

Over 30 Years of Scientific Innovation

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FOR OVER 30 YEARS EUROFINS HAS REGULARLY BEEN THE FIRST IN THE WORLD TO DEVELOP NEW TESTING TECHNOLOGIES THAT HAVE HAD AN IMPACT ON LIFE

OVER 30 YEARS 800 LABORATORIES 45,000 EMPLOYEES

**33 MILLION TESTS PERFORMED EVERY MONTH.
WE'RE HERE TO KEEP OUR WORLD SAFE.**

Since its foundation in 1987, Eurofins has contributed significantly to the advancement of science in testing for life. We are proud of the discoveries and advancements our specialists have made in myriad sectors, ranging from food to pharma to forensics testing.

Our laboratories around the world are helping to improve cancer treatments, fight the growing problem of antibiotic resistance, catch criminals, safeguard the health of bees, ensure our food and water is safe, determine paternity, and beat viruses like Zika. Underpinning our work in these areas and more, Eurofins has one of the world's largest and most varied databases, rich with fingerprints of thousands upon thousands of foodstuffs and drugs.

The complexity of our analyses, often searching for the minutest traces of a substance, mean our activities in these areas necessarily sometimes take years of painstaking research and ongoing improvements of our numerous methods. In other areas, we have responded overnight to global health crises.

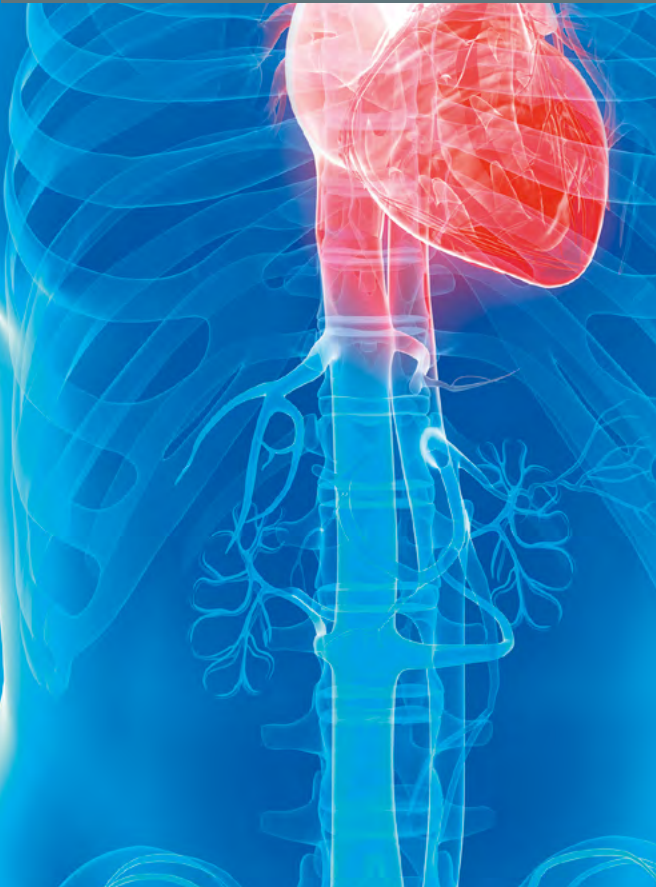
Wherever we are and however long it may take, our work helps to make life safer.

This booklet presents a mere fraction of the hundreds of stories from across our activities about our scientists' great work for over 30 years to push the frontiers of Testing for Life.



PROTECTING TRANSPLANT PATIENTS AND
SAVING LIVES

Immunity at the heart of the matter



Surgeons worldwide perform more than 100,000 solid organ transplants annually, with successful transplantation of hearts, lungs, livers and kidneys saving and changing thousands of lives. After complex surgery, the risks of infection and organ rejection mean the patient's fight for recovery starts immediately; even anti-rejection medication can lead to serious adverse effects. Eurofins' work in immunity is helping to change patient recovery outcomes.



Adverse reactions that can hamper patient recovery are managed by understanding the exact state of a patient's **immune cell function** and using that knowledge to balance the immune system. Experts at Eurofins Viracor developed ImmuKnow® assay, a test that provides a biomarker to help doctors to evaluate a patient's existing immunity levels and to optimise treatment.

In 2015, a study published in *Transplantation* showed that Viracor's assay improves outcomes in solid organ transplant patients. The study showed that not only was ImmuKnow® able to detect the level of immunity provided by cells in immunosuppressed patients, but it ultimately improved patient **survival rates** by helping to optimise immunosuppression. This benefit is critical when balancing the risk of organ rejection caused by insufficient suppression and the risk of infection caused by excessive suppression.

The study also showed that using ImmuKnow® in solid organ transplant

patients helped decrease bacterial and fungal infections, particularly in the critical two-week post-surgery period. It also reduced immunosuppressant drug use and improved patient survival by 13% in the first year post-transplant. ImmuKnow® is cleared for use by the US Food and Drug Administration.

Eurofins Viracor has developed additional, innovative tests with **fast, accurate results** to help surgeons care for their transplant patients. It now works with approximately 60% of US transplant programmes and has earned its leading position in the field.

Eurofins is deeply committed to contributing positively to global health. With technological and medical advances leading to continual increases in the number of transplant operations occurring worldwide, Eurofins is focused on the development of further specialised and fast-turnaround tests that will play an important role in patient outcomes.



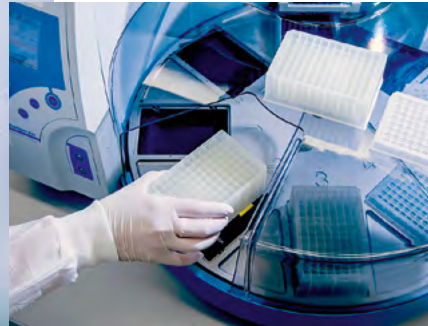
the science behind

ImmuKnow® is an immune cell function assay that detects cell-mediated immunity in an immunosuppressed population by measuring the concentration of adenosine triphosphate (ATP) synthesis from CD4 cells following stimulation. The technology combines cell stimulation, cell selection, and quantification of metabolic markers (ATP) to measure cell-mediated immunity. Since the CD4 lymphocytes orchestrate cell-mediated immunity responses through immunoregulatory signalling, the measurement of CD4 activation reflects the degree of immune function.

For references, please see bibliography

Disclaimer: ImmuKnow® is FDA cleared for the following Intended Use: Detection of cell-mediated immune response in populations undergoing immunosuppressive therapy for organ transplant. Product Insert 2015. Eurofins Viracor, Inc., Lee's Summit, MO.

TACKLING GLOBAL ANTIBIOTIC RESISTANCE



Testing for multiple resistance genes

Since the accidental discovery of penicillin by Alexander Fleming in 1928, the world's reliance on antibiotics has increased dramatically. Yet overuse and misuse across the world has seen antibiotics become ineffective against an increasingly large number of bacteria, and antibiotic resistance is now a globally ticking time bomb. Eurofins is at the forefront of efforts to tackle this crisis.

The Centers for Disease Control and Prevention reports that, each year, more than two million people in the US alone become infected with bacteria that are resistant to many antibiotics and attributes at least 23,000 deaths to these infections. Even the World Health Organization has warned that the world may be entering a 'post-antibiotic era' in which some of the most dependable medicines become useless and common infections once again threaten to prove fatal.

Knowledge of the bacterial resistance genes is key. In 2016, Eurofins Diatherix launched the ABRx™ Antibiotic Resistance Panel, an exciting development that sees diagnostic tests able to simultaneously detect 17 clinically problematic and prevalent genes that lead to resistance to some

of the most commonly prescribed antibiotics.

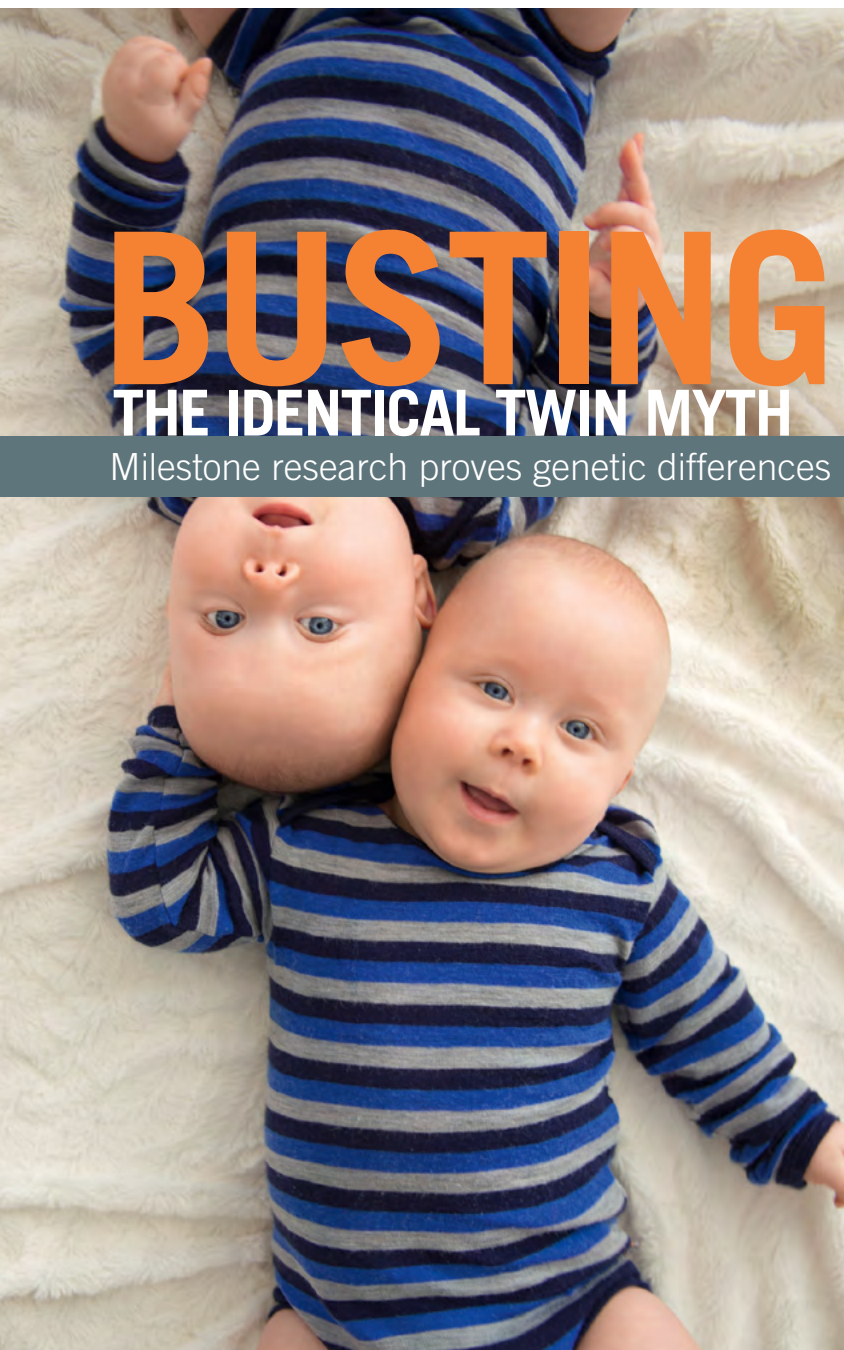
With specimens taken directly from the patient, the ABRx™ Antibiotic Resistance Panel provides **rapid-response** results within one day. It also offers doctors and pharmacists clinically-important and accurate patient-specific information to aid their decision-making and ensure the prescription of an effective antibiotic in each clinical case.

