RESEARCH WITH

IMPACT

Centre for Research in Biosciences

UWE BRISTOL

University of the West of England

Welcome

Dear colleagues,

I am delighted to introduce *Research with Impact*, a new publication from the Centre for Research in Biosciences (CRIB) at the University of the West of England (UWE) in Bristol.

CRIB is a vibrant multidisciplinary research centre. It has over 120 members who bring together expertise in biosciences, bio-sensing technology, plants, the environment and agri-food. Applied and translational research is at the heart of what we do; we aim to address real-world challenges as well as contributing to the Government's main goal of economic growth. We achieve this by driving forward innovative ideas working closely with the stakeholders.

At CRIB, we have extensive links and close collaborations with industry, health organisations, charities and government agencies across the UK, Europe, China, Malaysia, the United States and other parts of the world. Our research has been supported by a range of funding bodies including the UK Research Councils, Innovate UK, industrial companies, the National Institute for Health Research, the European Commission and the British Council. The development of UWE's Enterprize Zone, that provides space for new businesses to form and flourish, will open up new opportunities for wider engagement with industry, and will accelerate the impact of our research.

In this publication, we highlight some exciting research projects that are already making a difference to society as well as the projects that are accelerating towards the market.

I hope you enjoy reading about our exciting research. You can read more about our work at www1.uwe.ac.uk/hls/research/biosciences

With best wishes,

Olena Doran

Professor Olena Doran
Director, Centre for Research in Biosciences

Get in touch with CRIB

Do you have any project ideas you would like to develop with the Centre for Research in Biosciences? We would be delighted to hear from you. Get in touch via **CRIB@uwe.ac.uk**

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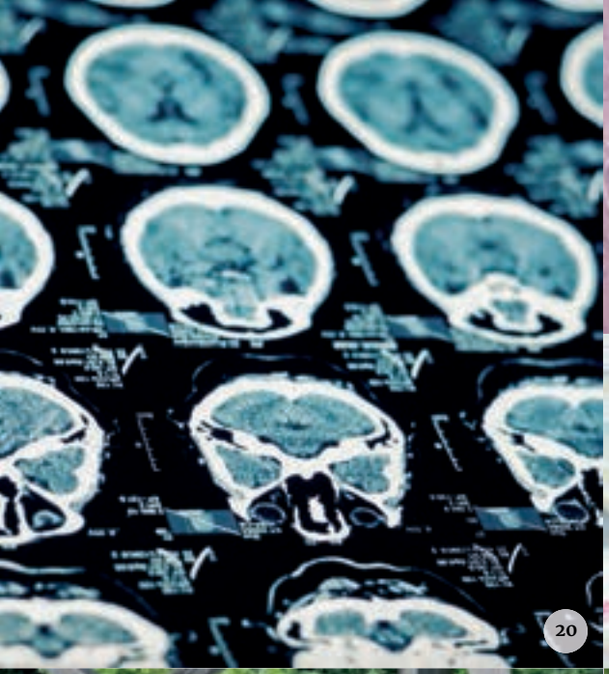
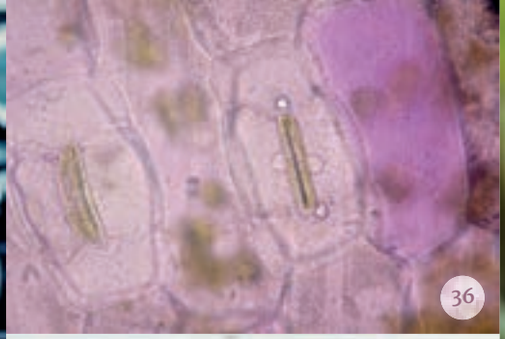
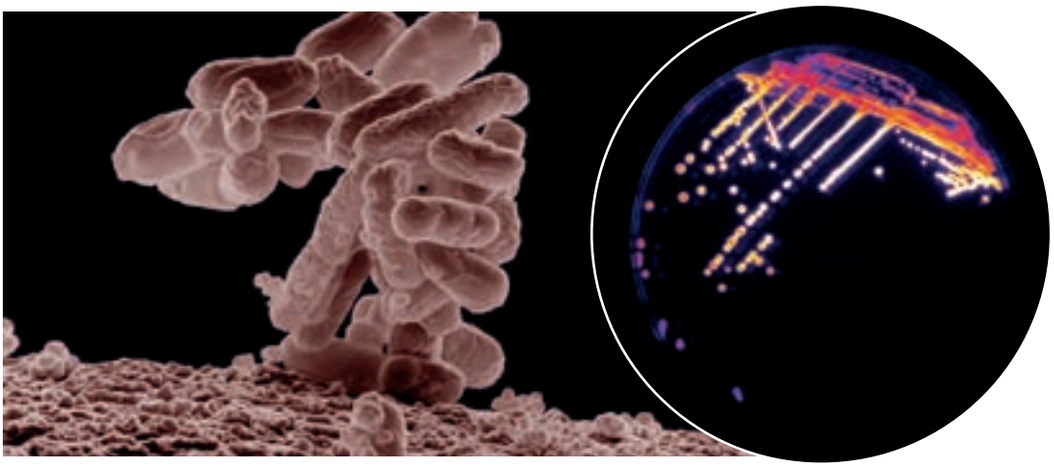




Bacteria glowing the way to better cancer treatment

NEW TESTS USING BIOSENSORS COULD PREVENT INEFFECTIVE CHEMOTHERAPY AND REDUCE SIDE EFFECTS



The Tommies of World War One called it 'angel's glow'. Limbs torn by shells or bullets, they would be carried back across the battlefield, though the interminable mud that clung to everyone and everything. Once back in the trenches they were at serious risk of infection. For some, though, came a strange sign that luck was on their side: at night, their wounds would begin to glow.

At first, it must surely have been terrifying, but in time the soldiers began to welcome it, for those blessed with the angel's glow seemed to have a better chance of healing.

"The glow was actually from a type of soil bacteria in the mud, *Photorhabdus luminescens*, which may have helped prevent the more serious gangrene infections by out-competing other bacteria," says Dr Elizabeth Anderson, a lead scientist in Professor Vyv Salisbury's research group within CRIB. Harnessing this bioluminescent quality to develop new, innovative, applications has been the focus of the team for the past 12 years. Working with companies, hospitals and patient groups, the team has brought its expertise to bear on real-world problems; from decontamination procedures to antibiotic drug development and cancer treatment.

DRUG TESTS

The initial research was prompted by collaboration with Professor Alasdair MacGowan, Professor of Clinical Microbiology and Antimicrobial Therapeutics at the University of Bristol and an Honorary Consultant at North Bristol NHS Trust. A new test was needed that could indicate how effective antibiotics were; those available at the time relied on leaving the microbial cells for some time and then counting the numbers left alive. The research group wanted to improve on this by creating a test that could show the impact of the antibiotic drug or disinfectant as it happened.

To do this, they took inspiration from the angel's glow — genes to be exact. The *lux* genes of *P. luminescens* create two enzymes, one which powers the light-producing reaction, and another that produces and recycles the fuel for it. The team obtained a plasmid — a simple ring of DNA which can be taken up by other bacteria — with these genes inserted. To stimulate the uptake process, the bacteria that were to receive the DNA were given electric shocks. This kills many bacterial cells, but some survive. The success of the gene transfer becomes evident when the bacteria that have survived the process begin to glow.

This means that once a culture of bacteria that has the *lux* genes from *P. luminescens* has been treated with an antibiotic or disinfectant, the effects are instantly evident: as the cells die, the lights go off.

The team has now used this technique on a range of important pathogens such as *Salmonella*, *E. coli*, *Staphylococcus aureus* and *Streptococcus pneumoniae*. The biosensor for *S. pneumoniae*, a bacterium which causes pneumonia, provided Glaxo SmithKline with the first real-time test of their newly developed antibiotic, Gemifloxacin. It also helped the company calculate the correct dosage, eliminating waste of the drug while ensuring it remains effective against the infection.

ABOVE
***E. coli* bacteria magnified at 10,000 times**

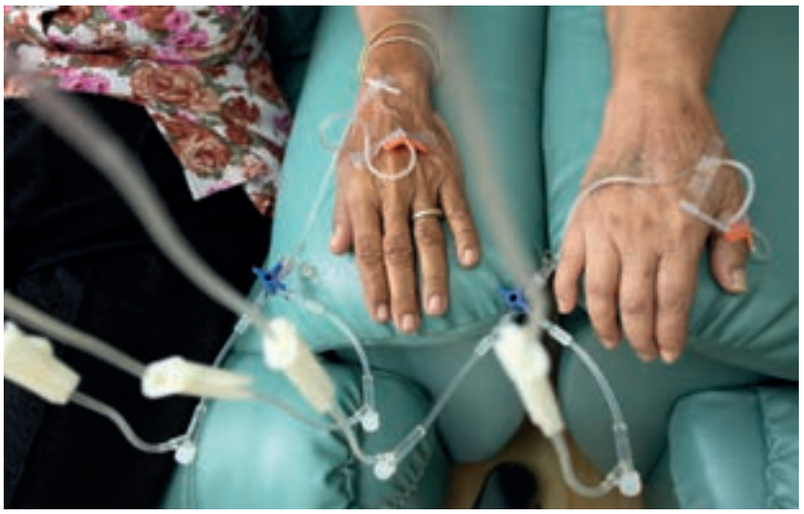
ABOVE RIGHT
False-coloured image of the glowing *P.luminescens*

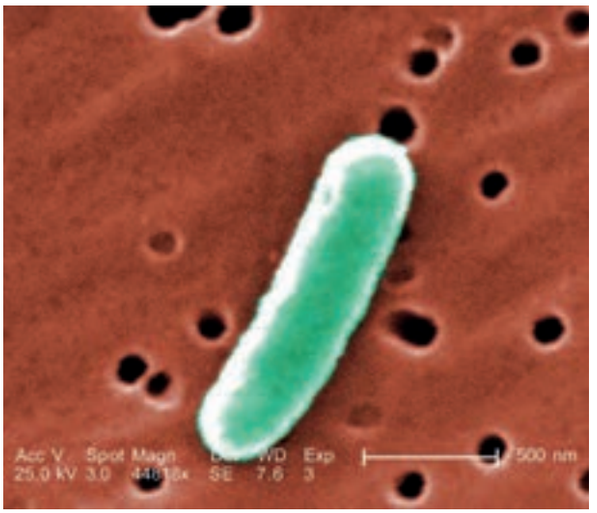
OPPOSITE
(From left) Dr Gareth Robinson, Professor Vyv Salisbury and Dr Elizabeth Anderson

BELOW
***P.luminescens* became a welcome sight to wounded WWI soldiers**

CANCER QUESTION

It was the drive to address another real-world problem that led the team to their next breakthrough. After a seminar by Prof Salisbury on the use of their biosensors for testing antibiotics, a haematologist in the audience, →





ABOVE
Electron micrograph of *E.coli*

ABOVE LEFT
Chemotherapy could be transformed with biosensor screening

Professor Ann Smith, at the time a senior lecturer in haematology and immunology at UWE, raised her hand to ask an intriguing question: would it be possible to create a glowing bug that responded to chemotherapy?

Acute myeloid leukaemia (AML) is an aggressive and often fatal blood cancer that requires powerful chemotherapy, and treatment typically begins within 24 hours of diagnosis. The problem is that this treatment not only has a powerful effect on the cancer, it is also highly toxic for the patient. The side effects are such that the treatment itself brings substantial risks.

Immediately after diagnosis, a patient's 'fitness' is assessed using criteria based primarily on age but also accounting for sex, the stage of the cancer and any other diseases. For some patients, the doctors must come to the difficult conclusion that they will not be able to withstand the chemo.

The sad fact is that because AML most commonly affects people in their 60s or older, a significant proportion of patients are deemed unsuitable for treatment. However, although doctors must currently go on the fitness data alone, there is another crucial piece of information that could help them to fine tune their decision. This is where the UWE bioluminescent biosensor — a patented strain of *E. coli* carrying *lux* genes being developed in collaboration with the clinical diagnostics company Randox Laboratories — could provide a real breakthrough.

RACE AGAINST TIME

The key piece of information needed is simple: how responsive will the patient be to the chemotherapy? Once the drug reaches the patient's cancerous cells it must be pumped into the cell and then converted to its active form for it to be effective. In some patients, a high proportion of the drug is converted, and that may mean that they can receive a reduced dose — and therefore reduced side effects — but still benefit from the same cancer-killing properties. For elderly patients this could be their only chance of putting the disease into remission without risking the life-threatening side effects of the drug.

However, determining which patients are likely to convert a high proportion of the drug into its active form is far from simple. For starters, it is extremely difficult to measure levels of the active-form drug. Secondly, even if you were to bypass measuring the levels themselves and record the proportion of cancer cells that die after exposure to the drug, as in conventional cytotoxicity tests, it wouldn't work for AML. "A conventional test takes three days," says Dr Anderson. "That's too long." Treatment must often begin very soon after diagnosis, so the test itself needs to be rapid. So the team has developed an entirely different technique.

A sample of cancer cells from the patient is initially exposed to the chemotherapy for an hour; enough time for the cells to convert the drug to the active form, if they are able. These cells are then put in what is essentially a blender to break down the cell walls and release the contents, including any active-form metabolite. There is now a twist: the active-form metabolite is converted back into its original form. Once this has been done, the biosensor is added, and the light show can begin. Had the drug not been converted to its original form, it would not have been able to pass through the cell walls of the bacteria and crucially, the level of original form drug is a good measure of the amount of active-form drug that was created.

As the drug passes into the bacterial cells it begins to have the same effect as it does in the human cancer cells, damaging the DNA. "This is where it is not just simply, 'if light goes out, the drug is effective,'" says Dr Gareth

'The test provides the speed that's required: whole the process takes less than eight hours'

Robinson, a member of the research team. In fact, as the damage begins to accrue — but before the cell dies and the lights go out completely — the glow intensifies. This effect is thought to be linked to the cell's attempts to repair the damage caused by the drug, and it is this peak of light that is used for the test.

The team compare this light peak to a control that exposes the biosensor to exactly the same patient sample, but rather than converting the active-form metabolite back into original drug, it is left as it is. As the active form cannot cross cell walls, it is harmless to the bacteria and the glow should remain at a low level. It is the ratio of these two light levels that reveals the amount of active-form metabolite the patient's cells were able to produce.

A large difference in the two peaks indicates a good rate of conversion to the active form of the drug, and so the patient is likely to be responsive to the chemotherapy. Furthermore, the test provides nuanced data beyond a simple yes/no result. The differing light levels can be used to tell the doctor: this patient has a 90% chance of responding, the other a 20% chance.

TRANSFORMATIVE TEST

The test provides the all-important speed that's required: the whole process, from taking a sample of the patient's blood to giving a result, takes less than eight hours. This is because the bacterial cells divide every 20 minutes, accruing DNA damage at a rapid rate. Using human cells like this wouldn't work, as they only divide every 24 hours.

