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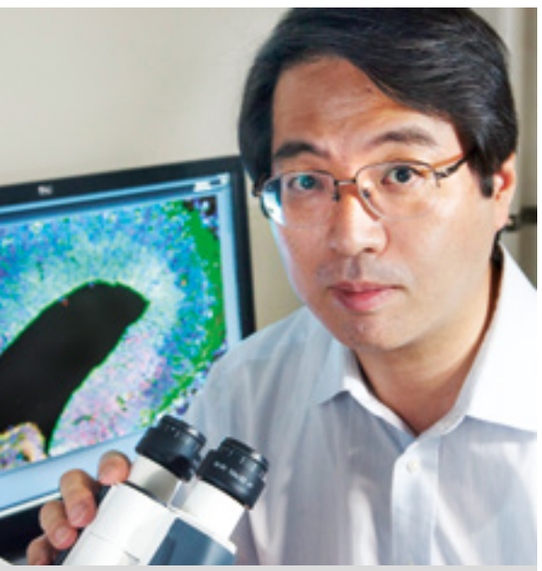

Yoshiki Sasai The image on the monitor shows one of his optic cups grown from iPSCs.
Picture: © Hans Sautter / Agentur Focus

Sasai's Death

By Prof. Dr Paul G. Layer

It was a Tuesday morning in early August. I'd just returned to my office from a trip to the US. I'd been at an eye conference, ISER 2014 in San Francisco, where I'd heard about recent work in growing human retinal tissue from stem cells. Still reeling from all the new data and numbed by jetlag, I'm trying to marshal my thought processes, writing endless mails to colleagues and making a pig's ear of my travel expense forms. As I am putting together a figure for a manuscript (fig. 1), one of my doctoral candidates appears at the door, somewhat pale, not his usual self. "What's up?" He takes a deep breath: "Sasai is dead. Looks like suicide. My wife read about it this morning in the Japanese news [author's note: she is Japanese]. It's not 100 % certain that it's our Sasai, but it probably is him."

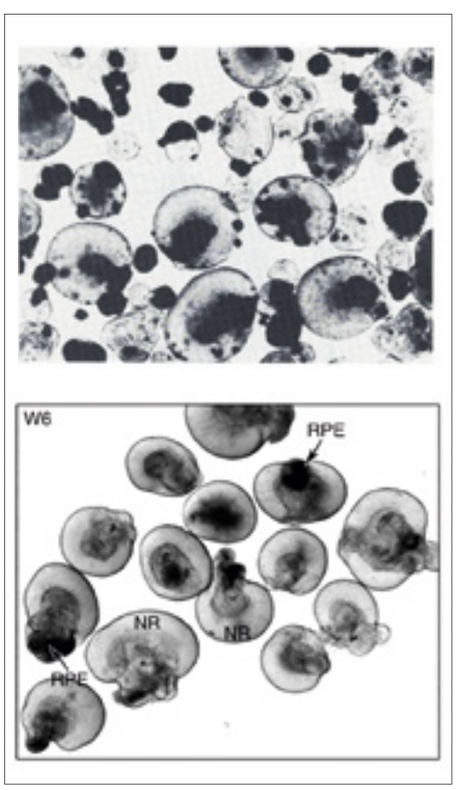

Fig. 1 Surprising similarity between the highly structured retinal spheroids originally grown in the 1980s from embryonic chick retina by the team led by Paul Layer, top, (Prog Ret & Eye Res, 1994, 13, 197–230) and those now grown by Zhong et al. 2014 from human iPSCs, bottom, (Nature Commun 5, doi: 10.1038/ncomms5047). The black clumps of cells are retinal pigment epithelial (RPE) cells.

Yoshiki Sasais state as a stem cell biologist had rocketed overnight when he and his team had produced artificial optic cups from a novel type of murine stem cells. His publication in Nature stunned the fields of regenerative medicine and eye research [1], and was also the reason that the name Sasai had been on everyone's lips at the ISER congress. One starting point Sasai cited for his work was a study by Nakagawa from 2003, which itself had been based on our "mini-eyes" from chick retina [2] (see also q&more 2/11). The genealogical proximity of our work meant we therefore thought of him as "our Sasai".

One can easily imagine how thrilling all of this was for me: after all, we had been the first to show – as early as the 1980s – that isolated stem cells of embryonic chick retina can be used to grow balls of cells with complete cell sheathing (we dubbed these retinal spheroids or "mini-eyes", fig. 1), and had thus delivered a proof-of-principle for these most recent research developments [3–5]. The ultimate step – implantation of this tissue into blind patients – was to be taken very soon. Dr Magdalene Seiler has been researching the transplantation of foetal retinal tissue into the eyes of blind experimental animals for many years, which has led to several clinical successes [6] (fig. 2).

And now, the news that Sasai has taken his own life. We're shocked, but the chain of events is clear to me immediately. Sasai's death isn't about his artificial optic cups, nor is it about a scandal involving Rosi: it's all to do with the controversy that's been raging since early this year over Haruko Obokata, principal investigator at the renowned RIKEN Centre for Developmental Biology (CDB). It's about her results, which she was also able to publish prominently in Nature [7, 8]. Before we go on, one should first know that in regenerative medicine, the search is still on for the most suitable stem cells, which can then be multiplied quickly and grown into tissue – so as to be able to replace diseased tissue or organs. But which kind should be used? Embryonic or adult stem cells (ESCs, ASCs), pluripotent or multipotent stem cells? All have their individual problems. Only with cells known as "induced pluripotent stem cells" (iPSCs) did it seem as if the Holy Grail had been found. Once again, it was a Japanese team that in 2006 published a method for manufacturing iPSCs from adult cells. In 2012, Shinya Yamanaka received the Nobel Prize for this work – an entirely justified decision given its expected medical and economic significance. This method is now being used worldwide in many labs, although it is a highly complex procedure. Accordingly, the world is all ears when someone claims that they can do the same thing much more simply. Which was just the angle taken by Haruko Obokata in her Nature paper: take a good look, everyone, at how easy it can be! Instead of many separate and meticulous steps, she just sprinkles her cells with citric acid

and, hey presto, they rejuvenate themselves – like the phoenix from the ashes – unaided into iPSCs, which she then terms STAP cells, for "stimulus-triggered acquisition of pluripotency".

Many stem cell researchers immediately tried to replicate the method but their attempts failed. Experts in the field then looked at the work in more detail, and soon uncovered methodical and factual inconsistencies. Queries were raised, the leading scientific organisations got involved and top journals had reason to think hard about their peer review protocols. And all eyes were naturally on the researchers at the CDB. The Japanese government demanded urgent clarification of the facts. Although Ms Obokata first attempted to defend her results, she quickly became overwhelmed, being described by colleagues and the media as both difficult and emotionally unstable. While Sasai had not been her direct mentor, he was formally her supervisor as Director of the CDB. He had also proofread the work in question and was probably its co-author for this reason. After protracted wrangling, the ill-fated author was found guilty of scientific misconduct, her results were adjudged falsified and both Nature papers were retracted in July of the same year. While no evidence of misconduct was proven on the part of Sasai himself, both the organisation and management of the CDB as a whole were called into question and have now seen sweeping changes.

So much for the scandal itself. Many questions remain, which Sasai's death has brought into even sharper relief. In what kind of a system do we research scientists live today, exactly? Was Sasai's death – similar to that of Danton [1] at the time – also a consequence of aberrant developments and embroilments in society, from which Sasai believed he could no longer extricate himself? Why did he think he could no longer go on living? What led him to commit suicide? Can it "simply" be ascribed to the Japanese soul, which perhaps finds it particularly difficult to bear a (presumed) loss of face? No – the case reveals much more and is therefore an occasion for all of us to stop and think for a moment about our role as researchers.

Why is a young research scientist tempted to engage in deceitful behaviour and falsify her data, and why is it even possible for such revolutionary results – which are, let's face it, "too good to be true" – to be published so quickly in prestigious journals? One the hand, there is a huge pressure to perform in contemporary scientific research, on the other, we have the media, always lusting after the next headline-grabbing news story. All of us, especially junior researchers, are under constant pressure to publish ever more frequently and in ever more prestigious journals. And why this obsession with impact factors? Those making research applications – ourselves included – now consider a publication with an IF of less than 4 as no longer being fit to list there, the work is thus de facto seen as worthless. Just think about that word – "worthless" – again for a moment: years of work from entire teams are rated as worthless, as null and void. Effectively, we're saying: "You've spent years working on nothing!" Imagine telling hardworking tradespersons or Parliamentary backbenchers that the years of work they've put in have all been for nothing! And this damning verdict only comes about because the team isn't quite as well networked as another or because it's researching something that's not (yet) really "cool". All these goddamn rating systems, everything has to have its "metric" – a capitalist performance mentality applied to academia and the intellect. Yet originality and the power to innovate – i.e. quality – cannot be quantified (which is precisely why we have these two terms, quality and quantity). During my trip, I also had the chance to discuss the question of university ratings with my former boss at Stanford Medical School. His exact words on the topic: "This is all nonsense." And he should know, as the long-standing Chairman of one of the world's best universities. Time and again, we find that new breakthroughs continue to be fuelled by precisely those discoveries that did not make it into the flagship journals. Examples of this abound in every field of research. The tale of the retinal spheroids, outlined above, is just one such story from my own research, which with the availability of human stem cells was suddenly plastered over the covers of the daily newspapers. And an example that is now, in labyrinthine fashion, also bound up with Sasai's death.

Young researchers in particular are under almost unbearable pressure. Today, if you've set your sights on a research career with prospects of a permanent position, you'll publish like there's no tomorrow. Given this attitude, it's hardly surprising that we're regularly confronted with such cases of scientific misconduct from researchers. And we can set up as many improved oversight mechanisms for supervisors or journals as we like: it's all useless if the system and the mentality it supports do not change. Improve the career prospects for young researchers. Look in particular at the whole person when rating their work, and not just their IQ and IF. Don't pursue research solely from a career perspective

Fig. 2 Dr Magdalene Seiler (right) of the UC Irvine Medical Centre has spent decades researching the transplantation of foetal retinal tissue (see article). Pictured here with one of her colleagues (Anuradha Mathur, left) and the author at lunch in Irvine, August 2014.

but for the satisfaction that comes from understanding the natural world. Besides our cogitations on the sublime, this is after all the most human of our pursuits. Perhaps this, too, was one of the points that poor Sasai wanted to make with his death: Stop and think – this way is wrong! Indeed, let us reflect well, before – as in the time of Danton – yet more heads have to roll.

"Danton's Death" is the title of a famous German play by Georg Büchner.

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market view

2014 Eppendorf & Science Prize Research on skilled limb movement

The US scientist Eiman Azim, Ph.D., Postdoctoral Research Fellow at Columbia University in New York has won the 2014 Eppendorf & Science Prize for Neurobiology. Dr. Azim's work offers fundamental new insights into the neural mechanisms that enable skilled limb movements to be both smooth and precise. His research has provided direct support for long-standing theories about the roles of internal feedback pathways within the central nervous system and external feedback from the muscles in regulating fine motor control.

→ **www.eppendorf.com**

Picture: Eppendorf AG

Glionova Therapeutics secures $5.7 million to take glioblastoma therapy into clinical trials

Glionova Therapeutics, an oncology-focussedbiopharmaceutical company, announced that it has closed a SEK 42 million (US$5.7 million) Series A financing deal, co-led by Novo Seeds and the company's founding investor HealthCap, to take its lead compound GLN-1001 for the treatment of glioblastoma into clinical trials. The project is based on recently published research from the Department of Medical Biochemistry and Biophysics at the Karolinska Institute and the Swedish national research infrastructure organisation Chemical Biology Consortium Sweden.

→ **www.glionova.com**

Alliance: GE Healthcare and Takeda join forces

GE Healthcare and Takeda Pharmaceutical Company Limited announced that they have entered into an alliance agreement for research and development. This will focus on imaging modalities in the field of hepatic fibrosis, a key factor in the diagnosis and treatment of liver diseases. Early stages of liver disease have almost no clinical symptoms, a progress of the disease is characterised by hardening of the tissues due to fibrosis accompanying the inflammation of the liver and a worsening of symptoms due to cirrhosis.

→ **www.takeda.com**

Merck reports strong top-line growth in Q3

Merck delivered sales growth of 9.3%, reporting sales of 2.9 billion€ in the third quarter of 2014 (Q3 2013: 2.7 billion€). Apart from organic sales growth of 4.6%, sales increased by 5.1% as a result of the acquisition of AZ Electronic Materials (AZ). A weaker euro led to slightly negative foreign exchange effects of -0.5%. EBITDA pre one-time items increased by 3.1% to 857 million€ (Q3 2013: 831 million€). Owing to the good business performance in the first nine months and the very good organic sales growth in the third quarter, Merck is assuming a slight organic sales growth for the full year 2014.

→ **www.merck.de**

Ireland offers potential for manufacturers

Representatives from eight companies in the field of analytical, bio- and laboratory technology visited Ireland in October to build business contacts to Irish industrial partners in the laboratory, research and pharmaceutical sectors and to thus boost their exports. The positive developments in the Irish economy open promising business opportunities for German manufacturers in these branches. The Irish budget deficit has been continuously reduced since the economic crisis. Between 2006 and 2013, a total of 8,200 million € has been invested in research and development.

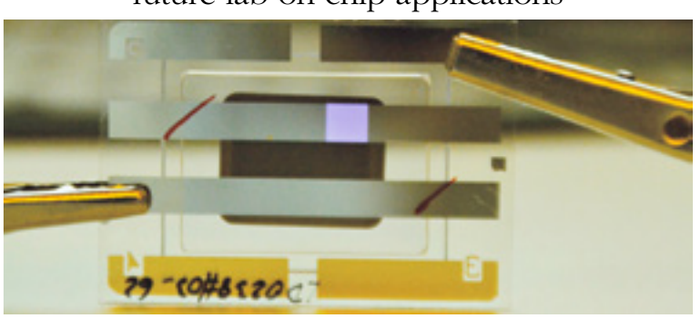
→ **www.spectaris.de**

Eurofins Genomics: A first GLP certification for DNA sequencing services in Germany

Eurofins Genomics, the genomics division of the Eurofins Scientific Group, has now achieved Good Laboratory Practice (GLP) certification for DNA sequencing services. It is the first genomic service provider in Germany to attain such a qualification. GLP is a required standard for laboratories conducting non-clinical safety studies such as toxicology and pharmacology studies in animals. It is also required for non-clinical safety studies for the development of drugs, small molecules, vaccines and gene therapeutics. GLP standards are also critical for the development of diagnostic kits and device validation (FDA and patent submissions), the analyses of production strains and GMO plants such as genetic stability testing.

→ **www.eurofinsgenomics.eu**

Electronic devices towards future lab-on-chip applications



Smart and portable medical equipment is essential for fast and easy point-of-care and point-of-use diagnostics. Lab-on-a-chip applications in handheld devices can help to save time for laboratory medical analysis in emergency scenarios. The combination of sub-micrometre-thick light-emitting devices and photo-detectors with tuneable spectral characteristics could play a key role in future sensing chips based on organic electronics. These applications implement excitation and detection of fluorescence or phosphorescence in a marker. Time-resolved measurements are also possible. The integration of both OLED and organic photodiodes into one chip could be a way to achieve low-cost personal diagnostics outside the laboratory.

Image: © Fraunhofer FEP

→ **www.fep.fraunhofer.de**

Roche's new production facility in China

Owing to the growing demand for diagnostic products in the Asia-Pacific region, over the next three years Roche will be investing 450 million Swiss Francs in a new production facility to manufacture diagnostic products in the Suzhou Industrial Park in China. As an extension of the global Roche production network for diagnostic products, the facility in Asia will focus on the manufacture of tests for immunodiagnostics and clinical chemistry. Workplaces for more than 600 employees will be created at the site over the next few years.

→ **www.roche.com**

nutriepigenetics

Cancer prevention and nutrition

What role does epigenetics play?