Not only does this hinder the worsening of symptoms and hospital re-admissions, but detection results can be used to define an antibiotic class avoidance strategy. Best of all, the ABRx™ Panel tests are helping to slow the frightening pace of worldwide antibiotic resistance.

the science behind

The ABRx™ Antibiotic Resistance Panel allows the molecular identification of antibiotic-resistant genes in patient samples. It is focused on the detection of 17 gene types, within seven gene classes which are associated with resistance to three major groups of antibiotics (β-lactams like penicillins, cephalosporins and carbapenems, fluoroquinolones, and macrolides). Offering the direct detection of clinical samples, without the requirement for bacterial isolation, the ABRx™ Panel has been validated on respiratory secretions, rectal/stool swabs, and urine specimens.

For references, please see bibliography



BUSTING THE IDENTICAL TWIN MYTH

Milestone research proves genetic differences

Amid abandoned legal cases, high-profile acquittals of serious crimes and paternity suit dismissals, scientists were increasingly questioning the dogma that identical twins could not be genetically differentiated. Only theoretical approaches existed to solve this conundrum, until a Eurofins breakthrough provided an answer to the puzzle of how to tell identical twins apart.

Monozygotic twins derive from one zygote (the fertilised egg) that splits into two, meaning that the basis of the two twins, at first, comprises exactly the same genetic material. By pushing the limits of genetics and forensics, a multi-disciplinary team at the flagship Eurofins Genomics laboratory in Ebersberg, Germany, developed a **world-first methodology** in 2014 that showed what happens next.

Using a whole genome sequencing approach, Eurofins demonstrated that rare mutations occur early during the embryonic stage whenever a cell divides as part of the development process. Eurofins also provided evidence that, because these changes happen so early on, the mutations can be found in all the DNA of the person, including the DNA of its sperm (in the case of a male) or its egg (in the case of a female).

Eurofins' first test case used this new understanding of embryonic genetic mutations to assess identical twin

adult males as the potential father of a child. While one was found to carry an identical mutation to the child, the other was not. These results were used to prove **conclusive paternity**.

The first application of the test in a criminal case supported the Boston Police Department and Suffolk County District Attorney's Office in a serial rape case. The authorities were confident of the guilt of two male offenders; however, one had an identical twin and could not be convicted Eurofins received saliva samples from both twins, and a crime scene sample. The test delivered a successful result in just two weeks, successfully and unambiguously matching one of the twins to the crime.

Through the use of state-of-the-art chemistry and ongoing process refinements, Eurofins has already cut the test costs by 50%, ensuring **accessibility** of one of the most important tools in the search for the truth.

the science behind

The process involves ultra-deep next-generation sequencing. Researchers first read and decipher the genetic information found in the DNA, before analysing the data collected through intelligent information technology. The focus is on the appearance of specific mutations, also referred to as single nucleotide polymorphisms, or SNPs.

For references, please see bibliography





NEW GENERATION OF NON-INVASIVE PRENATAL TESTING METHODS

Breakthrough genetic disorder determination

For over 30 years Eurofins NTD, has held a leading position in the research and development of **prenatal screening protocols** to help detect birth defects, genetic diseases and other conditions. For example, Eurofins NTD was among the world's first clinical laboratories to provide an alpha-fetoprotein screening programme. This test is still used today to identify any risk in the foetus for open neural tube defects.

Eurofins NTD also offers tests to screen for Down syndrome, trisomy 18 and 13, and offers the only test that combines first-trimester aneuploidy risk assessment with Y chromosome and early onset preeclampsia in one report.

In December 2016, Eurofins LifeCodexx launched the worldwide first non-invasive prenatal test (NIPT) assay based on a quantitative real-time PCR for prenatal testing of foetal trisomy 21 (qNIPT). The novel and proprietary

qNIPT assay allows significant cost efficiencies and speed over other classical NIPT methods as it is based on polymerase chain reaction (PCR), the most widely used platform within the molecular diagnostic space. This test's significantly lower cost and faster processing time allows more pregnant women to opt for NIPT as a reliable and affordable prenatal testing method.

In 2017, Eurofins Genoma unveiled a step-change breakthrough in **NIPT** technology developing, for the first time ever, a NIPT that screens for both de novo – gene mutations, that are not inherited – and inherited single-gene disorders. NIPTs are simple blood tests that were previously only able to screen for aneuploidies, errors in cell divisions which see 'daughter' cells having the wrong number of chromosomes, and microdeletions, which are chromosomal deletions that are too small to be detected by light microscopy and can lead to genetic disorders. More recently, NIPTs also

Prenatal tests are conducted during pregnancy to provide information about the health of the mother and her baby. Eurofins has pioneered work to develop improved prenatal screening tests for conditions that might affect the foetus, and help inform healthcare decisions before and after the birth.

the science behind

Based on quantitative real-time PCR rather than on next-generation sequencing, the qNIPT foetal trisomy 21 test from Eurofins LifeCodexx is not only cheaper, but also significantly faster. Due to different methylation patterns of specific gene regions of the maternal and foetal DNA, positive and negative samples can be distinguished and the test provides rapid results in two to three days under highest quality standards.

The GeneSafe™ test is intended for patients who had an abnormal ultrasound finding which was suggestive of some form of monogenic disorder. The technology screens for new mutations that are common and cannot be detected by standard carrier screening, as these mutations are not present in the parents.

While traditional NIPT screens for conditions typically associated with advanced maternal age (e.g. Down syndrome), GeneSafe™ also screens for genetic disorders that are associated with advanced paternal age (men that are >40 years old), ensuring a more comprehensive screen for couples of advanced age.

For references, please see bibliography



screened for **rare aneuploidies** and segmental chromosome imbalances (gains and losses) in every chromosome in the foetal genome.

Not only does this new Eurofins test, GeneSafe™, identify prenatal conditions which traditional prenatal testing may miss, but it also detects over 40 severe genetic disorders that might occur in the absence of any family history of the condition.

The test is also the first ever NIPT to detect disorders with an increased likelihood associated with **paternal age**, something particularly useful as later-stage parenthood is increasingly common, especially in developed countries.

Simple and safe to administer, this test poses no risk to the foetus and works well as a complementary screen to traditional NIPTs, allowing healthcare professionals and patients to make more informed medical decisions.



Food fraud and the adulteration of what we eat and drink is a global problem and one which is only worsening. Fraudsters are substituting genuine, everyday ingredients with cheaper alternatives that mislead buyers and consumers, risk allergies, side-effects and even severe harm to human health. The desire to help put an end to such dishonest practices led to the birth of the company that became Eurofins.

Authenticity and origin testing to protect our food and drink

SNIF-NMR®

HOW IT ALL BEGAN



Back in 1981, Professors G. and M. Martin and their teams at the University of Nantes developed a **pioneering process** to detect the chaptalisation (or over-sugaring) of wine, and the enrichment of freshly pressed grapes. In 1987, this SNIF-NMR® method led to the creation of Eurofins. The Company has continued to develop the process, now using it to check the authenticity of wines, spirits, fruit juices, flavours, sugar syrups, and vinegar. Together with other tests, the technique makes it possible to verify the geographical origin of products and decide, for example, whether vanillin was chemically-produced or whether it is natural – useful information as the two have a huge price difference.

This technique is still state-of-the-art 30 years after its invention, and is a **gold standard** in its field. SNIF-NMR® is the official method of analysis for

a variety of organisations, including the European Union (EU) and the International Organisation of Vine and Wine (OIV) for wine; the Association of Analytical Chemists (AOAC) for fruit juices, maple syrup, and vanillin; and European Committee for Standardization (CEN) for vinegar.

In addition, Eurofins has committed significant resource to developing a comprehensive reference database of over 30,000 pieces of data for wine, fruit juice, natural flavours and other products. The company continues to pioneer and develop cutting-edge tools to empower customers to fight back against food and beverage fraud.

the science behind

Atoms exist as different isotopes. Sugar in sugar beet, for example, comes from a different biochemical pathway than sugar in grapes, and therefore contains different proportions of hydrogen isotopes (1H and 2H). SNIF-NMR® can measure the different hydrogen isotope ratios at each position in a molecule, thereby identifying the addition of sugar from another source. The SNIF-NMR® process allows the quantification, with very fine precision, of isotopic ratios across a molecule. A number of factors affect these, including botanical origin, climate and geography and so the resulting isotopic fingerprint provides in-depth information about the origin of the molecule.

For references, please see bibliography



THE FUTURE OF BEEES

Breakthrough science for supporting better bee health



THE FUTURE OF LIFE



Honey bees travelling from flower to flower are more than just a pretty sight; the species is the world's most important, and most threatened, pollinator. Environmental changes including increased pesticide-usage mean high levels of concern globally about pollinator safety in the present agricultural climate. Eurofins is leading efforts to support and protect bee health and reverse the trend. Some of Eurofins' innovative approaches in this area are now becoming part of standard industry protocols.



Eurofins' Agrosience Services division developed an innovative method of fully investigating the **effects of pesticides** on the homing behaviour of honey and bumble bees. Tiny **radio frequency identification transponders** with unique identification numbers are permanently and harmlessly attached to each bee's thorax. The transponders are registered by scanners at the hive entrance whenever a bee enters or leaves, allowing their homing rate to be assessed.

One sample of honey bees is fed different doses of an insecticide compared to an untreated control sample, and assessment is conducted within 24 hours of release. Available data covers the duration of foraging flights; the duration of return flights after treatment and during feeding studies; the return rate or homing success, and any confusion by the

bees over hive entrances as a possible indicator of disorientation. The study design can also be adapted to include realistic field-exposure scenarios and to observe delayed effects for up to two weeks post-release. The Eurofins method provides data crucial to understanding the impact of the insecticide and any direct links between test types and their impact on bee health. Eurofins participates in an international laboratory ring test aimed at development of a standardised test design based on this technique.

Just as innovatively, Eurofins pioneered a field application method to assess the impact of abraded dust from pesticide-treated seeds on honeybees. Varying particle size makes the dust inherently more difficult to test than liquid substances; however, abraded pesticide particles or contaminated dust contain high concentrations of

pesticide and can prove toxic to bees actively collecting pollen and nectar. Finding a solution was imperative, but previous trials proved labour-intensive and complicated and were affected by numerous factors including wind direction. Eurofins developed a **purpose-built dust applicator** which improved risk assessment methodology and opened up a range of field trials to investigate potential side effects on bees and other organisms.

Understanding about bees' behaviour is aided further by Eurofins' **bee breeding** programme. By ensuring the fittest possible queens, Eurofins supports the development of strong hives and standardised quality of bees for testing. With significant expertise in commercial queen production for the beekeeping industry, Eurofins is now able to produce mated sister queens, enabling proper trait-specific selection

for breeder queens, perfect nutrition during larval development, and appropriate mating with quality drones. Breeder queens are selected from winter survivor stock from the 1000+ colonies across Eurofins' Bee Health and Management Solutions farms.

Eurofins also takes an innovative approach to breeding methods for honey bees using genome-wide DNA analysis. Working alongside the Institute for Bee Research in Hohen Neuendorf, Germany, and deploying next-generation sequencing technology, Eurofins selected bees with **increased resistance** to deadly natural parasites and virus infections in combination with the **varroa mite**. This guarantees breeding success, helping to develop healthy bee populations and to maintain functional eco-systems.

the science behind

Eurofins' key role in safeguarding bee health includes solitary, honey and bumblebees. Quality bee-breeding is aided by testing queens and drones for fertility, reducing stressors and optimising nutrition. To aid selection of disease-resistant bees, Eurofins analysed the whole bee genome using next-generation sequencing to identify single-nucleotide polymorphisms (SNPs), small natural changes, in the bees' DNA. Bee variants are sorted according to the SNPs, using DNA chips developed to characterise gene variations in bees with known parasite resistance, productivity and docility. This allows beekeepers to identify the genetic basis for these positive traits and avoid time-consuming selection.