The team is keen to emphasise that although the science behind it is highly complex, the test is simple to use and interpret, and could become routine in hospital labs. "The bugs are freeze dried, for example," says Dr Anderson. "To use them you just add water, shake them for an hour and they are ready to go."

For the patients, this test could mean the difference between life and death. If an elderly patient has borderline fitness, but the test shows they are very responsive to the chemo, this would give a doctor enough information to go ahead with a lower chemo dose, in the knowledge that it is likely to be effective against the cancer but also result in fewer side effects. For younger people, it may mean getting the right treatment sooner. After the initial round of chemotherapy, the current procedure is to wait six weeks before testing to see whether the patient is in remission. "It's always been that way," says Dr Anderson. "You wait and see. But there is now a move to bring further treatment forward, because if you can see someone isn't responding earlier than six weeks, why wait?" The biosensor test could provide a crucial piece of evidence to inform this decision by providing an early warning of who is likely to be less responsive.

ABOVE
P. luminescens **give off a clearly visible glow in the dark**

The research team also collaborates with the patient group Bristol Blood Buddies, to gain insight from its perspective. Its members have been quick to suggest more advantages to the test in addition to the most obvious ones. Infertility in women, for example, is a common side effect of AML chemotherapy. However, if the biosensor shows that a female →

patient is highly responsive they may be able to receive a lower dose, treating the cancer effectively and yet preserving her fertility. Furthermore, they have pointed out that the test is likely to have wider social benefits. Lower doses of chemotherapy, reduced treatment for side effects and shorter spells in hospital will all provide important savings for the NHS.

The technique is currently undergoing retrospective trials; the team runs the test on samples from recently diagnosed patients and six weeks later compare their predictions to the actual outcome; in other words, whether the patient is still in remission or not. So far, the test has been 90% accurate, and there is now a move toward trials in which the test will inform treatment decisions. Although testing the responses of individual patients to AML chemo has previously been considered too complex and time-consuming, the growing evidence for the efficiency and accuracy of this test has garnered support from clinicians, with the UK acute myeloid leukaemia clinical trials working party and clinical collaborators in UK, US, Norway and Canada all adding their support.

ABOVE
Petri dishes containing colonies of *P. luminescens*

BELOW
Leukapheresis is used in the treatment of some AML patients

FUTURE DEVELOPMENTS

As well measuring patient response to currently available chemotherapy, the test will help with the development of new or modified drugs. For example, if there are patients who would respond to a drug, but are unable to pump it into cancerous cells, an obvious target for pharmaceutical companies is a new drug that could be given in conjunction with the chemotherapy and would ease the chemo's passage into cells. The biosensor test would provide a rapid indication of how efficient the new formulations are. In fact, the test has already been used to evaluate a new drug, Elacytarabine, that would do precisely this, in collaboration with the company Clavis Pharma.

In addition, new formulations of chemotherapy drugs can be extremely expensive, and the NHS must decide how best to spend the money, for the benefit of all. The biosensor test could also help inform these decisions. "It might be that we could cherry-pick the people for whom a targeted new formulation would be likely to be effective," says Dr Anderson.

Beyond pharmaceutical companies and hospitals, the team is keen to engage with the general public. As well as numerous visits to schools and science events, it was awarded £10,000 by the Biotechnology and Biological Sciences Research Council (BBSRC) to showcase its work at the Great British Bioscience Festival in London in 2014. It also represented the research council at Cheltenham Science Festival in 2015.

So while there will be no angel's glow for cancer patients — the bacteria go nowhere near the patient's body — the humble bacterium *P. luminescens* looks set to make a return as a life saver.

Investigating radiation's effects on plant life

WITH A GROWING NEED TO STORE NUCLEAR WASTE, UNDERSTANDING HOW RADIATION EFFECTS VEGETATION IS VITAL

We've got a pretty good idea of how plants and animals respond to large doses of radioactivity over a short period of time – they don't tend to cope well. But we're less certain about what happens when plants start to take up small amounts of radioactive isotopes from the soil over a long period of time — a growing problem in a world where nuclear power as seen as an important bridge between fossil fuels and renewable energy.

"The UK needs to build a nuclear waste repository to put all the nuclear waste that currently sits at Sellafield somewhere in secure storage," says Dr Neil Willey, who is Director of UWE Graduate School as well as leading this research. "We work on how plants might take up radioisotopes from the soil and then transfer them into food chains, but we also work on the effects that the radioisotopes might have on the plant."

The difficulty is that both of those things depend on not just the radioisotope, but the combination of plant and soil too. "For all practical purposes, there is an infinite number of combinations of soil type and plant species," says Dr Willey. "We're trying to find out which groups of plants have particularly high radioisotope uptake and which groups of plants have low uptake, so that from knowledge of where a plant sits on the taxonomy, you can predict what its uptake would be even though you've never measured it in the field."

ABOVE
Dr Neil Willey is measuring radioisotope uptake in plants

That means gathering data for soil types and plant species that were previously unrepresented in the data. "Things like the cereal crops are well-represented because they're widely grown and are important in food chains and so on," says Dr Willey. "But then there are other groups of plants that aren't well represented in the research." For those, his team performs its own experiments — contaminating soil samples in the lab that were collected from different locations, growing plants in them and measuring the radioisotope uptake.

As well as the immediate uptake, Dr Willey's group is also looking at the effects of radioactive contamination over the long term. "We're using molecular and biochemical techniques and looking at root growth to try and work out in a bit more detail what the effects of chronic radiation are," says Dr Willey. "Certainly in plants, there's some evidence that chronic low levels of radiation actually make them grow better, not worse."

The data will be invaluable to planners trying to model the effects of leaks from nuclear power plants and waste storage facilities, which will remain radioactive for generations. As such, it's important to look at not just the plants inhabiting the British Isles today, but those that might grow here in the future too. "We have quite a lot of representation from plants that are characteristic of Mediterranean climates," says Dr Willey. "If temperatures change and the climate changes, and the environment becomes polluted, stress to organisms is a really important."

'The data will be invaluable to planners trying to model the effects of radiation leaks'

***Rosa spinosissima*, a member of the Rosids group which have an average uptake of radioactive pollutants**

Sniffing out disease to speed up diagnosis

INSPIRATION FROM DOGS' SENSITIVE NOSES MAY TRANSFORM HOW MANY CONDITIONS ARE DIAGNOSED

The stories of dogs sniffing out cancer in their owners are tantalising. How do they do it? How can we harness this remarkable ability? These questions were the inspiration for research that has led to the development of an 'electronic nose' that can sniff out diseases ranging from the superbug *Clostridium difficile* to bladder and prostate cancer.

The research, led by Professor Norman Ratcliffe, began with the humble potato. By identifying the specific compounds given off by rotting potatoes, Professor Ratcliffe and his team were able to develop a hand-held device that could be used by potato sellers to locate sacks of potatoes that needed to be checked. However, the potential of their idea was far greater.

In 2009, consultant gastroenterologist Professor Chris Probert, now based at the Institute of Translational Medicine at the University of Liverpool, contacted the team. He was interested in investigating whether a similar sniffing technique could be used to provide a rapid diagnosis of an infection with the superbug *Clostridium difficile*, a bacterium that can cause severe diarrhoea. These infections can be fatal in the frail and elderly and a rapid diagnosis would allow treatment to be administered more rapidly and help hospital staff contain the spread of the disease.

But developing a *C. difficile* test was step up in complexity. Unlike with rotten potatoes, there is not a narrow range of molecules, or 'biomarkers,' that would allow instant identification of an infection. "It was challenging," says Professor Ratcliffe. "There aren't just a few key compounds, so we can't do a test with just a few sensors."

ABOVE
Dogs appear to be effective at detecting prostate cancer and other cancers too

The team — which, along with Professors Ratcliffe and Probert, included Dr Ben De Lacy Costello, Dr Richard Ewen, and Dr Paul White at UWE — was awarded a £1.5 million grant by the Wellcome Trust to develop a device capable of sniffing out a *C. difficile* infection using a patient's stool sample. Their work led to the development of the OdoReader.

HOW IT WORKS

Initially, the diarrhoea sample is heated to release the volatile compounds and a sample of these gases is then injected into 30 metres of coiled tubing. At the end of the tube is a sensor, whose electrical resistance changes as the molecules hit it. The molecules vary in size, which affects how long they take to travel through the tube; the smallest with the lowest boiling points are the fastest. It is this pattern of the sensor responses over time that gives the unique signature of the mix of volatile compounds, indicating whether the disease is present or not.

However, because people differ substantially in their diet and gut bacteria, the signatures of *C. difficile* vary between patients. To overcome this challenge, the team created software based on a technique called neural networking. This allows the programme to 'learn' which signatures are *C. difficile* by examining a large number of infected samples to find the characteristic patterns, and then applying that knowledge to new samples.

Importantly, trials using over 1,000 samples show the machine has a high level of accuracy. Moreover, the equipment can be used by hospital staff with minimal training. The sample bottle simply needs to be slotted into the machine and the rest is automated. "In half an hour a clinician will have the result," says Professor Ratcliffe. "Does the patient have a *C. difficile* infection: yes or no?" The speed of the

‘The potential of the device has become evident to collaborators and funders alike’

test is crucial. Patients can die of a *C. difficile* infection quickly and the current time for the test results to be returned (which can be a few days), is too long for vulnerable patients.

CANCER DETECTION

The potential of disease sniffing doesn’t end there. Around 10,000 people are diagnosed with bladder cancer each year in the UK. However, if a patient is showing symptoms, the only way to confirm the diagnosis is to insert a camera up the urethra; this is not only painful and invasive for the patient, it is costly for the NHS too. In fact, bladder cancer is said to be one of the most expensive cancers to treat, partly because of the need for repeated use of this test.

So Professors Ratcliffe and Probert have again been collaborating to adapt the OdoReader to provide bladder cancer diagnosis. A small urine sample from the patient is all that is required. Just as with the *C. difficile* diagnosis, this is heated to release the volatiles and the signature can be interpreted by a pattern recognition algorithm. The trials have gone well. “The results could hardly have been better,” says Professor Ratcliffe. “The test gave pretty much 100% correct diagnosis.”

Further development may even allow the OdoReader to detect bladder cancer early, if the differences in urine volatiles between the different stages of cancer can be determined. But Professor Ratcliffe says the test can already be beneficial for patients. “I had a call from a woman who told me her husband is desperate to use the OdoReader because he just can’t take having any more cameras put up his penis. It’s happened about 40 times every year since his diagnosis and it’s making him depressed.”

Since the principles of how the device works have been established, its potential has become evident to collaborators and funders alike and a wealth of new projects are being developed, including a new test for prostate cancer.

A grant application is also in progress in collaboration with a senior consultant urological surgeon, Mr Raj Persad, at the Bristol Urological Institute, to explore the possibility of using the OdoReader to diagnose kidney cancer.

As well as using stool and urine samples, the team is also investigating whether the OdoReader can detect signs of disease in breath. As part of a collaboration with Imperial College London, the team is applying for funding to find a way to use a patient’s breath samples to diagnose oesophageal cancer.

Professor Ratcliffe says the remarkable progress made is down to the multi-disciplinary nature of the team. “The OdoReader is the result of a successful collaboration of physicists, chemists, microbiologists and clinicians,” he says. “I’d be surprised if the system wasn’t in hospitals in some form in the next five years.”

ABOVE
The Odoreader may be used to test for several diseases

ABOVE RIGHT
Professor Norman Ratcliffe, leading the research into sniffing out cancer

(From left)
Dr Adrian Crew,
Professor John
Hart, and Professor
Olena Doran

Wiping a bad taste from the mouths of pork consumers

A NEW DETECTOR DEVELOPED IN COLLABORATION WITH THE PIG INDUSTRY PROVIDES A FAST, EFFECTIVE MEANS TO SPOT BOAR TAINT

There is a huge demand for pork around the world — it accounts for 38% of meat production globally and it is the most consumed meat in Europe. So maintaining the characteristic taste of pork is essential to ensure its continued acceptance by consumers in the UK and in international markets.

Boar taint is an unpleasant taste and odour that's found in some cooked pork from entire (non-castrated) male pigs. It occurs due to an excessive accumulation of some naturally occurring compounds in pig subcutaneous fat; it is linked to sexual maturity and can be prevented by the surgical castration of piglets which is the most common way of dealing with it in Europe.

However, due to animal welfare concerns, the UK introduced a ban on the castration of piglets. Therefore, to prevent or reduce boar taint, the majority of UK pigs are slaughtered at a younger age; before they reach sexual maturity. Most pigs here are slaughtered at an average weight of 78kg, in contrast to 93kg in most other European countries. This inevitably results in lost revenue to the UK pig producing sector.

But things are about to change elsewhere too. Surgical castration will be discontinued across the European Union (EU) and European Economic Area (EEA) countries as the EU is planning to implement a voluntary ban on surgical castration in 2018. This means that boar taint is now a major issue for pig producers and the food industry in many countries.

"The question is, how will the European meat industry address the challenge of detection and elimination of tainted carcasses from the food chain when the pig industry moves to an entire male pig production system?" says Professor Olena Doran, Director of the Centre for Research in Biosciences (CRIB) and one of the leaders of CRIB's boar taint research. "There is a strong potential threat to the quality of pork and its acceptance by consumers."

TAINT TESTING

There are already some methods to detect of boar taint compounds. But they are expensive, time-consuming, and require specialised equipment and staff training. Furthermore, they cannot be used 'on-line,' providing results at the point of test, in abattoirs and elsewhere. This has been recognised by the Scientific Panel on Animal Health and Welfare of the European Food Safety Authority which declared: "There are no harmonised methods of consistently identifying carcasses with boar taint in commercial slaughter houses. Investigation of possible processing techniques to reduce the offensive properties of boar taint is hampered by the lack of such methods to assess levels of the compounds contributing to the →

ABOVE
Boar taint is more noticeable in prime cuts rather than processed pork

BELOW
The way pigs are maintained has an influence on taint

Changing a pig's diet can reduce the chances of boar taint

KEY BOAR TAINT FACTS

■ **Globally, around 75 per cent of pork consumers are sensitive to boar taint.**

■ Men are less sensitive to boar taint than women because they also produce one of the compounds responsible for it.

■ **There are differences in sensitivity to boar taint between countries which could be down to habituation and due to differences in the way pork is prepared. Sensitivity to boar taint reduces after repeated exposure.**

■ People with two copies of a certain version of the OR7D4 odour receptor gene have been found to be more sensitive to boar taint than those with one or no copies.

ABOVE
Good animal husbandry helps to reduce the risk of boar taint

BELOW
Professor John Hart has developed a sensor that can detect pesticides in river water

phenomenon". Therefore, there is an urgent need for a rapid, reliable, cost-effective and user-friendly method for detection of boar taint compounds in the abattoir.

Research within CRIB aims to provide a solution to this problem. Professor Doran, Professor John Hart and Dr Adrian Crew have brought together complementary expertise in meat quality, genetics and technology development to produce prototype technology that can rapidly detect boar taint compounds. This unique technology, which has no analogues in the world, is now at the stage of evaluation and validation and this is being conducted in collaboration with commercial partners. "The novel technology could be used as a portable hand-held device or incorporated into a stationary device in an abattoir depending on the size, operational speed and other requirements of individual technology users," says Professor Hart. "We're working ahead of the EU legislation and aim to have this technology on the market by 2018," adds Dr Crew. "Since we started this work, interest in our technology from pig industry and instrumentation companies has grown."

