Institution: University of Nottingham

Unit of Assessment: 6; Agriculture, Veterinary and Food Science

Title of case study:
Commercialisation of Actiphage® - a low cost rapid diagnostic test for mycobacteria

Period when the underpinning research was undertaken: 2001 – 2019

Details of staff conducting the underpinning research from the submitting unit:

Name(s):	Role(s) (e.g. job title):	Period(s) employed by submitting HEI:
Dr Cath Rees	Associate Professor in Microbiology	1993-present

Period when the claimed impact occurred: 2014 – present

Is this case study continued from a case study submitted in 2014? N

1. Summary of the impact

Research by the University of Nottingham (UoN) has led to the development of a new test to detect Mycobacteria. The method, patented by UoN, is low cost, simple, sensitive, rapid (results within 6 hours), amenable to automation, and can be tailored to detect different mycobacterial pathogens. The test has been commercialised as Actiphage® by PBD Biotech Ltd, a spin-out company of UoN. Since the company formed in 2014, PBD Biotech Ltd has secured investment of [redacted] to commercialise the technology; created 8 new jobs to support research, development and international commercial activity; and achieved cumulative sales of [redacted]. Actiphage® has been approved for exceptional use in UK bovine tuberculosis control programs and is supporting veterinarian and farmer efforts to achieve official TB free status on farms in England and Wales. In recognition of its potential to support control of mycobacterial diseases of livestock including bovine tuberculosis and Johne's disease, Actiphage® has received numerous industry awards.

2. Underpinning research

Mycobacterial disease causes a significant burden in terms of morbidity and mortality in both clinical and agricultural settings globally. The two most prominent mycobacterial diseases in animals include bovine tuberculosis (bTB), caused by *Mycobacterium bovis* and Johne's disease (JD), caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). In the UK, bTB is the most pressing animal health problem with numbers of affected herds increasing. Furthermore, UK Government spends approximately GBP70,000,000 per annum on bTB disease control in England, with economic losses to industry as a result of disease estimated to be a further GBP50,000,000 (Bovine TB Strategy Review, 2018). Effective diagnosis of mycobacterial infection is the cornerstone in the control of mycobacterial diseases of livestock such as bTB and JD. However, detecting Mycobacteria remains challenging since culture methods, considered to be the gold standard, take up to 3 months to deliver reliable results as the bacteria are slow growing. Other available methods offer more rapid detection, however they do not differentiate between live and dead bacteria and are limited by either low sensitivity (PCR-based methods) or specificity (antibody-based methods).

Research undertaken at the University of Nottingham (UoN) has used bacteriophage to develop sensitive and specific methods to identify viable mycobacteria. In 2007, initial work exploited a bacteriophage amplification assay, to indicate presence of viable MAP in milk samples **(G1)**. Presence of mycobacterium in a test milk sample was indicated by plaques on a medium layer of bacteria after overnight incubation. The identity of the specific mycobacterial cells that were lysed by the bacteriophage was then confirmed using polymerase chain reaction (PCR) specific to MAP **(1)**. UoN researchers also showed this phage-PCR approach could identify MAP in blood samples **(2,G2)**. The phage-PCR approach was a significant step forward as it showed identification and enumeration of slow growing mycobacteria was possible within 48 hours **(1,2)**.

Subsequent UoN research considered whether the method could be improved by using bacteriophage to lyse the mycobacterial cells within a liquid sample, and then using PCR to sensitively detect the DNA released from the bacteria cells **(G3)**. This would simplify the assay format, remove requirement for overnight incubation and significantly reduce the time

to deliver results. Results showed that specific mycobacteriophage D29 could be used to detect a range of mycobacteria from blood samples including both bTB and MAP using the adapted assay **(3)**. Furthermore, in a study of *M. bovis* infected cattle, the adapted assay showed high sensitivity (95% with 95% confidence interval of 0.84–0.99) and high specificity (100% with 95% confidence interval of 0.92–1) **(3)**. The new assay format that offered the potential for rapid, high-throughput detection of mycobacteria within 6 hours, generated significant interest amongst the microbial research community **(3)**. The new assay format was the basis of patent application PCT/GB2014/052970 filed by UoN in 2014 **(I1)** and the diagnostic test commercialised as Actiphage®.