For references, please see bibliography



HELPING TO STOP ZIKA IN ITS TRACKS

Rapid real-time Zika test results



Summer 2016 could be remembered as the summer of Zika, with thousands of confirmed and suspected cases of the virus, real fear among travellers to affected regions, and withdrawal from the Rio Olympics by dozens of worried athletes. Eurofins responded with the fast-track development and emergency launch of a test to help stop the spread of this heart-breaking and virulent virus.

The Zika virus was first linked to a fast growing number of cases of **microcephaly** (a condition resulting in smaller-than-normal head size) in newborns, particularly in Central and South America. Global concerns grew when travellers to these areas, and to others endemic to the virus, began to test positive for Zika. Spread primarily through mosquitos, Zika virus can also be passed on by some forms of sexual contact.

Determined to assist its clients and patients prior to the onset of the mosquito season, Eurofins Viracor became one of the first commercial laboratories to develop and offer a real-time polymerase chain reaction (PCR) test for Zika to hospitals and health care providers. Working in partnership with the US Food and Drug Administration, the Company obtained **emergency use authorisation** in July 2016, making the test swiftly available for use during a declared public health emergency.

Eurofins' Zika assay tests human blood serum, blood plasma, or urine (the latter collected alongside a patient-matched serum or plasma specimen) from individuals presenting Zika-virus signs and symptoms. It also tests those who have a history of living in, or travelling to, affected areas. This **rapid-response** test gives doctors and healthcare professionals a result within just eight to twelve hours of receipt of the sample by Eurofins' laboratory.

The assay's exceptional sensitivity safeguards against cross-reactions with other viruses in the same family (Flaviviridae), including Dengue, Japanese encephalitis, West Nile, and St. Louis encephalitis, or with other viruses known to cause similar clinical symptoms, such as Chikungunya.

the science behind

Eurofins' real-time PCR test for Zika virus is designed to detect and measure ribonucleic acid (RNA). RNA is extracted from the specimen and reverse transcription of viral RNA to DNA is used. With the help of the polymerase chain reaction (PCR), a laboratory method used to make very large numbers of copies of short sections of DNA from a very small sample of genetic material, the number of DNA copies obtained is amplified and enables specific genes of interest to be detected or measured.

For references, please see bibliography

A PIONEER IN DIOXINS DETECTION

Detecting one part out of 1,000 billion is not the limit

Dioxins and polychlorinated biphenyls (PCBs) are toxic chemicals that persist in the environment and accumulate in the food chain. Even at the level of 1/1000000000 to 1/1000000 of a gram, they are considered to pose a **serious hazard** to humans and the environment. National and international treaties and directives have been passed to minimise or stop their circulation.

Eurofins has led the way in testing and analysis to aid understanding of the levels of dioxins and PCB to which we are exposed. In 1985, the Company was one of the first to start dioxin analysis and, by 2010, became the very first laboratory in Europe able

to test for all the **persistent organic pollutants (POPs)** identified in the 2001 Stockholm Convention. One of Eurofins' specialities within this operation is the full analysis of 209 separated PCB isomers in one method. This ability is of particular importance for the fish oil industry, as PCBs and dioxins are lipophilic, i.e. soluble in fat, not in water, and therefore accumulate in fat/oil at the end of the food chain in fish.

Dioxin analysis is important across a number of different matrices, including food and feed, human samples, animals, plants, air, industrial products, residues, sludge, and water. Eurofins analyses all of these. However, dioxin

analysis is extremely difficult to perform, and the requirement for the detection of very low levels of substance (one part out of 1000 billion others) and the distinction of each substance among the very similar dioxins and PCB molecules necessitates the use of highly sophisticated techniques and advanced equipment.

Heavy investment in research and development has helped Eurofins achieve a market leading position globally, and the Company now analyses around 30,000 dioxin samples per year, performing the highest throughputs worldwide. Eurofins has released more than

To protect human health, understanding the levels of dioxins in the environment and the food chain is imperative. Eurofins is at the forefront of global efforts to limit our exposure to dozens of dangerous chemicals.



750 publications on dioxins and POPs testing to date.

One of the sources of dioxins can be waste incinerators and other "chimney industries". These burn substances containing chlorine at very high temperatures, creating the conditions for dioxins formation. For this reason, emission gases are sampled and analysed to check that filters and other technical means to avoid dioxin formation are properly functioning. Eurofins companies are world leaders in the area of sampling and analysis of emission gases.

the science behind

PCBs are a compound class of chlorinated aromatic hydrocarbons which consist of 209 individual components. They started being commercially produced in 1929 but were recognised to be harmful in the 1970s and have since been banned or severely restricted. It is estimated that around two million tonnes of PCBs have been produced, about 10% of which still remain in the environment today. Twelve of the PCBs are similar, in terms of molecular biological behaviour, to dioxins and are known as dioxin-like PCBs.

For references, please see bibliography

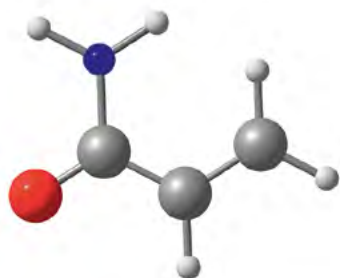




First to detect acrylamide in food

ACRYLAMIDE ON THE TIP OF EVERYONE'S TONGUE





Some rather unusual discoveries in Sweden in the 1990s led to the finding that acrylamide is not only toxic, but also present in a high number of foodstuffs. Acrylamide is understood to be probably carcinogenic to humans and both the World Health Organization and the Food and Agriculture Organization of the United Nations view the levels of acrylamide in foodstuffs as a major concern. Eurofins has played a leading role in detecting and tracing occurrences of the substance and working to increase understanding of its dangers.



Soon after the walls of a new train tunnel through a mountain range in southwest Sweden were sealed in 1997, fish in nearby waterways started to die and cattle showed signs of paralysis. An immediate investigation by the authorities saw the Eurofins team in Lidköping, at that time AnalyCen AB, perform a large number of initial tests on water, soil and sediment. The cause of the problems in fish and cattle was found to be a **significant leak** of un-polymerised acrylamide and methylolacrylamide (acrylamide is the starting material to produce the non-toxic polymer polyacrylamide).



Further investigations showed widespread contamination in creeks and groundwater across the area, and the grouting material used in the tunnel construction was found to contain both acrylamide and

methylolacrylamide. Concerns grew for both the local population and for the construction workers who had been exposed, but tests conducted by Stockholm University's Department of Chemistry showed 'relatively high' background levels of acrylamide even in their control group. This meant there had to be another **exposure source**.

This source became clear during 1998, when Eurofins Sweden completed comprehensive feed and food analysis, making the **breakthrough discovery** that acrylamide was forming in heated food. A partnership with Stockholm University saw a huge project involving extensive analysis and the frying, baking and boiling of all kinds of foodstuffs to better understand what was happening. The team discovered that when the amino acid asparagine – found in many vegetables, and in higher concentrations in some varieties

of potatoes – is heated to high temperatures in the presence of certain sugars, acrylamide can form. High-temperature cooking methods such as frying, baking or roasting were more likely to produce acrylamide, but only when heated to a temperature above 120 degrees Celsius (248 degrees Fahrenheit).

The team continued to work over several years to further identify the causes of acrylamide formation, and to refine the analytical methodology and advise food producers and consumers alike. Across Eurofins globally there are now 20 laboratories able to perform this analysis. The original team's commitment to staying curious, to exploring and to questioning has undoubtedly helped inform the whole acrylamide debate and supported the food processing industry globally to refine its practices.

the science behind

Acrylamide, a colourless, non-volatile crystalline solid, is a raw material (monomer) in the production of polyacrylamide polymer, used as a coagulant in waste water treatment and to clarify drinking water, as well as a grouting agent for the construction of dam foundations and tunnels. Potential contamination from the environment meant that reducing the levels in food became even more critical and the European Commission for Food Safety has adopted a recommendation that acrylamide levels in food are monitored with a view to reducing them.

For references, please see bibliography



TESTING FOR TRACEABILITY OF MEAT

Clarity and confidence for the food chain



FROM FARM TO FORK DOWN TO INDIVIDUAL ANIMALS

Food safety crises, from bovine spongiform encephalopathy (BSE, or "mad cow disease"), to the European horsemeat scandal, have rocked the food industry and driven consumers to demand safer food products and reliable information about sources and production processes. Eurofins' innovative testing approaches have helped provide true "farm-to-fork" traceability and much-needed confidence to the sector.

BSE was first identified in cattle in 1986 and, unfortunately, necessitated the slaughter of over four million animals in the UK alone. In 1996, the disease was acknowledged to have jumped the species barrier to humans, via the consumption of infected beef and beef products. The first cases of a new variant of Creutzfeldt-Jakob disease (CJD), were registered and over 220 people are believed to have succumbed to the disease in Western Europe.

was stored in a database and compared with control samples taken post-slaughter. Large-scale adoptions of the technology for DNA traceability projects included the Pays de la Loire region of Western France.

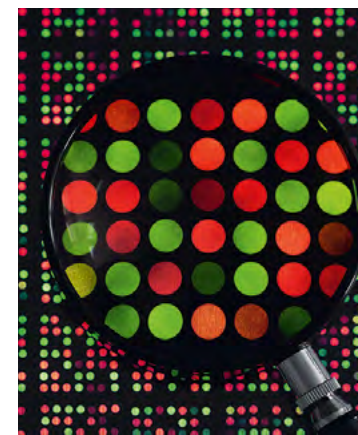
Technologies have continued to evolve, and Eurofins is still leading the way in rapidly reacting in times of crisis. One such cutting-edge technology – **Animal ID DNA Chips** – was quickly

In addition to these human and animal fatalities, the global news headlines resulting from the BSE scandal and other food safety crises cause pressure across the supply chain and highlight the very real importance of being able to reliably trace the origin of meat products.

developed and launched in the wake of the horsemeat crisis in 2013. Consumers, retailers and producers were demanding the verification of product composition, proof of its compliance with relevant regulations, and proof that it was entirely consistent with the claims on the label.

The technology to achieve this lagged behind until, in 2001, Eurofins developed its innovative patented method, **Eurofins TAG**. For the very first time, genetic fingerprints were used for the precise identification of cattle in the food industry that had been tested free of BSE with each individual animal matched to one genetic profile. Simple to administer, reference samples were taken from animals before they reached the slaughter house, and the information

Eurofins recognised that existing analytical methods to detect single species were no longer sufficient to meet these requirements; Animal ID DNA allows the precise **simultaneous identification** of 21 specific animal species in food products, including pig, chicken, rabbit, cat, sheep and goat, as well as horse.



the science behind

The Animal ID DNA chip technology uses mitochondrial DNA targets to enable the identification of 21 animal species in food and feed through a single test. Fully-accredited, it makes specific and cost-effective detection of a large panel of species possible for the first time.

Eurofins-TAG is based on the multiplex analysis of a set of 11 microsatellite markers recommended by ISAG (International Society for Animal Genetics). A unique genetic fingerprint is obtained for each animal and can be used for traceability studies of BSE tested animals of known origin or paternity testing.

For references, please see bibliography



THE FOREFATHERS OF PESTICIDE TESTING

First multi-pesticide detection method in Europe

Pesticides were launched in the 1930s, and the protection they afforded crops was lauded. However, the hundreds of pesticides sold worldwide, with more developed each year, plus cases of the use of banned pesticides like DDT and other persistent organochlorine compounds, mean ongoing pressure to ensure that foodstuffs are safe for consumption.

It was only in the 1960s that concerns for the impact of pesticides on human health began to be raised, but the first methods devised for pesticide analysis were slow, expensive and imprecise. This all changed when Dr Wolfgang Specht recognised that all of the pesticides in use were relatively similar, and that analysis of several of them at the same time would be significantly more efficient as well as

more effective. In 1964, he launched the ground-breaking S19 pesticide analysis test as the first ever multi-method in this area.