The team has filed a European Patent Application and are working closely with the UK and international industry and policymakers on the strategies to take the boar taint detector to the market as soon as possible.

ABOVE
The boar taint detector will help farmers sell their pigs at higher weights

COMBINED EXPERTISE

Professor Doran, an expert in molecular biology and genetics, started her boar taint research over 15 years ago. At the time, she was investigating the genetic control of the deposition of boar taint compounds in fat. "We identified a number of genes we believe may be responsible for controlling boar taint but the issue is that the mechanisms controlling boar taint appear to be breed-specific and therefore it is very difficult to develop a universal strategy to deal with this pork quality issue," she says.

Professor Doran became actively involved in international consortia on boar taint research, has been working closely with the European Commission, European Association for Animal Production and established the UK/China Partnership on novel technologies for food quality. "Boar taint is an international issue and it has to be addressed via the joint expertise of researchers and stakeholders across Europe and the world," she says.

Professor Hart leads the electrochemical biosensor/sensor research group within CRIB. He and Dr Crew, a Senior Research Fellow who is part of Prof Hart's team, have a strong track record in developing platform sensor technologies for the agri-food industry as well as for the biomedical and pharmaceutical sectors. "The technologies we developed helped to address real world challenges," says Professor Hart. "This includes portable instruments for the rapid detection of organophosphate pesticides in food and water, analysis of progesterone in milk, novel approaches for screening drugs, a hand-held instrument for the detection of ammonia in environmental samples and many other applications". Dr Crew adds: "Bringing together our expertise and skills was essential in solving the issue of boar taint detection which required an inter-disciplinary approach; we have all contributed."

The critical part of the boar taint detector development was starting discussions and consultations with industry at the very early stages of the technology's development. "All our research during this journey has been guided by industry and other stakeholders which was the key to our success," says Professor Doran. "There has been huge interest in the boar taint detector in Europe as well as in China →

"By working with instrument companies and end users, it enables us to maximise the impact of what we develop." **PROFESSOR JOHN HART**

ABOVE
Dr Adrian Crew, Professor John Hart and Professor Olena Doran

and the US," says Dr Crew. "The boar taint detector can be used as a platform technology for analysis of other meat quality traits and can potentially be used on live animals which will be really exciting," adds Professor Hart.

The initial pilot studies were supported by British Pig Executive and the European Commission Tender which was followed by a number of research grants from the Biotechnology and Biological Sciences Research Council (BBSRC) and CASE PhD studentships in collaboration with the pig industry. In parallel with the technology development, the research team has conducted an evaluation of the market and cost-effectiveness of the technology. "Our activities have been greatly supported by the Biosciences Knowledge Transfer Network which facilitates collaborations between academia and industry. We would also like to acknowledge strong support from the University, Faculty of Health and Applied Sciences and the Department of Biological, Biomedical and Analytical Sciences," says Professor Doran.

IMPACT

The main purpose of this journey from fundamental cross-disciplinary research to producing the prototype technology and then taking it to market is to address a real-world challenge. The research team anticipate that the technology will have a strong impact on the international pig producing sectors, the environment, consumers, animal welfare and bring other benefits to the UK and international community.

In particular, implementation of the boar taint detecting technology in the UK will help British pig producers recoup a significant proportion of the cost lost during the slaughter of pigs at lower weight. The manufacturing of a boar taint detection system will also allow instrumentation companies to grow their businesses via a sustainable income.

Finally, the boar taint detection system will prevent the entry of tainted pork into the food chain, enhancing consumer satisfaction and increasing the volume of repeat purchases.

Research Council UK report Big Ideas for the Future, 2011, featured the applicants' research quality among the 100 top research directions which will have profound effect on our future. (http://bit.ly/1N7OJ9N — p60)

> "Our research has been guided by industry and other stakeholders." **PROFESSOR OLENA DORAN**

Lessons learned from the greater water-parsnip

HOW KNOWLEDGE GAINED FROM A HUMBLE WETLAND PLANT WILL TRANSFORM SPECIES REINTRODUCTIONS IN SENSITIVE HABITATS

Reintroducing a species into an ecosystem that it was once a part of is an increasingly common way for conservation groups to enhance biodiversity. But often there's no guide or manual on how to do it, and it's not as simple as just planting a load of seeds or opening a cage and hoping for the best.

Dr Mark Steer has discovered the challenges of plant reintroduction the hard way when trying to reintroduce the greater water-parsnip (*Sium latifolium*) into the Somerset Levels, and he's now working to ensure that others benefit from the knowledge he gained. "If you want your plant reintroduction to have knock-on ecological benefits," says Dr Steer, "then just reintroducing one plant species on its own is unlikely to do very much".

The greater water-parsnip is a two-metre tall plant which produces white clusters of flowers that look a bit like cow parsley. It's suffered a massive decline over the past century, largely due to the increasing use of mechanical diggers to manage waterways, something that's disturbed its habitat, as well as poor water quality.

INSECT MAGNETS

Dr Steer began a reintroduction program after seeing data showing that in areas where greater water-parsnips were still present, the flowers were being visited by more than half of the local pollinators. The plant appeared to play a crucial ecosystem role — not just as an important source of food in its own right, but also acting a little like a waterhole in the Serengeti; drawing prey (in this case insects) into a small area so that other species could eat them.

But an assessment of the effects of the greater water-parsnip reintroduction on the Somerset Levels showed that things didn't quite go according to plan. "The plant comes into flower and a large majority of the local insects go 'Weeey, fantastic!' and most of them visit this plant instead of spreading themselves out on all the other flowering plants," says Dr Steer. "We couldn't find any evience that the plant was actually helping to increase populations of pollinating insects."

That finding changed how Dr Steer now approaches reintroductions. "Yes, species reintroductions are important, but you need to ally those to a much wider program of works," he explains. "You need to look at where you can make changes at the habitat level."

The key to changing plant reintroduction practice is communication, says Dr Steer. "There's so much information coming out, and some of it can be contradictory, it's just a question of making sure we try and bridge the gap between researcher and practitioner, which is really difficult to do. Even if we produce a nice easy-to-read report, the number of people within conservation organisations who will actually read it is relatively low. So we need to make sure we get out and talk to people and disseminate the messages, so when people are then thinking about a project, they can implement the advice that's coming out of the scientific literature."

ABOVE
The greater water-parsnip attracts many species of pollinator

BELOW
The Somerset Levels, home to the greater water-parsnip

IDS photos

From traumatic brain injury to an Alzheimer's treatment

HOW PATIENTS WHO HAVE HAD SUDDEN BRAIN INJURIES MAY INSPIRE A NEW TREATMENT FOR THE MOST PREVALENT FORM OF DEMENTIA

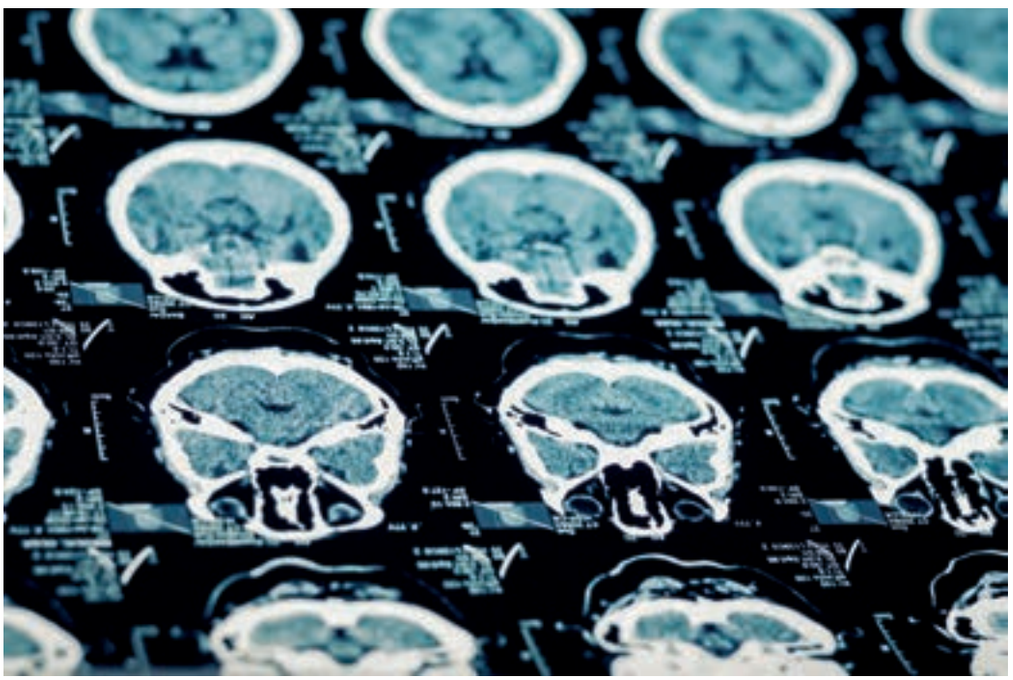
There is currently no cure for Alzheimer's disease and even diagnosing the condition can take years of tests. For a disease that affects more than 520,000 people in the UK and several million people world-wide, this is not good news.

But research taking place within CRIB could lead to a new, faster way to diagnose Alzheimer's as well as a treatment that would simply involve taking a supplement that would delay the condition's development.

Many of the symptoms of Alzheimer's early stages, such as short term memory loss and mood swings are signs of normal ageing or other conditions. Only one third of patients with these symptoms will go on to develop Alzheimer's — and currently it is difficult for clinicians to tell whether they are witnessing the early signs of Alzheimer's or something different.

SLOW DIAGNOSIS

Patients suspected of having Alzheimer's first take a questionnaire to assess their mental abilities and may go on to have a brain scan. It can take months, or even years in some cases, for an Alzheimer's diagnosis to be confirmed. However, Professor Myra Conway at CRIB is working towards a blood test that would benefit early diagnosis.



Professor Conway has found early evidence that the levels of three 'redox proteins' increase in the cerebrospinal fluid of those with Alzheimer's. She is now looking to see whether the levels of these proteins also increase in the blood. If they do, checking levels of these proteins would provide doctors with a new diagnostic tool. The research is being supported by £50,000 from the Freemasons' Grand Charity, after an application for funds supported by the UK charity, BRACE.

ABOVE
Brain scans are part of the sometimes lengthy Alzheimer's diagnosis process

ABOVE RIGHT
A blood test would provide a rapid disgnosis

A separate line of research is inspired by patients who have suffered traumatic brain injuries such as having been involved in a road traffic accident. "It's been found that levels of certain components of the blood drop in those who have suffered a traumatic brain injury and glutamate levels increase," says Professor Conway. "Glutamate is toxic to the nerve cells in the brain,

"The impact of this treatment on the lives of Alzheimer's patients would be phenomenal. To be able to maintain their cognitive reserve would give them back their lives." **PROFESSOR MYRA CONWAY**

resulting in neuronal loss." It's been found that after giving supplements to the patients who have suffered a traumatic brain injury that contain the depleted blood components, some cognitive abilities are regained. Some patients have even come out of their vegetative state.

MAINTAINING RESERVE

This raises some intriguing questions. "Is there an indication that the profile of these blood components also changes as patients with mild cognitive impairment get older?," says Professor Conway. "Is there a way that if levels of these components start to drop, we can supplement what's naturally there?"

Supplementing these components would not cure Alzheimer's, but it might delay its progression if the treatment starts early enough; a patients' cognitive reserve may be preserved, says Professor Conway. "It could improve their quality of life."

Early pilot research is looking at the biochemical processes involved with the regulation of glutamate levels in cell models in the lab. Professor Conway and her team also plan to measure levels of blood components in Alzheimer's patients to see whether, just like those with sudden brain injuries, they are relatively low. It's already known that glutamate levels cause toxicity and contribute to the destruction of neuronal cells.

If these supplements are found to slow the progression of cognitive decline, the long term implications are huge. "The impact of this treatment on the lives of Alzheimer's patients and their families would be phenomenal," says Professor Conway. "To be able to maintain their cognitive reserve would give them back their lives."

Professor Myra Conway (left) with her research team

Myra Conway

Sleep pill alternative

LEMON BALM MAY PROVIDE THE REST WITHOUT THE 'HANGOVER'

Sleeping pills can provide a welcome respite for anyone with sleeping problems. But researchers within CRIB are investigating an alternative sleep aid — lemon balm — that may have a distinct advantage over conventional pills.

Tests carried out with healthy elderly people found that aromatherapy with lemon balm (*Melissa officinalis*) led to dramatic improvements in their sleep. A more recent study, carried out with The Research Institute for the Care of Older People (RICE) and the University of Bath, showed similar improvements in the sleep of the elderly with mild cognitive impairment and dementia.

"We know that a lack of sleep exacerbates dementia symptoms," says Associate Professor Chris Alford, who is leading the research. "So it's well worth trying to sort out their sleep."

Conventional sleeping pills can leave a residual 'hangover', a reduction in awareness, after a period of sleep. That can be particularly problematic for the elderly. "They tend to wake more in the night," says Associate Professor Alford. "They may need to get up and go to the bathroom and there can be an increased risk of falls with conventional hypnotics. So if we can find things that can help their sleep but are not associated with an increased risk of falls, that would be great."

Years of research on aromatherapy treatments indicate that lemon balm may be the solution. "With aromatherapy we don't see these residual effects," says Associate Professor Alford. "We don't know why something can improve your sleep but doesn't have residual effects."

The next stage of the research, working with Professor Andrew Scholey at Swinburne University in Australia, will be to work out the mechanisms of the active ingredients of lemon balm. This may reveal why there are no residual effects and if it is possible to take it alongside other important medications older people may need. In the long term, the research may lead to the development of a pill or capsule containing the active ingredients from lemon balm that could be a sleep aid.

Hunting the oak tree assassin

THE ICONIC OAK IS UNDER THREAT BUT NEW RESEARCH WILL TELL US MORE ABOUT TWO OF THE PRIME SUSPECTS

It is a symbol of strength and endurance that has been adopted as the national tree of several countries including England and Germany. But the oak is under attack from a condition that can kill a tree off in four to six years.

Exactly what's behind Acute Oak Decline (AOD), a syndrome affecting thousands of oaks in the UK, has been unclear despite the fact that it has been present here for the past 30–35 years. However, recent research has identified a few possible culprits.

Among them are bacteria. About 20 bacterial species have been isolated from lesions in the bark of trees with AOD that ooze dark fluid, one of the characteristic symptoms of the condition. Initial work at CRIB has focused on characterising these bacteria and about 10 species that were previously unknown to science have been identified.