UoN researchers have shown practical application of the UoN technology in the area of animal health, using it to rapidly detect bacteraemia in animals suffering from both bTB and JD **(2-5,G2,G3)**. The results gained when testing blood samples indicate that the method can detect infection before the standard diagnostic immunological tests **(2,4,5)**. Results from a large trial carried out in the UK indicate that the method can identify animals infected by bovine TB missed by both Defra's routine surveillance test (Single Intradermal Comparative Cervical Tuberculin - SICCT) and interferon-gamma test (a supplementary blood test used by Defra alongside SICCT) **(6)**. The test has been successfully used to screen exotic animals (specifically lions) for TB infection and was found to perform better than other existing commercial tests **(7)**. In addition, research undertaken in collaboration with clinical partners at University of Leicester demonstrated UoN technology could be used to diagnose human tuberculosis (TB) and identify those most at risk of developing the disease **(8)**. The potential for application in human health is significant and as a result the research publication **(8)** was within the top 5% of all research outputs ever tracked by Altmetric.

3. References to the research

University of Nottingham UoA6 staff are **bold**.

Underpinning references:

1. Stanley, E.C., R.J. Mole, R.J. Smith, S.M. Glenn, M.R. Barer, M. McGowan, **C.E.D. Rees** (2007) Development of a New Rapid Combined Phage and PCR method for the detection and identification of viable Mycobacterium paratuberculosis within 48 h. Appl. Environ. Microbiol. 73; 1851–1857. doi: 10.1128/AEM.01722-06
2. **Swift, B.M.C.**, Denton, E.J., Mahendran, S.A., **Huxley, J.N., Rees, C.E.D.** (2013) Development of a rapid method for the detection of viable *Mycobacterium avium* subsp. *paratuberculosis* in blood within 48 h using a phage-based assay. J. Microbiol. Meths. 94; 175-179. doi:10.1016/j.mimet.2013.06.015
3. **Swift, B.M.C.**, Meade, N., Barron, E.S., **Bennett, M., Perehenic, T.**, Hughes, V., Stevenson, K., **Rees, C.E.D.** (2019) The development and use of Actiphage to detect viable mycobacteria from bovine tuberculosis and Johne's disease-infected animals. Microbial Biotechnology 13; 738-746 doi:10.1111/1751-7915.13518
4. **Swift, B.M.C., Huxley, J.N.**, Plain, K.M., Begg, D.J., de Silva, K., Purdie, A.C., Whittington, R.J., **Rees, C.E.D.** (2016) Evaluation of the limitations and methods to improve rapid phage-based detection of viable *Mycobacterium avium* subsp. *paratuberculosis* in the blood of experimentally infected cattle. BMC Veterinary Research, 12 (1), art. no. 115. doi: 10.1186/s12917-016-0728-2
5. **Swift, B.M.C.**, Convery, T.W., **Rees, C.E.D.** (2016) Evidence of *M. tuberculosis* Complex bacteraemia in intradermal skin test positive cattle detected using phage-RPA. Virulence, 7; 779-788. doi: 10.1080/21505594.2016.1191729. See also editorial '*A bloody evidence: Is Mycobacterium bovis bacteraemia frequent in cattle?!*', Maggioli, M.F. USDA National Animal Disease Centre (NADC, Ames, Iowa). http://dx.doi.org/10.1080/21505594.2016.1213477
6. Research evidence submitted by **Dr Cath Rees** and **Dr Ben Swift** to the 'Bovine TB strategy review' (August 2018).
7. Molenaar, F.M., Burr, P.D., **Swift, B.M.C.**, **Rees, C.E.D.**, Masters, N. (2020) Conservation challenges: the limitations of antemortem tuberculosis testing in captive Asiatic lions (*Panthera Leo Persica*). Journal of Zoo and Wildlife Medicine. 51; 426-432. doi: 10.1638/2019-0084
8. Verma, R., **Swift, B.M.C.**, Handley-Hartill, W., Lee, J.K., Woltmann, G., **Rees, C.E.D.**,