Used to analyse the contamination in foodstuffs caused by insecticides and other toxic substances, the multi-residue S19 method later evolved to become the official European enforcement method, and the laboratory that developed it – part of the Eurofins family since 2000 – is the recognised **Competence Centre** in the field of pesticide analysis.

Demand for multi-residue methods in plant protection analysis is huge, but more than half of existing pesticides can be assessed with just one method. Moreover, multi-residue methodology means not only detection, but also direct simultaneous quantification, of residues in order to prove that maximum residue levels are not

exceeded. This makes significant contributions to consumer safety.

In 1979 the first method using S19 was published – for tobacco. This test's **real innovation** was the first-ever use of gel permeation chromatography (GPC), allowing the separation of the pesticide analytes from the matrix, in particular fat, by size exclusion via a soft gel. At first, around 200 molecules, mainly organochlorine and organophosphorus pesticides which were primarily used during that time, could be analysed; Eurofins is now able to analyse more than 900 pesticides and their metabolites, processing over half a million samples each year.

Thanks to Eurofins' pioneering spirit, a portfolio of validated methods for the analysis of residues in fruit and vegetables, baby food, tea, and tobacco is now available.



the science behind

The GPC technique is the most notable step within the Specht-Method and this was further evolved and utilised for the clean-up of polycyclic aromatic hydrocarbons, ubiquitous environmental pollutants generated mainly through the incomplete combustion of organic materials.

Their testing requires the removal of fat without removing fat soluble pesticides. To do this with a solvent is not possible, however GPC works instead on assessment of the similar molecular size of pesticides.

Further developments have seen a new extraction and partition step introduced into the well-established multi-residue DFG method S19, replacing dichloromethane with ethyl acetate/cyclohexane. In addition, working processes were further refined. The latest development is the reduction in use of solvents in order to protect the environment and save costs.

For references, please see bibliography



30 YEARS OF DATA FOR LIFE

Building rich databases before Big Data



Many of the tests that Eurofins performs around the world rely on one of the Company's extensive **proprietary databases**, not simply to compare results to, but very often to obtain the results themselves.

Eurofins' **DNA fingerprint** database, for example, contains unique identifying characteristics (the "fingerprints") of foodstuffs and enables proof of authenticity that had not previously been available. Not only does this prevent unscrupulous suppliers gaining an unfair market advantage, but it also prevents them endangering the health of the consumer.

Eurofins developed its proprietary DNA fingerprint databases for several specific analytical requests (e.g. **basmati / fragrant rice authenticity** or determination of different pine nut species), using proprietary or published DNA fingerprint methods and reference samples which were made available by authorities or via Eurofins' laboratory network and its customers. The databases allow identification of pure and mixed samples, but also of



Over the last three decades, Eurofins has earned its place as a leading provider of analytical services. The Company now performs more than 150 million tests each year to establish the safety, identity, composition, authenticity, origin, traceability, and purity of biological substances and products. Its extensive databanks offer years' worth of information about pharmaceuticals and food and their properties.



the presence of as-yet unknown or unapproved species or varieties. The testing methods meet the need for traceability – a major requirement of EU food legislation – and can be adapted very fast to the needs of the market, for example if new species or varieties are approved by authorities and reference material is available.

Another hugely exciting step-change in proprietary databases came with the launch in 1999 of Eurofins' **BioPrint™ databases** to improve and optimise the selection of drug candidates. The database comprises a large and homogeneous set of experimental data, generated in-house and containing more than 2,400 compounds, including marketed drugs, compounds which failed in clinical trials, and reference compounds.

Each compound has hundreds of pieces of information stored, with the BioPrint™ database covering in vitro assays as well as in vivo characteristics such as drug reactions, pharmacokinetics and therapeutic indications. On average there are 400

records for each compound, meaning more than 1 million records are stored. High-quality and high-content datasets, combined with modelling and mining tools, place new drug candidates in the context of well-understood drugs. This allows scientists to **anticipate adverse drug reactions** and supports lead compound characterisation and prioritisation (a lead is a possible drug candidate but may still have suboptimal structure and characteristics).

A further development is an analytical solution from Eurofins QTA that provides a major advancement in the way customers perform their infrared (IR) analysis. **IR spectroscopy** allows for fast analysis, even outside a laboratory environment, for simple parameters and is widely applied for raw material checks. The backbone is a proprietary database of (IR) spectra. Spectroscopy is the study of the interactions between matter and electromagnetic radiation. When an electromagnetic wave passes through a molecule, the molecule absorbs the energy at a specific frequency and, because different molecules absorb energy at different

frequencies, the collected spectrum can be used to identify the materials. QTA® uses near infrared (NIR) spectroscopy and mid-infrared (MIR) spectroscopy technologies in its qualitative analysis services.

The QTA® methods provide a simple interface for non-skilled users, thereby overcoming the challenges of traditional infrared analyses which have to be developed and maintained by experienced spectroscopists or chemometricians.

Instruments at the testing site scan a sample and send the light spectra of the sample to the secure server via the internet. The analysis and data interpretation are completed within minutes on the server, and the results are returned in real time. Eurofins QTA has developed customised testing methods for beer, hops, sugar cane juice, feed, fertilisers, and formulated pesticides.

the science behind

Information about the origin of a food product is often encrypted in its chemical composition, and rapid developments in science and technology over the last few decades allow its analysis and interpretation. Two of the most important techniques within DNA fingerprinting are DNA fragment length analysis and microsatellite or short tandem repeat (STR) analysis. DNA fragment length analysis considers changes in the length of a specific DNA sequence to indicate the presence or absence of a genetic marker. Eurofins used this technique to successfully detect a species of poor-tasting pine nuts which had triggered 39 biotoxin notifications in the EU Rapid Alert System for food and feed. STR analysis is used to compare specific areas on DNA from two or more samples. This technique, again based on Eurofins' extensive proprietary database of specific DNA fingerprints, was used by the Company to prove the authenticity of Basmati rice when the market was flooded with cheap imitations.

The BioPrint project was started with the hypothesis that the in vitro pharmacological profiles of new drug candidates generated in Eurofins' laboratory could act as a fingerprint, capturing information on the in vivo activity of the compound. The Company found that hierarchical clustering of the drug and reference compounds based on their in vitro pharmacological profiles, achieved the grouping of many of them by their therapeutic areas or biological actions; for example, antidepressants clustered with other antidepressants, and antifungals with other antifungal drugs. Using this "fingerprint", a drug candidate's potential therapeutic, and adverse actions could be placed in the context of all the drug and reference compounds present in the database by performing simple profile similarity analysis to identify "neighbour" compounds.

The backbone of the QTA® solution is a proprietary database developed using Chingometrics methods which cover all Eurofins QTA applications. Chingometrics are unique data treatment methodologies applied to calibration models for spectroscopic qualitative analysis applications. The proprietary database and algorithms are dynamically maintained for superior accuracy and precision, with primary data generated using industrial standard methods and stored in a highly-secure central server.

For references, please see bibliography



FINDING ADULTERATION EVEN WHEN NOT LOOKING FOR IT

Leading the way with non-targeted screening

When you search for something specific, that's probably all you are likely to find. By removing the constraints of targeted analysis and searching without restriction, Eurofins has opened up a whole world of new possibilities.



Eurofins' evolution of non-targeted screening methods is one of the Company's most exciting developments. Teams from across the Company have pioneered wider screening techniques covering a range of sectors, from food and pharmaceutical, to environment and water. A very prominent example of an unexpected, and hence missed issue that evolved into a scandal was the addition of the industrial chemical melamine found in baby milk in China in 2008. Another was the addition of horse meat to beef in Europe in 2013. Such fraudulent practices were never expected and so were not explicitly searched for. Non-targeted screening tests are enabling a whole variety of industries to better guard against health crises.





the science behind

High-resolution nuclear magnetic resonance (NMR) testing is one example for such an **untargeted screening approach**. Eurofins' method is now able to provide additional insights to those offered through conventional approaches. This investigative technique allows the detection of hidden anomalies, such as the addition of sugar to honey, or the mislabelling of geographical or botanical origins in the composition of a complex matrix. The approach can be applied to liquids or extracts from solid foods, requiring only limited preparation of the sample.

Another of Eurofins' pioneering techniques is a new non-targeted screening method, based on **16S rDNA screening** and next generation sequencing. Used to profile the

microbiome of food, pharmaceutical and environmental samples, the test focuses on identifying the micro-organism communities present, helping to recognise a broad spectrum of bacteria. For example, the test can be used for the control of fermentation processes or the analysis of biofilms (a thin microbial layer). The tests require no assumption to be made about a given species of interest, and all species included in the database can be identified in one test.

These sophisticated testing methods also have numerous applications in the environmental world. Eurofins Vejen is working with the University of Copenhagen, Denmark, to develop an untargeted method, **ChemFingSoil®**, for assessing environmental hydrocarbon pollution.

The first commercial project using this technique, examining the origin of hydrocarbon pollution in soil, was launched in February 2017. In the near future, the project is planned to be extended to involve chemical fingerprints of hydrocarbons in indoor air and also of soil and sediment pollutions with polycyclic aromatic hydrocarbons.

And this is only the beginning. Eurofins' laboratories globally are developing a whole range of other non-targeted screening techniques. Eurofins' insights are helping to ensure that consumers and the wider world at large are fully-informed about exactly what is in the products we all consume.

Eurofins' techniques are based on multivariate data analysis, multiple analytical approaches and extensive use of computer models.

NMR is carried out on the protons or carbon atoms of the molecules to be analysed, a full resonance spectrum becoming available within minutes. This technique allows the non-targeted detection of all organic compounds, and the NMR profile can be regarded as a unique fingerprint of the sample. Databases of authentic samples allow the establishment of statistical models for checking the integrity of foodstuffs.

Eurofins' launch of its next generation sequencing test service has opened up non-targeted bacterial screening for food and feed testing. Used for raw or processed food matrices, it provides a view of flora in the product thanks to bioinformatics and database tools. 16S rDNA is the commonly used and recommended DNA target region for bacterial species identification.

ChemFingSOIL™ was designed to describe the hydrocarbon source archetypes in soil by use of multivariate statistical analytical tools. Eurofins has, together with University of Copenhagen, patented the data processing method, with six archetypes validated and so described by the model: natural C5 hydrocarbons, petroleum / turpentine, engine oil / lubrication oil, gasoline / fuel oil, and pyrogenic hydrocarbons. Together they account for a vast majority of the pollution found in soil samples tested. The model is built on the chemical pattern of hydrocarbons in soil and is derived from GC chromatograms, which provides a quantitative analysis of volatile and semi-volatile organic compounds. Now an objective and quantified determination of the contents of any soil sample can be given.

For references, please see bibliography



Farming and herd improvement testing – in six seconds

Dairy farming had been undergoing evolution since the 1960s. Large and highly-specialised farms are now the standard, and dairy farmers are ever-more focused on herd improvements and introducing value-added tests to their farm management. Through its highly-automated milk laboratories, Eurofins is helping farmers produce increased and higher-quality yields from healthier herds.

Eurofins has expanded its services from stand-alone testing to supporting the whole value chain at the farm, in order to help dairy farmers to make informed decisions in favour of food safety and animal welfare. The Company has developed a range of tests to provide insights into the origin of milk, the total yield, cow's health, feed efficiency, environmental impact, quality, and adherence to all relevant regulations. Eurofins' approaches have reduced test times from weeks to just days and hours, providing results for more than 20 different parameters for the management of the farm.

The dramatic **milk yield increases** achieved since the 1960s are testament

to the effectiveness of this precision farming and precision testing, with the average yield increasing from 4,200 litres per year to over 10,000 litres per year for an average dairy cow in Denmark.