Dr Clarissa Gerhäuser
Epigenomics and Cancer Risk Factors,
German Cancer Research Centre, Heidelberg

Detoxification, anti-inflammatory agents, radical scavengers, antioxidants, anti-hormonal effects, cell growth inhibition, programmed cell death – all are terms that have been connected with the prevention of cancer by drugs or nutritional factors over the last thirty years. For the last ten years or so, the focus has been turning to a new field: epigenetics.

Researchers working in epigenetics study the heritable effects on gene expression that occur independently of changes in the DNA sequence. Epigenetic mechanisms play a pivotal role in embryonic development, in tissue-specific gene expression and the memory formation, to name just a few examples, and enable the organism to adapt to changes in the environment. Disruptions to these processes contribute to ageing and the genesis of (chronic) disorders – also including carcinogenesis. Essentially, there are three epigenetic mechanisms that control gene expression (fig. 1). Histone modification involves the attachment of small chemical methyl or acetyl groups to histones (cell nucleus proteins, around which the DNA is wound): this influences the packing of genetic material in the nucleus and plays both a positive and negative role in gene transcription. DNA itself can also be methylated: if this occurs in promoter regions not normally methylated, then expression of the gene is inhibited. Non-coding (micro-)RNAs, of which over 2,500 have been identified in recent years, are each

thought to regulate several hundred genes and have more of a modulatory function. They affect the translation of messenger RNA (mRNA) into proteins, since they either influence mRNA stability or block protein synthesis by binding to the mRNA's 3' untranslated regions.

Natural substances switch genes on

The first pioneering research showing the potential influence of natural substances and nutritional factors on epigenetic mechanisms was completed in the US in 2003. A team from Rutgers University described how a constituent of green tea was able to inhibit DNA methyltransferases (DNMTs) and thus prevent the deactivation of tumour suppressor genes in a laboratory study. DNMTs are the proteins that attach the chemical switches to the DNA, thus making these less accessible for transcription factors. These initial findings were followed by a large number of other studies reporting on the capabilities of natural substances as methylation inhibitors. These include polyphenols from apple juice, coffee, soya beans and curry powder, selenium, vitamins, and sulphur-rich constituents in onions, garlic and brassicas (fig. 2). Cell culture experiments have shown that these nutritional constituents inhibit promoter methylation, thus enabling the reactivation of a multitude of genes that are silenced during carcinogenesis. These genes play a key role in detoxification processes, in DNA repair and during cell differentiation, as well as influencing uncontrolled cell growth via regulation of the cell cycle and the induction of apoptosis. With hindsight, however, one must concede that the inhibitory potential of certain substances was overestimated – probably as a result of the methods used for the detection of changes in methylation – and that some of the results of earlier experiments cannot be reproduced by using modern quantitative methods. Furthermore, since most of the studies were in vitro experiments designed with the intention of analysing a few DNA regions at most, they do not offer a genome-wide view of these substances' effects on DNA methylation.

Information is generally scarce concerning the impact of nutritional factors on the methylation pattern in animal models or in human pilot studies. One exception is the research being conducted by Gary Stoner at Ohio State

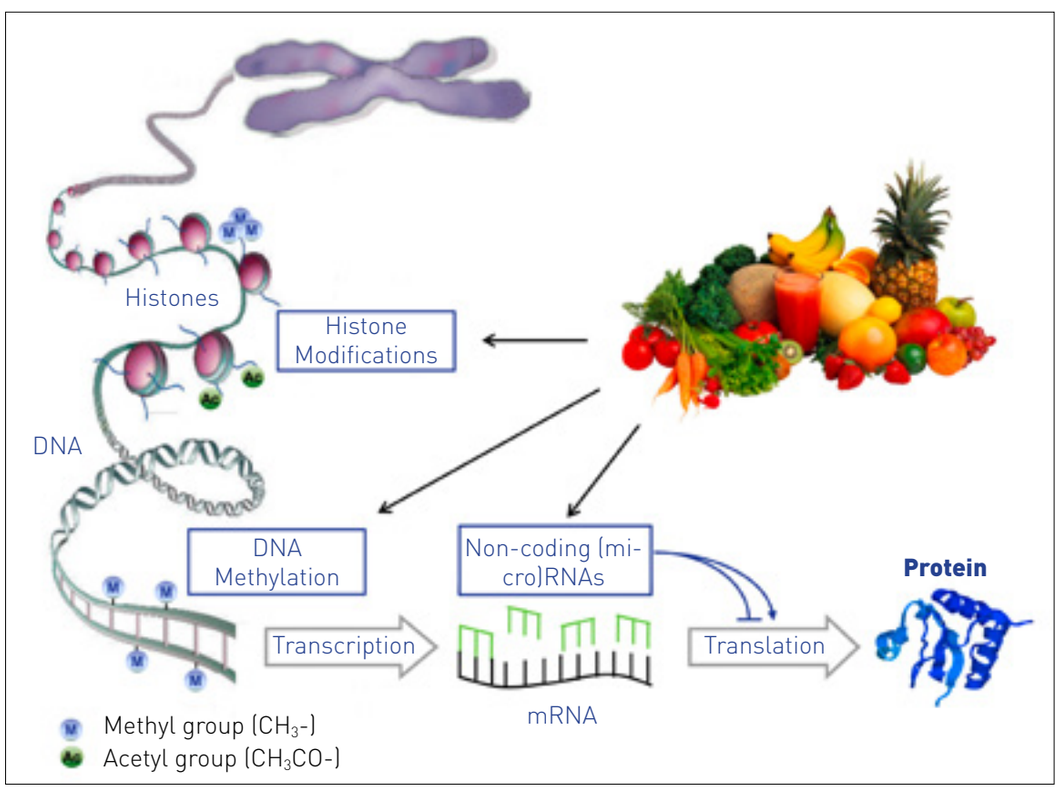


Fig. 1 Overview of gene expression regulation via epigenetic mechanisms. Histone modifications, DNA methylation and non-coding (micro)RNAs present attack vectors for natural substances sourced from nutrition.

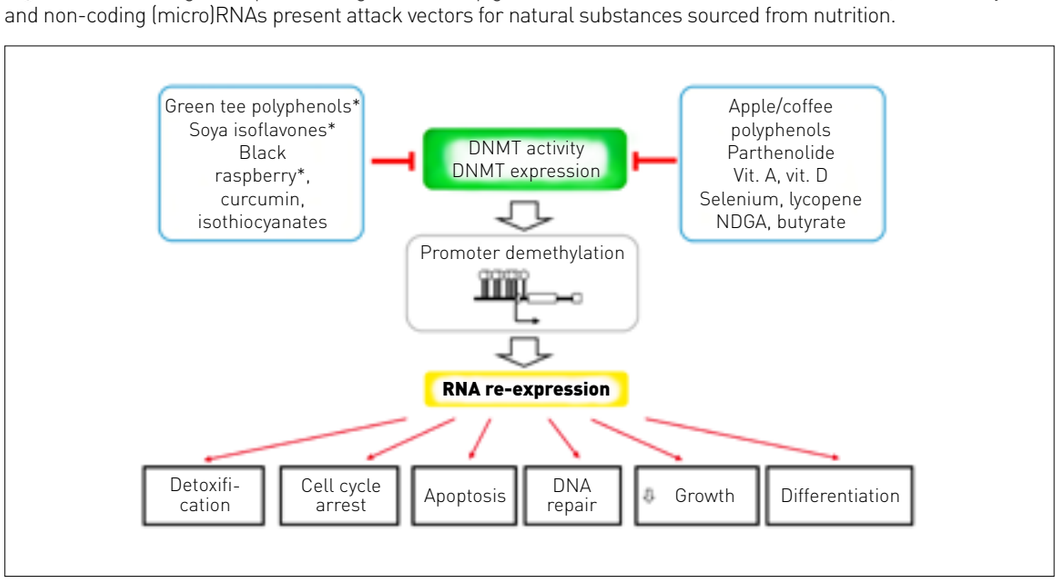


Fig. 2 Inhibition of cancer-associated DNA methylation. In cell culture experiments, constituents from nutritional components, vitamins and trace elements such as selenium inhibit the activity or expression of DNA methyltransferases. As a result, silenced genes performing key functions in cellular processes such as detoxification, cell growth control or DNA repair are reactivated and contribute towards cancer prevention.

University (USA). For over 20 years, Stoner has been investigating the anticarcinogenic properties of black raspberry, a raspberry variety that is especially popular and widespread in the USA. In one study involving patients with colorectal cancer who consumed 45 g of freeze-dried black raspberry daily for up to nine weeks, silenced inhibitor proteins of the Wnt signalling pathway – often disrupted and permanently activated during colorectal cancer development – were reactivated, thus retarding cell growth. These findings were subsequently confirmed in rodent models for genetic or chemically-induced ulcerative colitis. The effect is ascribed to the anthocyanin pigments, which are also to be found in other blue and red fruit, including blueberries, cherries and grapes.

Retarding the inflammatory response

In 2004, researchers at the Linus Pauling Institute in Oregon (USA) were the first to show that a metabolic end product of the broccoli constituent sulforaphane inhibits the activity of histone deacetylases. These proteins are responsible for removing acetyl groups from histones. As a result, key proteins that control the cell cycle and programmed cell death can be reactivated, and the growth of cancer cells can be suppressed, as demonstrated both in vitro and in rodent tumour models. Activities similar to sulfora-

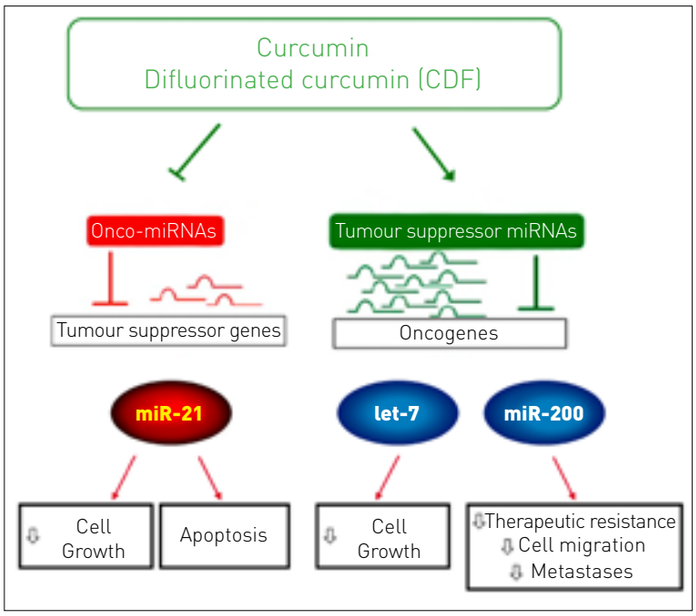


Fig. 3 Influence of curcumin on microRNAs. Curcumin and a synthetic curcumin analogue inhibit onco-miRNAs and activate tumour suppressor miRNAs. As shown by in vitro and animal model studies, not only is cell growth retarded, but genes are suppressed that participate in the development of therapy resistance, migration of cancer cells and the development of metastases.

Clarissa Gerhäuser studied Pharmacy at the University of Würzburg and obtained a Ph.D. (summa cum laude) in Pharmaceutical Biology at the University of Munich in 1993. She has worked as a postdoc and research assistant professor in the area of cancer chemoprevention with Prof. J.M. Pezzuto at The University of Illinois at Chicago, Chicago, IL, USA from 1993 to 1996, funded through a Feodor-Lynen Fellowship awarded by the Alexander-von-Humboldt-Foundation. In 1996, she joined the German Cancer Research Center (DKFZ) in Heidelberg and currently heads the group Cancer Chemoprevention and Epigenomics. Her major research interest is the investigation of molecular mechanisms associated with breast and prostate cancer, with a strong focus on epigenetic mechanisms. In 2003, she was awarded with the European Association of Cancer Research (EACR) Young Cancer Researcher Award Highly Commended and the Phoenix Pharmacy Scientific Research Price for 'Pharmaceutical Biology'. Clarissa Gerhäuser has authored more than 90 research articles, reviews and book chapters and holds four patents. Also, she has co-edited a comprehensive reference book on 'Chemoprevention of Cancer and DNA damage by dietary factors' (Wiley Press, 2009).

phane are seen with garlic constituents and the short-chain fatty acid butyrate, which is produced in high concentrations in the gut by the bacterial fermentation of dietary fibre. Another series of natural substances – including curcumin (from curry powder), anacardic acid (from cashew nuts), delphinidin (from pomegranate) and catechin (from green tea) – inhibits the histone acetyl transferase P300. P300 is an enzyme that transfers acetyl groups not only to histones but also to non-histone proteins such as the tumour suppressor protein P53, the androgen receptor or the transcription factor NF-kB. This could work to retard the hormone-mediated growth of prostate cancer cells and inflammatory responses, for example.

miRNAs as biomarkers

microRNAs (miRNAs) – small, non-coding RNA molecules – block messenger RNA transcription or influence their stability, thus regulating its translation into proteins. Since they are highly stable and can be detected in blood or urine, their suitability as biomarkers for the development or progression of diseases is also being investigated. The expression of some miRNAs in tumour genes increases during carcinogenesis. These "oncomiRs" – such as miR-21 – inhibit tumour suppression genes, promote tumour growth and inhibit apoptosis. Equally, tumour suppressor miRNAs such as let7 or miR-200 are often deactivated during carcinogenesis. This promotes the propagation of cancer cells, the formation of metastases and – importantly – the development of therapeutic resistance. In a series of cell culture experiments, a research team at Wayne State University (USA) showed that curcumin – and a chemically-modified form of curcumin in particular – was capable of normalizing the expression of these miRNAs and thus counteracting these processes (fig. 3).

Interestingly, miRNAs are also affected by tobacco smoke. In a study by the University of Genoa (Italy) conducted in 2010, rats were exposed to tobacco smoke daily for four weeks. Subsequent examination of lung tissue using microarray analysis demonstrated that, of 488 miRNAs investigated, around 50% were less strongly expressed in the animals exposed to tobacco smoke than in the control group. It was shown that the influence of tobacco smoke on miRNAs was reduced in animals that had received broccoli/watercress constituents or chemical substances promoting the detoxification of tobacco smoke carcinogens for three days prior to tobacco smoke treatment.

Outlook

As these few examples show, nutriepigenetics – i.e. the influence of nutritional components on epigenetic mechanisms – offers an interesting new approach for researchers working in cancer prevention. To date, available data are generally limited to in vitro investigations: few human studies are available that substantiate the functional relevance of epigenetic mechanisms for the cancer prevention efficacy of natural substances. Future research will need to identify best strategies for chemopreventive intervention targeting the epigenome.

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Precisely targeted regulation

lab&more in conversation with Stefan Miltenyi, CEO of Miltenyi Biotec GmbH, Germany

The biomedical community is euphoric about recent results in clinical trials of new treatment modalities for genetic diseases and oncological indications: T cells with modified chimeric antigen receptors (CAR) have been genetically modified with the aid of lentiviral technology and hold out the prospect of a novel form of cancer immunotherapy. Lentiviral technology itself is currently regarded as the most effective way of inserting genetic material into a cell in order to modulate its function. Miltenyi Biotec GmbH, which is one of the oldest and largest biotechnology companies in Germany, announced in August that they had acquired the lentivirus business segment of the US company, Lentigen Corporation.

Lab&more was in conversation with Stefan Miltenyi, the founder and CEO of Miltenyi Biotec, and asked him about the potential of lentiviral vectors.

Mr. Miltenyi, what are the advantages that lentiviral vectors have over other gene therapy vectors that made you decide to acquire the lentiviral production technology of one of the leaders in this field?

The main objectives of all gene therapy vectors are to deliver a specific genetic payload safe and efficiently to target cells where the transgene is to be expressed. In contrast to other gene delivery systems lentiviral vectors can transduce target cells very efficiently with very little toxicity. A gene of interest is then stably expressed in the transduced cell and in its daughter cells. This is required if the transgene has to be expressed for a prolonged time (e.g. for cancer therapy). Moreover, lentiviral vectors serve as a very broad gene delivery platform as pseudotyped vectors can transduce all types of mammalian cells, including non-dividing cells which cannot be transduced with gamma-retroviral vectors.