Haldar, P. (2020) A novel, high-sensitivity, bacteriophage-based assay identifies low-level mycobacterium tuberculosis bacteremia in immunocompetent patients with active and incipient tuberculosis. Clinical Infectious Diseases, 70 (5), pp. 933-936. doi: 10.1093/cid/ciz548

Underpinning grants:

G1. PhD Studentship: The Development Of A Rapid Detection Method For Mycobacterium Avium Subspecies Paratuberculosis In Milk. Sponsor: BBSRC CASE studentship, Biotech Laboratories Ltd, 2001-2005, BBSRC CASE7359. GBP11,700, Supervisor: Cath Rees.

G2. PhD Studentship: The development of phage-based assays for the identification of Mycobacteria. Sponsor: Lab21, 2010 – 2014. GBP 42,000. Supervisor: Cath Rees

G3. Developing the Commercial Potential of a Novel Mycobacteria Detection Assay in Human Cystic Fibrosis Patients. Sponsor: Hermes Fellowship administered by UoN, January 2015 – July 2015. GBP26,044. PI: Cath Rees and Ben Swift

Intellectual Property Rights:

I1. UoN have patented the technology (*Mycobacteria detection using bacteriophages*; PCT/GB2014/052970 / WO2015/049516/Al filed by UoN Oct 2014; Granted in Europe EP3052650B1 April 2019. Granted in US US10344339B2 July 2019). PBD Biotech Ltd was established to commercialise the test (2014) under exclusive licence. UoN has an equity stake in PBD Biotech Ltd.

4. Details of the impact

Actiphage® is an innovative new test for the rapid detection of bovine TB (bTB), Johne's disease (JD) and other mycobacterial infections. The unique selling point of Actiphage® is that it rapidly and precisely detects live Mycobacteria in a range of sample types including blood and milk within 6 hours. This is a significant innovation over the current rapid diagnostic Mycobacteria tests that are based on immunology (e.g. IDEXX antibody test) that do not distinguish between exposure or infection and standard PCR-based tests that do not distinguish between viable or non-viable cells. The ability to identify live cells is a unique feature of the test and also allows the test to be used to differentiate between infected and vaccinated animals. Furthermore, the test can specifically identify different species of Mycobacteria in any host including humans.

Commercialisation of Actiphage®

In **2014**, a patent application to protect the UoN technology to detect Mycobacteria using bacteriophage was filed (PCT/GB2014/052970) and a spin-out company, PBD Biotech Ltd (PBD), was established to commercialise the intellectual property under exclusive licence from the University of Nottingham (UoN). Subsequently, PBD have invested [redacted] to maintain the patent, granted in Europe (April 2019) and USA (July 2019), and to trademark the technology as Actiphage® (2017) **(a)**. In **2017**, PBD launched Actiphage® as a kit-based laboratory test targeted at a range of markets from research to diagnostics, with a primary focus on the detection of bTB and *paratuberculosis* (MAP) infections in farmed animals. The technology has been recognised by nominations for several industry innovation awards, winning: the Crop or Livestock technology at Ag-in-motion Expo (Canada, 2017); the Royal Dairy Innovation Award at Dairy-Tech (UK, 2019); the West Suffolk Award for Innovation (UK, 2019); The Cream Award (UK, 2020) and made the Cognitive top #21toWatch list (top 7 in the 'Things' category; UK, 2020) **(b)**.