Following pathogenicity testing by Forest Research, the research agency of the Forestry Commission, in which young trees were infected with the bacteria isolated from lesions, two species, *Gibbsiella quercinecans* and *Bronneria goodwinii*, are now principle suspects. Now the research at CRIB, funded by the charity Woodland Heritage in collaboration with Forest Research, will focus on uncovering exactly how these bacteria harm the trees they infect. "Both species are new species; we don't know anything about them," says Dr Carrie Brady, who researches microbial taxonomy. "We don't know how they get into the tree and how they cause the disease. So my task is to figure out what makes them pathogenic."

BELOW
The mighty oak may be felled by tiny bacteria in AOD

Dr Brady has been inserting genetic material into the DNA of the two species of bacteria. This 'foreign' genetic material becomes incorporated into the bacterial DNA at different random points in a range of genes, preventing them from producing proteins. Finding which proteins are inactivated in different bacteria which have foreign DNA inserted at different points, may help to reveal what individual genes do. "We're then looking for things that may affect the bacteria's pathogenicity," says Professor Dawn Arnold, who is supervising the research.

This means that genes found to enable the bacteria to enter the walls of oak tree cells, to be mobile and to produce proteins toxic to the cells will be particularly significant. "In the long term, identifying a really critical gene in the bacteria may enable us to develop a control measure other than genetics to limit that bacteria," says Professor Arnold. "You could target that gene. If you found a chemical that could disrupt that function but didn't harm the tree, that could be an effective way to fight AOD." Getting a better understanding of the

Images: Forest Research

TOP, LEFT & ABOVE
Bacteria found in dark lesions like these on oak trees are being investigated at CRIB

"Identifying a critical gene may enable us to develop a control measure" **PROFESSOR DAWN ARNOLD**

biochemical processes involved is crucial to this approach.

If these bacteria are found to be a significant factor behind the decline, then a rapid system of detection would be useful to foresters. "If the bacteria could be detected in trees that are not showing advanced symptoms, infected trees could be removed," says Professor Arnold.

AOD mostly affects the pedunculate oak (*Quercus robur*) and sessile oak (*Quercus petraea*) in the UK and is most prevalent in the Midlands and South East England.

The research taking place within CRIB compliments the AOD studies being carried out by Forest Research's own scientists and researchers in other universities and research organisations. One avenue of research is the relationship between the bacteria found in lesions and the larvae of the buprestid beetle (*Agrilus biguttatus*) which are usually found close to lesions and may spread the bacteria.

Our French bean crops are under attack

Fighting the French bean arms race

THE HUMBLE BEAN NEEDS HELP FENDING OFF A DEVIOUS FOE

A silent arms race is taking place between the French bean and a bacterium that can kill them off. It's a race that researchers within CRIB are trying to tip in the bean's favour.

Halo blight in French beans (*Phaseolus vulgaris*) is caused by the bacterium *Pseudomonas syringae pv. phaseolicola* and has huge economic implications, killing off crops — or at least leaving the beans with unsightly lesions on their pods. "It can wipe through a crop or make it unmarketable," says Professor Dawn Arnold.

Research led by CRIB and involving Reading and Oxford universities is investigating the genes within the bacterium responsible for its pathogenicity. The team is focusing on the arms race between bacterium and bean. "The plants are able to recognise some strains of the bacteria, and when they do, they kill off some of their own cells to stop the bacteria spreading," says Professor Arnold. "This creates a hostile environment for the bacteria."

However, the bacteria detect early stages of the plants' response and modify their genetic material, ejecting a lump of it from their genome and producing a 'genomic island'. Crucially, this DNA codes for proteins the plant can detect, so the bacteria become undetectable. It's what's known as a 'stealth episome'.

"We would like to be able to manipulate the plant so the bacteria are not able to recognise that the plant is doing something," says Professor Arnold. This would involve determining what it is within the plants' response that the bacteria recognise and eliminating this through selective breeding or genetic modification.

What happens between the French bean and the halo blight bacteria is unusual from a research point of view. "It's one of the only systems where it's possible to see these genetic islands moving," says Professor Arnold. "So it's a good model for other systems. A lot of other bacteria show evidence of these islands, so this research has a broader impact."

Impact brief

A ROUND-UP OF TRANSFORMATIVE RESEARCH FROM ACROSS CRIB

RENEWABLE FUEL

Turning algae into diesel to power cars

Could the petrol in your car one day be replaced by fuel made from algae? Research within CRIB is focusing on whether microalgae, single-celled algal species, will make a practical source of biodiesel.

Microalgae produce algal oil, which is similar to vegetable oils such as soy and can easily be turned into biodiesel. But producing the oil on a wide scale is tricky, partly because microalgae often grow better in cooler water.

But what if the algae lives in an environment that's warm? "We decided to look at The Roman Baths in Bath," says Dr Heather Macdonald, who collaborates with Bath University's Professor Rod Scott. Their research was funded by the South West of England Regional Development Agency, UWE and Bath University.

The study found that most of the algae collected were not as productive as they would have been if grown at a lower temperature. So the focus now is on finding ways to increase the oil content of microalgae by using stress. Research already conducted shows that low nitrogen stress increases the amount of oil within the microalgae.

ABOVE
The Roman Baths are home to several microalgae species

PHARMACEUTICAL DEVELOPMENT

New technique for testing drugs

Before a new drug is launched, it has to be subjected to rigorous testing to ensure it is safe and effective. But pharmaceutical development can be costly, involving lengthy laboratory tests and clinical trials.

A novel method of testing new and established drugs for impurities is being developed by Professor David McCalley, who works within CRIB and was named as one of the world's 100 most influential analytical scientists by *The Analytical Scientist* magazine in 2013.

The technique employs HILIC (hydrophilic interaction liquid chromatography), a method associated with high performance liquid chromatrography (HPLC) in which compounds are separated by passing them down a column of absorbent particles. HILIC is particularly useful for the separation of compounds which are very soluble in water.

"We've been looking at the application of HILIC to compounds like antibiotics," says Professor McCalley. "It's important that you monitor the levels of antibiotics circulating in patients because if you give them too little, they may be ineffectual; if you give them too much, they can have very damaging effects. Scientists and clinicians are also interested in how drugs are metabolised — what happens to them in the body. HILIC is a good method for that."

ORTHOPEDIC SURGERY

Uniting bone and implant

Titanium is strong, light, and ideal for surgical implants because the body's natural healing processes often create a strong join with the patient's bone. However, one in 10 titanium joint replacements fail when the bone doesn't join firmly and the implant works loose. This means more surgery. It's a problem that costs £300m a year in the UK.

But a new implant coating could help. Working with colleagues at the universities of Bristol, Brighton and Cardiff, Dr Jason Mansell and his team have been testing a coating of lysophosphatidic acid, a component of the tissue healing process. Lab tests have confirmed it acts with vitamin D to promote bone cell maturation, an important event for enhancing bone formation and bonding with titanium.

The coating also appears to inhibit the attachment of bacteria, such as MRSA. "This is significant since surfaces that enhance the actions of vitamin D yet deter the attachment of bacteria have not been forthcoming," says Dr Mansell. Orthopaedic implant manufacturer Corin are on board for the next stage, which involves making sure the coating can be applied consistently and can resist sterilisation.

The coating is relatively cheap. It would add just tens of pounds to the cost of a typical implant which retail around £7,000. "If everything slots into place and goes very smoothly, then in theory within the next three to five years we could be looking at clinical trials." Soon after his appointment at UWE in September 2013, Dr Mansell received a grant from the Severnside Alliance for Translational Research and, as of August 2015, the NIHR have agreed to support the next stage of the developmental process.

BELOW
A simple coating on implants could save millions

One avenue of research, conducted with Professor Andrew Lovering at the Antimicrobial Reference Laboratory at Southmead Hospital Bristol, is how to test antibiotic levels in tuberculosis treatment. Professor McCalley has received two major grants from the Engineering and Physical Science Research Council (EPSRC) for his HILIC research; one was partly funded by GlaxoSmithKline.

ABOVE
HILIC could transform how new and existing drugs are tested

NEW THERAPIES

Pigs could hold the answers to bladder problems

People who suffer from bladder conditions face some uncomfortable challenges. Incontinence products can be expensive and embarrassing, and the need to keep a toilet nearby can hamper everyday activities. But research into treating these conditions could receive a boost.
A technique has been developed by researchers within CRIB to maintain entire pigs' bladders so new drugs can be tested on them.

"Pigs have a similar bladder to humans," says Dr Bahareh Vahabi, who is leading the research. "They're the same size, have similar wall structure, and a similar receptor density."

Once the bladders are extracted at an abattoir, they are placed in a custom-built jacketed bath that keeps them warm and supplied with nutrients. It took a lot of time and troubleshooting to perfect this system.

The next step is to work out how to simulate medical conditions in these healthy bladders, but there's already interest from pharmacological and devices companies. "It's one of the most unique models out there," says Dr Vahabi.

HEALTH MONITORING

Credit card-sized cholesterol lab

Accurately measuring your cholesterol as a patient currently requires a bulky meter, into which you insert a strip with a blood sample on it. But a credit card-sized, disposable 'lab on a chip' has been developed at CRIB that can do the same job.

The diagnostic device could be used by those with high cholesterol and has been developed as part of the Smart Integrated Miniaturised Sensor (SIMS) project. It uses organic and printed electronics. "At the moment, most of these devices are your typical sensor strip and meter configuration, and this ties people to a particular lifestyle," says Professor Tony Killard, who led the SIMS project. "SIMS was designed to improve compliance both in terms of measurement and drug treatment. If you're monitoring you're managing, and if you're managing you're medicating," he adds.

"It's about increasing complexity to decrease complexity for the user," he explains. "You take it out of the packet, you add your blood sample and it does everything else for you. It gives you a high quality, quantitative diagnostic measurement that can then be transmitted to your mobile phone, and then to your healthcare manager."

The work was funded by the EU Seventh Framework Programme, and conducted in partnership with other universities, research institutes, and companies across Europe. The University of Liverpool contributed expertise on the development of organic electronics, and making circuits on a flexible substrate. Dublin City University worked on sensor development, while the VTT Technical Research Centre in Finland did a lot of the printed systems integration. There were also partnerships with the Fraunhofer Institute of Electric Nano Systems in Germany, which worked on the battery technology, and Alere, a company that makes medical diagnostic devices.

Within CRIB, the work involved figuring out how to integrate all these technologies. "It was about how you bring all these components together and make them work, because nobody had actually done that level of printed electronic integration, so nobody knew what the challenges were going to be," says Killard. "Key issues are things like production yield — you've got to get hundreds of thousands of devices to work — while also integrating the components. There weren't a lot of design rules and design principles that were able to mitigate a lot of those challenges."

ABOVE
Cholesterol testing typically involves a sensor strip and meter

The hope is that the device for measuring cholesterol will just be the start, and the lessons learned in the project can be used to develop other health monitoring tools. Killard's group has also worked on a small device to detect the presence of ammonia in a patient's breath, which can be indicative of liver or kidney problems.

Testing new treatments for breath odour

HOW AN ARTTICIAL NOSE IS LEADING THE FIGHT AGAINST WHAT IS A SURPRISINGLY COMMON AFFLICTION

Mondays are breath-testing days at CRIB. Tuesdays and Wednesdays are too. Each week, between Monday and Wednesday, at least 20 people show up on the second floor of the biosciences building at the University of the West of England (UWE), home to CRIB, ready to have their breath analysed by a fancy machine.

Many of those who turn up on the second floor have some degree of what is politely termed 'oral malodour'. To use the less polite term: bad breath. They take part in clinical trials that will determine how different formulations of toothpastes, mouthwashes and other products affect breath odours. Their results feed directly back to well-known companies like Johnson & Johnson, Philips, Colgate-Palmolive, Proctor & Gamble and SME Helperby.

SERIOUS PROBLEM

Dr Saliha Saad is the researcher running the trials. While the prospect of working on bad breath may not sound appealing, it is important, rewarding research. Nearly all humans have some degree of bad breath or halitosis at some time or other around 30% of us have levels that are detectable by others, so it can be a cause for concern either when it leads to social awkwardness or indicates an underlying condition.

It's a Thursday, so Dr Saad isn't welcoming any participants today, but a quick glance around the lab leaves little doubt as to what her team is currently testing. On the bench-top next to the machine is what appears to be an large electric toothbrush with an oddly shaped head. Dr Saad introduces it as a prototype of a new Philips tongue cleaner with built-in mouthwash delivery. For obvious reasons, it can't

be photographed. It might sound like a gimmick. But talking with Dr Saad about her research, it becomes clear that the design for the new device is based on solid science.

TONGUE ECOSYSTEM

Whilst not all of us are in the habit of brushing our tongues, there is good reasons for doing so. Its surface is covered in about 100–1,000 billion bacteria. It's these bugs that may have the biggest impact on whether a person suffers from oral malodour. But they're difficult to get rid of because they're knitted together in a tight microbial community called a biofilm.

As Dr Saad explains, a person's tongue biofilm is a unique mix of microbial species, very diverse in composition, and it is largely determined from birth. "Wherever you are born, you'll acquire the flora of that environment. From your mother first, then from nurses and doctors, close family and carers. You can reduce the numbers but you will never remove them completely. Most of them have a beneficial effect and they protect us."

Some don't, though, and some release gases that contribute to malodour. These volatile chemicals — some of the worst culprits being sulphides because of their tendency to 'escape' — can hit the nose to produce intense smells. Dr Saad is trained to assess the intensity of these smells based on a standard scale invented by Canadian-Israeli halitosis researcher Professor Mel Rosenberg. The current version of the organoleptic scale, which puts odours into five categories, from 'no odour' to 'extremely strong odour,' was modified at UWE following a workshop of international experts (including Rosenberg) in 2003.

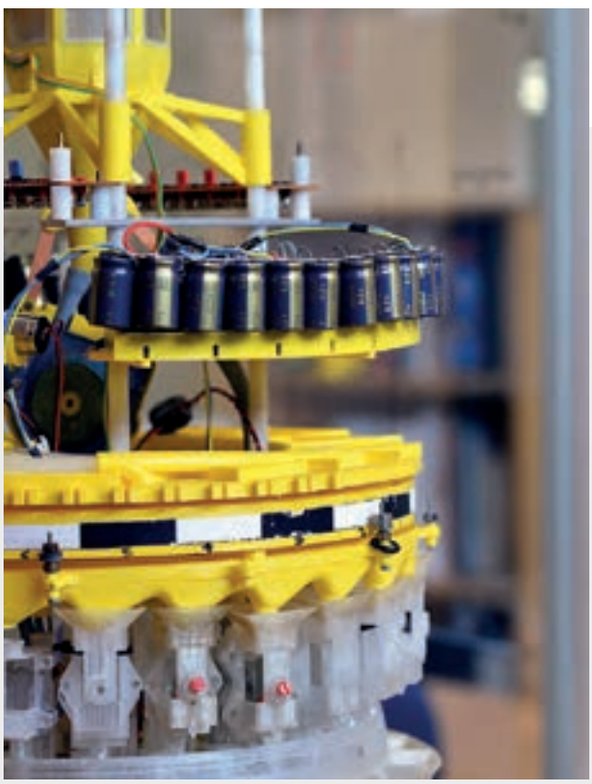
SCRATCH 'N' SNIFF

Dr Saad and colleague Professor John Greenman have also hosted courses at the University in which they train health professionals to use their noses to identify compounds in breath and learn organoleptic assessment methods. At her desk, Dr Saad pulls out a small, flexible book about the size of a child's flip book. This, she says, is how they assess whether a potential trainee breath judge has a good enough base level of 'smell acuity'. She flicks through; down the right-hand side of each page is a series of coloured boxes. Each one signifies a different smell that can be released with the scratch of a pencil — it's a scratch 'n' sniff test. The first one she turns to provides options of watermelon, peanut, rose or paint thinner and upon scratching, the solvent-like smell of paint thinner immediately escapes. There's another one that smells just like smoky bacon and there are 70 test smells in total.