Whenever viruses are discussed, their ability to mutate is usually a central topic, especially in the case of retroviruses. What precautions are being taken in your company to prevent replication-competent viruses from being formed?

In contrast to the genome of a virus that contains all viral genes, the genome of a lentiviral vector is modified so that every viral gene is deleted or truncated, which prevents the formation of new viral particles upon transduction. For the production of lentiviral vectors the viral components are split on several different constructs, which then are added in trans – a system called split packaging. During all the years of experience with lentiviral vectors, a replication-competent lentivirus has never been detected. Lentiviral vectors are generated from the master copy of the plasmids used for split packaging, and subsequent cell transduction is a single event. Therefore, there is no accumulation of mutations as in the case of a replicating virus.

Probably the two greatest challenges for gene therapy are to guide the integration of the therapeutic gene into the host DNA correctly and to prevent the expression of other genes on the host chromosome from being changed by the integration process. What progress has been made to meet these challenges and how effective is your vector system?

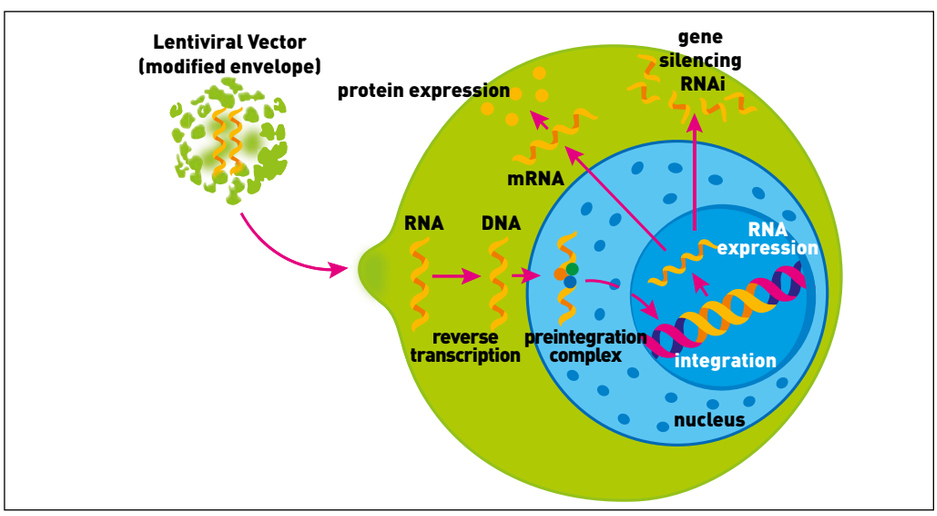
One of the features of lentiviral vectors is their ability to efficiently deliver their payloads into the genome of target cells. Unlike gamma-retroviral vectors, which contain strong promoters that can activate neighboring enhancer elements, such as oncogenes, lentiviral vectors do not contain strong promoters to upregulate their expression. The lack of strong promoters in lentiviral vectors prevents the genotoxic effects seen with the gamma-retroviral vectors. In addition, the 3' LTR has been deleted to generate vectors with further decreased genotoxic potential. Several pre-clinical studies have confirmed that lentiviral vectors have no genotoxic potential, in contrast to gamma-retroviral vectors. Also the accumulating safety data from various clinical trials further support these findings.

In the future, we anticipate to have a range of lentiviral vector products that address the evolving needs of the translational medicine scientist. These include various pseudotypes and commercial-scale manufacturing.

What are the new therapeutic options that will be made available through lentiviral technology?

The technology will provide a robust method for stable gene delivery into mammalian cells of interest. These include hematopoietic cells, such as T cells, and hematopoietic stem cells (HSCs). T cells provide, as an example, new options for cancer therapy, as indicated by the promising results seen with CAR-T cell therapy for acute lymphocytic leukemia. HSCs provide therapeutic options for a variety of diseases, including genetic diseases like ALD, beta thalassemia and SCID. This is just the beginning for therapeutic applications of this technology.

(Interview: Claudia Schiller)



Transduction mechanism of lentiviral vectors. The lentiviral vector transfers genetic material into the target cell, where it is inserted into the host genome. This can lead to the expression of a particular protein. Alternatively, the expression of a particular protein can be inhibited through formation of a specific RNA molecule (RNAi).

Stefan Miltenyi studied physics and medicine in Cologne, Germany. In 1989 he founded Miltenyi Biotec to commercialize MACS Technology for magnetic cell separation, which he developed through his master thesis. Today, Miltenyi Biotec has 1400 employees and focuses on tools for biomedical research and cellular therapy.

Trust, but verify

Isothermal amplification in food analysis – a real alternative to conventional PCR

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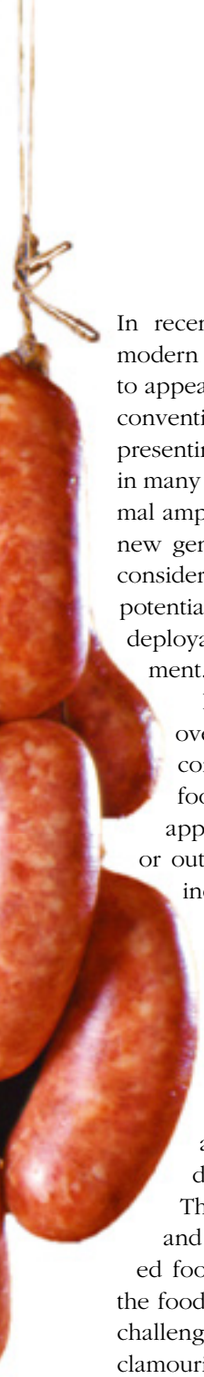
Inherited genetic material – deoxyribonucleic acid or DNA for short – is unique to every living thing and can therefore be utilised like a fingerprint for identification purposes. DNA analysis has thus become an indispensable tool within food safety, and in the real-time testing of both raw materials and finished products. The established method for confirmation of specified target DNA in a sample is the polymerase chain reaction (PCR).



In recent years, however, several more modern and simpler methods have started to appear, with serious potential to replace conventional PCR in certain fields while presenting at least an interesting alternative in many other areas of application. Isothermal amplification of DNA has ushered in a new generation of tests that are not only considerably faster than traditional PCR but potentially offer the option of being deployable outside a laboratory environment.

If we glance at media reporting over the last few years we quickly come to the conclusion that many foodstuffs are not always what they appear to be. In addition, negligence or outright attempted fraud in the food industry can often result in serious repercussions for consumer health. While there are many methods available for detecting pathogens, allergens or other undesirable constituents, one problem is common to them all: they are expensive, time-consuming and must almost always be conducted under laboratory conditions. The rising demand for food products and an increased need for quality-tested foodstuffs continues to present both the food industry and regulators with new challenges. All of these stakeholders are clamouring for rapid, simple detection methods for specific DNA sequences.

Question marks for dinner

The label on a food product must specify its country of origin, expiry date and ingredients. We are given no information as to whether the product is contaminated with pathogens, whether all of its constituents are actually listed or whether the product contains substances that trigger allergies or have genetically-modified components. While many of the food products that end up on our table have already been analysed, tested and evaluated, this requires a laboratory and trained personnel capable of performing the relevant analytic procedures. There is a lack of rapid, simple detection methods capable of returning a result in less than a couple of hours.

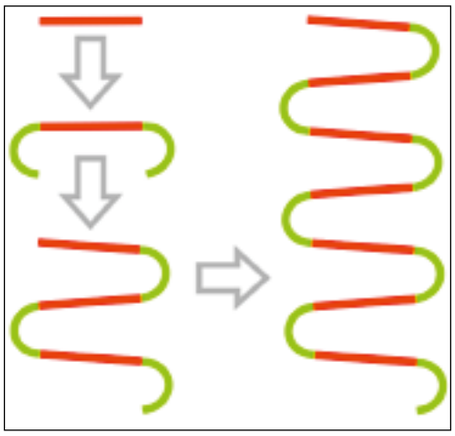


Fig. 1 Diagram of the isothermal LAMP reaction workflow. From the starting-point of a DNA target range (red), "loop" structures are then formed (green). Unlike PCR, LAMP does not result in the formation of a multitude of single, short products (amplicons) but in long product chains with continuously iterating sequences. The amplification of DNA is not cyclical but continuous: this results in the generation of a large quantity of product DNA. These fragments can attain lengths of over 20 kilobase pairs.

Fig. 2 Simple visualisation of test results from isothermal amplifications: the addition of a DNA intercalating dye causes positive samples to fluoresce.

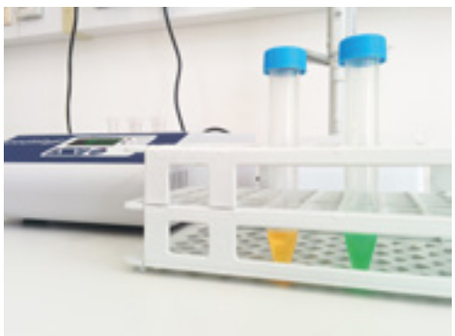


Fig. 3 For isothermal amplification, only basic lab equipment is required, namely a pipette and a standard heating block. Sample colour change is readily identifiable: positive samples fluoresce bright green, negative samples retain their orange hue.

Listeria in dairy products

In late 2009, the bacterium Listeria monocytogenes was discovered in sour milk cheese. Some 33 people contracted listeriosis, eight of whom died. This corresponds to a mortality rate of just under one in three patients, itself demonstrating the danger presented by contamination with these pathogens. The outbreak was caused by a failure to follow hygiene protocols and the use of out-of-date enzymes and other ingredients.

Horsemeat in meatballs

In 2013, Europe was shaken by the horsemeat scandal. Horsemeat was discovered in many meat-based ready meals – where it was naturally not listed as a product ingredient. One of the commonest drugs deployed within veterinary medicine – and thus by equine vets – is phenylbutazone, which is used for pain relief and to combat inflammation. The EU had already prohibited the use of this drug in animals destined for the meat production industry, since its use carries a number of health risks. The consumption of undeclared horsemeat can therefore have repercussions for health. Quite apart from the fact that it also constitutes outright fraud by mislabelling, since beef is considerably more expensive.

Kurt Brunner studied technical chemistry at TU Vienna, where he received his doctorate in 2003 from the Institute of Chemical Engineering. While preparing his dissertation, he worked in the field of fungal molecular biology, with periods of research at the University of Naples. This was followed by postdoc work in several projects investigating plant-pathogen interactions at the University of Natural Resources and Life Sciences and TU Vienna. He has led the "Molecular Diagnostics" group in the Department for Agrobiotechnology (IFA-Tulln) since 2008. His research work focuses on the development of DNA-based test systems for the analysis of foodstuffs and water.

Celine Zahradnik studied biology at the University of Vienna, where she received her Diplom in 2010 from the Department for Systematics and Evolutionary Biology. During her degree, she worked as a research assistant in the fields of molecular biology and fungal chemodiversity. Since 2011, she has worked on her dissertation, entitled "Isothermal DNA Amplification Methods", in the Department for Agrobiotechnology (IFA-Tulln).

Genetically-modified maize

One topic that has attracted heated debate over the years is the addition of genetically-modified maize to a range of products and animal feeds. The risk posed to consumer health by the consumption of genetically-modified organisms (GMOs) has not been exhaustively investigated to date and therefore remains an open question. Notwithstanding this fact, many countries have introduced national legislation concerning the threshold value of GMO content above which a product must be appropriately labelled. Even in these cases, however, labels can still be economical with the truth.

Allergens in food

Although most allergens can be detected via antibodies using an ELISA (enzyme-linked immunosorbent assay) test, this method cannot be used in some situations. Celery offers one such example: it is too closely related to other plants such as the carrot and parsley, which are often included side by side with celery in recipes for sauces, condiments and pre-packaged meals. Celery has been classified as a potential allergen since 2005, however, and must therefore be declared on the label. This, in turn, can be achieved only with recourse to a DNA-based detection method.

All of the potential ingredients named above are sourced from living organisms and therefore contribute their specific DNA to a product. Not only can this be used for specific detection but DNA also possesses key benefits compared to other analytes. It is highly stable, i.e. analysis is possible even for food that has been heavily processed. Even after prolonged boiling, DNA suffers only minor damage and can still enable positive identification. In addition, it can even distinguish between organisms that are very closely related – such as celery and parsley – and only a few copies are sufficient for reliable identification.

Revolution in a test tube

Kary Mullis developed PCR in 1983, thereby fundamentally changing the future of molecular biology and molecular diagnostics. From this point onwards, it became possible to make millions of copies of a single target DNA molecule within a few hours – thus facilitating its detection. One major disadvantage of conventional PCR is the necessity to use a thermal cycler, which must periodically heat, cool and re-heat the reaction mixture in cycles of a few seconds. The machine is not only expensive itself but incurs costs in terms of its maintenance and operation by trained personnel. In addition, the DNA, which is extracted from a complex sample matrix, must also be properly purified, since co-isolated inhibitors can disrupt the reaction procedure.

Isothermal DNA amplification as an alternative to PCR

The discovery of thermostable DNA polymerases exhibiting "strand displacement activity", i.e. with the ability to take the DNA double helix apart by "unzipping it" and thus simultaneously permit the extension or de novo synthesis of an individual strand, has rapidly led to the development of many novel amplification techniques. One of the most fundamental benefits is the fact that these reactions can be conducted in isothermal – i.e. constant – conditions of temperature, therefore dispensing with the need for cycles at different temperatures. This property also obviates the need for the thermal cycler familiar from conventional PCR: instead, isothermal amplification reactions can be carried out on a simple heating block. Energy consumption for this constant-temperature process is also much lower than that required by alternate heating/cooling cycles, thus offering potential for a small, battery-operated instrument.

LAMP: the isothermal racehorse

One isothermal amplification method in particular has gained a stalwart following, namely "loop-mediated isothermal amplification" (LAMP) [1]. This method not only offers the appealing features of ruggedness and stability but is also very simple to perform (fig. 1). The reaction itself is complex, however: six separate primers bind to the target DNA and modify the strand in such a way that the ends bind to themselves in loops, forming a dumbbell-like structure. As agglomeration of the primers to this structure successively continues, a longer and longer chain of consecutive target sequences is cre-

ated, enabling the amplification of a tremendous amount of DNA in under half an hour. The advantage to this process is that the required reagents can be ready-mixed and freeze-dried beforehand: the only components then required to start the reaction are water and the extracted sample. The reaction vessel is simply heated to a predefined temperature and the reaction then starts immediately. After the prescribed period of roughly 30 to 45 minutes, a fluorescent intercalating dye is added. This type of dye can interact only with double-stranded DNA, which can itself be produced only by successful amplification of the target molecule. If this dye is able to insert itself between double-stranded DNA, then a colour change takes place from deep orange to fluorescent green (fig. 2). Neither a UV lamp nor any other equipment is needed for detection: the colour change is clearly visible to the naked eye.

"Pocket PCR"

If there is no need to procure, maintain and operate a thermal cycler, this offers enormous savings in terms of time and money. One heating block measuring about 10x15cm is sufficient for performing an isothermal reaction and the result can be interpreted immediately using the naked eye (fig. 3). The possibilities for developing tests for problematic issues such as horsemeat, bacterial contamination, GMO maize, etc. are almost unlimited. In the future, it should be possible to conduct such tests on-site: all that will be required for this is a small carrycase containing all of the necessary reagents and equipment, with which all of the procedures – from DNA extraction to the test results – can be performed in less than two hours.

The Molecular Diagnostics Group at IFA-Tulln has already developed isothermal amplification tests for celery [2] and genetically-modified maize [3]. Current developments are focusing on distinguishing between different animal species (horse, cattle and pig) using these highly promising methods. All of these experiments indicate that this simple analytical procedure is the equal of conventional PCR both in terms of selectivity and sensitivity. The focus of future test development work will broaden from detection methods for foodstuffs to include tests for water. Simpler, faster tests would also be necessary in this area to guarantee the long-term safety of drinking water consumption – particularly in the developing economies. The possibilities here are almost unlimited and it is entirely conceivable that conventional PCR will be supplanted by novel, isothermal methods in many fields in a matter of years.

