

## Impact case study (REF3b)

<p><b>Institution:</b> University of the West of England (UWE), Bristol</p>
<p><b>Unit of Assessment:</b> 3 – Allied Health Professions, Dentistry, Nursing and Pharmacy</p>
<p><b>Title of case study:</b> Accelerating the development of new chemotherapy drugs using bioluminescent bacterial biosensors</p>
<p><b>1. Summary of the impact</b></p> <p>Novel bioluminescent bacterial biosensors developed at UWE, Bristol, and commercialised by Randox, have been used by a range of companies to demonstrate effectiveness of drugs and decontamination procedures. This has improved development processes at companies including Clavis Pharma, Purest Solutions and Dycem, leading to new manufacturing processes and quality control test methods. The biosensors are used in novel applications to give pharmacodynamic data on effectiveness of drugs and real time <i>in-situ</i> demonstration of effectiveness of decontamination processes. These biosensors, pioneered and developed by Vyv Salisbury's group, have been commercially adopted and used for evaluation by at least six collaborating companies.</p> <p><b>2. Underpinning research</b></p> <p>This research arose following a meeting in July 1997 between Vyv Salisbury (UWE Senior Lecturer 1988-2003, Reader 2003-07, Professor 2007-present) and Alasdair MacGowan, Consultant Microbiologist at The Bristol Centre for Antibiotic Research and Evaluation, North Bristol NHS Trust, investigating effects of new antibiotics on bacterial pathogens. The problem was that standard antibiotic testing methodology used viable counts to study bacterial recovery from antibiotic challenge, but this is an indirect method and does not reflect real-time, <i>in-situ</i> recovery of individual bacterial cells. Salisbury's group, funded by Glaxo SmithKline, developed a clinical bacterial isolate expressing <i>lux</i> genes and used it to show real-time recovery of bacteria after antibiotic dosing, rather than relying on indirect viable counts, and resulted in the first real-time data on the post-antibiotic effect of a new quinolone antibiotic, Gemifloxacin [R6]. The data showed extended suppression of bacterial pathogens by the antibiotic and informed dosing policy, allowing reduced doses while still maintaining efficacy. It became clear from this initial project that bioluminescent bacterial biosensors give real-time, <i>in-situ</i> information on bacterial viability and thus have a wide range of applications [R5 and grants D, E, F and G].</p> <p>Salisbury, Shona Nelson (UWE Senior Lecturer 1999-present), and postdoctoral researchers Steven Beard (2000-2002), Habib Alloush (2002-2007), Gareth Robinson (2007-2012) and Elizabeth Anderson (2007 onwards), have over the past 12 years genetically engineered notable bacterial pathogens (including <i>Salmonella</i>, <i>Pseudomonas aeruginosa</i>, <i>E. coli</i> O157, <i>Neisseria meningitides</i> and <i>Staphylococcus aureus</i>) to express the <i>lux</i> genes so that they are self-bioluminescent, emit light in response to chemotherapeutic agents and also indicate bacterial viability in a range of environments. The <i>lux</i> genes are inserted on a plasmid, to maintain the pathogenic and environmental survival properties of the biosensors. This allows real time, <i>in-situ</i> response to be accurately monitored using a luminometer or low-light imaging system [R5 and grants D, E, F and G].</p> <p>The construction and use of these whole-cell bioluminescent bacterial biosensors has enabled the group to rapidly detect response of mammalian, plant and bacterial cells to a range of environmental factors and insults, including cytotoxic drugs and chemical and physical decontamination procedures. The light given out by the biosensor is non-cumulative; multiple readings can be taken from the same sample to show <i>in-situ</i> response over a particular time period. The research has enabled the group to monitor survival of <i>Salmonella</i> on food surfaces during heat treatment, <i>in-situ</i> [R4 and grant E] and in real time, and to track light given out by bioluminescent bacteria within the gut of <i>Nematode</i> worms [R3 and grant D]. The project resulted from an approach from researchers at the Center for Infectious Disease Dynamics at Penn State University. We led the collaborative research demonstrating that worms can act as vectors of mammalian disease, because the bioluminescent bacteria can be visualised causing disease inside laboratory mice that are fed on infected worms [R3 and grant D].</p> <p>A key outcome of this research is an <i>E. coli</i> biosensor (patented as strain UWE1) and test platform to detect nanomolar concentrations of the active form of chemotherapeutic drugs within leukaemia patient cancer cells in less than 8 hours [R1, R2 and grants A, B, C and F]. This provides the first</p>