Testing itself has also developed markedly, with initial tests providing only limited, simple analysis of fat content with moderate accuracy. Eurofins now offers multiple testing, at **high throughput** in automated specialised laboratories and delivers approximately 200,000 results each day from 30,000 milk samples. Tests can be conducted on an individual cow, or on the milk bulk tank, and routine tests now include fat and protein to

evidence quality and assess price. The **somatic cell count** – consisting primarily of the white cells – are measured too, as increases in these can be a sign of a response to pathogenic bacteria and lead to illness or disease in the animal.

Eurofins is also able to run bovine diagnostics to measure factors such as beta-hydroxybutyrate, which indicates ketosis or possible energy deficiency of the cow, and Paratuberculosis (Johne's disease), which is a contagious and chronic disease reducing the milk yield. Other factors covered in the diagnostics include Bovine Viral Diarrhoea, which is a highly contagious infection

causing diarrhoea; Infectious Bovine Rhinotracheitis, which causes generalised systemic infections, and Bovine Spongiform Encephalopathy – commonly known as BSE – a fatal central nervous system disease. These all have significant implications for the animals' welfare, fertility and yield, as well as creating potential issues around food safety and adherence to legislation.

Eurofins is already working on more dynamic and interactive testing to include differential somatic cell count, genetics, environmental information, feed efficiency, and differentiation of spores.

the science behind

Four primary tests are used to test milk:

(i) the Fourier Transform infrared test (FTIR), using mid infrared spectrum to predict within six seconds more than 18 different parameters: including fat, protein, lactose, urea, casein, ketosis, freezing point depression, solids, adulterants;

(ii) flow cytometry for detection of somatic cells in milk. Flow cytometry is a technique used for counting and characterisation of particles and cells, and can be described as "the measurement of cells while they are flowing". The test speed is six seconds per sample;

(iii) flow cytometry for detection of the total count of bacteria in milk. Bacteria are counted through specific staining of the DNA and the use of a fluorescent marker, and at a test speed of 24 seconds per sample, and lastly,

(iv) diffusion testing for screening for inhibitors in milk. This test looks for antibiotic residues in milk and is performed using 96-well microtiter plates with agar and a coloured pH indicator. Spores from the test organism *Bacillus stearothermophilus* var. *calidolactis* will have a reduced growth when an antibiotic is present in milk. Results are qualitative and either positive or negative, and the test speed is 96 samples per three-hour period.

For references, please see bibliography

the science behind

Detecting drugs in hair has been possible since the early 1980s using forensic toxicology, a useful diagnostic tool in proving past drug use or exposure. Two Eurofins forensic toxicology laboratories – Toxlab in Paris and ChemTox in Strasbourg – were involved in the ongoing evolution of testing techniques for several years and together achieved an important breakthrough that assists the judicial system and victims of drug-assisted crimes.

Identifying knock-out drugs in hair

BEATING DRUG-ASSISTED RAPE—FORENSICALLY

Psychoactive substances are being used to facilitate crimes such as thefts and sexual attacks. Administered surreptitiously and without the knowledge of the victim, such crimes often involve drugs with a very short half-life. The drugs, including **hypnotics, sedatives and antihistamines**, can rapidly impair consciousness and cause loss of memory of events that occurred after drug intake. This meant laboratories and law enforcement agencies were struggling to assess drugs with a short 'life', and victims of crimes such as rape, sexual assault or theft were often left not immediately or fully understanding what had happened to them. Delays of more than two or three days in reporting such crimes only compound the problem, as most of the drug molecules were undetectable through the techniques classically used in forensic toxicology (gas and liquid chromatography).

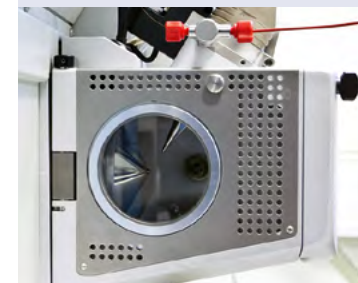
Presenting a solution, Eurofins used **liquid chromatography** coupled with **tandem mass spectrometry** and with triple-quadrupole, for the determination of these molecules in biological fluids and, more particularly, in hair. Essentially, they were able to scientifically and conclusively determine the presence of a drug in hair samples provided as evidence in drug-facilitated crimes, even when

there was a delay in the submission of evidence for analysis. It could also show that the drug detected was only ingested once on the date of the crime, eliminating defences that the victim was a regular user of that drug.

Given the variety of analytes, the limited amount of hair samples, and the need for a fast result, the Company limited the number and the complexity of the extractions, and focused on the technique of **selected reaction monitoring** (see right). Once the technique had been validated, Eurofins helped the French Ministry of Health and the French Ministry of Justice to publish a circular outlining how to obtain biological samples (blood, urine and hair) in such cases. They also participated actively in the organisation of external quality controls and the creation of a committee on drug-facilitated crimes, and they continue to provide their data to the French Health Agency to help it build an accurate epidemiological database.

Traditional radio-immunoassay testing evolved into liquid chromatography testing coupled with tandem mass spectrometry to meet the requirements of the Courts of Justice for analysis by two independent methods. Liquid chromatography coupled with mass spectrometry is applied in Forensics mainly for the analysis of thermally-unstable molecules in complex samples such as biological fluids. A triple-quadrupole mass spectrometer is a tandem mass spectrometry method in which the first and third quadrupoles act as mass filters, and the second causes fragmentation of the analyte through interaction with a collision gas. Selected reaction monitoring is a method used in tandem mass spectrometry where an ion of a particular mass is selected in the first stage and an ion product of a fragmentation reaction of the precursor ion is selected in the second stage for detection. Having information on the mass of the molecule (ion) itself and its fragmentation products allows for unambiguous identification of molecules.

For references, please see bibliography





HIGH-FLYING INNOVATION

Unconventional inspiration for DNA synthesis

Ready access at realistic cost to short DNA strands is crucial to many areas of scientific investigation. Turning to aerospace for inspiration, Eurofins made a vital ingredient more affordable and, in doing so, helped enable huge numbers of scientists elsewhere continue with valuable research.



the science behind



Nucleotides are the building blocks of life, and a form of these – oligonucleotides (or oligos) – are short, synthesised DNA strands required by scientists for almost all polymerase chain reaction (PCR)-based research. PCR sees stretches of synthesised DNA (oligo synthesis) amplified by creating a number of identical or near-identical copies and is used in a huge set of basic and applied research applications, across all areas of life science and a whole range of genomic investigations. In most cases, the synthesised oligos are used to determine whether a searched-for DNA sequence is present in a product or biological system.

The uses for oligos are broad but, when Eurofins was founded in the 1980s, oligos' high price rendered these **critical research materials** unobtainable for all but the most well-funded scientists. Eurofins Genomics US developed an innovative new platform enabling the production of oligos at an attractive price-point,

essentially democratising DNA and making oligos available to everyone for research.

Since that time, there have been many different iterations and advancements in **synthesis technology**, culminating in a further developmental leap in 2017. A major shortcoming of oligo synthesisers was their wear and the frequency of breakdowns with associated down-time. Here, Eurofins Genomics US turned to an unlikely field for the model – aerospace engineering – completely rethinking the DNA manufacturing process and, once again, succeeding in reshaping the marketplace.

Building a **DNA synthesiser** to manufacture these oligos is almost like building a small jet engine – both utilise the science of air pressure, air flow, vibration, fluidics, and thermodynamics, inside complex, mechanical engineering. But it was the adoption of aerospace engineering's

reaction to downtime – the sector considers it unthinkable – that facilitated the greatest step-change.

To engineer its next generation DNA synthesiser, Eurofins Genomics US partnered with a commercial-grade, aerospace engineering company. The result was the production of high-quality oligos in a shorter time. Also, owing to the incredible power and speed of the machine, the Eurofins team raised the ceiling on scalability and volume, its **innovative design** making the production of even extremely small volumes of DNA affordable.

Not only has this new device filled a critical gap in today's synthesis environment, but the advancement has the potential to help fuel deeper investigations in the biological sphere, revealing important new insights and ushering in a new era of research and scientific progress.

Scientists who buy oligonucleotides demand several conditions: high quality and reliability, rapid turnaround time, low cost, and the right quantity. Unfortunately, previous DNA synthesis technologies failed to accomplish all of these at once. Traditional high-throughput oligonucleotide synthesisers use a lot of reagents and make more DNA than that required in most applications. By contrast, emerging microarray or chip based methods generate too little DNA that, besides requiring additional downstream work before being useful, is only suitable for specific applications, predominantly, gene synthesis.

For references, please see bibliography

TESTING FOR TAILORED CANCER TREATMENT

Empowering cancer research through innovation

Cancer is a complex and multi-dimensional disease, driven by genetic instability and influenced by a multitude of factors. Each patient is unique, and each tumour ever-changing, yet treatment historically has taken a generalised approach with limited success. Advances by Eurofins are helping researchers to “personalise” cancer treatment and better target care for better outcomes.

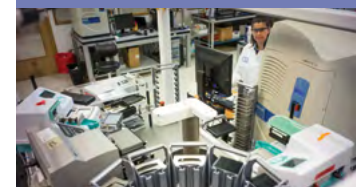
The Eurofins Pharma Discovery Services team in the US launched its OncoPanel™ Cell-Based Profiling Service comprising more than 300 genomically-diverse human cancer cell lines across 18 different tissue types. The service screens and profiles **anti-cancer compounds** in drug discovery, and also predicts potentially beneficial responses.

This means that drug candidates can be assessed in the very early stages of their development. By statistically correlating the genetic profile of each tumour cell with its response to the potential drug, predictive biomarkers can be identified. Drug discovery researchers can now determine which genomic features may predispose patients to successful treatments or identify **potential resistance mechanisms** that may diminish the effect of treatment.

In addition, Eurofins offers a 3D cell culture service that adds even greater insights by more closely modelling tumour structure. These cells are grown as three-dimensional tumour spheroids and designed to mirror key attributes of human tumours. Innovative, high-content imaging allows the comparison of 2D and 3D cell cultures to assess the efficiency and effectiveness of various drugs in penetrating each tumour.

Truly personalised insight into drug discovery means benefits for patients through the possibility of **faster and better results** from cancer treatment plans and the potential for health care services to reduce waste through inappropriate or inefficient drugs and therapies.

the science behind



High-content imaging enables simultaneous detection of a potential drug's ability to inhibit tumour cell growth and other biological responses. Depending on data requirements the services can be performed as short and long-term cell culture, as well as 3D models. Such 3D cultures, as opposed to standard 2D models, are particularly interesting, because they take account of the fact that a tumour in real life is not a flat assembly of cells. The 3D cultures hence mimic morphological and phenotypic characteristics of real tumours. They are grown in a way to ensure that the majority of the cells within the culture exist within spheroid or loose aggregates at the time of treatment.

For references, please see bibliography

NURTURING CELLS TO GROW

Culture media monitoring with Spedia-NMR™

Solid understanding of cell growth patterns and behaviours is central to the development of effective vaccines and antibodies. An innovative analysis service from Eurofins has enhanced researchers' access to the data they need to optimise research and production conditions when using cell cultures.

The production of biologic therapeutics, such as antibodies or vaccines, requires cell cultures and fermentation bioprocesses. A full understanding of the cell media used allows scientists to first establish those factors that most impact on growth and then to determine the best way to optimise the cell upstream process. This in turn allows for the development of more effective bioprocesses, improved yields and cost efficiency, while reducing variability.

This focus on understanding and controlling the **cell growth conditions** as early as possible, particularly during the fermentation upstream development phase, led to the launch of Eurofins' Spedia-NMR™. This nuclear magnetic resonance-based (NMR) analysis service is able to very rapidly monitor a wide selection of feed components, metabolites and toxic compounds in the culture media.