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Picture: © Fotolia.com | photology1971

ebola

A mysterious fever

Ebola outbreak in West Africa

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Dr Markus Eickmann and Prof. Dr Stephan Becker
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In early March this year, deep in the forest region of southeast Guinea, cases started to accumulate for a mysterious fever that claimed many lives among its sufferers. Muscle pain, high fever, vomiting and severe diarrhoea – all symptoms that doctors often observe in Africa's hospitals, but this time, all attempts to treat them proved futile.

Fig. 1 Doctors and care workers can enter the isolation ward only with appropriate protective clothing. For medical personnel, the work is extremely exhausting and stressful, both physically and mentally. With temperatures outside exceeding 30 °C, focused work in the plastic protective suit with mouth guard, goggles and gloves is possible for only about an hour at a time. Children in isolation wards in particular are fearful of the masked helpers; the protective clothing makes communication with patients difficult.

Initially, patients were thought to be suffering from a severe form of malaria, which occurs throughout the year in this region. Accordingly, the local people went about the business of caring for their sick relatives and burying their dead as normal. Medical personnel from the NGO Doctors Without Borders, who had been providing support to a regional malaria project for a number of years, soon realised that this was not a typical malaria outbreak, however. The disease spread much too quickly, and whole family groups were falling ill almost simultaneously. On some occasions, patients from bodily orifices and died only a few days after falling ill. Doctors Without Borders alerted the Ministry of Health in the capital city of Conakry and the World Health Organisation (WHO). Epidemiologists were sent to the affected regions. By mid-March, the facts were clear: virologists from Lyon and Hamburg confirmed the outbreak as the first occurrence of Ebola fever in West African Guinea. This was the official starting-point of what has been the largest and most complex Ebola epidemic to date.

Previous Ebola outbreaks had been reported only in Central African countries. Over the last 38 years, repeated outbreaks were reported in the Democratic Republic of the Congo (formerly Zaire), South Sudan, Uganda and Gabon. The Ebola virus takes its name from a tributary of the Congo River. It was along this river that the first documented Ebola outbreak occurred in 1976: a total of 318 people from 55 villages fell ill, of which 280 died – equating to a fatality rate of 88%. The first case occurred in a Belgian missionary hospital. Shortly afterwards, almost all of the nuns and nurses had fallen ill, as well as most of the hospital's patients. The sisters possessed just five hypodermic needles, which they had used for hundreds of patients – without sterilising them after each use.

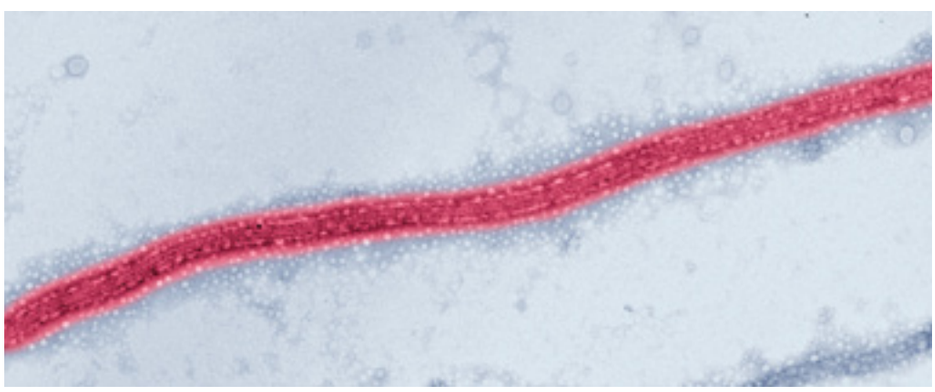

Fig. 2 Electron micrograph of an Ebola virus. In this colourised EM image of a Guinea isolate, the characteristic thread-like structure of the Ebola virus can be seen (image used with the kind permission of Dr L. Kolesnikova, University of Marburg).

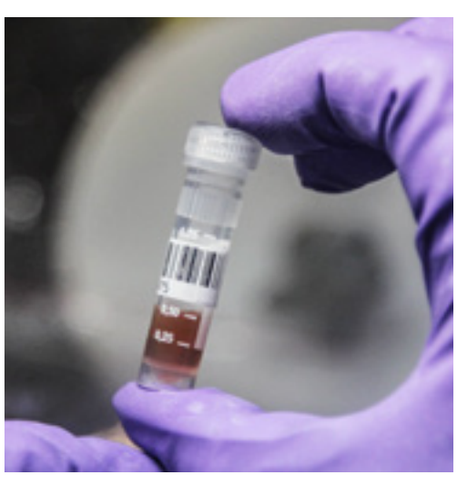

Fig. 3 Patient blood samples are tested for Ebola virus in the EMLab in West Africa. Stationed in Guinea, the tent lab is staffed by experts from all over Europe.

Fig. 4 All of the equipment, chemicals and consumables required for diagnostics in the laboratory are packed into plastic crates. The EMLab project (http://www.emlab.eu/) is financed by funds from the European Union.

From reservoir host to humans

Ebola viruses are zoonotic viruses. The natural reservoir for Ebola is thought to be various species of fruit bat. While the bats do not themselves fall ill, they excrete large quantities of the virus in their saliva and excrement. Normally, ebola viruses circulate unnoticed within the fruit bat population. Animals such as apes or forest antelopes can also become carriers as a result of their eating habits, however, if they pick up fruit partly eaten by fruit bats and infect themselves with the virus. Interestingly, the fruit bats are not only to be found in Central African regions. Their range also stretches as far as Western Africa. Every year, millions of fruit bats fly several thousand kilometres, chasing the rainy season, since the bounty of fruit to forage for is especially great at this time. While it is still unclear how Ebola came to be present in western Africa, it is logical to assume that fruit bats from Central Africa brought the Ebola virus to West Africa as part of their migrations. Transmission of Ebola viruses to humans is a relatively rare occurrence. In such cases, the infection occurs during the preparation and consumption of contaminated bushmeat – such as that from fruit bats or infected apes. As regards the current outbreak, it is assumed that the index case was an infant who probably caught the infection from a fruit bat and then proceeded to pass the infection on to family members. The spread and person-to-person transmission of the Ebola virus is achieved by direct contact with infectious bodily fluids, such as while caring for sick individuals or performing traditional burial rites.

Response of the human body to an Ebola infection

The incubation period of an Ebola infection ranges from 2 to 21 days. People fall ill suddenly and the body first responds by exhibiting non-specific 'flu-like symptoms such as fever, headache and muscle pain, followed by nausea with vomiting and severe diarrhoea. When the virus enters the body, it first infects immune cells such as dendritic cells, macrophages and monocytes: this enables the virus to spread rapidly throughout the organism. This primarily affects

vital organs such as the liver, spleen, lungs and kidneys; these suffer from necrosis and organ failure. The massive secretion of pro-inflammatory cytokines also causes endothelial permeability while disrupting blood clotting processes. As a result of the blood loss observed in a proportion of the patients, the Ebola virus is also termed a hemorrhagic fever virus. If disease progression is severe, the infection typically causes the patient to die from shock and multiple organ failure within 14 days after the first symptoms appear.

Currently, there is no specific treatment option available. The symptom-oriented measures are merely supportive in nature and consist of the administration of infusions against fluid loss, pain relievers and antibiotics, so as to prevent possible secondary infections. The poor adaptation of humans to this virus of animal origin, combined with the lack of availability of a licensed and properly tested form of therapy, means that almost 70% of patients with Ebola are dying in the current outbreak in West Africa.

Three major factors are responsible for continuous propagation of Ebola in West Africa

Despite all of the efforts by the international community, the outbreak has yet to be brought under control. Currently, there are three primary factors that are promoting the spread of Ebola within the affected countries. The first is the transmission of Ebola in heavily populated urban areas in the capital cities of Conakry (Guinea), Monrovia (Liberia) and Freetown (Sierra Leone). Many of those who fall ill migrate to the capital in hope of receiving better healthcare, thus introducing the Ebola virus into the city. Attempts to break the Ebola epidemic's chains of infection in major urban areas with populations of several million people constitute a major challenge for the local health authorities and international aid organisations. Due to limited facilities in terms of beds and personnel, large numbers of patients can no longer be accepted at treatment centres. The second factor is the transmission of Ebola in rural areas. Many Ebola patients continue to be cared for by family members, although strict isolation and quarantine protocols must be observed to prevent the virus spreading further. Many people also contract Ebola from funeral ceremonies. In accordance with local customs, the corpse is washed and touched during burial in a rite of farewell. Since the viral load of the deceased is extremely high and any virus on the dead person's skin remains infectious for some time, many people become infected at funerals. The third factor driving widespread propagation is the cross-border transmission of Ebola into the frontier areas of Guinea, Liberia and Sierra Leone. Restrictions on travel, intended to keep populations within their own state boundaries so as to counter the spread of Ebola, are essentially unenforceable within this tri-border area. Trade and social interaction are two reasons for frequent and unverifiable border crossings. Since many people travel to visit their sick relatives or attend funeral services across the border, this makes patients and their contacts extremely difficult to pin down.

Reasons for the scale of the current outbreak

How did the virus manage to spread from a few dozen infected individuals to affect some 7,157 people (official WHO figure as on 1 Oct 2014)? A number of specific cultural factors have been decisive in promoting the spread of the disease. Many people become infected during traditional burial ceremonies: the customs of washing the dead before burial and touching them in a rite of farewell are particularly problematic. Nor is there universal acceptance within the various populations that Ebola hemorrhagic fever is really an illness and not some sort of magic curse.

In the field lab, work with infectious patient samples is conducted in a glovebox. Internal glovebox pressure is negative and intake/exhaust air is filtered to ensure no viruses can escape to the environment. Once the samples in the glovebox have been deactivated, isolation of the viral RNA and subsequent PCR-based diagnosis can be performed without any special safety precautions.

The team of authors is participating in Ebola field diagnostics in Guinea as part of the European Mobile Lab project.

Marburg virologists Dr Svenja Wolff, Dr Thomas Strecker, Anne Kelterbaum and Dr Gordian Schudt (l to r) have each spent several weeks in Guinea as part of the EMLab project, so as to conduct on-site diagnostics of suspected cases of Ebola. Another virologist from Marburg is currently deployed in Guinea.

Thomas Strecker is a principal investigator in the Institute of Virology at the University of Marburg. His research interests focus in particular on investigating the molecular mechanisms of virus release in arenaviruses and filoviruses.

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Gordian Schudt is a postdoc in the Institute of Virology at the University of Marburg. He is particularly fascinated by the microscopic investigation of living cells that have become infected with Ebola or Marburg viruses. Within these cells, he investigates viral transport, assembly and release.

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Author photos: Katharina Kowalski

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Markus Eickmann is Head of the BSL-4 Containment Lab at Phillips University Marburg. His fields of research include biology and the diagnosis of high consequence viruses.

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Local people also harbour a strong distrust of Western medical personnel. There have been occasional attacks on vehicles and personnel operated by national or international aid agencies. Rumours and myths, which spread like wildfire, work to further fuel peoples' anxieties, with the result that many refuse medical help after falling ill. Shortly after the outbreak started, for example, one rumour was circulating that white doctors had spread the disease to tempt people into treatment centres, where their organs would be harvested for use in wealthy countries. The fact that the dead are returned to their relatives only in sealed body bags or caskets adds weight to this hypothesis, causing families to doubt whether they are really receiving their loved ones back for burial. As a consequence, families then hide away their sick relatives – and small children in particular – which in turn leads to further cases of Ebola and deaths within the family.

Another factor promoting the spread of the disease is the poor quality of the public health-care systems in the affected areas. This Ebola outbreak is the first in West Africa. The countries were entirely unprepared for the outbreak and simply have no experience that tells them how health authorities should be handling this disease. No action plans for epidemics had been prepared and opportunities for the laboratory-based diagnosis of disease are extremely limited. The provision of basic medical care is virtually non-existent in the tri-border area of Guinea, Sierra Leone and Liberia in particular. Still affected by the legacy of the earlier civil war, this area lacks the medical infrastructure and trained personnel needed to stop the further spread of the Ebola epidemic.

European experts join the fight against Ebola

In Germany, laboratory diagnostics for a suspected case of Ebola must be performed in a biosafety level 4 containment lab. There are only two facilities of this kind in Germany: the Bernhard Nocht Institute for Tropical Medicine in Hamburg and Philipps University Marburg both operate this class of containment lab. These sites work with extremely dangerous viruses while observing very strict safety protocols. For protection, workers wear a totally encapsulating suit with its own air supply. If an outbreak occurs in a developing country such as Guinea, however, logistical problems prevent the shipping of samples to Europe for analysis in such specialist laboratories. Furthermore, a local diagnostic unit is also essential for making timely decisions about whether a patient is suffering from a severe form of malaria (for example) and not Ebola. This has important repercussions: if the patient has Ebola, s/he must be placed in quarantine; if s/he has malaria, however, then quarantine would risk exposing the patient to Ebola. To bring an outbreak under control quickly, a lab diagnostics facility at the epicentre of the outbreak is of prime importance. In 2011, a number of institutions from all over Europe – including the Marburg Institute of Virology – joined forces to establish the European Mobile Lab (EMLab) project. The aim of the project was to set up lab units for rapid deployment during the diagnosis of dangerous pathogenic organisms in Africa. Conceptually, the mobile laboratory project involved designing a way to pack a fully-functional laboratory into 12–15 transportable boxes. These boxes contain all of the technical equipment and lab materials necessary to perform molecular diagnostic experiments. This concept has the major benefit of enabling the entire set of equipment to be shipped using normal civilian air transport: the scientists can take the lab with them as their "luggage". At the destination, only small vehicles are needed to transport the equipment. Once on-site, the laboratory can be operated using a local power supply running off car batteries or small generators.

Shortly after the outbreak began, the WHO requested help from EMLab in the fight against Ebola. In late March, only a few days after the outbreak went public, the first EMLab team set up the mobile lab in the town of Guéckédou, in the forest region of Guinea's southeast. Direct collaboration between EMLab and Doctors Without Borders makes it possible to find out whether a patient is infected with Ebola in just four hours. The diagnostic procedure used here is real-time quantitative PCR. Over a period of six months, some 2,500 samples have now been tested for Ebola. European experts have been deployed in Guéckédou continuously since March, so as to perform diagnosis of suspected cases of Ebola. Five virologists from Marburg have also participated, in the form of multiple four-week foreign assignments in one of the European Mobile Lab projects funded by the European Union.

Low risk of introduction to Germany

All of the countries affected by Ebola operate international airports with direct flights to other countries in Africa, the Middle East or Europe. In early October, the Atlanta-based CDC public health agency confirmed the first case of Ebola imported into the United States. The man in question boarded a plane in Liberia without symptoms before falling ill a few days after arriving in the USA. The patient's travel history meant that the illness was identified quickly, with the introduction of quarantine measures and the identification of possible contact persons being completed without delay.

The likelihood of the disease spreading via air travel can be calculated statistically for each international airport around the world (http://rocs.hu-berlin.de/D3/ebola/). These figures show that direct flights to Paris Charles de Gaulle in France or Brussels Airport make the likelihood of a passenger introducing Ebola higher for these airports than for airports in Frankfurt or Berlin. As the case imported into the USA shows, the possibility of the virus being introduced into Germany cannot be entirely excluded. As in the US, however, precautionary measures have also been taken here: contingency plans are in place at both airports and health authorities, and these have been supplemented by diagnostic procedures, trained personnel and isolation/treatment wards. Last but not least, the German population has a better understanding of the infectious origin of certain diseases and the hygienic precautions that need to be taken. It is nonetheless important to remember to ask travellers from West Africa who start suffering

ebola

National and international aid workers do not receive such a hearty welcome in every village. In some areas, anxieties, rumours and ignorance fuel aggressive tendencies that sometimes give rise to violent attacks: only a few weeks ago, the inhabitants of a village in Guinea ambushed members of a reconnaissance team, ultimately killing several of them.