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same-day phenotypic test for leukaemia patients, which is carried out on a small blood sample and used to decide what treatment they are likely to respond to. This ground-breaking diagnostic test system is currently being developed in collaboration with Randox Laboratories.

### 3. References to the research

- R1. 'New ways of treating cancer' in *Big Ideas for the Future* (2011) report published by RCUK/UUK highlighting the top 100 pieces of university research 'that will have a profound effect on our future'. UWE Work with bioluminescent microbial biosensor described on Page 16 <http://www.rcuk.ac.uk/documents/publications/BigIdeasfortheFuturereport.pdf> (Grants A,B,C and F)
- R2. Alloush HM, Anderson E, Martin AD, Ruddock M, Angell J, Hill PJ, Mehta P, Smith MA, Smith JG, **Salisbury VC** (2010) A bioluminescent microbial biosensor for *in vitro* pre-treatment assessment of cytarabine efficacy in leukaemia. *Clinical Chemistry* **56**, no. 12, pp.1862-1870 [doi: 10.1373/clinchem.2010.145581](https://doi.org/10.1373/clinchem.2010.145581). (Grants A,B,C and F)
- R3. Lacharme-Lora L, Perkins SE, Humphrey TJ, Hudson P, **Salisbury V** (2009) Use of bioluminescent bacterial biosensors to investigate the role of free-living helminths as reservoirs and vectors of Salmonella. *Environmental Microbiology Reports* 1 (3), pp.198–207 [DOI:10.1111/j.1758-2229.2009.00031.x](https://doi.org/10.1111/j.1758-2229.2009.00031.x) (Grant D)
- R4. Lewis RJ, Baldwin A, O'Neill T, Alloush H, Nelson SM, Dowman A, **Salisbury V** (2006) Use of Salmonella enterica serovar Typhimurium DT104 expressing *lux* genes to assess, in real time and *in situ*, heat inactivation and recovery on a range of contaminated food surfaces, *Journal of Food Engineering* 76: pp.41-48 <http://dx.doi.org/10.1016/j.jfoodeng.2005.05.023> (Grant E)
- R5. Alloush, H.M., Lewis, R.J. and **Salisbury, V**, (2006) Bioluminescent Bacterial Biosensors: Applications in Food and Environmental Monitoring. *Analytical Letters*, 39: pp.1517-1526 <http://dx.doi.org/10.1080/00032710600713172> (Grants E and F)
- R6. Beard, S., **Salisbury, V.**, Lewis, R., Sharpe, J. and MacGowan, A. (2002) Expression of *lux* genes in a clinical isolate of streptococcus pneumoniae: Using bioluminescence to monitor gemifloxacin activity. *Antimicrobial Agents and Chemotherapy*, 46 (2). pp.538-542. ISSN 0066-4804 <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC127039/pdf/0459.pdf>