Between 2017 and 2020, PBD raised a total of [redacted] investment funding to commercialise Actiphage® **(a,c)**. To date, through the commercialisation of Actiphage®, PBD have created 3 new jobs (PBD employees) and 5 contract positions (total 8 FTE) to support R&D and international commercial activity **(a)**. PBD offer two laboratory test kits; Actiphage® Rapid for detection of mycobacteria within 6 hours and Actiphage® Core for highly sensitive and specific detection of mycobacteria within 2 days. In addition, PBD sell PCR kits for use with both Actiphage® kits. PBD has exported test kits to over 10 countries including USA, Canada, Australia, New Zealand, Ireland, France, Korea and Italy **(a)**. Kits have been supplied free of charge for research and validation studies, which are required for certification under World Organisation for Animal Health (OIE) standards and approval for

use in bTB diagnosis in the UK and in other countries. PBD also offers a diagnostic service to detect Mycobacteria species in samples from multiple animal origins including bovine, non-bovine and exotic species. PBD Biotech's sales to date have been [redacted] (laboratory test kits) and [redacted] (diagnostic services) **(a)**.

Application of Actiphage® in management of exotic species

TB is a growing problem for zoos and wildlife parks and Actiphage® has been used to test animals including bison, deer, goats, sheep, alpaca, tapir, takin, lions, parakeets and beluga wales, offering a new way of identifying TB and informing management of protected animals **(a)**. In 2018 Actiphage® was used to inform decision making in an Asiatic lion conservation programme. As part of extensive ante-mortum TB testing, Actiphage® gave positive results in the case of two lions imported from India who were in quarantine at Whipsnade Zoo **(7;a,d,pg2)**, and in the case of a female breeding Asiatic lion based at Paignton Zoo **(a)**. In both cases, the animals were euthanized to prevent TB being spread to other breeding lions thus protecting the UK Asiatic lion collection.

Application of Actiphage® in bTB management of cattle (UK)

All testing for bTB in the UK is done under strict government control. Testing can only be carried out using tests validated to OIE standards that have been authorised for bTB testing by Defra's Animal and Plant Health Agency (APHA). The authorised tests are the tuberculin skin test (used for statutory bTB testing across the UK) and the interferon-gamma test (used as a supplementary test alongside the tuberculin skin test in specific TB breakdown herds). An additional OIE validated test, the IDEXX antibody test, may also be used in chronic bTB breakdowns under instruction by APHA where repeated tuberculin and interferon-gamma testing has already occurred.

The availability of Actiphage®, and other new tests, has stimulated discussion in the media on the testing and control of bTB in the UK. The potential role of Actiphage® in management of bTB was highlighted on BBC Radio 4 Farming Today (February 2017; November 2017; September 2018) and BBC One Six O'Clock News (December 2019) **(e)**. Dr Rees was invited to talk about the test at the Bovine TB Symposium (March 2017), organised by the 'Save Me Trust' and attended by UK experts, and was subsequently invited to contribute evidence **(f,g)** to the review of the UK Government's Strategy (led by Sir Charles Godfray) to eradicate bovine tuberculosis (bTB) in England **(October 2018)**. The published review highlighted the requirement for diagnostics test, noting:

"The last few years have seen new tests proposed which are currently in the early stages of commercialisation. […] Research and development to characterise the performance of novel tests and explore new options is, we believe, of high priority" **(g, pg.41, 3.50)**.
Development and validation of new diagnostic tests require access to materials and samples from infected farms. Such access is currently restricted by notifiable disease legislation which risks hindering bovine TB control and the development of new commercial products for the international market. Altering the legislation so that it eased the provision of research material while maintaining appropriate levels of biosecurity would be helpful **(g, pg.41, 3.54)**.
Actiphage® was highlighted in the review as one of the additional tests that some farmers "*would like to employ […] to accelerate the removal of infected individuals and to better manage within herd transmission*" **(g, pg.43, 3.40)**.