The courses are mostly attended by those who want to become trained organoleptic judges for clinical trials and anyone wanting to enrol has to score at least 80% in the series of scratch card tests. These judges are trained to give a quick breath assessment before and after a treatment that's being tested, to monitor how effective it's been. The →

OPPOSITE
The microbes in our mouths can cause odours

BELOW
Ecobot III, developed at Bristol Robotics Laboratory, uses microbes to break down organic material

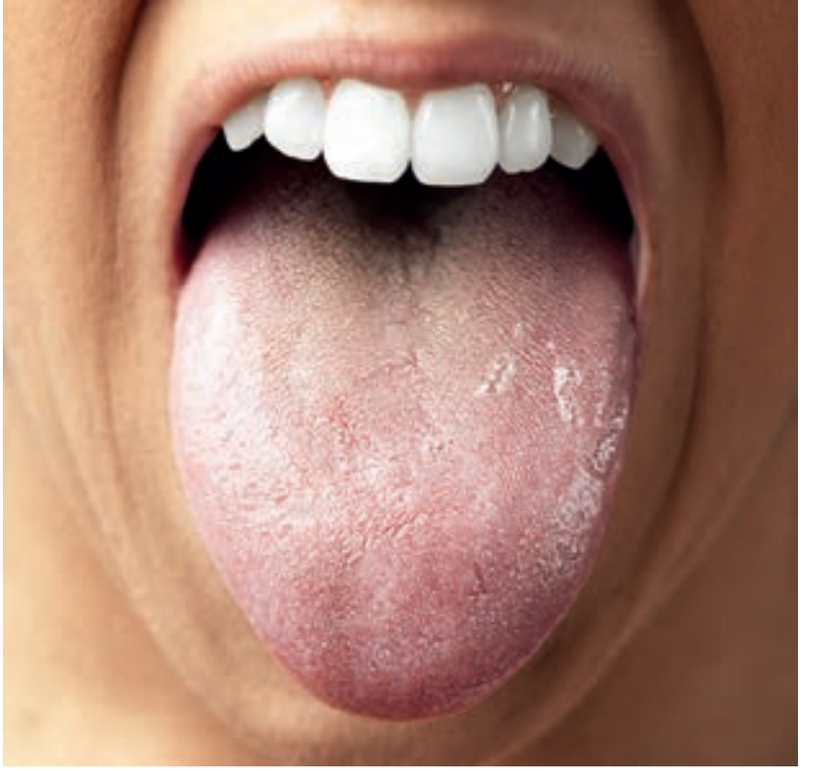


Bristol Robotics Laboratory

Robots fuelled by microbes

BIOFILMS CAN PRODUCE USEFUL POWER AS WELL AS SMELLS

The microbes in biofilms on the tongue may well be the source of some unwanted odours, but some biofilms can be useful. For example in microbial fuel cells, it is the biofilms that grow around the electrodes that break down the 'fuel' which consists of waste organic materials. At the same time they abstract electrons that can be carried into an electrical circuit. By joining together large numbers of these cells, it's possible to power all sorts of devices. "Our first ideas were ways of energising robots," says Professor John Greenman of CRIB, who works on the cells with Professor Ioannis Ieropoulos from the Bristol BioEnergy Centre (BBiC). "But the fuel cells we developed for running our robots are the sort that can be made larger by multiplication. If you put enough together, you could take any sort of domestic waste and produce electricity from it and clean up the waste at the same time. Each house would be able to produce sufficient electricity to drive a few things around the home." The group has used urine to provide the energy for lights in urinals on the university campus. Aid organisations are exploring the technology with UWE as a way to provide lighting for toilets in refugee camps.



human nose is generally considered to be the gold standard, better than any machine.

However, Dr Saad and Professor Greenman have also compared human assessment with various gas-analysing instruments and found that they measure up well. Older instruments only detect total sulphides, or different types of sulphides. But the machine that's so in demand in their lab uses a technique called selected-ion flow-tube mass spectrometry (SIFT-MS) to detect a whole bunch of other gases thought to contribute to oral malodour, including amines, acids and indoles. This detailed analysis capability is one of the reasons a lot of big companies want to work with the team.

ABOVE RIGHT
The tongue surface is home to billions of bacteria

ARTIFICIAL TONGUE

It's not just that companies are able to test their own formulations and products. The research that Dr Saad and her co-workers are doing is helping to uncover a more detailed picture of the processes that lead to oral malodour. Drawing on the work of others, they've developed their own way to model and monitor these processes outside the human mouth. They use a 'tongue' made from a cellulose matrix that's fed continually by a medium representing saliva. They populate the tongue with odour-producing bacteria with tongue scrape inocula from biofilm donors. "We mix the scrape in a fluid and then we inject a certain volume on to the matrix," says Dr Saad.

After two or three days growth and 'maturing' under the flow of artificial saliva, the bacteria on the tongues — housed in small plastic containers — reach a steady state and are ready for assessment. Dr Saad can use the SIFT-MS machine to analyse the gases being produced, and to see what happens to the levels of these gases when she introduces various antimicrobial compounds. Another technique involves measuring gases from inside the system itself. In one 2012 study, Dr Saad, Professor Greenman and their colleague Dr Keith Hewett showed that it was possible to get real-time sulphide measurements from biofilm models containing integrated sensors based on microbial fuel cell technology (see 'Robots fuelled by microbes,' on p27).

BIG PICTURE

The idea is not to find compounds that target specific bacteria, but to find ways to tackle biofilms as the whole complex ecosystems they are. "We want to look at the whole picture," says Dr Saad. "We want to know how much the biofilm is going to be reduced." By looking at how low the levels of gases drop when antimicrobial compounds are introduced, and how long they take to recover, they can calculate approximate kill rates for bacteria and get an idea of what kinds of compounds might work.

Their growing understanding of the tongue biofilm is also leading companies to consider other treatment approaches to mouthwashes and toothpastes. Mechanical approaches could help to gently break up biofilms and reduce any offending odours. The new Philips product Dr Saad is testing, for example, brings together both chemical and mechanical approaches, delivering mouthwash at the same time as the physical cleaning.

As knowledge grows about the communities of bacteria in our mouths and on our tongues, it may become possible to dampen the activities of those that produce the worst smells. Dr Saad, though, doubts there will ever be a cure for bad breath, if only because what smells 'bad' for one person doesn't necessarily for another. To her, the indoles in breath odours, which some people's just can't stand, smell like mothballs. "I don't mind it," she says. "It's a childhood smell that reminds me of the clothes wardrobe in my grandmother's house."

> 'This detailed analysis is one of the reasons a lot of big companies want to work with the team'

An alternative way to treat prostate cancer

CONTROLLING GENE 'SPLICING' MAY PROVIDE A NOVEL AVENUE FOR THE TREATMENT OF CANCER

Genetic research that focuses on the 'alternative splicing' of RNA could reveal a new way to treat prostate cancer that is less harmful than some of the current therapies.

In alternative splicing, single genes code for different proteins thanks to the different 'messgenger RNAs' they produce. Over 94% of human genes show alternative splicing, and aberrant splicing has been associated with diabetes, neurodegenerative disease and cancer.

Scientists within CRIB at the University of the West of England (UWE) have focussed on VEGF, a gene which codes for a growth factor that drives angiogenesis — the formation of blood vessels. Alternative splicing means that VEGF can be either pro- or anti-angiogenic. In prostate and other cancers, the balance of its splicing is shifted to favour pro-angiogenic VEGF, providing the tumour with the blood vessels it needs to grow and spread.

ABOVE
Dr Michael Ladomery is hoping to find a new prostate cancer treatment

With backing from several sources, including the Wellcome Trust and Prostate Cancer UK, molecular geneticists within CRIB have the key molecules in this process, and are collaborating with prostate cancer specialists and clinicians at the University of Bristol with a view to developing a new way of treating the disease.

The researchers discovered that the protein SRSF1, a splice factor, is required for the production of pro-angiogenic VEGF. SRSF1's activity is enhanced by SRPK1, a protein kinase (kinases are enzymes that add phosphate groups, in this case to SRSF1). They have demonstrated, that by inhibiting SRPK1 in vivo, the splicing of the VEGF gene can be switched to make more anti-angiogenic VEGF, thus denying the tumour the blood vessels it needs.

Dr Michael Ladomery, who is leading the research at CRIB, says: "We have shown that targeting the machinery of VEGF splicing has theraputing potential."

Research is continuing in collaboration with universities nationwide to further examine the consequences of targeting this molecule, with a view to eventually moving towards clinical trials. This includes basic research work by PhD students at UWE, research at the Universities of Bristol (led by Dr Sebastian Oltean) and Nottingham (Professor Dave Bates) where further in vivo studies and pharmacokinetic studies are taking place, trying to create a more potent compound for treatment.

"There is a need to derive additional treatment options for patients, because current therapies are limited," says Dr Ladomery. "Our aim is to put alternative splicing on the radar of clinicians and pharmaceutical companies and give patients additional options in the next 5 to 10 years and beyond."

Targeting alternative splicing could also help the treatment other types of cancer, Dr Ladomery hopes. "We are confident it that it can be targeted in other contexts, including leukaemia," he says. "Professor Bates' work at Nottingham shows powerfully that targeting this pathway is also showing great promise in the treatment of eye disease. This latter point illustrates how easily basic research into fundamental pathways can help us to tackle multiple biomedical problems."

BELOW
Focusing on RNA could provide a new way to fight cancer

(From left) Dr Robin Thorn, PhD student Bethany Fox and Professor Darren Reynol

Going off grid with water treatment to save lives

FORGET MILES OF PIPES AND HUGE SEWAGE TREATMENT PLANTS, A NEW FILTRATION SYSTEM CAN PROVIDE CLEAN WATER IN DISASTER ZONES

Behind a little wooden gate, not far from the science building at the University of the West of England (UWE), is a shed. It's a dull shade of green, unassuming and, being partly masked by leafy foliage on one side and the tall grasses surrounding the pond on the other, barely noticeable to the droves of students and staff passing by each day. But as soon as the gate has clicked and the shed door is unlocked, it becomes apparent that this is no garden variety shed.

The shed belongs to the research team of Professor Darren Reynolds. Inside, the walls are covered in pipes, sockets, electronic devices and meters of various kinds. Just inside the open door stand two tall, blue plastic containers full of water – one either side.

"Essentially it's a TARDIS," Professor Reynolds had joked earlier in the day when describing the shed-based water box technology he's experimenting with here. The similarity becomes slightly more apparent as he starts flicking switches with the casual dexterity of a Time Lord.

Shed, TARDIS, or box, its purpose is simply demonstrated. The blue plastic container on the right-hand side of the door receives water from the outside environment — in this case, pumped in from the pond. A quick peak under the lid of that right-hand container reveals that it is indeed full of murky-looking pondwater, probably containing goose guano. Seconds later, the same water pours out of a spout into the left-hand container, looking clean and clear enough to drink: Reynolds' shed turns dirty water into clean water.

The utility of small-scale water treatment may not be particularly apparent on a university campus in the UK, where clean water comes out of a tap. "In the UK, we have thousands of treatment plants," says Professor Reynolds. "We have hundreds of thousands of kilometres of pipes, which are either carrying dirty water away or providing us with clean drinking water. You turn the tap on and the water is immediate, it's fit to drink and you don't even think anything about it." Elsewhere in the world, though, the reality couldn't be more different.

In 2014, 748 million people around the world were living without access to clean, safe water. Water might be abundant on planet Earth, but just 3% of it is fresh water and less than 1% of that is available to us as drinking water. In the developing world, the choice is too often between dying of thirst and dying of a water-borne disease. Drinking unsafe water is thought to account for a large proportion of the 760,000 deaths a year caused by diarrhoea in under-fives.

The solution, says Professor Reynolds, lies not in building large-scale water distribution networks like those in the UK, but in developing small, off-grid water supplies. Although his shed may only be an experimental unit, what it generates signifies a potential lifeline to someone in rural, sub-Saharan Africa or in the aftermath of a natural disaster. So how does one of these mysterious water boxes work?

ABOVE
Access to clean water is vital in the aftermath of disasters such as the 2015 Nepal earthquake

LEFT
The water treatment system is effective against *Staphylococcus aureus*, a common cause of food poisoning

EMBRYO OF AN IDEA

The key technology at the heart of the filtration system is a novel type of disinfectant that kills the bacteria in contaminated water whilst having minimal impact on the environment. Working with colleagues Dr Robin Thorn and Dr Gareth Robinson at the Centre for Research in Biosciences (CRIB), Professor Reynolds has developed and proven a system based on electrochemically activated solutions (ECAS), known to be a greener variety of biocide. Although the system uses chlorine, the same chemical used to disinfect swimming pools, it is generated on site at very low concentrations.

"Anything related to producing chlorine is usually horrible," →

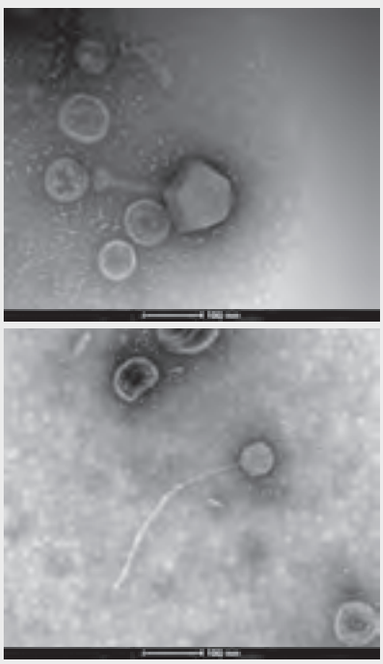
explains Professor Reynolds. "Our system still uses chlorine, but the currency of chlorine is slightly different. It doesn't have a long life. So we've toyed around with using it to produce drinking water, and that's essentially been the embryo, if you like, of the idea." To create their biocide, they take salty water and, using electricity in an electrochemical cell, separate it into two solutions: an analyte, containing chlorine ions, and a catholyte. The analyte, chlorine-containing solution is a powerful biocide, but it's easily neutralised by blowing air through it.