### Key grants

- A. **Vyv Salisbury** (UWE PI) Development of a rapid *in vitro* multi-drug test device to predict response to combined drug chemotherapy in leukaemia patients, before treatment. MRC Developmental Pathway Funding Scheme Industrial Collaboration Award. June 2012 – December 2014. £370,000 with additional £500,000 from Industrial Collaborators, Randox
- B. **Vyv Salisbury** (UWE PI) Pre-screening test for chemotherapy efficacy. National Institute of Health Research. September 2009 – May 2010. £97,000. (Outcomes published in references R1 and R2 above.)
- C. **Vyv Salisbury** (UWE PI) Bacterial biosensors for rapid pre-screening of chemotherapy efficacy. Department of Trade and Industry – Technology Strategy Board. September 2007– August 2009. £530,408. (Outcomes published in R1 and R2 above.)
- D. **Vyv Salisbury** (UWE PI) A study of helminths as novel vectors and reservoirs of pathogens in the environment, using *in-vivo* real-time imaging systems. The Joint Environment and Human Health Programme (NERC, EA, DEFRA, MOD, MRC, The Wellcome Trust, ESRC, BBSRC, EPSRC and HPA), NE/E009085/1. August 2007 – April 2008. £123,000. (Outcomes published in R3 above.)
- E. **Vyv Salisbury** (UWE) EU Framework V 'Bugdeath'. EU Framework V. September 2003– August 2005 £98,690 (Outcomes published in R5 above.)
- F. **Vyv Salisbury** (UWE PI) Evaluation of *lux*/GFP reporters. BBSRC. September 2003– August 2005. £239,488 (Outcomes published in R1 and R2 above.)
- G. **Vyv Salisbury** (UWE PI) "Lighting up biomedical research", Wellcome Trust – Engaging

Science – Promoting public involvement. 2003-2004. £30,000.

#### 4. Details of the impact

The work of Salisbury's group to develop and use bioluminescent biosensors has revolutionised analytical procedures and allowed rapid direct *in situ* testing of bioactive compounds and formulations, giving real-time, accurate data. The outcome of the research with bioluminescent biosensors is to allow, for the first time, the direct effects of physical and/or chemical challenge on a living cell to be visualized and quantified *in situ* and in real time. The main beneficiaries are pharmaceutical and clinical diagnostics companies including Radox Laboratories, where the biosensors have led to improved validation procedures, accurate data on drug efficacy and novel manufacturing processes [see source S1].

Summary of impacts:

- **Guidelines for antibiotic dosing.** By using our real-time bioluminescent *Streptococcus pneumoniae* biosensor, Glaxo SmithKline has been able to determine bacterial recovery after challenge with Gemifloxacin, a novel quinolone antibiotic they had developed. This has helped them determine the correct dosage more directly and quickly than was possible using their previous technique of using indirect bacterial colony counts to show bacterial viability after antibiotic treatment. It therefore contributed to the overall guidelines for dosing of 1 tablet every 24 hours.
- **Real-time demonstration of decontamination.** Several companies have made use of videos of the bioluminescent bacterial biosensor technique made by UWE. They have used the videos as vivid promotional tools for their products demonstrating very clearly, in real-time and *in situ*, how rapidly the products kill bacteria. Examples include Purest Solutions [S2], Clavis Pharma (demonstrating the effectiveness of their antimicrobial patches against Staphylococcal infection) and Dycem Ltd (demonstrating the effectiveness of their antimicrobial flooring compared to normal flooring material). Video imaging (used on Dycem's company website) of our bioluminescent Salmonella bacterial reporters showed inactivation of bacteria in 1 hour on Dycem flooring compared to 4 hours on normal flooring.
- **Bugdeath project,** predicting microbial death kinetics: Bioluminescent bacterial biosensors, developed at UWE were used in this project, leading to a software application (Bugdeath 1.0) that simulated the effect of heat treatment on the surface of a range of foods.
- **Predicting the effectiveness of cancer chemotherapy.** Here the bioluminescent bacterial biosensors are part of a test platform that is currently under evaluation. The full impact of the test platform will not be evident until it can be used in a clinical trial. Meanwhile, however, the bioluminescent biosensor research has been used to evaluate a novel chemotherapy drug, Elecytarabine, in a consultancy project with Clavis Pharma (2012). UWE's tests have enabled them to establish that the new drug is effective for treatment in these situations and has given the company a means of comparing their new drug to standard treatment regimens. The biosensor has also provided a rapid means of quality control testing between drug batches. It provides accurate data on the time-course of drug uptake and conversion to active form by leukemic cells. This has contributed to full drug evaluation during the development of novel chemotherapeutics which has, in turn, helped in the recent re-launching of the company [S3].
- **Changing clinical attitude to phenotypic testing and patient-centred chemotherapy.** Even though current genetic testing to predict outcome in leukaemia is simple and rapid, it has limited value due to the wide range of genetic changes that can give rise to the disease. There is no single genetic marker that will accurately predict response to chemotherapy. The alternative of looking at the response of actual patient cells (phenotypic testing) has been regarded by clinicians as too complex and time consuming for routine use. Our rapid test platform has renewed clinical interest in phenotypic testing. This is evidenced by the support of the UK AML clinical trials working party and clinical collaborators in UK, USA, Norway and Canada. Consultants have changed their outlook in favour of pressing for the completed development and approval of this technique as a method that meets real clinical needs. The research has therefore effected a change in the received wisdom amongst clinicians on the most appropriate kind of clinical tests to pursue [S4].