By analysing and comparing media profiles from cell cultures, Spedia-NMR™ delivers information on how cells consume and metabolise the

media along the culture process. This, in turn, allows fine-tuning of the composition of the media to improve **cell viability**, reach the highest yields of biologic product and facilitate rapid process troubleshooting.

Spedia-NMR™ technology offers the biotech research and manufacturing communities significantly enhanced access to detailed quantitative data, across the broadest scope of nutrients and metabolites. This potentially allows for a substantial reduction of the upstream process development timeline by several weeks or months, greatly improving bioreactor capacity access.

The true adaptability of Spedia-NMR™ analysis has led to its application across a range of cell systems and production platforms including bacterial fermentation, stem cells and virus production. Spedia-NMR™ is designed to perform rapid, robust and **quantitative analyses** of spent media samples in a very cost-efficient manner, using semi-automated data acquisition and processing.

the science behind

A range of NMR methods and techniques for spent cell media bioanalysis has been developed, to support the characterisation of biologics and biosimilars (including peptides, small proteins, oligo-/ polysaccharides) or complex mixtures. Unlike standard methods, Spedia-NMR™ does not require systematic calibration and has a built-in matrix effect compensation. The method is not dependent on the user or machine, delivers highly reproducible data, and analyses over 50 different compounds in the media.

For references, please see bibliography



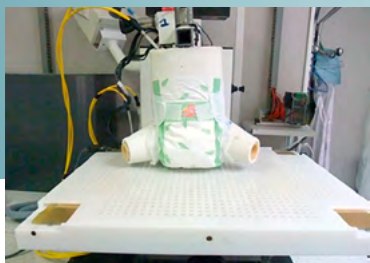
OUT OF THE BOX

PRODUCT TESTING

When nothing is available, build your own robot



The market is brimming with a vast array of consumer hygiene products, from diapers for babies to feminine sanitary items. Eurofins' sophisticated and realistic testing device revolutionised leak protection testing, providing manufacturers with reliable information about the effectiveness of products.



In 1991, Eurofins launched SCAN 1, a first-of-its-kind testing device for diapers (nappies). This exciting dynamic "model baby" offered a valuable validation tool and the first alternative to costly and hard to implement consumer panels. Not only did it allow Eurofins to provide very real technical feedback, but also to simulate **realistic user conditions**. Since its birth, the device has evolved into a third iteration – SCAN 3.

Leak protection is the best quality indicator for diapers, and this forms the core focus of the SCAN 3 device.

The parameters can be set for a wide range of realistic uses, focusing on known criteria around pressure, the quantity and number of instances of urine discharge, gender, and positions during use. Furthermore, the dynamic nature of the model's software allows testers to reproduce typical 'day-time' positions and 'night-time' positions for both sexes.

This **innovative device** is able to test not only the core absorption of the diaper but also other factors that may affect its efficiency, such as the shape of the product during wear, the degree

and manner of "gathering" during movement to a standing position, and tape resistance.

Validated since 1998, the SCAN range has seen technical advances including an anatomical ability to move the device's "legs", and the availability of devices in a range of sizes. Testers are also now able to quickly switch the device from one position to another, a feature that has further enhanced the degree of **confidence** that manufacturers, and consumers, can have in the absorbency of their products.

the science behind

The pioneering new SCAN 3 simulation device can be used in the debugging phase of a product test to provide useful and reliable technical feedback, based on qualitative indicators, and so reduce R&D costs. Later in the product development process, the tool can facilitate the pre-selection of prototypes. As part of this innovation, Eurofins has developed a further enhancement to the way in which pressure is tested on products. Now completely independent of the weight of the model, pressure is still exerted from top down, but is completely variable for the first time and can be adjusted for the weight desired via a secured arm with vertical mobility.

[For references, please see bibliography](#)

INVENTING THE MOST SENSITIVE HEAVY

Testing for safety in soil, water, food and pharmaceuticals

METALS DETECTION METHOD

Heavy metals in foodstuffs such as fish and seafood, fruits and vegetables, and nuts and cereals are a serious threat to health. Exposure to even small amounts of cadmium, lead, mercury or arsenic can prove to be extremely toxic as they accumulate in the liver and other organs. Eurofins' pioneering tests for these substances can detect the minutest traces.

Heavy metals in the environment, in substances such as soil and water, can find their way into the food chain and pose a serious risk to consumers. The products most likely to contain these heavy metals are biological – those wholly or partly derived from biomass such as plants, trees and animals – and pharmaceuticals. Comprehensive detection of the presence of toxic materials in such products helps ensure the safety of the foodstuffs we consume and the medicines we take. By working with the latest available technology, using state-of-the-art instrumentation, and refining best practice methods, Eurofins Frontier Global Sciences has helped to pioneer some of the industry's **lowest detection limits** for toxic trace metals in the environment. Operating in the cleanest-possible testing environment is paramount, and the lower the detection level, the greater the importance of avoiding contamination, even at minute levels.

The US' Environmental Protection Agency (EPA) hired Eurofins as its trace metals and metals speciation reference and validation laboratory,

asking the Company to perform the final validation of what developed into the US EPA 1600 series methods for waters, soils/sediments and biological tissues. Today, these principles are adopted for **compliance monitoring** around the world.

Eurofins' presence and expertise in numerous other sectors meant that these methods had applications in a number of markets and industries outside environmental testing, and the company has expanded the tests' reach to dietary supplements, the food and beverage sector, and pharmaceutical products.

Furthermore, Eurofins' work became a catalyst for both industry and the regulatory community in **shaping policy** in several of these areas. For example, the value of measuring **metals speciation** to differentiate between total arsenic and the actual most harmful or toxic forms of arsenic – such as arsenate and arsenite (or inorganic arsenic) – helped to provide the scientific means to develop policy in the food and environment sectors.



the science behind

The team was able to achieve lower detection limits through a combination of expert sample handling to greatly reduce laboratory-related contamination; training of customers to take samples to reduce environmental contamination, and additional proprietary measures to digest and/extract elements of concern which will allow the laboratory to use higher sampling sizes (which reduce detection limits) as well as minimise interferences. The laboratory uses a closed vessel high-heat pressurised digestion for both solids and liquids as well as microwave technology.

For references, please see bibliography



A PASSIVE REVOLUTION IN WATER SAMPLING

From spot-checking to continuous environmental monitoring

Traditional water-sampling techniques are complicated, time-consuming, and often expensive. Worst of all, they provide either only snapshots of pollution events or need expensive sampling stations that require electricity and servicing. Eurofins Sorbisense came up with a unique alternative.

Eurofins' ground-breaking Sorbisense™ **passive sampling** system is designed to ease groundwater and surface water contamination assessment, and provide better and more reliable water-quality testing data.

SorbiSystems™ comprises a small cartridge, the SorbiCell, which contains an adsorbent and a tracer salt. SorbiSystems™ is suitable for installation in groundwater, surface water and wastewater environments. Once installed, water passes through the unit at a **controlled rate**, retaining the accumulation of specific chemical groups and solutes such as pesticides, nitrate, and volatile organic compounds. These solutes are collected throughout the entire

installation period of the sampling cartridge.

As water moves through the unit, the tracer is dissolved proportionally to volume, regardless of the sampling rate. This principle allows a very accurate measurement of the sample volume and solute mass concentration. The cartridge is then easily removed and sent to a laboratory for analysis.

Data obtained through SorbiSystems™ represent an **accumulated average concentration** rather than a single snapshot, providing significantly more consistency of results and enabling an exact assessment of risks and the effect of remediation.

The technology can be used to assess groundwater, drains, drinking water, lakes, ponds and streams, and industrial wastewater. It has particular appeal for companies in sectors like aviation, oil and gas, and utilities where security issues and access to sites can be a challenge. Efficient for all types of risk screening and risk assessments, the system is estimated to save 50-80% of the previously required time in the field.

In another example of Group innovation in water sampling, Eurofins is the first laboratory network to make the **POM (polyoxymethylene) passive sampling** technology commercially available in Europe. Developed by the Norwegian

Geological Institute, POM is a passive sampling method which can be used to **monitor organic contaminants** such as PAH and PCB in water.

Thin strips of the polymer polyoxymethylene are fixed to ropes or other sampling gear and deployed from an anchor line to monitor fresh water, sea water, wastewater, groundwater and pore water, across various applications, over a four week period or longer. POM sampling is simple to use, does not require special deployment of a field cage and avoids sample contamination or saturation.

the science behind

The Sorbisense™ sampling tool is suitable for sampling virtually all chemical solutes (contaminants). No equilibrium-based assumptions are made, and no diffusion calibrations required, just direct and transparent volume-based calculations of solute concentrations. Specially-designed mounting units allow for easy installation in a wide range of water environments.

POM is an equilibrium passive sampler where strips of polyoxymethylene are submerged in water, via an anchor line or placed in an underwater sediment bed via an aluminum frame. The POM strips monitor for organic contaminants in water over a period of four weeks or more. The state of equilibrium that the strips achieve during this time allows for the concentration in the water to be calculated, and extremely low concentrations of PAHs and PCBs can be detected. The detection limit can be as low as 1 pg/l for PCB and 1 ng/l for PAH. The testing method does not require any information on the salinity, temperature, water velocity or viscosity of the sample.

For references, please see bibliography





ALGAE, ALGAE, EVERYWHERE

TOXIC AND WHAT A STINK

First rapid test for cyanobacteria toxins in water bodies

Warm weather brings people outdoors and often to recreational waters such as beaches, lakes, ponds and rivers to enjoy those beautiful days. It can also bring about potentially toxic algae to these same bodies of water. While a number of people are familiar with "red tides," blue-green algae, more correctly known as cyanobacteria, can be just as dangerous.

Toxins produced by certain strains of cyanobacteria found in these freshwater, estuarine and marine bodies, can cause liver and kidney damage, have been linked to Alzheimer's and may be carcinogenic. Higher levels of the ratio of nitrogen to phosphorus; increased organic matter availability; higher temperatures, and decreased light intensity attenuation are among the factors that influence the formation and persistence of these cyanobacteria.

In addition, scientists have found that climate change may be a catalyst for a global increase in harmful cyanobacterial blooms. All these impacts have led to increased calls for improved testing methods for **cyanobacterial toxins**. Eurofins Abraxis responded with the development of rapid **detection test kits** for microcystins, cylindrospermopsin, saxitoxin, and the world's first detection kits for anatoxin-a and β -Methylamino-L-alanine (BMAA).

Cyanobacteria are often confused with green algae, because both can produce dense mats that can impede activities such as swimming and fishing, and may cause odour problems and oxygen depletion which can affect fish. However, unlike cyanobacteria, green algae are not generally believed to produce toxins. Cyanobacterial blooms, on the other hand, are known to produce highly potent cyanotoxins that can damage the liver, kidneys and the reproductive system, and even cause death.

These toxins can enter the body through accidental ingestion, inhalation or dermal contact, or can end up in drinking water through the water processing system, as occurred at Lake Erie in Ohio in 2014 and which badly affected the city of Toledo. Some bacteria blooms can overwhelm the filter or capacities of water treatment plants and there is evidence that the toxin can enter the food chain via

irrigation systems. Cyanobacteria do not always produce toxins however, and the presence of toxins can only be detected through water sample analysis. The toxin microcystin was detected at Toledo by the use of kits from Abraxis, part of Eurofins Technologies since 2017, and the test has since become EPA method 546.