The isolation ward in Guéckédou in Guinea's forested region has been run since March by Doctors Without Borders (Médecins sans Frontières). The large number of patients and the widespread propagation of the outbreak has pushed national and international aid agencies to their limits – additional support is now urgently needed.

from a high fever if they could have had contact with sick individuals. In such cases, an Ebola diagnostic procedure must be performed immediately. The patient must be moved to a special isolation ward, where s/he can receive the best-possible treatment without infecting other people. If these precautions are taken, then an outbreak of Ebola in Germany is very unlikely.

Far-reaching consequences of the outbreak

The end of the current Ebola outbreak is very difficult to predict. Cautious estimates published by the WHO assume that the epidemic could be brought under control by mid-2015. The further progression of the epidemic depends on a large number of factors. Direct action must be taken without delay. Medical personnel, logistics staff and more field hospitals must be transported to Africa, alongside large quantities of protective clothing. This is possible only with the concentrated effort of many countries. Financial aid alone is not sufficient. International attention and the recognition that a humanitarian crisis is currently occurs in West Africa – and one which could destabilise the entire region – have come very late. In affected regions, attempts at translation work must be redoubled, so as to integrate the concept of infectious diseases into the local inhabitants' cultural background. Effective interventions to protect against infection must be established that are compatible with day-to-day life. Local aid workers not only have to deal with a lack of acceptance from the population but also face severe physical and psychological challenges; they are often overwhelmed by the workload and the dire nature of the outbreak. To alleviate this situation, greater numbers of trained personnel are required.

Drugs for treating Ebola – as were deployed to treat medical personnel flown out of the country – are likely to remain unavailable to sick individuals in West Africa for the foreseeable future, since they can be manufactured only in small quantities at this time.

The World Health Organisation is now eagerly awaiting the results from two ongoing vaccination trials. These studies are designed to confirm both immunogenesis and harmlessness for a vaccine that has previously not been tested in humans. In the best-case scenario, several thousand doses of the vaccine could be made available to the West African population in 2015, stopping Ebola from spreading further.

Seven months have passed since the low-profile start to this outbreak. Since then, more than 7,000 people have contracted the virus, with well over half of all patients ultimately succumbing to their infection. Initially affecting a handful of villages in the forested region of eastern Guinea far from the capital city of Conakry, the outbreak has since spread to the West African countries of Liberia and Sierra Leone, with cases also being reported in Africa's most populous country, Nigeria, as well as Senegal. The WHO expects to see about 20,000 cases by the end of 2014. Ebola is no longer a health problem that is limited to isolated countries: it threatens to destabilise West Africa. The global community must now mobilise all of its available resources, so as to finally bring the worst Ebola outbreak in history under control.

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Pictures: Thomas Strecker

water analysis

In a class of its own

OMI (Organic Molecule Identification) in water using LC-MS(/MS): Steps from "unknown" to "identified": a contribution to the discussion

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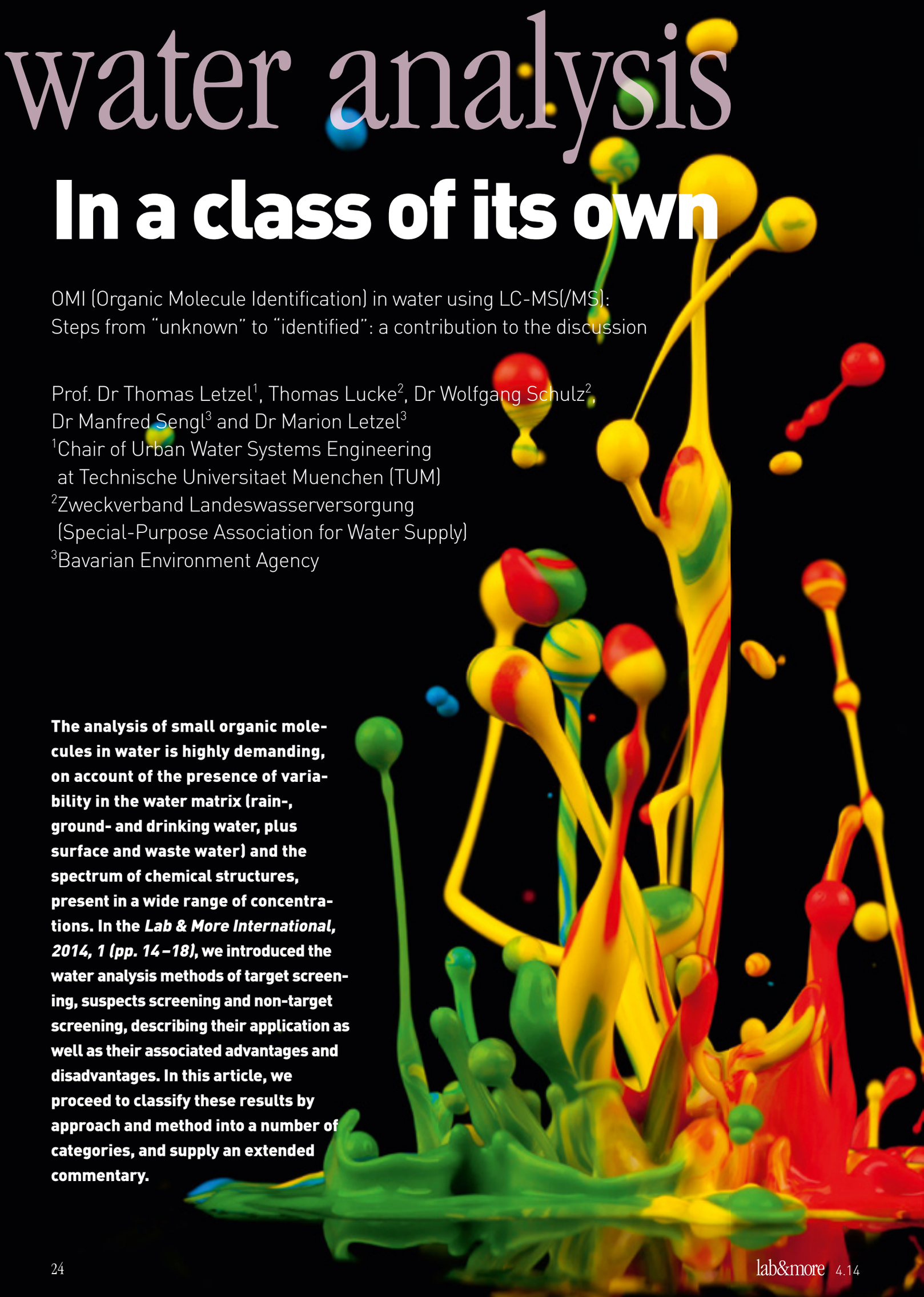

The analysis of small organic molecules in water is highly demanding, on account of the presence of variability in the water matrix (rain-, ground- and drinking water, plus surface and waste water) and the spectrum of chemical structures, present in a wide range of concentrations. In the *Lab & More International, 2014, 1 (pp. 14–18)*, we introduced the water analysis methods of target screening, suspects screening and non-target screening, describing their application as well as their associated advantages and disadvantages. In this article, we proceed to classify these results by approach and method into a number of categories, and supply an extended commentary.

Present-day analytical detection systems have not only shown rapid development but are also becoming more specific and more sensitive. One could of course imagine the task of testing water to detect molecules dissolved in it to be a very simple one – especially when using liquid chromatography (LC) coupled with (tandem)-mass spectrometry (MS(/MS)) [1, 2 fig. 1]. This conclusion is also suggested by the prevalence of LC-MS(/MS) equipment in water analysis labs.

This coupling is indeed very successful when deployed for the quantification of known small organic molecules in water, as it is a superlative analysis method for molecules that are water-soluble and easily ionised. This is especially true if one can separate the molecules from one another chromatographically prior to detection with mass spectrometry [3, 4]. Such work involves the use of conventional reverse-phase liquid chromatography (RPLC; for medium-polar and non-polar molecules) and, more recently, hydrophilic interaction liquid chromatography (HILIC; for polar molecules) [5].

As a result of the increased use of accurate, high-resolution mass spectrometry (HRMS), qualitative analysis targeting the presence of organic trace substances is also possible. The task here is to analyse the sizeable dataset thereby generated, which presents analysts with substantial challenges: goal-oriented dataset analysis on the one hand and – in particular – the resultant analytical findings on the other. The original assumption – that mass spectrometry results are themselves sufficient to unambiguously characterise the molecules – proved ultimately untenable, not least due to the proliferation of isomeric compounds [6]. Current consensus states that, without further background knowledge, complex samples of this type are effectively beyond comprehension or evaluation [2, Fig. 2]. Unavoidably, therefore, one must evaluate the results obtained before then assigning them into categories. Categorisation takes place depending on the level of organic molecule identification (OMI) and the strategic orientation. Accordingly, the category thus results from the analytical method used on the one hand but also from the databases and reference standards used on the other.

Baseline situation for OMI categorisation

In recent years, the analytical techniques and strategic approaches within water analysis – while initially discrete – have moved slowly but surely to a more uniform perspective. In the water community, there is consensus not only on the identification strategy with instrumental analysis but also on the use of databases and reference substances for the full identification of trace substances.

Just recently, the team headed by J. Hollender published a classification of measurement results that is subdivided into levels 1 to 5 (incl. 2a/2b) [7]. The assignment is primarily based on the use of high-resolution, accurate-mass mass spectrometry. Other details are also considered as part of the evaluation strategy. Readers should take the time to consult this "Viewpoint" article since knowledge of its content is assumed by discussions in the sections below.

The complementary categorisation presented here accords with the one referred to above. Supplementing this approach with comparison testing from multiple (here: two) labs extends it while also employing it as a further basis for interpretation.

Principles of OMI categorisation with multiple associated laboratories

Organic molecules are viewed as being identified in the proper sense of the word when one has decoded these initially unknown substances to the extent that one was able to produce a reference substance and confirm this physically/chemically by using synthesised reference materials. Following the definition of this OMI as category 1, category 2 is then utilised for two types of molecule without an explicit reference substance: ones unambiguously assignable either via analytical chemical

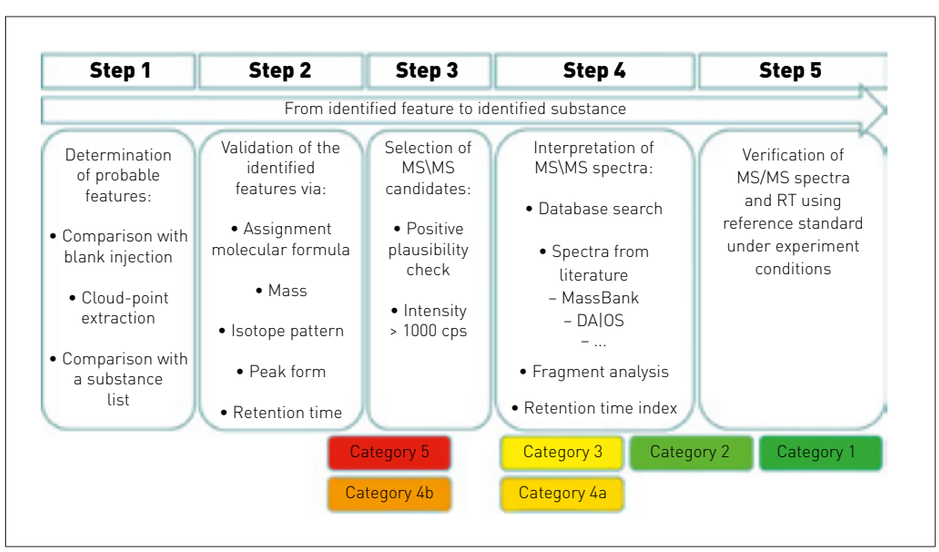

Fig. 1 Identification workflow, incl. categorisation of results

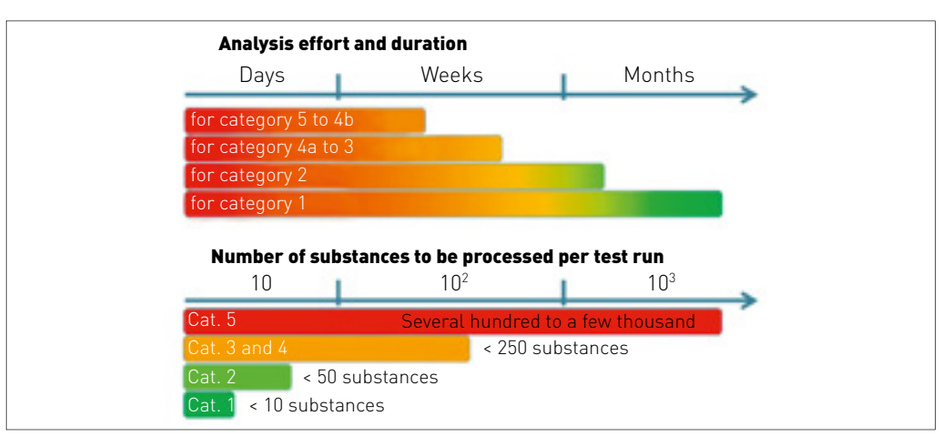

Fig. 2 Analysis effort and duration

RISK-IDENT project group at the last annual meeting in February 2014. Article authors are circled in red; from left to right: Prof. Dr Thomas Letzel, Dipl.-Ing. (FH) Thomas Lucke, Dr Wolfgang Schulz, Dr Marion Letzel, Dr Manfred Sengl

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data or with the aid of substance databases (2a); or ones that can be viewed as confirmed via what are termed "diagnostic fragments" from the mass spectrometry used (2b). If both criteria are satisfied, then classification of the OMI as category 2 can be maintained until the OMI is promoted to Category 1 by comparison with a reference substance. While category 2 can be achieved by a single laboratory (or mass spectrometry system), it can also be obtained as a result of combining the data from multiple laboratories (or multiple mass spectrometry systems). The OMI is assigned to category 3 in cases where even the participation of multiple labs (or mass spectrometry systems) does not generate consensus on an unambiguous molecular structure. Such cases occupy a "grey area" within identification, so to speak: while some evidence speaks for the OMI concerned, this evidence is not entirely unambiguous. While the participation of multiple laboratories can corroborate a conjecture, it may not necessarily produce unambiguous evidence. In this process, an MS/MS result can be coupled with the accurate mass from a second mass spectrometer. If the second system does not refute the structure – e.g. via a different molecular formula – it can then be assigned to category 3.

Category 4 is subdivided into two sub-categories (4a and 4b). By definition, a substance is typically assigned to category 4 (a or b) in cases where a) it could be measured only by a single mass spectrometer (incl. MS/MS measurement) or b) could be determined by multiple laboratories (accurate mass, but without any MS/MS results). As such, the use of suitable additional laboratories (or technologies) can mean category 4 is rapidly superseded, with promotion possible to at least category 3. To category 5 are assigned what are termed "masses of interest". This category can also contain (non-) accurate masses of molecules from individual laboratories.

Categorisation using the following levels of organic molecule identification (OMI):

- Category 1: OMI confirmed by reference substance
- Category 2: OMI via multiple unambiguous indicators (without reference substance), possibly with restrictive subdivision: 2a: OMI unambiguously confirmed solely by using databases
  2b: OMI unambiguously confirmed solely by using "diagnostic fragments" (from production spectra)
- Category 3: OMI with results from multiple laboratories (incl. MS/MS and accurate mass) but without unambiguous indicators
- Category 4: OMI with initially non-assignable results from 4a: from a single laboratory (incl. MS/MS)
  4b: from multiple laboratories (without MS/MS) with molecular formulas
- Category 5: OMI with "masses of interest", which are non-assignable

Strategic methodology for OMI categorisation

What, then, do we need for the categorisation of analytical data? What is the underlying workflow for OMI?

