Flashbulbs popping. Magazines asking for my favourite recipes. I must confess, my arrival is a bit of a distraction from the task of trying to make hadrons collide. Especially when the Swiss TV guys ask the scientists if they could film me at the control panel. If they are not careful they could miss the elusive Higgs Boson, AKA The God Particle – the particle that explains why things have mass in the first place. They are hoping to detect it any day now. A hadron, in case you are wondering, is a composite particle made of quarks held together by the 'Strong Force' – so called because it is 100 times stronger than the electromagnetic force that holds atoms together. (I think I've got that right.) And the thing about hadrons is, it's very hard to make them collide. You wouldn't believe the trouble the boffins have gone to. At 17 miles in circumference the Large Hadron Collider is the biggest machine in the world. At minus 271.25°C it's also one of the coldest places in the Solar System. But when the particles collide it becomes 100,000 times hotter than the inside of the sun. Before you can even consider getting your hadrons to collide, you have to whizz them up to 99.9999991% of the speed of light. Then you put them on a specially constructed track of magnets, set them off in opposite directions, and take cover. And then bang! All hell breaks loose, right? Screams, falling masonry, police blue tape and a cop shouting through a megaphone, 'OK folks, nothing to see here, back to your lives.' No, not really. It takes thousands of computers linked together around the world in a giant network to analyse the data looking for the tell-tale traces of a hadron collision. But it's well worth the effort because it should enable us to answer a very fundamental question, namely what sort of universe have we got? There are two options. The standard model (boring!). Or… drum roll! The Higgsless variant! This one is full of goodies, including super symmetry. All I can say is, if you like symmetry, you'll love supersymmetry.

They also hope to find out where all the gravity has gone. If you've ever seen a grand piano fall on a cat you may dispute this, but the force of gravity is very weak. They think most of it is leaking away into other dimensions. So if all the gravity is leaking away and we just end up with the slops, then it seems pretty clear to me that we need to take extra special care of the bit we get. We need to measure it properly. Cherish it even. Which is precisely what Kern Scales do. Just saying. Anyway, everything turns out splendidly in the end. The hadrons collide like dodgems at the fun fair. And the Kern scales perform flawlessly. All in all, it promises to be quite a year at CERN, with two magnificent discoveries to record in the history books. The famous Higgs Boson. And a new dwarf star. Ahem!

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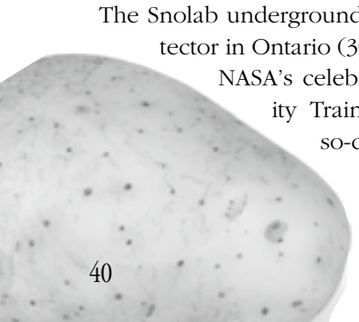
Next stop – the British Library to attend my very own TED talk. Follow my adventure at GnomeExperiment.com.



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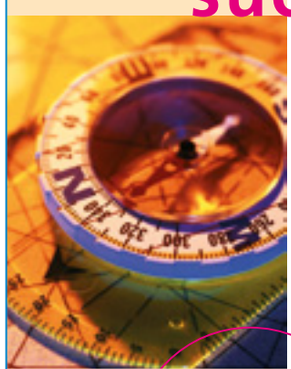

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