Following extensive lobbying by the 'Save Me Trust', leading UK veterinarians and other interested groups, Defra gave permission for Actiphage® to be used within a project to eradicate bTB on a farm with a chronically infected dairy herd. Within this Gatcombe Farm (Devon) project (2017), conducted under veterinary supervision, Actiphage® was used to detect bTB positive animals, not detected using the statutory tuberculin skin and interferon-gamma tests, and inform decisions on animal exclusion (by isolation or culling) from the herd. This formed part of a comprehensive disease management system, including stringent farm hygiene practices. The herd of 350 cows was declared Officially TB Free (OTF) in **May 2018 (h)** allowing the farmer to trade for the first time in seven years **(e)**. Following

successful demonstration of the potential of the test to enhance a control program at Gatcombe, APHA allowed exceptional private use of non-validated tests, including Actiphage®, for control of bTB in cattle in England **(April 2018) (i)**.

In 2019, PBD Biotech were invited to speak to the Welsh Government about opportunities for the use of Actiphage® testing in Wales. In Spring 2019, the Office of the Chief Veterinary Officer for Wales granted permission for the first Welsh trial of Actiphage®, conducted under veterinary supervision. This enabled detection of bTB in cattle considered at high risk of having bTB that had tested clear using the current government approved methods and informed decisions on herd management (animal exclusion). Acknowledging the need for new accurate bTB blood tests, the Vet supervising the Welsh trial stated *"Actiphage® is able to identify the presence of relatively low numbers of M. bovis in the blood stream of infected cattle. It is not dependent upon an immune system response to the pathogen - in contrast to current validated tests - and so has greater sensitivity than such as the official Tuberculin SICCT skin test. The benefit of using Actiphage® is that it offers the potential for eradicating the disease from the farm; as early identification of animals at risk of bovine TB enables heightened disease management and control."* **(j)**. Subsequent to the trial, in **November 2019**, the Welsh Government issued a new policy setting out terms and conditions for the exceptional use of non-validated tests, including Actiphage®, as a supplementary approach to managing problem cattle herd breakdowns in Wales **(k)**. Under the supplementary testing regime, APHA monitor the results and decide whether it is necessary to remove any direct contact animals from the herd under the Tuberculosis (Wales) Order 2010 (as amended) with compensation.

Summary
Actiphage® is an innovative new test for the rapid detection of bTB, JD and other mycobacterial infections. The test has been commercialised through the formation of PBD Biotech Ltd, a UoN spin-out company. PBD Biotech Ltd has secured [redacted] to commercialise the technology, created 8 new jobs to support R&D and international commercial activity, and achieved cumulative sales of [redacted]. Actiphage® has been approved for exceptional use in UK bTB control programs and is supporting veterinarian and farmer efforts to achieve OTF status on farms in England and Wales. In recognition of its potential to support control of mycobacterial diseases of livestock, Actiphage® has received numerous industry awards.

5. Sources to corroborate the impact

a. Letter of support from the CEO, PBD Biotech Ltd (December 2020) [PDF]
b. External webpages detailing PBD/Actiphage awards [PDF]
c. External webpages detailing investment raised and details (2017-2018) [PDF]
d. British Veterinary Zoological Society (BVZS) Conference article, pg2 references Actiphage use in Asiatic lions at ZSL Whipsnade Zoo case (November 2018) [PDF]
e. Actiphage media coverage on BBC Radio 4 Farming Today (September 2018; November 2017; February 2017) and BBC One 6 O'Clock (December 2019) [available on request]
f. Invitation for Dr Cath Rees & Dr Ben Swift to contribute to Defra Bovine TB Strategy Review (June 2018) [PDF]
g. Bovine TB Strategy Review, report to Secretary of State, DEFRA, (October 2018) and stakeholder engagement (accessed 16th January 2020) [PDF]
h. Farmer's Weekly article, '*Brian May's Gatcombe Farm project secures TB-free status*' (October 2019) accessed 29th October 2019 [PDF]
i. APHA protocol for exceptional private use of non-validated tests for TB on cattle in England (April 2018) accessed 23rd May 2018 [PDF]
j. PBD Biotech article, '*Welsh farm pioneers first trial of breakthrough Actiphage® blood test*' (January 2020) accessed 7th February 2020 [PDF]
k. Welsh Government Policy on 'Use of non-validated tests in cattle herds' (November 2019) accessed 7th February 2020 [PDF]