First, we establish an analysis on the basis of LC-MS/MS. Identification strategies are also available for other separation/detection techniques, with correspondingly similar variants. The strategy described in detail here defines a workflow comprising five steps. The category to which a "mass of interest" can be promoted in the course of these five steps depends on the results obtained within each individual work step. Note: the five categories used for an iden-

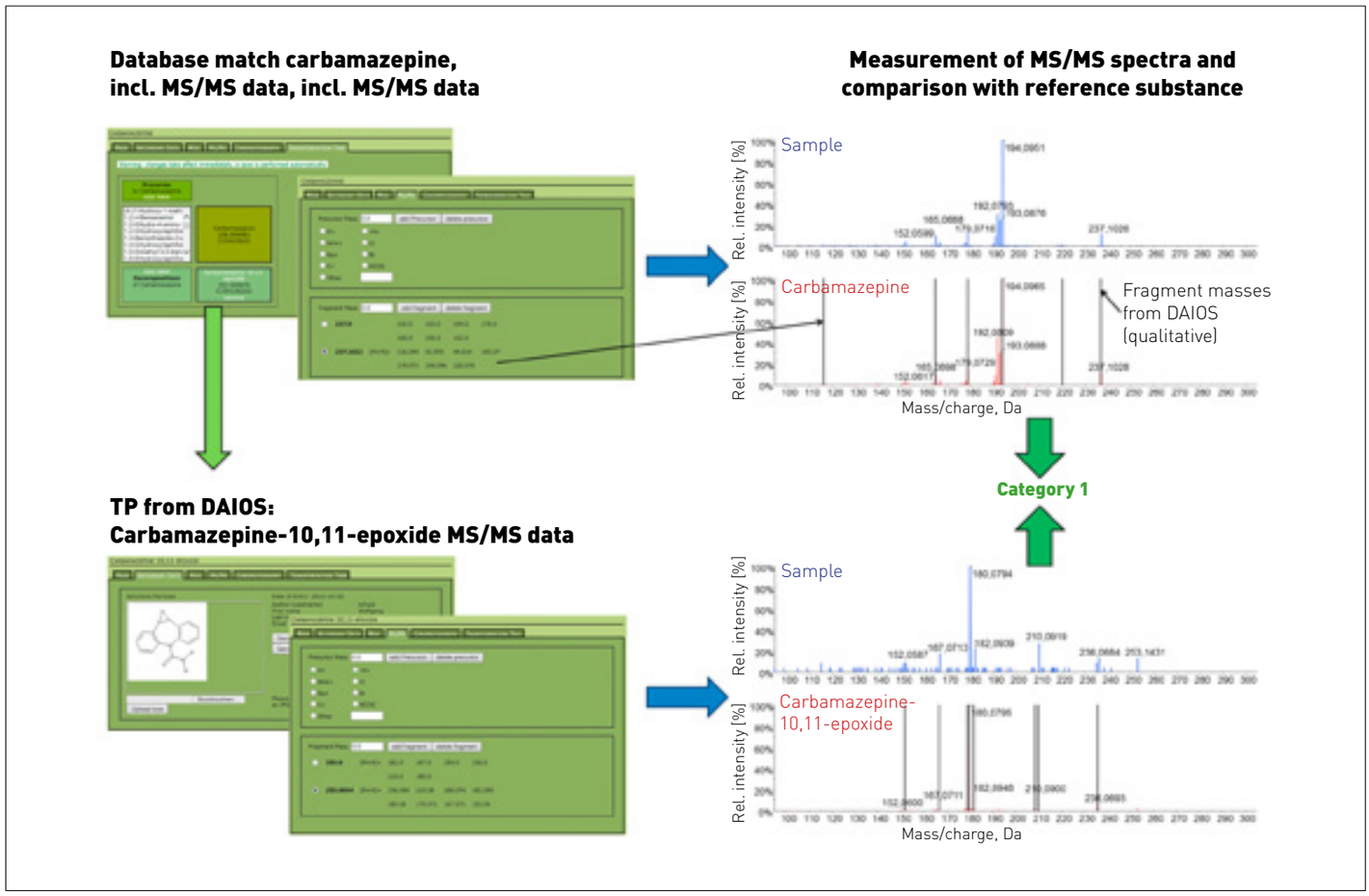

Fig. 3 Database search and subsequent identification of database matches using LC-MS/MS

tification do not correlate with the five work steps to be performed (see fig. 1).

Step 1 comprises the fundamental data processing, such as "feature" recognition, accounting for blank values and graphical reproduction. In this step, blank value correction is to be seen as the most important step, taken in order to filter out false positives. The features determined (exact mass peak with corresponding retention time) can then be compared even during this step with a database, in order to obtain some initial suspected compounds. If this option is skipped, then the features are processed further without assignment to suspected compounds.

Step 2 involves plausibility checking of the analytical data obtained in step 1, with the aid of this data's physical and chemical properties. Features without the additional information of a suspected compound can be assigned to a molecular formula here, by taking into account the exact mass and the isotope pattern.

In step 3, predetermined parameters are used to select features that are referenced for the separate performance of experiments using (tandem)-mass spectrometry (e.g. QTOF-MS). Here, the selection of the threshold (i.e. the minimum height of the selected peak) is a key parameter, necessary in order to obtain meaningful and sufficiently high-intensity production spectra. This step is also the first in which the OMI categorisation is decided, since the MS/MS spectrum constitutes an essential decision-making aid for assignment. If recording of MS/MS data is possible in only one participating lab, then the only possible assignment is to category 4b. Category 5 contains all components for which no fragment ion spectra could be obtained.

At this stage, it is also entirely possible to record qualitative data from regular samples by using a triple quadropole mass spectrometer. Assuming the MS units stem from the same maker and have identical source geometry, this involves applying the fragment masses recorded in the product ion spectrum for identification to a triple quadropole method with corresponding LC method. This offers a highly-sensitive and selective procedure for the qualitative determination of unknown substances even before final assignment to a category with modest effort for larger numbers of regular samples. At the same time, this enables an initial prioritisation by environmental relevance.

Currently, step 4 is still time-consuming and may also require additional measurements to obtain meaningful results following interpretation of the measurement data. The results from this step are definitive for the identification of a compound, since they form the basis for decision-making as to the reference substance with which step 5 must be performed. Further validation typically utilises data from separate laboratories; this requires additional effort in terms of time and coordination.

If step 4 is successful, then the 5th step can proceed with the unambiguous assignment of the previously unknown substance. This requires the availability of an existing or – in the more complex case – newly-synthesised reference substance.

Assignment of OMI categorisation

The time taken depends on the category and is highly variable (see fig. 2 Top), ranging from just a few days to as long as several months for the full and complete identification (category 1) of a compound. The procurement or synthesis of a reference standard is one of the most time-consuming and costly steps in this strategy.

At the same time, the number of components to be processed falls drastically during the course of analysing a sample, from the first step (feature finding) through to potential identification by use of a reference substance (see fig. 2 Bottom). The scope of

component reduction work is strongly dependent on the choice of parameters during plausibility checking (step 2) and the selection of candidates for additional experiments, such as the recording of product ion spectra. The extent to which a dataset must/can be reduced is decided in accordance with the number and intensity of components obtained by feature finding. It is certainly advisable to select the highest-intensity or most striking components for further analysis. A useful aid to initial decision-making here is to query the matches in substance lists and to use the associated metadata, such as (e.g.) toxicity data, degradation rates when evaluating technical processes or specific issues of interest arising from the context of the investigations performed. One example of such a process-driven issue might well be the discovery of theoretically determined transformation or degradation products for previously identified compounds during a specified process.

Application of OMI categorisation

– using the example of carbamazepine and its transformation product carbamazepine-10,11-epoxide

The methodology for database searching and further analysis of regular samples is based on an LC-QTOF screening measurement with positive electrospray ionisation. Suitable application software is used to extract features from the MS scan data. These features are characterised by their exact mass and retention time. A comparison of the features detected with a materials database such as STOFF-IDENT [8] or DAOIS [9] using the exact mass results – in our sample case – in a match for carbamazepine.

By consulting the database entry for carbamazepine in DAOIS, the corresponding transformation product (TP) carbamazepine-10,11-epoxide entered is then used for investigating the raw data. Prediction tools such as the University of Minnesota's Predicted Pathway System (UM-PPS) [10] also offer a further option for locating transformation products. These proposed formulas and the exact masses that are calculated from them are used for the investigation of the raw data. In this way, further analysis not only uses suggested known starting compounds – sourced from the database searches – but supplements this on an ad hoc basis with potential transformation products. Identification of the starting compounds and their TPs is now conducted jointly using the same analysis strategy. Once the defined parameters have been satisfied in terms of plausibility checking and intensity, the next step is then to record and interpret the product ion spectra.

The (tandem)-mass spectrometry results then serve as the basis for assigning the substances to the respective categories. By recording the MS/MS spectra solely in the regular sample and without measurement of the MS/MS spectra for a reference substance, the fragment masses listed in DAIOS would already enable an assignment of both substances to category 2. The highest degree of confirmation (category 1 – see figure 3) is achieved if the reference substance is present, and product ion spectra are recorded for both sample and standard – thus enabling confirmation of the compound.

Summary

An OMI categorisation based on LC-MS(/MS)-based data obtained from water samples is possible from a number of perspectives. One such perspective published recently takes as a starting-point a lab and its facilities, i.e. is oriented on the mass spectrometer, databases and/or reference substances.

The categorisation presented in this article also takes into account the analytical environment of individual laboratories, utilising the association of this – in the "best case" – supplementary and complementary information. To be able to compare data, one crucial factor in this approach is naturally the adoption of shared standards by the laboratories. Retention times on dissimilar columns can thus be harmonized or – even more usefully – TOF-MS data (besides the accurate mass) can be validated by QTOF data and extended by tandem MS measurements (with structural information) as required. Experience has shown that the relaying of these MS/MS data is a useful tool in the qualitative analysis of unknown substances in regular samples with the aid of a triple quadrupole method. Whether case-based or generic, both databases and reference standards can be both shared and updated very simply among networked laboratories.

This is another reason why we firmly believe that the future of screening techniques is to be found in the networked lab environment. Ultimately, this will lead to the adoption of lower-end LC-MS systems with low-resolution MS technology by routine lab testing, with these units being extended by expensive, high-resolution, MS systems as needed. In addition, work on producing a shared data repository in this field will have the effect of increasing the informational density of existing substance databases, thus further improving the professional use of such databases. We are looking forward!

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The meaning of peak capacity

Does increased LC peak capacity equal increased value for complex sample analysis?

Dr Frank Steiner, Thermo Fisher Scientific

Despite the strength of liquid chromatography (LC) to separate compounds through well tunable selective retention, its efficiency lags behind gas chromatography (GC) that can deliver up to several millions of plates per column. Resolution improvement in LC should thus be addressed with selectivity optimization first. If samples get too complex, however, this approach is no longer feasible, as improving selectivity in one part of the chromatogram will automatically negatively affect peak resolution in the neighborhood. Hence, higher efficiency is the best way to resolve complex chromatograms. Small particle phases in long columns make best use of modern UHPLC pressure capabilities to boost efficiency further. Two-dimensional LC (2D-LC) is another powerful way to a significant increase of separation power. A common measure for the separation potential in chromatography is the peak capacity n_C. This is the maximum number of peaks that can be separated with a resolution of R = 1.0 if equally distributed over the entire chromatogram. Moreover the peaks should be symmetrical and of similar size to estimate n_C directly from column plate numbers (N). As real life separations are mostly far away from this ideal world, peak capacity is often challenged for its practical relevance. We will assess examples for one-dimensional and a two-dimensional method on how peak capacity translates into analytical benefits.

Estimating peak capacities from column plate numbers

Gradient methods can generate peak capacity higher by a factor of two than isocratic methods on the same column in a similar analysis time. The reason is that in isocratic elution peak width steeply increases with retention time, whereas in gradient elution, peak width is not as much affected by retention time. In fact, peak width is often regarded as quasi-constant throughout the chromatogram. Hence, gradient elution is a must to resolve complex samples. Formulas to calculate the theoretical n_C in gradient methods are advanced and depend on retention parameters that may differ for the individual components of a sample. To estimate n_C, in a common wide range reversed phase gradient for complex samples, where the organic content changes by at least 60% and the gradient volume V_G exceeds by at least 30 times the column flow-through volume V_M, the following formula can be applied:

$$n_C \approx 2 \cdot \sqrt{N}$$

Considering maximum plate numbers of 100,000 < N < 150,000 for long UHPLC columns or coupled columns, it is possible to achieve peak capacities of 600 to 800 on a column length of 40 to 50 cm. Under a pressure of 1200 to 1400 bar, such columns can be run at their maximum efficiency with approximately 5 mm/s linear velocity. This allows for a t_M of about 1 min so that gradients with $V_G = 30 \times t_M$ can be run in about half an hour. While this is an impressive peak production rate of 25 peaks/min,

the absolute peak capacity remains limited in 1D-LC.

Extending to a second chromatographic dimension enables peak capacities of 3000 to 5000 because the total n_C is now a combination of both dimensions according to the following formula:

$$^{2D}n_C \approx {}^{1}n_C \times {}^{2}n_C$$

This rule only applies if the following three prerequisites are fulfilled:

- Combination of two orthogonal separation mechanisms, through independent retention and selectivity criteria in both dimensions
- Coverage of entire retention space in both dimensions, by distribution of peaks from lowest to highest retention in first and second dimension
- Optimized sampling rate from 1st dimension by collecting twice as many fractions as its peak capacity

None of the three criteria can be easily met, but the third mainly affects the overall analysis time. Even with a very fast method of 2.5 min cycle time in the second dimension, analyzing 100 fractions from the first dimension to preserve $^{1}n_C$=50 would result in more than 4 hours analysis time. Such a 2D-LC setup could theoretically come close to the impressive number of n_C = 10,000, which would be equal to a production rate of 40 peaks/min. Besides the significant instrumental challenge involved, such 2D methods can hardly be achieved in practice. Typical 2D peak production rates are lower than in 1D-UHPLC. Normally much lower numbers of fractions than 100 are transferred from the first dimension. Therefore the peak capacity in the first dimension is relatively meaningless and always limited by the number of fractions in practice.

1D-UHPLC example for the untargeted exploratory assay of traditional Chinese medicine (TCM)

Like most natural products, TCM samples are normally very complex and can contain hundreds of compounds that are relevant for a screening assay to characterize them. Figure 1 demonstrates how the use of 2.2 µm particles packed in long columns facilitates such TCM analyses. The original method on a 250 mm column with gradient duration of 18 min yielded a peak capacity of n_C = 590 and created a pressure of 915 bar at the start of the gradient. To fully exploit the 1500 bar pressure capability of the Thermo Scientific™ Vanquish™ UHPLC system, the column length was extended to 400 mm and the gradient time to 29 min to further increase the peak capacity. The pressure was now at 1360 bar, but a peak capacity of n_C=720 was determined for this method. This increase of 22% followed well the theoretical expectation of a peak capacity increase with the square root of the column length extension under respective gradient adaptation. A zoom into a particular part of the chromatogram shows how the improved method provides better resolution. Of interest was the overall benefit of the method with increased peak capacity and how this can be quantified in such a screening method where peaks are not identified. The table in Figure 1 shows some figures of merit to compare both methods. Under defined integration parameters the overall number of peaks, as well the number of peak pairs with a certain resolution is depicted. The number of identified peaks was more than 60% higher on the 400 mm column setup. Even more relevant are the statistics on the peak pair resolution. At all resolution levels the improved method provided more than 50% additional peak pairs to meet this criterion. At the relevant R = 1.5 level the gain was even more than 50%. At least for this TCM example, the result is a clear proof of the practical value of n_C to improve methods for complex samples.