The idea of using ECAS as biocides has been around for decades and, during the 1990s, some water purification devices were invented based on this approach. However, in a 2010 study, Professor Reynolds, Dr Robinson and colleagues from CRIB demonstrated the fast-acting effectiveness of a salt-water ECAS against important disease-carrying microbes, including methicillin-resistant *Staphylococcus aureus* (MRSA) and spores of *Clostridium difficile*, which can cause diarrhoea. They showed that it was effective at lower concentrations than previously thought and suggested that the biocide could be exploited as a low-cost, environmentally friendly, on-demand disinfectant — exactly how they now use it. Although the chlorine ions work fast to kill bacteria, the biocide retains its green credentials by virtue of there being no residual chlorine. By contrast, in water systems in the developed world, the chlorine has to be long-lasting in order to keep water clean whilst it travels long distances through underground pipes.

ABOVE
Professor Darren Reynolds, PhD student Bethany Fox and Dr Robin Thorn outside 'Stanley' the shed

CLOSE COLLABORATION

In recent years, the team has worked with a range of industrial partners to combine their ECAS-based biocide with other water treatment technologies to



Hunting bacteria-slaying viruses

BACTERIOPHAGES MAY BE OUR SAVIOUR AGAINST ANTIBIOTIC RESISTANCE

"You could describe them as the dark matter of the biosphere," says microbiologist Dr Dann Turner. He's talking about bacteriophages — viruses that infect bacteria. There may be as many as 10 nonillion (10^{31}) of these phages in the environment at any one time, yet little is known about them. But Professor Reynold's research team is trying to find out more.

What's exciting about bacteriophages is that now antibiotics are becoming increasingly redundant against bacteria, phages could provide the solution. The CRIB research team is working with Public Health England to isolate and characterise bacteriophages that infect multiple drug resistant bacteria.

Phages infect bacterial cells, hijacking them to produce more phage, before causing the cells to burst open, killing them. Turner is hoping to identify phage genes and proteins that are involved in taking over host cells. If he can do that, he may be able to find a new way to fight bacteria.

Antibiotics are limited in their modus operandi. "Current antibiotics act upon a limited repertoire of targets," says Turner. "It might be the protein synthesis or inhibition of cell wall synthesis and function. Hopefully our research can give us an idea of some new targets."

Phage are highly specific to the bacteria they infect, but Turner's sights are currently on a bacterium called *Acinetobacter baumannii*, which is resistant to several different antibiotics. One possibility for tackling the bug is packaging up bacteriophage proteins into tiny parcels that can be delivered as medicine — an approach that the group are exploring with nanotechnology company Blueberry Therapeutics.

"If you have an earthquake, then you have the ability to take a box, drop it down with a helicopter and produce drinkable water." **PROFESSOR DARREN REYNOLDS**

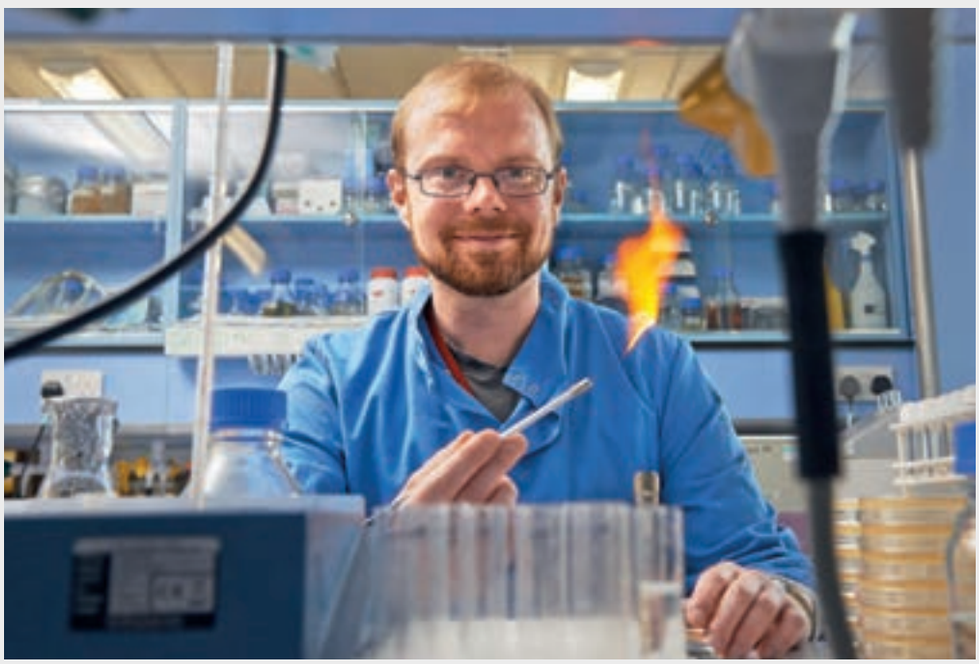
create a portable water treatment system. As well as the electrochemical cell that produces the disinfectant, the system includes a series of filters, whose pores decrease in size to remove particulate matter. While the science of each component is not new, the way they have been combined to produce an effective, portable system is. A water sensing system has also been developed, that can be used to monitor the water going in and coming out of the filters anywhere with internet access, to check that the water is being cleaned effectively.

Pentair, a global technology company specialising in water treatment, supply the microfiltration membranes. Bridge Biotechnology makes the electrochemical cells. Meanwhile, engineering firm Portsmouth Aviation helps with systems integration and manufacture. The CRIB team has been working with Portsmouth Aviation to design the system so it fits into shipping containers, making it easy to transport around the world. Already, the system has been tested in Romania, where water from biologically contaminated wells was cleaned effectively.

The system is now being marketed by Portsmouth Aviation, which does much of its work in the defence sector. And things are looking promising. "They have had interest from European and Middle Eastern countries," says Dr Thorn.

The current product is "battlefield robust," says Professor Reynolds, and it may have to be. One of the most immediate applications for the boxes is in disaster-stricken regions, meaning they could have to be dropped in by helicopter. "If you have an earthquake, or some other kind of catastrophe, then you have the ability to take a box, drop it down with a helicopter, stick a pipe in a body of water and then press a button to produce drinkable water," says Professor Reynolds. "It would be fit for purpose for something like that, now."

ABOVE
Very little of the fresh water we're surrounded by is drinkable



BELOW
Dr Dann Turner is uncovering baceteriophages' modus operandi

In the month following the earthquake that shook Nepal in April 2015, WaterAid distributed thousands of water purification tablets to affected communities. It was reported that the water supply in camps in the city of Kathmandu had become contaminated and therefore many people had restricted access to clean water. In desperate situations such as these, small-scale water treatment could provide much-needed relief.

LONG TERM HELP

The bigger problem, though, is the dirty water that millions have to drink and wash themselves in on a regular basis. Could the team's biocide-in-a-box make any real impact on such a massive problem? The figures suggest it could.

Based on the existing system, which can produce 18 cubic metres of clean water an hour, it's easy enough to work out how many people could benefit →

from just one box. 18 cubic metres is equivalent to 18,000 litres. Assuming the average person can live on five litres of water a day, one box running for an hour could supply 3,600 people with enough water for a day. But that's in just one hour. Running 12 hours a day, the same box could theoretically supply 216,000 litres of water a day — enough for over 43,000 people.

FOOD WASTE FIX

Tackling the world's biggest problems has become something of a mission statement for Professor Reynolds' research group, which brings together researchers from a range of disciplines. Not only are they addressing the water crisis, they're also trying to deal with food waste by extending the storage life of fruit and vegetables. Most recently, they have been involved in a project with the supermarket chain Sainsbury's and an agricultural consortium which included Thanet Earth. The approach they've adopted exploits the ECAS biocide for a different purpose — to stop the growth of spoilage organisms on food.

They're even attempting to deal with the growing problem of antimicrobial resistance by searching for alternatives to antibiotics (see 'Fishing for phage' on p32).

In another line of research, the team is studying the natural fluorescence, or glow, of the microbes in fresh water to understand how it could be used to monitor water quality in real-time. The idea being that if water quality changes, so will the health of the microbes within it and this will be reflected in changes in their fluorescence.

The water quality project, a collaboration between CRIB, Bristol University and Chelsea Technologies Group, which specialises in sensor technology, was awarded a prestigious CASE award by the Natural Environment Research Council (NERC) to develop a fluorescence sensor. The award has funded PhD student Bethany Fox. As many locations, such as the UK, experience an increase in the frequency and intensity of rainfall, water treatment systems are being overstretched. So technology to monitor water quality in real-time will be vital.

ABOVE
Professor Darren Reynolds, PhD student Bethany Fox and Dr Robin Thorn

BELOW
Clean drinking water is a vital resource

Despite juggling so many different projects, Reynolds' enthusiasm for new ones appears boundless. "I just don't have enough time!" he exclaims, as he marches full tilt towards the little gate and the hidden shed.

At the shed, known affectionately as Stanley, the sound of water purifying is audible but doesn't impinge on the quiet tranquillity of the afternoon. It's an idyllic and oddly relaxing way to carry out cutting-edge science and there's a familiar, yet, in a wider context, incredible, result: clean water.

Improving the prognosis for very premature babies

THE DISCOVERY OF A GENETIC LINK BETWEEN EARLY BIRTHS AND CEREBRAL PALSY COULD LEAD TO A TREATMENT

One in 12 babies in the UK and more than one in 10 worldwide are born very premature (less than 32 weeks of gestation) and some of these infants go on to develop neurological conditions early on in life. Until now, it has been far from clear why some very premature babies go on to develop these problems, whereas others don't.

But research being carried out by Professor Aniko Varadi within CRIB with colleagues at the University of Bristol, St Michaels' Hill Hospital and Southmead Hospital, is giving an insight into what's going on. Their work could lead to the development of bedside tests that could be carried out on premature babies to determine how likely neurological problems are later in life.

Professor Varadi and her colleagues have carried out genetic tests on over 800 samples of blood spots and umbilical cord tissue taken from premature babies over a 20-year period. They have been focussing their attention on a regulatory part of the DNA that controls the activity of a gene, EAAT2.

This gene encodes a protein that sits in the membranes of glial cells within the brain. Among other things, these cells soak up the neurotransmitter glutamate, which is important for transmitting signals from one brain cell to another but can become toxic to the brain if it reaches too high concentrations.

As well as the genetic data, Professor Varadi and her colleagues had details of the long term health of over 470 of the premature babies. By comparing the two it showed that babies born with certain forms, or 'variants', of the EAAT2 gene regulator have a higher chance of developing cerebral palsy — a group of conditions that affect muscle control and movement. They are also more likely to develop relatively slowly and scans of their brains are more likely to show lesions in the white matter. These problems may be caused by inappropriate regulation of EAAT2 protein production in the membranes of glial cells. The end result of this is that the brain becomes overloaded with glutamate, causing damage.

ABOVE
The rate of premature births is increasing in the UK

"This is the first research that appears to show that glutamate is linked to brain damage in very premature infants," says Professor Varadi. "There had been lots of hypotheses before, but this is the first physical evidence."

The consequences of having the wrong combination of genetic variants can be dramatic: babies born with certain combinations are four times as likely to develop cerebral palsy.

ABOVE
Professor Aniko Varadi, developing a genetic test for premature babies

As well as developing a bedside genetic screening test, in the longer term the research could be used to help children affected more directly. "Because we know the mechanism, the next stage would be to look at treatments," says Professor Varadi.

This research, funded by the University of the West of England and the David Telling Charitable Trust, is particularly important given that the rate of premature births is growing by 1.5% a year in the UK and the estimated cost of premature birth throughout childhood in England and Wales is around £3 billion per annum; that's more than the cost of obesity.

Globally, the World Health Organisation (WHO) states that of 65 countries with reliable trend data, all but three have shown a rise in premature birth rates over the past two decades.

From weapon of war to crop growth hero

HYDROGEN SULFIDE COULD PROVIDE US WITH A WAY TO INCREASE CROP PRODUCTION AS GLOBAL POPULATIONS GROW

With its foul, rotten-egg smell and a dark past as a World War I chemical weapon, hydrogen sulfide gas may seem an unlikely addition to a food crop's environment. Yet the chemical is produced naturally by both plants and animals and it could provide us with a means to improve crop yields as well as allowing us to develop new pharmaceutical products.

As part of a joint project with a team from CRIB, researchers at the University of Osijek in Croatia, are spraying trays of pepper plants with hydrogen sulfide in a bid to discover whether the chemical can give a better crop yields.

The rationale is that hydrogen sulfide is involved in cell signalling, particularly in times of stress, joining forces with other chemicals such as nitric oxide and hydrogen peroxide. "If you hit a plant, overheat it, cool it down, or give it a parasite or bacteria that it doesn't like, then levels of hydrogen peroxide, nitric oxide and hydrogen sulfide will increase," says Dr John Hancock, who is leading the research. One of the key challenges is working out exactly how plants respond to such stress conditions. "There must be subtle differences between these signals but we're not anywhere close to understanding that," he says.

ANCESTRAL ADVANCES

"What's really interesting is that we are making these chemicals all of the time but in fact they are really poisonous," says Dr Hancock. "It seems a bizarre thing for nature to do — to produce something that is poisonous and dangerous — and yet it seems to do it for positive reasons." He thinks there are evolutionary explanations for this. In our past, we evolved in a high sulfur environment, finding ways not only to tolerate the sulfur-based molecules but also to put them to good use.

"Both hydrogen peroxide and nitric oxide seem to work together in a signalling pathway involving proteins and probably gene expressions," says Dr Hancock. Tests involving spraying very low concentrations of the chemicals onto plants have revealed that they cause tiny openings on the underside of leaves — the stomata — to close, slowing water loss and gas exchange. "If you can modulate the process in a positive way you can, in theory, allow plants to survive drought stress," he says.

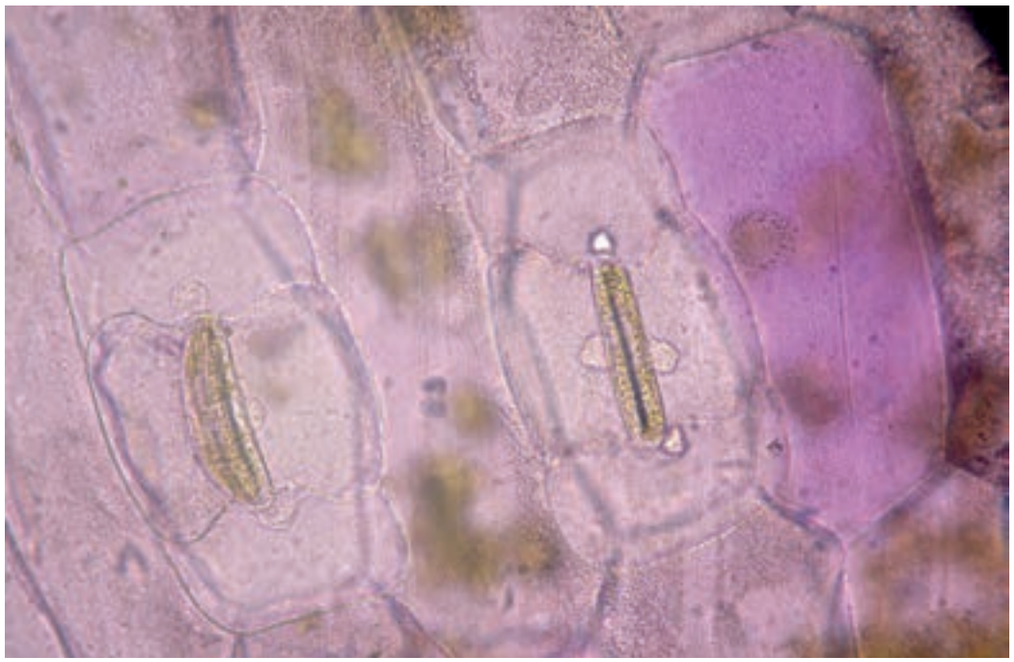
In tests on plants, it appears that the hydrogen sulfide has the opposite effect to nitric oxide, causing the stomata to open, allowing more water to flow through. "In some cases, this might have a better consequence for crops because more gas exchange and water flowing through them may be beneficial as long as they are kept well watered," says Dr Hancock.