Eurofins Abraxis is the world leader in rapid test kits for freshwater and marine toxins and continues to develop unique tests for cyanotoxins. Many of them are patent-protected and Eurofins Abraxis is shortly to commercialise the first ELISA – an enzyme-linked immunosorbent assay – for the cyanobacterial anabaenopeptins toxins. Eurofins Abraxis is also developing tests to help water companies to manage cyanobacteria that produce the compounds geosmin and methyl isoborneol which have a very strong taste and, particularly, odour.



the science behind

Cyanotoxins can be produced by a wide variety of planktonic cyanobacteria. The most commonly identified cyanotoxins are microcystins, cylindrospermopsin, anatoxins, saxitoxins, and BMAA. Microcystin-LR is the most toxic form of over 160 known toxic variants, and is also the most studied by chemists, pharmacologists, biologists, and ecologists. Microcystins contain several uncommon non-proteinogenic amino acids such as dehydroalanine derivatives and the uncommon β -amino acid ADDA. Microcystins covalently bind to and inhibit protein phosphatases PP1 and PP2A and can cause non-alcoholic liver disease, pancreatitis, and liver cancer. Eurofins Abraxis offers two cyanobacteria toxin test formats: a quantitative ELISA which takes 1.5-3.5 hours to produce up to 40 results, and a semi-quantitative lateral flow immunoassay (dipstick) that takes around 15-30 minutes depending on the analyte.

For references, please see bibliography



DNA profiling sensitivity has significantly increased over recent years, and forensic scientists are now able to detect DNA in situations where it was previously not possible. However, this super-sensitivity can mean the detection of low-level, background DNA, and mixed profiles in results. In response, Eurofins developed its own globally-leading software for determining the contributors to DNA mixtures in serious crime cases.

Eurofins' new DNA software heats up cold cases

SOLVING OLD CRIMES WITH NEW TECHNOLOGY



Autosomal DNA profiling was first used in criminal investigations in the 1980s. Techniques provided highly discriminating DNA profiles and compelling evidence of association, but did require several days, sometimes weeks, to produce a profile. Techniques have continued to evolve and have increased in sophistication. Now that DNA profiling is so sensitive, courts

sometimes call upon forensic scientists to give an opinion not only on the origin of blood, saliva or semen under examination, but also on how it got there – for example, by hand, from a weapon, or via a kick or a punch. Often, the levels of transferred DNA are as small as any **background DNA**, bringing extra challenges to the interpretation of any **mixed profiles** found.

As DNA profiling becomes ever more sensitive, the likelihood of detecting DNA from multiple sources is increasing. Interpretation of some of these profiles is straightforward, while others can be very complex. Eurofins responded with the development of LiRa, its own sophisticated statistical software, to help courts and investigating teams to understand the strength of the evidence in cases involving complicated profiles.

The first version has been in use for just over two years by a core group of Eurofins scientists. Accepted in the UK courts, this version is capable of evaluating mixtures consisting of up to three **DNA contributors**. The next version of LiRa extends its capability to calculate likelihood ratios for profiles with up to four contributors and has been available since February 2018.

Depending on conditions and the quality of conservation of the material, DNA can be present years later. Eurofins' sophisticated software has already been used to solve a number

of cold cases, including a 1984 violent sexual assault in Bradford, England, for which the assailant recently received a conviction, 33 years after the attack. Forensic scientists found a tiny speck of blood on a knife recovered from the scene and obtained a low-level, partial DNA profile. While the low-level nature of the result meant that the forensic scientist was unable to give a weight of evidence using conventional means, i.e. the binary model, she was able to see that the prominent components matched that of the suspect. Using **LiRa (discrete model)**, it has now been possible to calculate a likelihood ratio – the evidence is 490 million times more likely if the DNA came from the suspect and an unknown, than from two unknowns.

The software's capability is strengthened by Eurofins' own statistician and IT development team who, if necessary, can attend court to explain complex statistics and defend challenges. In addition to cold cases, LiRa is used in the investigation of current crimes.

the science behind

Traditional methods take a binary approach to analysis and Eurofins continues to employ such a method with single-source profiles and two-person DNA mixtures. The early version of LiRa used the more advanced discrete approach, taking into account the presence or absence of DNA alleles, but not their heights. The latest version of the software, LiRa V2, uses a continuous model and takes a quantitative approach, measuring the peak heights and treating them as continuous variables to calculate a likelihood ratio following comparison with a nominated individual.

For references, please see bibliography



Commercial development of genetically modified organisms (GMOs) is on the increase, with maize and soy just two examples. The emergence onto the market of the first GM animal for human consumption – a salmon – means increased calls for clarity on the sale of GM produce. Eurofins responded with the introduction of the world's first commercial test for detection of this genetically modified fish.

Fast, reliable testing for genetically modified fish

LEADING THE WORLD IN GM FISH DETECTION

GM salmon must be reared in closed fish farms without access to open water, to guarantee separation from non-GM fish. The first GM salmon, **AquAdvantage®**, found its way onto the Canadian market, and consumers' plates, in 2017, sold by producer AquaBounty Technologies. Atlantic salmon was modified with the introduction of a gene from another salmon species, the Pacific Chinook, which regulates growth hormones

allowing AquAdvantage® salmon to grow both faster and year-round instead of just during the spring and summer.

This GM salmon received approval for sale in Canada in May 2016 and, according to Canadian law, does not need to be labelled. In both 2017 and 2018, 5 tons of the GM salmon, AquAdvantage®, were sold in Canada. In 2019, the GM salmon was not only

approved for sale in the USA but also for cultivation in a salmon farm in Indiana. However, in EU member states and other countries, the marketing of AquAdvantage® salmon remained unapproved. As a result of this situation, a specific method to detect AquAdvantage® salmon was required.

Eurofins responded with the rapid introduction of a very specific and highly sensitive DNA-based test to

detect the genetic modification of AquAdvantage® salmon. The method is able to detect minute traces of the material in a sample, and to distinguish genetically modified salmon from unmodified.

The method, developed by Eurofins GeneScan Technologies, is also available as a full commercial detection kit for the modified salmon. In combination with an efficient DNA extraction method, the detection of GM material is possible even in highly processed samples. The test kit can be used flexibly with existing common DNA extraction methods and alongside one of the world's broadest and fastest-growing portfolios of existing GMO tests developed by Eurofins GeneScan.

Eurofins GeneScan, located in Freiburg, Germany, represents Eurofins' **GMO testing** competence centre and offers tests for all commercialised GMOs, including GM maize, soy, canola, rapeseed, cotton and rice.



the science behind

The Eurofins GeneScan Technologies test is based on real-time polymerase chain reaction (PCR). The high specificity of PCR is achieved by the use of so-called primers and probes, short single-stranded DNA fragments, which are specific for the target DNA sequence and help amplify the target DNA sequence that is located between the two primer sequences. Whereas conventional PCR uses gel electrophoresis to make the amplified products visible, state-of-the-art real-time PCR methods use a so-called probe which is a further single-stranded DNA molecule labelled with a fluorescent reporter dye at one end and a quencher molecule at the other. The DNA polymerase, an enzyme necessary for the PCR process, degrades the probe, releasing the fluorescent reporter molecule and therefore leading to an increase in fluorescence which can be measured in real-time.

For references, please see bibliography

NOT WANTED! DEAD OR ALIVE!

MOULD AND BACTERIA

Eurofins' new molecular methods enhance mould analysis

Mould and bacteria growth indoors can lead to illnesses. Work by Eurofins to improve testing methods to determine the presence of fungal and bacterial spores in indoor environments has resulted in much faster, more specific and detailed analytical tests. These tests increase the ability to detect a wider range of mould and bacteria species, including dead or dormant, that can develop inside buildings.

Awareness is growing of the threat to human health from mould and bacteria indoors, including **dead or dormant proliferations**. Modern buildings with higher energy efficiency may have limited air circulation and, consequently, higher humidity levels. Unfortunately, such conditions can lead to mould formation and bacterial growth. Higher numbers of these microorganisms both in air and in construction materials may affect the mucous membranes, causing respiratory ailments such as asthma, and symptoms resulting from an activation of the immune system, such as itching, eye discomfort, headaches and fatigue. In 2009, the World Health Organization (WHO) stated, that mould and bacteria in indoor environments should be minimised.

Traditional testing methods, where species determination analysis is based primarily on morphology, pigmentation and growth characteristics on media, were limited to living mould and bacteria cells. In fact, dormant mould can proliferate again under the right conditions, and may also produce harmful toxins (e.g. *Streptomyces geosmin*). Traditional testing methods are also laborious and time-consuming, with samples collected either by extracting a piece of the construction material or by air sampling, before cultivating mould over seven days. Moreover, this cultivation test does not take into account any dormant/dead bacteria or mould spores.

Eurofins Environment Sweden developed a superior method to detect

total mould/bacteria biomass, so including living and dead mould. A **new DNA test**, developed together with Eurofins' Genomics team in Ebersberg, Germany, uses a DNA probe designed to attach to most mould found in the environment. The company also offers another similar probe for bacteria and, in addition, specific probes for some species of mould and bacteria whose odours make them undesirable. One test option produces total mould/bacteria numbers, while another identifies bacteria and mould species by strain.

Although attribution of specific microorganisms that may occur in damaged building materials to specific illnesses remains scientifically and medically difficult, these new tests mean customers are better placed

to make informed decisions about their buildings' condition and their employees' health.

The tests can help distinguish between new ongoing moisture damage and old damage from previous mould or bacteria growths and, if total mould and/or bacteria numbers are above acceptable levels, damaged material can be removed from the building's structure and substituted with new. The tests on living and dead mould can also help evaluate different sorts of building materials for their qualities in terms of mould susceptibility.

Regulatory developments in a number of countries are likely to result in the testing method becoming more widely applied.



the science behind

An ability to identify total numbers of mould and bacteria present, whether living or dead, gives scientists a much deeper understanding of the extent of bacterial presence. Applying a simple criterion that a "damaged" material contains levels of mould or bacteria more than ten-fold higher than a reference material, Eurofins researchers detected a substantial number of "damaged" samples with 10-1000 fold higher levels than the reference sample. Bacteria were responsible for a far higher number of the "damaged" findings, and were sometimes solely responsible. The experiments also showed that living microorganism numbers were considerably lower than the total numbers, demonstrating the importance of detecting dead and dormant mould and bacteria as well as living, in order to define moisture damages causing indoor air complaints

For references, please see bibliography




FUELLING THE FIRST ASTRONAUTS

Fortifying food for space

Have you ever wondered what astronauts eat when they are in space? With a relationship with NASA spanning over five decades, Eurofins (as WARF Institute, Hazleton Laboratories, and Covance) has played a crucial role in ensuring the food that fuels astronauts as they travel to outer space is nutritious, safe and palatable.

During the 1960s, The National Aeronautics and Space Administration (NASA) was heavily involved in a programme to send manned rockets into outer space, to land on the moon for the very first time. Preparation required hundreds of extremely challenging research and innovation projects, including developing the products that the astronauts would eat during the first journey to the moon.

Food scientists needed to develop fully **fortified foods**, which could remain stable for long periods of time in outer space. These foods would require lyophilisation, or freeze drying, for stability and storage, and needed to contain substantial nutrients, to sustain the astronauts, while also tasting good.

NASA partnered with Eurofins scientists, at the time WARF Institute, Hazleton Laboratories, and Covance, to determine the correct steps required to fortify these foods. The vitamins that were to be used needed to be stabilised

using an **encapsulation process**, where vitamin molecules are confined within larger molecules. Testing for these encapsulated vitamins posed a particular challenge. However, new methods were required to accurately determine how nutritious the stabilised food products were across the board, in terms of proteins, vitamins, carbohydrates, fats and minerals.

As part of the project, Eurofins scientists developed a range of **new test methods**, utilising High Performance Liquid Chromatography (HPLC) and Inductively Couple Argon Plasma Atomic Emission Spectroscopy (ICAP), and many of the methods are still in use today. Methods developed for NASA to test for vitamins and minerals in these foods have since become Official Methods of AOAC International (formerly the Association of Analytical Communities), and are used in food testing laboratories across the world.

After the extraction techniques and analyses were developed, Eurofins laboratories also put them into practice and tested all of the foods developed by NASA for their nutritional content and stability. Many of these test methods became official methods and are used today for food testing beyond the purposes of space travel.