2D-LC/MS method comparison for proteomics workflows

Bottom-up proteomics workflows use LC/MS peptide mapping after digest of the sample with a protease. They then identify proteins from characteristic peptide m/z. Bacteria cells like *E. coli* contain more than 1000 characteristic proteins in a cytoplasmic fraction and, hence, up to 10,000 peptides in a tryptic digest. Figure 2 shows the application of two different 2D-LC methods applied to the same tryptic digest of *E. coli*. One method combines strong cation exchange (SCX) with reversed phase (RP) separation in the second dimension. The other method uses RP in both dimensions but at significantly different pH values of the mobile phase (9.6 and 1.9). The second dimension provided in both methods $n_C \approx$ 220. Given that the cycle time for the 2nd dimension was close to one hour, only 10 fractions were cut from the first dimension. Hence the maximum peak capacity of both methods was about 2200 (10 x 220). A closer look at the plots reveals that the patterns of the retention space coverage are different between both methods. The RP-RP method showed a pattern where most peaks were oriented along the diagonal which indicates higher correlation and thus limited orthogonality. The SCX-RP method showed more random distribution, but another difference between both methods is also obvious. Most peaks elute in the central fractions from the SCX run and are not so well distributed, compared to the 1st dimension run of the other method (RP at pH 9.6). The effective peak capacity of both methods needs correction by a surface coverage factor, which was graphically determined to 60% for the RP-RP method and only 47% for the SCX-RP method. Therefore the effectively usable peak capacity of the RP-RP method would be approximately 1320 (2200 x 0.6) and for the SCX-RP meth-

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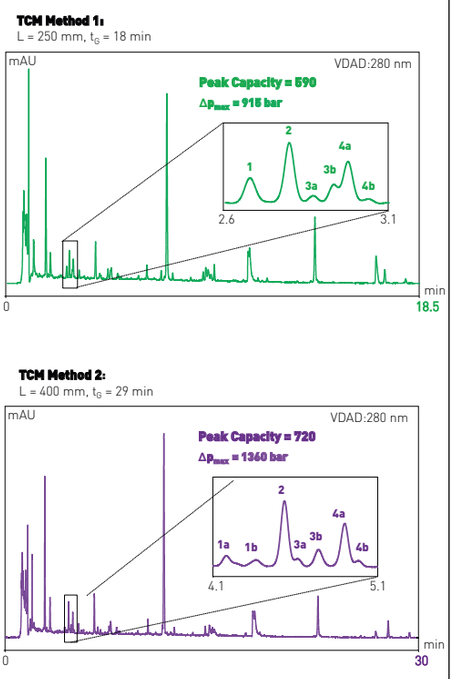


Stationary phase: Acclaim RSLC C18, 2.2 µm
Value of both methods:

Criterion	n_C = 590 method	n_C =720 method
Total peak number	125	206
Number of peaks with RS > 0.8	118	187
Number of peaks with RS > 1.0	114	179
Number of peaks with RS > 1.2	108	168
Number of peaks with RS > 1.5	101	163

Fig. 1 Comparison of generic exploratory method for traditional Chinese medicine run on a Vanquish UHPLC system with two different column lengths. Other conditions were as follows. Column id: 2.1 mm, L = 400 mm setup : coupling 150 mm and 250 mm columns with Thermo Scientific™ Viper™ coupling device, gradient : from 0% to 100% acetonitrile, flow rate : 670 µL/min, column temperature : 45 °C.

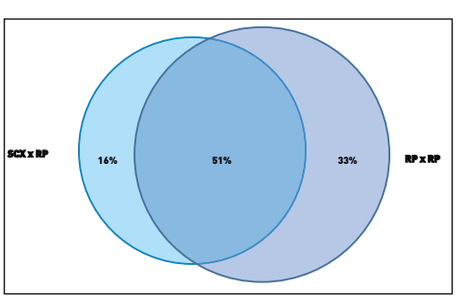


Fig. 3 Venn digram to show the complementary performance of the two methods from Figure 2. Ratio of proteins are shown as identified from MS/MS identficiation using an ion trap mass spectrometer and search against a Mascot™ data base (Matrix Science Inc.).

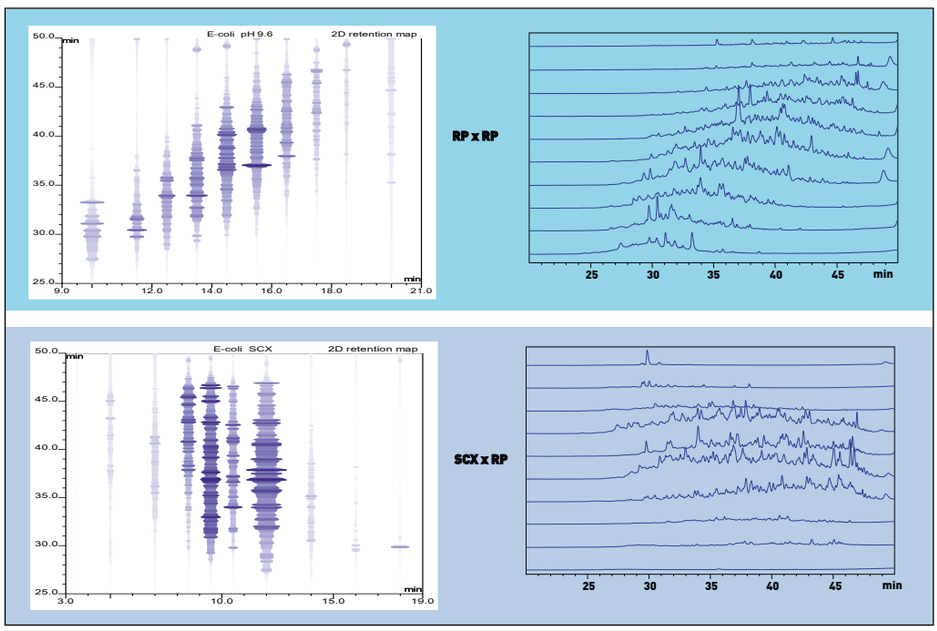


Fig. 2 Comparison of two different automated off-line 2D-LC analyses of *E. coli* tryptic digests run on a Thermo Scientific™ UltiMate™ 3000 RSLCnano system, with WPS-3000TFC autosampler with fraction collection and re-injection option. 10 fractions of 1 minute were collected from 1st dimension into 384 well plate. 1st dimension run on 300 µm id, 150 mm length capillary columns at 6 µL/min flow, 2nd dimension run on 75 µm id, 150 mm length nano columns at 300 nL/min flow. SCX column was a PolySULFOETHYL Aspartamide, 5 µm run in a phosphate buffer pH 3 with salt gradient up to 0.6 M NaCl in 15 min. All RP columns were Thermo Scientific™ Acclaim™ PA2 5 µm. First dimension RP was run at pH=9.6 with triethylamine and gradient to 50% acetonitrile in 15 min. Second dimension RP (both methods) was run at pH=1.9 with trifluoroacetic acid and gradient to 50% acetonitrile in 30 min.

od only ca. 1030 (2200 x 0.47). The ratio of identified proteins and how the results match between both methods is shown in Figure 3. The method with 28% higher effective peak capacity identified a 25% higher number of proteins (84% vs. 67% of total identified proteins). It is important though, that 16% of all identified proteins could only be found with the apparently less powerful SCX-RP method.

So what can we conclude on the practical value of peak capacity?

A column chain of 400 mm length using a 2.2 µm stationary phase yielded a peak capacity of n_C = 720 in a 36 min analysis cycle time when run at 1360 bar maximum pressure and supporting a productivity rate of 20 peaks/min. When comparing two different column lengths the number of well resolved peaks in a complex TCM mixture increased with the increase of column length. This was exceeding expectations based on peak capacity increase.

A 2D-LC method comparison in proteomics using nano-LC/MS in the second dimension was done with conventional 5 µm columns and a very shallow gradient in the 2nd dimension. The overall analysis time was 600 min. Peak capacities of up to 1300 could be achieved (after correction with retention space coverage rate). This yields a factor of 10 lower peak production rate but an almost twice as high peak capacity relative to the 1D-UHPLC method. In a nutshell:

- Peak capacity in 1D- und 2D-LC proved to translate into value for complex sample analysis in different application fields.
- Peak production rates are higher in 1D-UHPLC and peak capacity is usually sufficient, but 2D-LC is the only way to significantly exceed a peak capacity of 1000.
- 2D-LC combines the selectivity from two different methods and improved separation efficiency can only be achieved if the chosen combination is complementary, as in the example shown in this article.

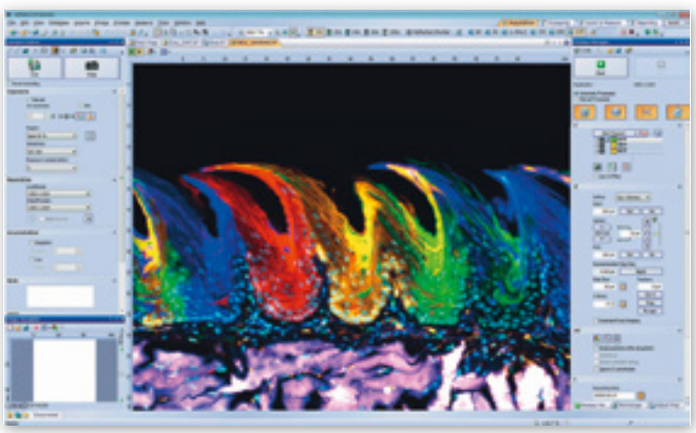
→ **frank.steiner@thermofisher.com**

Picture: © istockphoto.com | PaulCowan

Extremely durable lab identification labels from Brady

Brady Corporation R&D teams developed extremely durable and on-site printable identification labels for laboratory samples, engineered to stay attached when frozen with liquid nitrogen or heated in an autoclave. The ink used to print these labels can resist chemicals typically used in laboratories to ensure their legibility and to avoid sample loss. Brady researchers created a white paper explaining their point of view and outlining any governmental requirements regarding sample traceability. Technical data sheets on Brady's lablabels are also available. Contact Brady at emea_request@bradycorp.com for the white paper 'Sample Certainty' or for technical data sheets on laboratory labels.

www.bradyeurope.com/lab



Observe and react

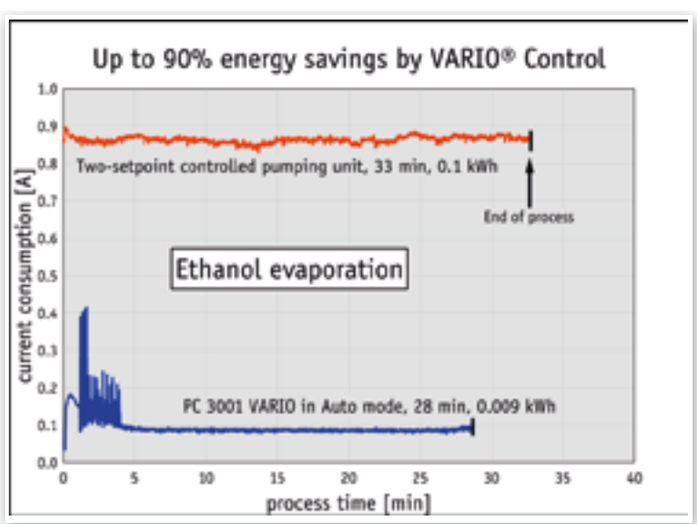
Assisting researchers in gaining a deeper understanding of dynamic biological processes, the new cellSens imaging software (version 1.12) ensures the most efficient use of valuable time-lapse experiments and the latest microscopy hardware. Building on the capabilities introduced by Olympus with its unique Graphical Experiment Manager (GEM) interface, cellSens 1.12 allows the user to truly get in touch with their sample. Enabling effortless setup of complex acquisition sequences and protocols, the GEM presents an intuitive method to seamlessly control motorised hardware, delivering outstanding ease and efficiency for advanced live cell imaging applications.

www.olympus-europa.com

Fully automated enzymatic bio-analysis – on less than 200 cm²

The enzymatic bio-analysis of organic acids and sugars plays an important role in quality control during wine making or dairy production. Automation has always been an option but typically involved large and expensive automates. R-Biopharm now introduces the RIDA®CUBE SCAN – probably the worlds smallest automate for enzymatic bio-analysis. With only 16 x 13 x 15 cm^3 the RIDA®CUBE SCAN is as precise as its big brothers. Every test kit contains ready-to-use cartridges with all reagents: Pipet your prepared sample in the tube, put the tube in the RIDA®CUBE SCAN and read the result within 15 minutes. It is as simple as that. Interested? Please take a look at our website or contact your local R-Biopharm representative.

www.r-biopharm.com



Time, energy-saving and low-maintenance

VARIO®-diaphragm pumps and chemistry pumping units optimize the vacuum automatically and accurately by adjusting the speed of the diaphragm pump. In a conventional two-point control system with solenoid valve the vacuum pump runs continuously at 100 % speed and the solenoid valve is opened and closed as needed to achieve programmed vacuum levels. With the Vacuubrand VARIO® control, the speed of the pump is adjusted automatically to the vacuum requirements of the process. Thereby, the power consumption and the cost of energy depending on the process can be reduced by up to 90 %. Due to lower motor speed and less strokes per minute the service intervals are extended significantly. VARIO® pumps are almost maintenance free in many applications. The vacuum controller CVC 3000 supports both fully automatic distillation with automatic boiling pressure detection and adjustment without any user pre-settings, as well as controlled and reproducible program sequences. The evaporation rates are maximized by the hysteresis free boiling pressure control which results in shortest process times. Typically more than 30 % time saving is achieved in comparison to two-point control.

www.vacuubrand.com

The Ukrainian Forum Complex Support of Laboratories

The Exhibition Center "KyivExpoPlaza" successfully finished the VII International Forum Complex Support of Laboratories, which once again confirmed the main event status of laboratory industry of Ukraine. The Forum is the unique and the only event in Ukraine that brings together all segments of the laboratory industry, scientific researches, and also informative scientific-practical and business programs, covering the most important and urgent issues of the industry sector. Organized by the LMT Company, the Forum is supported by the Committees of the Verkhovna Rada of Ukraine, the Cabinet of Ministers of Ukraine, the Ministry of Agrarian Policy and Food of Ukraine, and further Ukrainian institutions. The Forum's scientific-practical program included events such as 18 conferences, seminars, open discussions, practical master classes, 15 technical excursions that were oriented on advanced training of specialists in laboratory fields, development and improvement of practical and theoretical skills, familiarization with the latest technologies and modern

equipment for the professional areas: pharmaceutical, food industry; agricultural industry; veterinary medicine; science and education; ecology; water and water treatment etc.

120 leading specialists of laboratory field performed as expert speakers. Each year, a particular interest from visitors get special program days of the food industry and agricultural sector. In the framework of this program the roundtable Agroindustrial complex-FEA in 2014 was held: Losses from trading "war" with Russia and gains from the FEA with the EU. Problems and Prospects. The event was organized by the Ukrainian Agrarian Confederation. Moreover, the seminar Innovative technologies in agroindustrial complex organized by Odesa National Academy of Food Technologies kindled great interest among the agrarians. Perspectives and problems of European legislation on food safety were discussed in the framework of scientific-practical seminar, which was held by the National Codex Alimentarius Commission of Ukraine, L.I. Medved's research Center of Preventive Toxicology, Food and Chemical Safety, Ministry of Health of Ukraine, SI.

→ **www.labcomplex.com**

A particular note of optimism

At the 2014 edition of the JIB, despite an expected decrease in attendance, more than 3,300 participants from 51 countries demonstrated the attachment of laboratory professionals for this event. French and foreign medical biologists and lab professionals did participate in the JIB. They have met with exhibitors with whom they have discussed, spent extended time and given valuable availability: a favourable business context appreciated by all the suppliers. They have filled up their training obligation with special Continuing Medical Education programmes. The attendance to such training reveals the orientation of the lab actors to become more and more prone to a medical practice of their field. At last but not least, they have shown great interest for the French-German Health Forum. This special binational initiative was organised in a full room where discussions, debates and comments were as intense as they were fruitful. The future of the discipline, the evolving practices of lab specialists and the conditions of biomedical exams reimbursement were truly questioned in both countries. Beyond their respective two models of organisation, professionals in laboratory medicine from the two sides of the Rhine answer unanimously: medical laboratories activity must be considered as an efficient tool for a model of care in Europe to be more oriented toward prevention, screening and early diagnosis. The fidelity of international attendees, although not as many as usual, from 51 countries (vs 70 in 2013) is also a great marker of this 2014 edition. If foreigners did represent 35 % only of the exhibition visitors, they were 55% of conference delegates. Many of them consider important to present their works through the call for abstracts organised every year by the JIB. A session of oral presentations of the nine very best communications was for the first time implemented in the programme and open to all professional public.