CHEMICAL REFEREE

His focus has been on understanding the mechanisms of these plant signalling chemicals and his work has gained extra depth by linking to animal studies. At a conference several years ago, Dr Hancock met Professor Matt Whiteman from the University of Exeter's Medical School, who was also working on the signalling pathways, but in mammalian systems. The plant and animal systems are "frighteningly similar," says Dr Hancock, and the two researchers forged a collaboration to

ABOVE RIGHT
Dr John Hancock, who is leading the research on hydrogen sulfide in CRIB

BELOW
Pepper plants are being used to investigate the effects of hydrogen sulfide



study the pathways in both. Within CRIB, for example, researchers are using *Caenorhabditis elegans* as a model organism to find out more about how hydrogen sulfide is used in animals.

Studies already conducted by the teams suggest hydrogen sulfide helps to protect cells, especially during diseases such as atherosclerosis and diabetes, which are exacerbated by stress. Together, Professor Whiteman and Dr Hancock have developed a theory that increased levels of hydrogen sulfide in humans may reduce the risk of disease developing. "It is implicated in the onset of disease but is more likely to be trying to reduce the onset," says Dr Hancock.

In both plants and animals, the researchers think that hydrogen sulfide is acting as a 'referee,' regulating levels of hydrogen peroxide and nitric oxide, so that they do not over-accumulate and cause damage to cells and tissues.

Before they could begin their studies, the researchers had to find a way to deliver hydrogen sulfide gas to plants. For this, Professor Whiteman worked with a team of chemists to develop donor molecules that will hold onto the hydrogen sulfide in solution, releasing it in small amounts when needed. The work was so successful that he is now looking to use the system in pharmaceutical products.

Dr Hancock is aware of cases in China where hydrogen sulfide is already being sprayed on crops, post harvest. In addition to having concerns about flavour taint from the chemicals, he remains to be convinced that the process is worthwhile.

"My gut feeling is that if we can understand what hydrogen sulfide is doing and then mimic this in a less dangerous way, that would be a better way forward. I think we are quite a long way from that at the moment but there is potential; hydrogen sulfide is a really interesting compound."

Dr Hancock's next plant research priority is to look at the effects of the signalling chemicals on their roots given that many soils have high concentrations of hydrogen sulfide. "It's interesting to understand what the effects of hydrogen sulfide are on root development and growth," he says.

Dr Hancock is also interested in how bacteria are affected by hydrogen sulfide and how it might alter their interactions with plants. He has teamed up with Professor Dawn Arnold, an expert in molecular plant pathology within CRIB and who studies DNA movement in and out of pathogenic bacteria, to carry out the research.

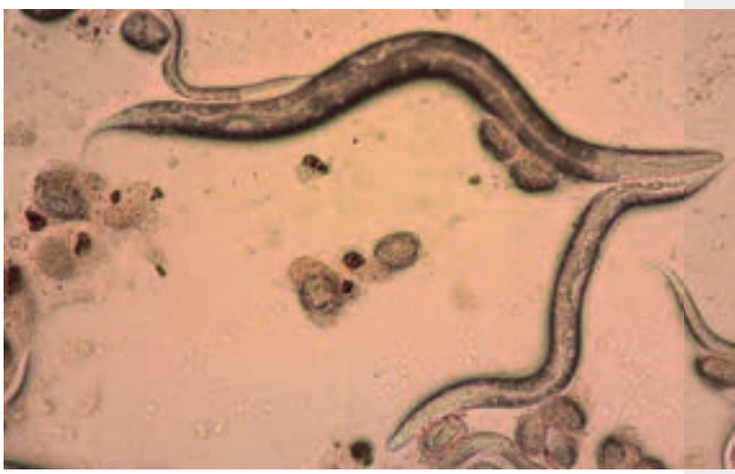
One area they will consider is whether bacteria have better access to plants if the stomata are more open thanks to hydrogen sulfide. "Also, if hydrogen sulfide affects the genetics of a bacterium, that might make it more or less likely to invade the plant," says Dr Hancock.

With so many possible effects of hydrogen sulfide, unlocking how to put it to use will be complex. But instead of being a villain, it could help to keep us healthy and well fed in future.

ABOVE
Hydrogen sulfide and nitric oxide together cause stomata to close

BELOW
***C. elegans* is helping to reveal the role of hydrogen sulfide**

'Advances in our understanding of hydrogen sulfide could help us improve drought resistance'



The battle to save our chocolate supplies

A VIRUS IS THREATENING THE TREE THAT'S THE SOURCE OF THE ALL-IMPORTANT COCOA BEAN, SO THE RACE IS ON TO COMBAT IT

Around the world, our appetite for chocolate has been growing. The steady recovery in the global economy and a growth in spending on chocolate in emerging markets such as China and India mean that demand for cocoa has never been higher. But there's a threat to our growing love of chocolate in the form of a virus — a virus that's killing off cacao plants, the source of the cocoa bean.

Cacao swollen shoot virus (CSSV) has been affecting chocolate production since the 1930s, but the growth in demand has brought the issue into focus. What makes the situation worrying is that CSSV has taken hold in West Africa, where 70% of the world's cocoa production takes place. Chocolate produced from cocoa here has the flavour consumers in the emerging markets favour.

"Chocolate is produced in Central and South America, West Africa and it's moving into South East Asia and places like Malaysia," says Dr Joel Allainguillaume. "But they haven't been able yet to get the same flavour as in West Africa. So any disease that affects cocoa production here will have a huge impact."

ABOVE
Dr Joel Allainguillaume is finding ways to fend off CSSV

BELOW RIGHT
Cocoa beans could be in short supply if we fail to act now

BELOW
The mealybug spreads CSSV between cocoa trees

COMPLEX PICTURE

The virus, a member of the family Caulimoviridae, is spread among cacao trees (*Theobroma cacao*) by mealybugs — small sap-sucking insects. CSSV resistance breeding programmes are currently underway in West Africa. These use mealybugs to inoculate candidate plant material. What makes things complicated is that there are at least 61 species of mealybug but, so far, only 16 of these are thought to be vectors of CSSV. So it's vital to be able to identify the mealybug species used in the breeding programmes. Working with colleagues at the University of Reading, Dr Allainguillaume has shown how a technique using DNA barcode analysis can be used to rapidly identify different mealybug species; something that is just not possible by eye.

The technique will prove invaluable in supporting the breeding programmes. "In the long term, we would be able to use cacao cultivars that are more resistant to the virus as parents to produce a breeding population of resistant plant material," says Dr Allainguillaume, a geneticist. This would mean that over time, cocoa production in West Africa would switch to plants that are less susceptible to CSSV compared to the ones we currently rely on for our chocolate fix.

The research on the genetic identification of mealybug species has been funded by Cocoa Research UK, a non-profit research association, and Mars. In their study, Dr Allainguillaume and his colleagues received samples of mealybugs from seven countries, including the Ivory Coast and Ghana; the countries most affected by CSSV. Using a technique known as High Resolution Melt Analysis (HRMA), they assessed DNA sequence variation in the barcoding gene cytochrome oxidase1 to pinpoint differences between mealybug species. Given that HRMA is far simpler and cheaper than DNA sequencing, it will

Image: Q. Holdman / US Agricultural Research Service

be a boon to research to identify and confirm true vectors of the disease.

BETTER DIAGNOSIS

Dr Allainguillaume and his research partners are taking another tack in their fight against CSSV too, looking into a new way to diagnose the disease, something that's tricky right now. "The symptoms of CSSV are similar to those of physiological stress," says Dr Allainguillaume. "So it's a bit subjective. The only symptom that gives you any certainty is the swelling of the shoot, but that happens late on in the infection and by that time, the plant is about to die and will have spread the disease to other plants."

The problem of diagnosis is exacerbated by the fact that much of the cocoa production takes place on small farms. "The loss of a single tree is a big deal to these farmers," says Dr Allainguillaume. Understandably, this makes farmers reluctant to remove trees unless they are absolutely certain they are infected. Genetic test can be developed to detect the presence of viral DNA in the cacao tree. But for this to be efficient, an extensive knowledge of CSSV genetic diversity is necessary.

> "We would be able to use plants that are more resistant to the virus to produce seeds" **DR JOEL ALLAINGUILLAUME**

So far, six strains of the virus responsible for CSSV have been genetically sequenced. Working with colleagues at the University of Reading and CIRAD in Montpellier, France, Dr Allainguillaume is working to sequence viral strains from all infected areas of cacao production in West Africa. The work is funded by CAOBISCO, the Association of Chocolate, Biscuit and Confectionary Industries of Europe. In the longer term, the ambition is to develop a test that could be used in the field to provide a definitive diagnosis of CSSV, giving farmers the certainty they need to remove infected trees quickly. This would help to monitor and control the disease's spread.

ABOVE
Cocoa trees are under threat thanks to CSSV

"We want to sequence a wider range of strains from the Ivory Coast," says Dr Allainguillaume. "Within that 70% of world production in West Africa, the Ivory Coast probably contributes 60–70%. We want to characterise the strains across the whole country better because if you are going to develop a system that detects the strains, you need to know which regions of the genome are genetically similar."

There is some indication that different strains of the virus are more virulent. "If we know more about the genetic differences between strains, we can start to see why some are more virulent," says Dr Allainguillaume, and this may provide an insight into the virus's modus operandi.

So next time you tuck into a chocolate bar, it is worth sparing a thought for the research going on to ensure that you, and millions of other chocolate lovers around the world, can continue to do this long into the future.

How 3D cell models will transform treatments

GROWING CELLS IN MORE LIFE-LIKE CONDITIONS WILL CHANGE HOW DRUGS ARE DEVELOPED AND ENABLE PERSONALISED MEDICINE

Testing how effective a drug treatment may be and anticipating any possible side effects is far from straightforward. One of the major challenges is that current tests carried out in the lab may be too simplistic and often fail to predict what will happen when a drug is inside a patient's body.

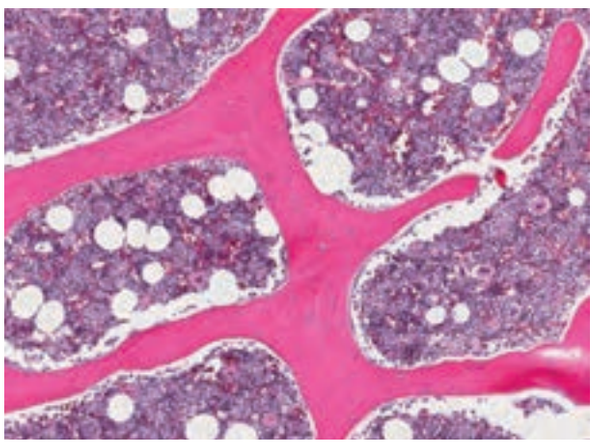
But researchers within CRIB are looking to overcome that by mimicking what goes on in the body by creating 3D models of cell structures. As well as helping with drug development, this may also help doctors determine which treatment is best for individual patients. The CRIB researchers have already set their sights on one nasty condition — leukaemia.

Developing 3D models is an important step. "If you make a simple *in vitro* model of cells, which is classically what has always been done, it's very two dimensional," says Dr Ruth Morse, who is leading the research. "If it's bone marrow cells you're growing, they stick to the bottom of the flask. But inside the body, the bone marrow cells sit inside a honeycomb structure and blood flows through the pockets. If cells are grown flat, their morphology changes, it changes the structure of their nuclei and therefore the position of the DNA and gene expression. So it changes lots of things, including how they respond to external stimuli, such as drugs."

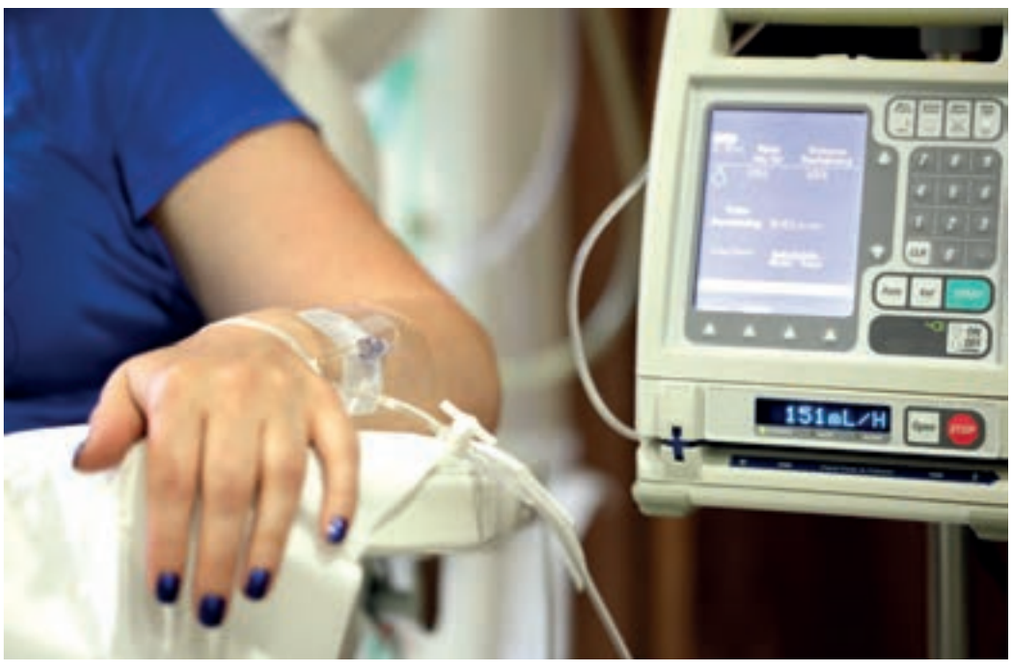
This is what is at the heart of this team's work; the need to recreate as accurately as possible what goes on inside the body, so it creates as realistic an environment as possible for testing the effects of drugs.

ENHANCED 2D

The team started its work by enhancing a 2D model, adding 'spheroids' of liver cells to 2D bone marrow cells. "In a body, drugs are metabolised, becoming active and being broken down too," says Dr Morse. "A lot of drug toxicity tests in labs have a tendency to overestimate the effects of the drugs because there is no mechanism to get rid of them. We were able to show that with liver spheroids producing liver enzymes, we got a better link with what happens in patients."