Eurofins' close working relationship with NASA continues today as they consistently develop new, more stable, and better tasting foods for their astronauts. As NASA develops food to be used in the International Space Station, and food to be used in the first manned space mission to MARS, which must remain stable for even longer, up to a five year period, Eurofins performs all quality control testing on these foods used in space.

the science behind

The food products developed by NASA had to be specifically fortified with nutrients and stabilised to ensure a longer shelf life. In order to ensure that the foods still contained the correct levels of vitamins and minerals needed, Eurofins was tasked with the development of new bespoke techniques which would allow for the extraction of these vitamins and minerals to facilitate testing.

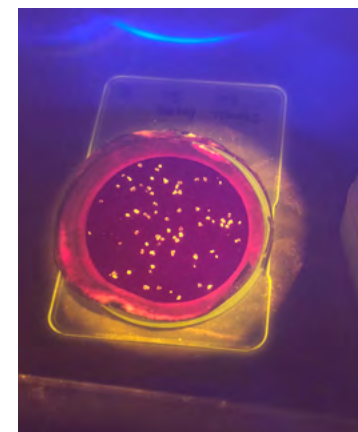
Methods used historically only had to measure natural levels of a vitamin. The NASA samples had additional vitamins added to the food, so the samples had to be treated with newly developed extraction techniques to isolate the natural vitamins and the added vitamins, a very delicate procedure.

The new analytical methods that were developed to test for vitamins in the foods created by NASA included, for example, High Performance Liquid Chromatography (HPLC) to separate, identify, and quantify each vitamin component in the foods created.

Those methods which tested for minerals made use of Inductively Couple Argon Plasma Atomic Emission Spectroscopy (ICAP-AES), a technique which is based on inductively coupled plasma to produce excited atoms and ions that emit electromagnetic radiation at wavelengths indicating a correlation to a particular element.

For references, please see bibliography

If not correctly recycled, plastic poses a major threat to our environment. Degrading into tiny particles, microplastics can remain in the environment for over hundreds, if not thousands, of years, infiltrating aquatic and marine ecosystems and contaminating the water we drink. Recognising the major global environmental problem that microplastics present, Eurofins is at the forefront of providing testing solutions to identify the prevalence of microplastics in our environment.



the science behind

FTIR spectroscopy and microscopy analyses use infrared light to scan test samples and observe chemical properties. In the dye imaging method, microplastic particles in water absorb dye and glow under ultraviolet light, highlighting their presence. The NOAA Method identifies larger microplastic particles in water which is considered to be dirtier. The water undergoes chemical treatment, density separation, mass determination and possible spectroscopic investigation.

Pyrolytic-GC-MS testing utilises heat to decompose samples to produce smaller molecules which are then separated by gas chromatography and detected using mass spectrometry. Pyrolytic-GC-MS can be applied to insoluble and complex materials including plastics, and when combined with FTIR spectroscopy provides optimal results.

For references, please see bibliography

Making microplastic testing commercially available

TACKLING THE
BIG
PROBLEM
OF TINY PARTICLES

Microplastics are any particles of plastic fragment smaller than 5mm. Polluting our environment every day, microplastics contain or carry hazardous chemicals that have a negative impact on nature and human health, and so their ingestion through water, or foods such as fish or shellfish, is of particular concern. In 2018, The World Health Organization announced it was to launch a review into the potential risks of microplastic particles in drinking water.

Eurofins Norway is at the forefront of developing testing methods to optimise microplastic testing, by combining the standard FTIR microscopic method testing with **Pyrolytic-GC-MS** testing. By their very nature, microplastic particles are just that – micro. Microplastic particles can be filtered from clean liquid samples with a vacuum filter, but the accuracy required to identify these particles is time consuming. The team is developing an innovative and **bespoke filtration process** to enhance process capacity by up to 6 times, and enhance process speed, delivering results in a commercially viable turnaround time,

where they can be applied to make a real difference.

Several methods can be used to identify individual microplastic polymers – the most common being dye imaging and NOAA methodology under **FTIR spectroscopy** and microscopy technologies. Eurofins SFA Laboratories, based in Wisconsin, is the first laboratory to make these analytical methods commercially available, offering testing across several matrices including marine and drinking water sources and finished bottled water. This lab is currently developing methods to determine microplastic contamination in other food matrices such as fish, shellfish, and aquatic plants like seaweed.

The Eurofins Group is currently undertaking research to better understand the threat that microplastics pose to the safety of our environment, utilising the testing methods developed by Eurofins Norway, to determine the prevalence of microplastics in ecosystems across the world.



SAFEGUARDING THE HEALTH OF

Setting the new standard in infant formula testing

THE WORLD'S MOST VULNERABLE CONSUMERS – INFANTS



In 2008, China was rocked by a national food safety scandal when it emerged that a chemical component called melamine, which is harmful to humans and is used in plastic and fertiliser production, had made its way into infant formula and milk. Of an estimated 300,000 victims affected by the compromised milk and infant formula, six babies died and an estimated 54,000 babies were hospitalised. The tragedy highlighted the importance of using the most stringent methods to test infant formula. Having screened tens of thousands of samples for melamine levels during the Chinese scandal, Eurofins has also played a crucial role in a global initiative to overhaul, redevelop and modernise infant formula testing.



the science behind

The Vitamin D, Iodine, Choline, and Carnitine methods developed by Eurofins made use of some of the most advanced analytical instruments at the time.

The assays for Vitamin D, Choline and Carnitine involved the use of LC MS-MS (Liquid Chromatography Tandem Mass Spectrometry) in vitamin analyses for the first time. This is an analytical chemistry technique that combines the physical separation capabilities of liquid chromatography with the mass analysis capabilities of mass spectrometry, far more accurate, precise and specific than the HPLC, colorimetric and ELISA methods used pre-SPIFAN.

The Iodine assay redeveloped by Eurofins utilised ICP-MS, where equivalent tests previously used ion specific electrode testing. ICP-MS, or inductively coupled plasma mass spectrometry, is capable of detecting metals and several non-metals at very low concentrations. This is achieved by ionising a sample with inductively coupled plasma and then using a mass spectrometer to separate and quantify ions.

For references, please see bibliography

Infant formula is often the sole source of nutrition for infants and must contain 100% of their required nutrients. By its very nature, it is one of the most highly regulated products in the world, and it is essential that the most stringent validated testing methods are used for product analysis. The 2008 Chinese milk scandal was described by the World Health Organization as one of the largest food safety events it had ever had to deal with, and noted that the crisis of confidence among Chinese consumers would be hard to overcome. At that time, the standard test methods for infant formula were those developed by AOAC International (formerly the Association of Analytical Communities) in the 1980's, which had become outdated. The scandal was the catalyst for an initiative to modernise and improve all infant formula testing methods.

As members of AOAC, leading infant formula producers such as Abbott, Danone, Johnson & Johnson and Nestlé, came together in 2010 to form the Stakeholder Panel on Infant Formula and Adult Nutritionals, **SPIFAN**, to develop a new uniform suite of stringent infant formula testing methods. Scientists from Eurofins Food Integrity and Innovation, at that time Covance Food Solutions, were chosen to lead SPIFAN, as Chair of the stakeholder panel, and were chosen to validate all new testing methods. Eurofins scientists also held positions as Working Group and Expert Panel Review Chairs, as well as method developers and reviewers.

SPIFAN identified and validated a modern suite of best practice analytical test methods, the current AOAC Official methods for nutrition in infant formula and adult nutritionals. 50 new AOAC

Official Methods have been validated by SPIFAN which are regarded as the world and industry standard in infant formula testing. **New methods** developed to test for fat soluble vitamins, A, D and E for example, are considered to be among the most accurate and precise methods ever developed.

Eurofins scientists participated in the collaborative study trials for over 15 of these new SPIFAN methods. The new approved SPIFAN methods for the analysis of Vitamin D, Iodine, Choline, and Carnitine were all developed and validated by Eurofins scientists, and are considered the international 'gold standard' in testing of this kind.

The redeveloped tests which are based on different methods than those used in the 1980s, are nowadays applied by every infant formula producer

across the globe. They are recognised by international standards groups such as the International Standards Organization (ISO), the International Dairy Federation (IDF), and Codex Alimentarius. SPIFAN, and the test methods developed by Eurofins, changed the landscape of infant formula testing, and showcased Eurofins' ability to create world-class testing standards.

PROTECTING CANCER PATIENTS

Identifying high-risk patients following stem cell transplant

For many cancer patients, the transplant of stem cells from a donor is potentially a life-saving treatment. However, there is a risk that donor cells can attack the patient receiving them in a disease called acute graft versus host disease (aGVHD), a potentially life-threatening condition following cell transplantation. Innovative tests, offered by Eurofins, can predict the risk of rejection so that the best patient care can be planned for.

Stem cell transplants, including peripheral blood, bone marrow, and cord blood transplants, can be used to treat cancer, most often those affecting blood or the immune system, like leukemia, lymphoma, or multiple myeloma. Bone marrow and stem cell transplant treatments use very high doses of chemotherapy, sometimes coupled with radiotherapy, to try to eliminate cancer cells. While the high dose treatment can kill cancer cells, it also can remove the stem cells in bone marrow that produce blood cells. Soon

after treatment, stem cells from a donor are given to a patient to replace those that were destroyed. The donor stem cells make their way into bone marrow and start to produce healthy blood cells again. A bone marrow or stem cell transplant from another person, either from a matched relative or an unrelated donor, is called allogeneic hematopoietic stem cell transplantation (HSCT).

While potentially life-saving, allogeneic HSCT can also lead to the

development of **acute graft versus host disease** (aGVHD). aGVHD is caused by immune dysregulation that is initiated when particular types of allogeneic white blood donor cells (the graft) react against the cells of the patient (the host) receiving them. The donated cells recognise the host as foreign and attack, damaging host tissue, and releasing inflammation fostering substances into the body. aGVHD typically occurs in the first three months post-transplant, has an incidence rate of between 19 – 66% and can be life threatening in severe cases.

Work over the past several years has investigated the use of **biomarkers** for non-invasive and predictive assessment of aGVHD risk. Biomarkers can determine whether aGVHD is likely to develop so that clinicians can accurately identify, at an early stage, patients which are at high-risk for severe aGVHD and improve patient outcomes following allogeneic transplants. In August 2018, Viracor Eurofins became the first laboratory

to make a predictive aGVHD assay commercially available.

The assay predicts the risk of aGVHD development in those patients who are beginning to display symptoms, those who have previously received aGVHD treatment and even those who are not yet presenting any symptoms indicating the disease. The scope of the test, made available by Eurofins, supplies healthcare providers with actionable data to definitively **identify at-risk patients** and guide decisions to improve treatment outcomes. High-risk patients can be preemptively treated for aGVHD, while low-risk patients may potentially avoid unnecessary treatment. The aGVHD predictive assay provides a faster, more cost effective and less invasive testing alternative to biopsy, and provides vital information to allow for preemptive intervention, minimising the risk of relapse and infectious complications.



the science behind

The aGVHD predictive assay was developed by Drs. James Ferrara and John Levine (both professors of Pediatrics, Medicine, Hematology and Medical Oncology at The Tisch Cancer Institute at Icahn School of Medicine at Mount Sinai), and validated in conjunction with 17 hematopoietic stem cell transplantation (HSCT) centers to help predict the risk of non-relapse mortality (NRM) and aGVHD in HSCT patients.

The assay is based on algorithms utilising serum levels of the so-called ST2 and REG3α biomarkers. These algorithms have clinically validated cutoffs that provide actionable insights within 24 hours. High-risk patients can be preemptively treated for aGVHD by adjusting immunosuppressive drug dosages prior to the onset of clinical disease, while low-risk patients may potentially avoid unnecessary treatment.

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