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• **Changing leukaemia patient expectations.** We collaborate with Bristol Blood Buddies group – their representatives attend our research group meetings. They have highlighted the many advantages of our assay system from the patient's aspect including a) improved quality of life, both during and after treatment; b) enabling high-risk patient groups who would otherwise not be eligible under current drug protocols, such as the elderly and children, to receive treatment; c) ensuring drug-insensitive patients receive the level of dose or combination of drugs they require for successful treatment of the disease; d) ensuring females in particular receive the minimum dose necessary to treat the disease whilst preserving fertility; e) limiting the number and severity of infections, which subsequently require hospital in-patient stays. The patient group have endorsed the project as follows “We consider the unique selling point of the Rapid *in vitro* test is the ability to conduct the test and receive results within hours to tailor the immediate treatment required for each individual. Many of us required immediate treatment upon diagnosis, so this test could have been extremely relevant to us. As a patient group it appears clear to us that tailor-made chemotherapy regimes will also lead to improved value for money in respect to treatment, as well as fiscal savings for both the government in general and the NHS, due to: a) shorter in-patient stays; b) lower doses of chemotherapy; c) fewer doses of additional treatments for chemotherapy side-effects; d) for working-age patients, less time off work and therefore fewer benefit claims; e) lower requirement for post-chemotherapy fertility treatment.” The patient group is committed to working with us and attend the project management board meetings.

• **Benefits of using the biosensor assay.** For both pharmaceutical and clinical diagnostics companies, including Clavis [S3] and Randox [S1], the benefits of using UWE- developed whole-cell bioluminescent bacterial biosensors are considerable. Because these companies see great advantages to them in this technique, they have created new in-house teams employing staff to develop it further, and have scaled up the technique into a fully deployed commercial production process. Large amounts of biosensor are produced very cheaply by growth in batch-culture (a new manufacturing process developed by Randox as a result of our biosensor assay) and then dispensed into 1 ml amounts for use and freeze-dried so that they can be stored indefinitely. The test procedure using the bioluminescent biosensors is simple and does not require the complex and expensive equipment used in HPLC or flow cytometry. The test response is measured by change in light emitted by the biosensor. This is sensitively measured with a luminometer or low-light camera, *in situ* and in real time, with no background interference. The biosensors have increased sensitivity, compared with other assay techniques; the biosensor assay is 10 times more sensitive than HPLC for measuring nanomolar amounts of Ara-CTP (the active form of the chemotherapeutic drug cytarabine) within leukaemic cells. This technology, because of its simplicity, high sensitivity, low cost and highly reproducible results, has been found to be ideal for batch testing by pharma, and also for use in clinical diagnostics.

**5. Sources to corroborate the impact**

S1. A **testimonial** from the Project Manager of Randox Laboratories Ltd is available from UWE Bristol.

S2. A **testimonial** from Director of Purest Solutions is available from UWE Bristol.

S3. A **testimonial** from a scientific advisor for Clavis Pharma is available from UWE Bristol.

S4. A consultant in Haematology at Frimley Park Hospital, Frimley, Surrey, UK may be contacted to corroborate the impact of the biosensor assay on treatment of leukaemia patients.