ABOVE
Bone marrow is the source of red blood cells



BELOW
Chemotherapy can occasionally cause tumours in cancer patients

Dr Morse and her team have been investigating different 3D scaffolds on which to grow bone marrow cells and the plan is to examine how similarly cells grown here behave to bone marrow in the body.

So far, samples of bone marrow taken from hip replacement patients at Southmead Hospital Bristol and patients undergoing cancer treatment at the Royal United Hospital Bath have been compared with cells grown in their 2D models. "We looked at how the cells grow and proliferate, and the expression of proteins on their surface," says Dr Morse. "The next stage will be to look at gene expression and morphological changes."

This work will be carried out at the same time as the development of the 3D scaffolds.

It's work that's well worth doing. When pharmaceutical companies are testing a new drug, they will often start with tests on single cells grown in 2D culture; typically bacteria or mammalian cell lines. The tests may then move into animal models. In some instances, where the initial single cell tests do not indicate any problems, in animal models the results can be quite different. "There is a gap between the current tests of new drugs, and we hope that the 3D model would fill that gap," says Dr Morse. "It could have a big impact on regulatory testing in the pharmaceutical industry."

The 3D models may also help us to understand what happens in therapy-related malignancies, where treatment for cancer causes another tumour to appear in a patient several years after the treatment has ended. Although relatively rare, it is still something doctors would clearly rather avoid. A greater knowledge of why it happens in some patients and not others would enable them to pinpoint at-risk patients before treatment begins, giving them a different treatment.

"There is a gap between the current tests of new drugs and the 3D model would fill that." **DR RUTH MORSE**

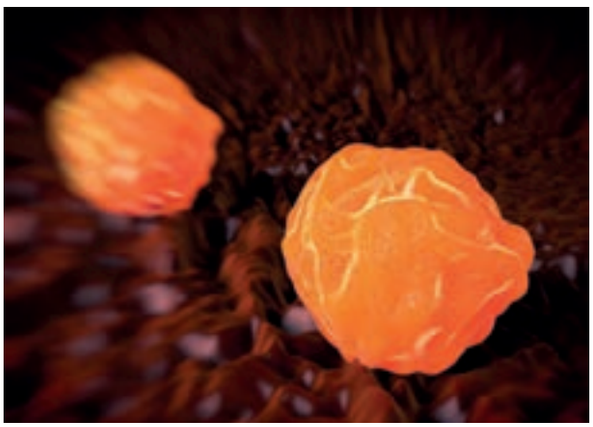
TARGETING LEUKAEMIA

A case in point is donor cell leukaemia. Some patients receive chemotherapy to 'kill' their leukaemia followed by a donation of bone marrow stem cells from a healthy individual to help replace the bone marrow damaged by the leukaemia and chemotherapy, and provide new blood cells. However, in 5% of patients, the donated cells become leukaemic whilst staying healthy in the donor. Dr Morse's group believes this is due to the patient's cells communicating with the donated cells.

"We are going to try to work out if the chemotherapy is causing these complications so we can work out alternatives and consider why a small sub-set of patients are affected but others are not," says Dr Morse.

Such therapy related malignancies are a particular problem for children who are robust and respond well to treatment, so are more likely to survive long-term. "The complications may occur 10–15 years after the treatment and the new tumours are often more drug resistant," says Dr Morse.

Survival rates for cancer are rising, so secondary complications such as donor cell leukaemia are coming more to the fore. First recognised in 1971, it had been thought to be rare. But a rise in reported cases suggests it is more common than was first thought, partly because more people are getting this sort of treatment as therapy approaches improve.



ABOVE
Bone marrow stem cells are sometimes given to cancer patients

TOP
New drug development could be helped with the 3D cell models

BELOW
Dr Ruth Morse, who is leading the cell model team

The team plans to investigate this condition using their 3D bone marrow structures and this should give a much better understanding of what happens than would be the case with simpler models. If they can get to the bottom of how the chemotherapy can cause DNA damage and how the cells communicate with each other, personalised treatment will become a possibility.

"Understanding the responses a tumour and a person are likely to have to a particular therapy means you can tailor their medicine accordingly," says Dr Morse. "We are looking to give people the best possible chance of surviving their cancer with minimal complications."

So while there is still work to be done, a 3D model could transform drug development and therapies.

Rapid tests will help patients and farmers

A NEW EARLY WARNING SYSTEM COULD REDUCE BOTH HOSPITAL ADMISSIONS AND VET BILLS

Imagine that every day you worried that a slight twinge or rough cough is the start of a flare-up of a long term condition that you have lived with for many years, or the prelude to a significant health issue such as the onset of heart disease. Or imagine you are a farmer and that one or two animals in your heard of pigs have started to show breathing problems which might spread to other animals and cause the loss of the heard.

New technology being developed within CRIB will help patients and farmers rapidly evaluate symptoms to decide if further treatment is needed, or if there is no need to worry.

It is this worry over what might be and the need for hospitalisation or expensive treatments that Professor Richard Luxton, Director of the Institute of Bio-Sensing Technology based at the University of the West of England, along with his colleagues and collaborators are hoping to prevent. They aim to develop an early warning system for flare-ups of disease or infections in animals that could be used by patients and farmers. The technology is being developed by bringing together the skills of bioscientists and engineers and working with hospitals and businesses.

ABOVE
Professor Richard Luxton, who is developing the technology

Health problems in people or animals can occur at any time and are currently difficult to predict. Although there are certain symptoms associated with infections, such as increased breathlessness or coughing, this could easily be the result of a normal cold. However, even in the early stages of a worsening or serious condition, the body's immune system will have already kicked into gear, producing molecules to help fight off the condition.

It is these 'biomarkers' that provide the key to the early detection system. Working with the collaborators in hospitals and with animal welfare scientists, suitable biomarkers are identified for particular conditions and a rise in their levels will predict the development of complications or an exacerbation of a disease needing treatment or hospitalisation.

"You can detect, using these biomarkers, whether the patient is actually having an exacerbation of the disease or whether they just have a cold, or if a farmer has a problem in the heard," says Professor Luxton. "This tells doctors whether they need to treat the patient or send them home, or if the farmer needs to call a vet." However, using conventional methods to detect the biomarkers takes several hours. Quicker tests are needed to ensure the right treatment is delivered as soon as possible.

BELOW
The prospect of a disease flare-up can be worrying for patients

SENSOR SOLUTION

To address this problem, Professor Luxton and his colleagues have developed a technology based on magnetic detection that can be adapted for different users such as patients or farmers. The system is based on the measurement of magnetic particles that are just a single micron in size.

These particles are coated with different substances, depending on the application, to which biomarkers in a sample (perhaps saliva) will quickly attach themselves, if they are present in the sample. A small

volume of sample is mixed with a small volume of magnetic particles for a few minutes. Once the mixing is complete, a magnet is placed under the sample tube. This draws the magnetic particles and their attached biomarkers down to a sensor coated with antibodies that also bind to the biomarkers. This forms a sandwich: the two antibodies, one on the particles and the other on the sensor surface with the biomarker sandwiched between them.

In the final stage, the magnet is moved away; at this point any magnetic particle that is attached to a biomarker will remain stuck to the sensor surface thanks to the bond with the antibody but the others, in the absence of the magnetic field, will diffuse back into the solution. The density of particles bound to the sensor surface is measured by a custom-built magnetometer and indicates the levels of biomarkers in the sample; with high levels indicating that a patient is at risk of disease flare-up.

"All this," says Professor Luxton, "happens in just four minutes. The process is so quick because the magnet pulls the particles down onto the sensing surface, unlike a conventional test in which the binding of biomarkers to a sensor surface is a slow process that must happen through diffusion".

> "Partnership is critical to the development of a commercial product." **PROFESSOR RICHARD LUXTON**

EASY TO USE

In healthcare applications the instrument has been evaluated against the conventional biomarker tests and performed well, mirroring their results. These successes have led to a number of high value grants from the Government and the NHS to take the instrument from the laboratory to the market.

In any development, the views of the end user are critical in terms of whether the technology is usable. Working with focus groups, designers and psychologists enables the development of these devices to be of value to the people who will be using the system. The results must be clear and point to further actions that need to be taken, if required.

For patients this simple tool for use at home could provide substantial health benefits and peace of mind — no more time-consuming trips to a hospital clinic for the sake of something that may just be a cold. Likewise for farmers, a negative result will remove worry about having to call in an expensive vet to test the heard.

Currently, prototypes are about the size of a can of baked beans and connected to a laptop. However, recent funding will enable pre-commercial prototypes of a self-contained, hand-held device to be developed. "Partnership is critical to the development of a commercial product and our commercial partners have done a fantastic job of taking this forward," says Professor Luxton.

ABOVE
A biomarker detection system would help to protect pig herds

BELOW
A saliva sample will provide a rapid test

This new assay shows how real-world solutions can be achieved using multidisciplinary approaches and by incorporating the requirements of users. "It is interdisciplinary working and the development of collaborations across technical, academic, commercial and healthcare sectors that have enabled these projects to move forward as they have done," says Professor Luxton.

Using the world's largest indoor rainforest as a lab

THE ECOLOGICAL CLOCK IS BEING WOUND FORWARDS TO SEE HOW CLIMATE CHANGE WILL EFFECT THESE TROPICAL HABITATS

They're known as the lungs of the world, producing about 20% of the Earth's oxygen. They cover less than 2% of the Earth's surface and yet are home to half of all the Earth's plant and animal species. Yet we know surprisingly little about how one of the principal threats rainforests are facing — climate change — will affect them.

But new research taking place within CRIB is helping to address that. It focuses on bird's nest ferns, which grow in the trees and play a pivotal role in rainforest ecosystems.

At the Eden Project in Cornwall, the world's largest indoor rainforest, research is taking place to understand the relationships between bird's nest ferns (*Asplenium nidus*) and the surrounding ecosystem. What's being learned here will help with experiments in the rainforest in Borneo that will involve winding the ecological clock forward to see what fate may await the ecosystem in 50 or 100 years' time.

ABOVE
Platforms at the Eden Project can be raised and lowered

'Knowing the roles of these ferns in rainforests could reshape the policies of governments'

At the world-renowned Danum Valley Field Centre in Malaysian Borneo, the field site of Dr Farnon Ellwood, who is leading the research, certain invertebrate species will be excluded from some of the bird's nest ferns to see what effect this has on the ferns and the surrounding ecosystem. Removing the invertebrates will simulate the extinction events that may take place if the planet heats significantly; the creatures that are small and therefore have a large surface area to volume ratio are likely to be particularly badly hit. Some of the bird's nest ferns themselves will also be removed from the trees. PhD student Julian Donald is currently working here and will be joined by another PhD student later in 2015.

"Bird's nest ferns contain very high numbers of invertebrate species but also things like snakes and mice," says

RIGHT
Bird's nest ferns grow harmlessly on trees

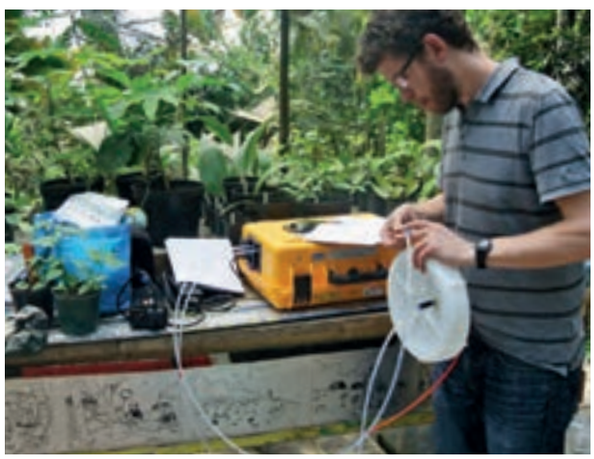
Dr Ellwood. "They are a genuine reservoir of biodiversity." As well as climate change, rainforests are threatened by logging, with vast swathes of trees being chopped down and replaced with oil palm trees; palm oil being used in everything from washing powder to mayonnaise. So knowing the roles of these ferns in rainforests could reshape the policies of governments who have control over these vital ecosystems.

"What we'll be doing is showing the effect of having removed what's there," says Dr Ellwood. If the ferns are shown to have a significant effect, it may mean that government policy is changed. "It could change the way that rainforests are logged so that trees with ferns have to be left."

Bird's nest ferns are an epiphyte, a plant that grows harmlessly on another, deriving their moisture and nutrients from the air, rain and debris that accumulates around them. These ferns are actually one of the largest epiphytes in the world, and can grow to be one fifth of a tonne. They are widely distributed in rainforests all over the Old World tropics, so Australia, South East Asia and Africa.

EDEN COLLABORATION

At the Eden Project, Julian is already making changes to small areas of the ecosystem to see what happens. "In one experiment, earthworms are being added to the soil surrounding the bird's nest ferns to see how that affects the number of microbes in the soil," says Dr Ellwood. "Earthworms decompose the leaves, allowing nutrients such as carbon and nitrogen to enter the soil and the microbes live on these nutrients." So some effect on microbial life is highly likely, the big question is what will the effect be?



ABOVE
PhD student Julian Donald setting up the bird's nest fern platforms at the Eden Project

BELOW
Dr Pete Maxfield at work in the Tropical Biome at the Eden Project

Other experiments involve adding nitrogen to the soil or changing the 'rainfall' within specific areas of the Eden Project's Tropical Biome using an irrigation system designed for the research by the Project's staff. Some of the bird's nest ferns are being exposed to drought conditions and this should prove particularly interesting — when rainforest is replaced with oil palm trees, rainfall in the region declines, so what happens within the Eden Project will provide a good indication of how rainforest ecosystems will react. Some of the bird's nest ferns used in the experiments at the Eden Project have been placed in trees found in the rainforests in Borneo, some in oil palm trees and others on a platform that can be raised and lowered to see what effects different temperatures have →

— the higher the platform, the warmer it gets. "We will perform analogous experiments in Borneo, scaling the experiments up in the real rainforest. The Eden Project is our indoor lab and training ground," says Dr Ellwood. It's in Borneo that the invertebrate exclusion research will take place too.

Dr Ellwood and Julian have forged a close working relationship with the Eden Project, which has provided accommodation for Julian and helped to develop many of the research materials. "The Eden Project has been brilliant," says Dr Ellwood. In return, our research is providing those who manage the Tropical Biome with new data on processes that take place there. "We've looked at micriobial biomass, organic matter and water content of the soil all through the biome. Eden staff are really interested as they want to know how mulching and their soil management affects things and we can tell them. For example, we've shown them that in the drier areas, there is less organic matter and vice versa."

So the research is already starting to pay dividends. But the potential to influence how rainforests are managed is immense.

ABOVE
Bird's nest ferns play a vital role in rainforests

RIGHT
Dr Farnon Ellwood on site in Borneo

BELOW
The Danum Valley Field Centre in Malaysian Borneo

'What happens within the Eden Project will provide a good indication of how rainforest ecosystems will react'