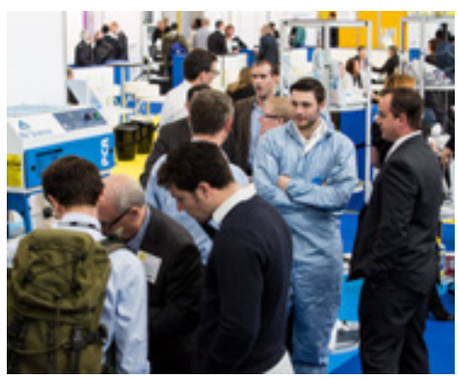
→ **www.jib-sdbio.fr**

Forensics Europe Expo, 21– 22 April 2015, Olympia, London

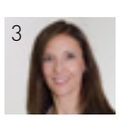
Forensics Europe Expo is the only premier dedicated international exhibition and conference for the entire forensic sector and supply chain showcasing the latest equipment and services, as well as providing the definitive source of education, best practice, training and networking.

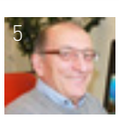
The 2014 edition of the show was a huge success with numerous show floor product demonstrations running alongside a top-level conference programme, and the 2015 event promises to be bigger and better, connecting the widest range of forensic equipment and services suppliers with over 3,500 international visitors.

→ www.forensicseuropeexpo.com

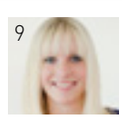










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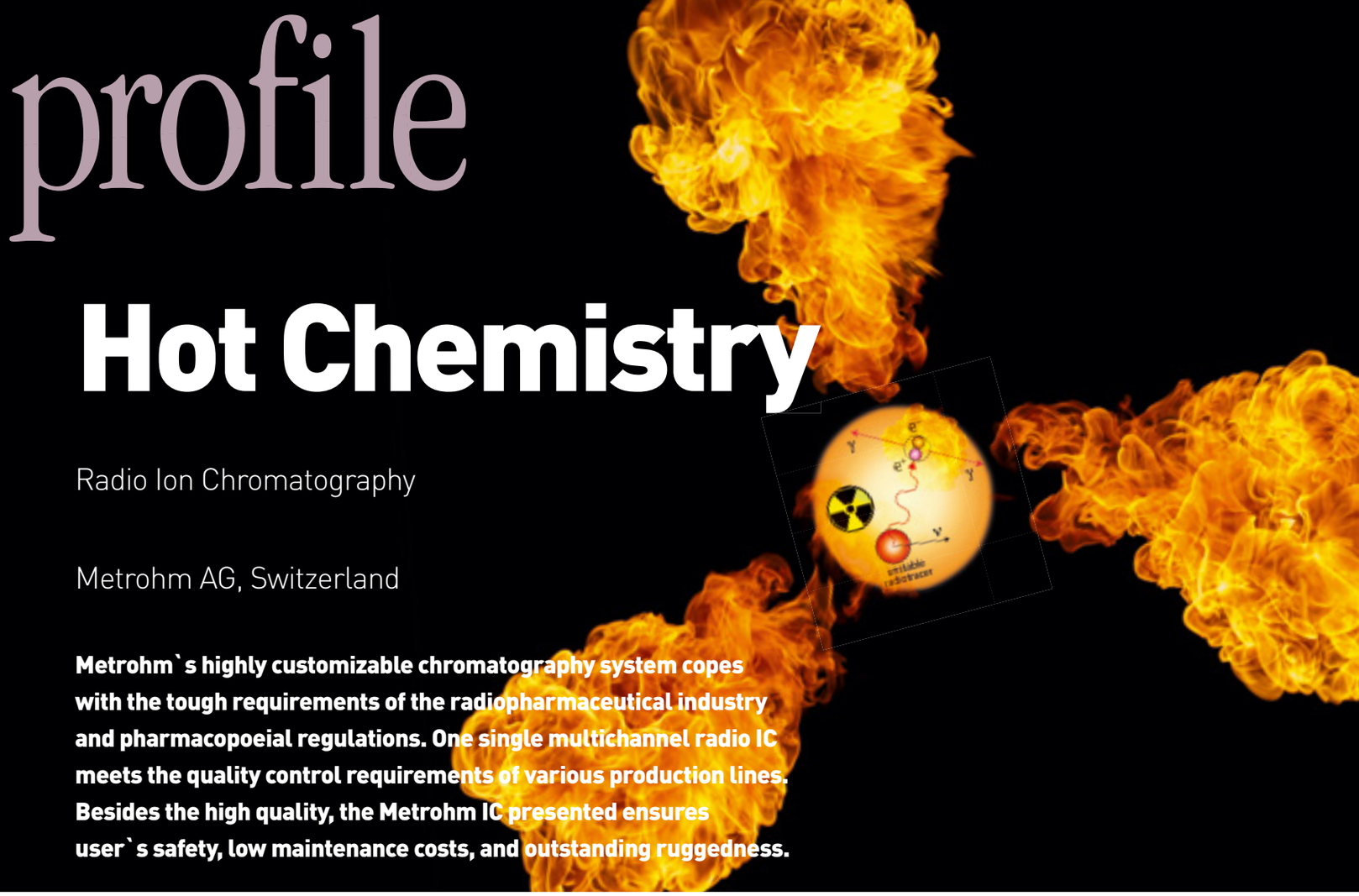
Hot Chemistry

Radio Ion Chromatography

Metrohm AG, Switzerland



Metrohm`s highly customizable chromatography system copes with the tough requirements of the radiopharmaceutical industry and pharmacopoeial regulations. One single multichannel radio IC meets the quality control requirements of various production lines. Besides the high quality, the Metrohm IC presented ensures user`s safety, low maintenance costs, and outstanding ruggedness.

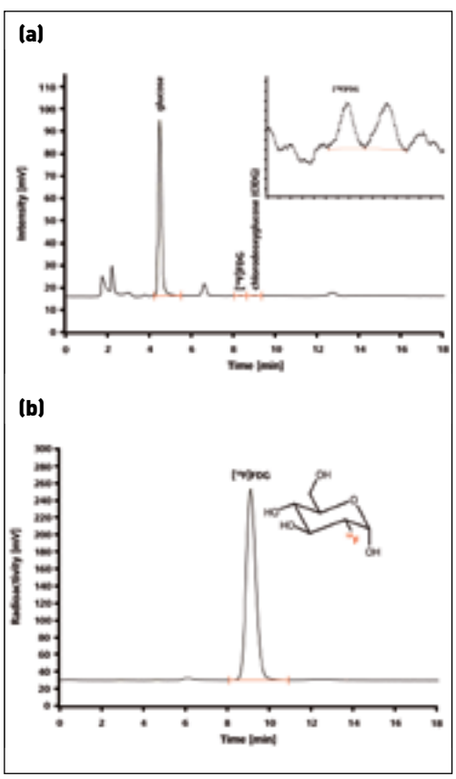
Radio IC aims to determine the radiochemical purity of radiopharmaceuticals. The latter are radioactive substances which are used for medical purposes, mainly in diagnostics, but also in the treatment and prevention of certain diseases. [^{18}F]fluorodeoxyglucose and [^{18}F]fluorocholine are two prominent examples of radiotracers which are used in diagnostics by positron emission tomography (PET). They are labeled with the radionuclide [^{18}F]fluorine. During the radioactive decay of the unstable isotope, a proton in the nucleus of [^{18}F]fluorine changes to a neutron. This process is accompanied by the emission of a neutrino and a positron. The latter combines with an electron in the surrounding tissue resulting in annihilation of both particles, and emission of two photons (gamma rays) in opposite directions, each with an energy of 0.511 MeV. From the data acquired through coincidence detection of the photon pair, the location of its emission in the patient's body is calculated. The latter coincides closely with the location of the original radiotracer molecule and thus reveals information on its activity.

The purity of radiotracers is crucial. The highly energetic gamma rays emitted during the combination of a positron with an electron are harmful to the human body; by using pure radiotracer, i.e., by avoiding injection of free [^{18}F]fluorine or other radioactive contaminants, the amount of radioactive substance administered to the patient can be kept to a minimum.

The quality control of the radiotracers is done by radio ion chromatography, in the short time between their synthesis and the recording of the three-dimensional PET scan. The separation step in radio IC is equal to that in regular IC – apart from it happening behind lead doors. What really sets radio IC apart from conventional ion chromatography is the detection step, in which a radioactivity detector is added to the setup. The radioactivity chromatogram reveals the presence of radioactive contaminants or, ideally, their absence.

→ **www.metrohm.com**

Picture: © istockphoto.com | skydie



(a) IC-PAD chromatogram with the glucose precursor, the carrier-free [^{18}F]FDG, and the impurity chlorodeoxyglucose. **(b)** Radioactivity chromatogram of the [^{18}F]FDG. The IC software converts the radiation units, counts per second (cps), to mV. Chromatographic conditions: Column: Metrosep Carb 1-50/4.0; eluent: 0.1 mol/L NaOH, 1 mL/min; column temperature: 25 °C; injection volume: 10 µL.

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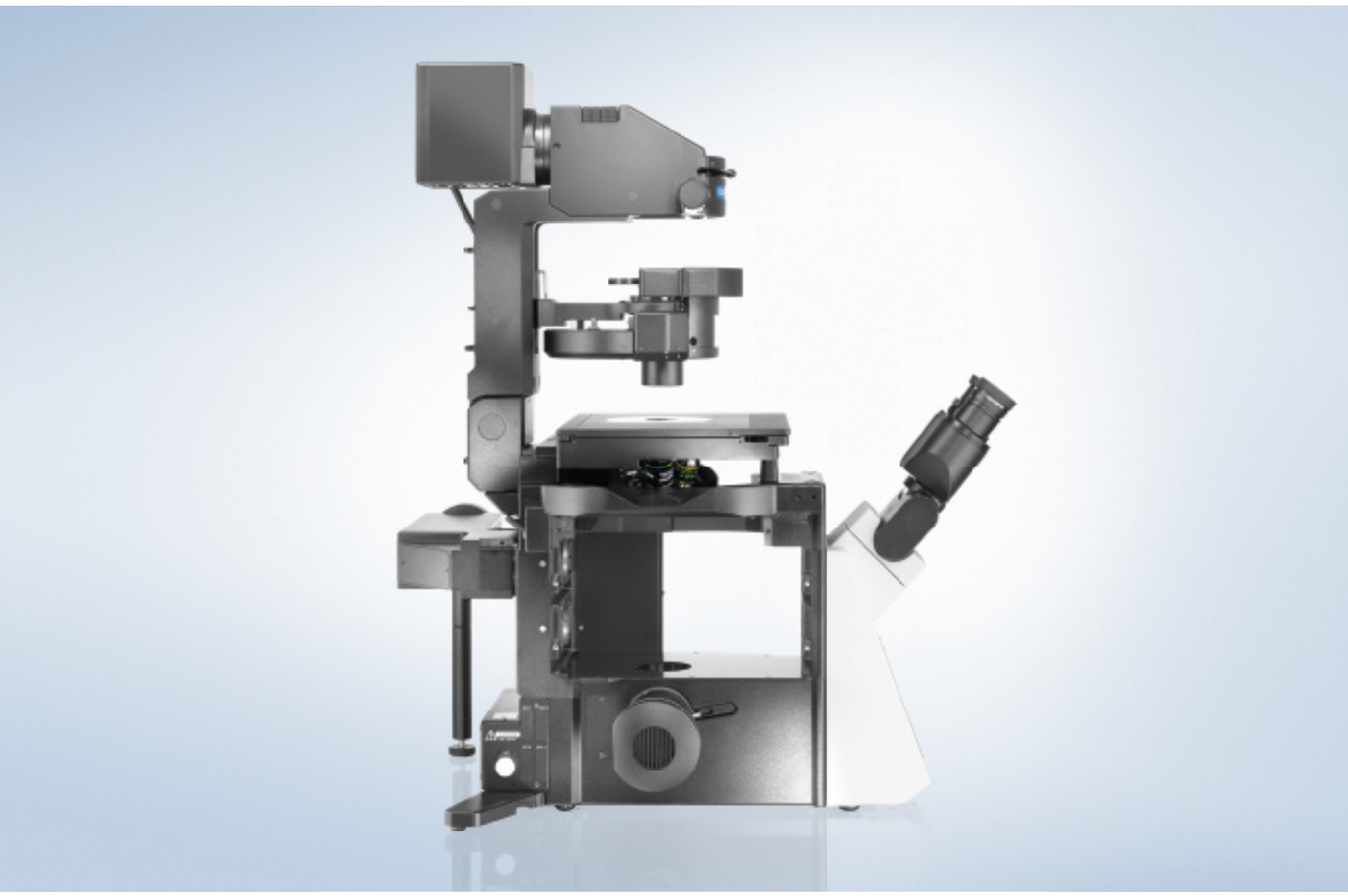

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Continuous and new contagious diseases pose a growing threat: Ebola, SARS, bird flu, swine flu, tuberculosis, etc. New high safety laboratories for biological pathogens are being built to prepare for such threats. No matter what type of laboratory you require, HT LABOR + HOSPITALTECHNIK AG is your expert full service supplier for multifunctional high-security laboratories.

The hermetically sealed laboratory systems are hygienically and microbiologically shielded units. Technical barriers, control and monitoring systems ensure compliance, control and traceability of the security policies set by national and international regulations.

All building services parameters are integrated into the control process, the alarm system and monitoring by probes and sensors. The result is a safe research environment for handling highly contagious and dangerous substances.

Types of laboratories

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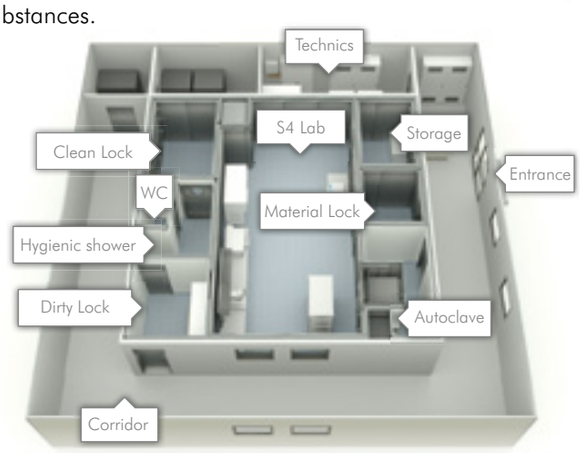
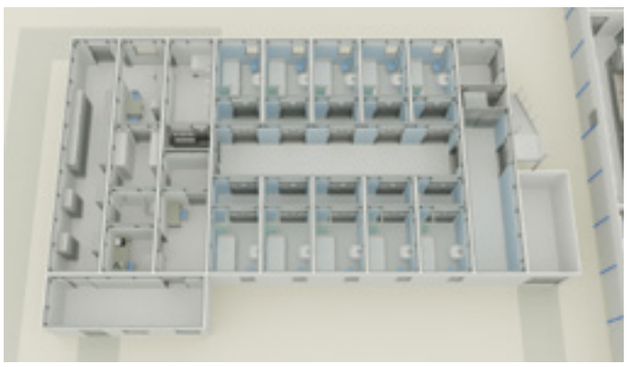
- Pharmacy
- Tuberculosis lab
- Genetic research
- Biomedicine
- Analysis
- Animal research lab
- Insulating rooms
- Epidemic research
- Epidemic test lab



BSL 4 LABORATORY

MEDICAL TREATMENT CENTER FOR EBOLA EPIDEMIC

This concept shows a self-sufficient EBOLA ISOLATION STATION, equipped with gas-tight doors, walls, sluice facilities and air filtration systems. Chemical wet-showers ensure the secure decontamination of the medical staff. The access is provided with electronically secured access control systems.



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Self-sufficient , fully equipped Safety Laboratory for biological safety levels 1 to 3 for dealing with highly infectious germs (e.g. Ebola, SARS, bird flue, ...).

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