Institution: University of Kent

Unit of Assessment: 3: Allied Health Professions, Dentistry, Nursing and Pharmacy

Title of case study: Pseudotype Viruses: A Translational Platform Facilitating the Development, Testing and Application of Therapeutics, Vaccines and Diagnostics for Emerging Viruses of Global Concern

Period when the underpinning research was undertaken: 2009–present

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:
Nigel Temperton	Reader in Molecular Virology	2009-present

Period when the claimed impact occurred: 2014-2020

Is this case study continued from a case study submitted in 2014? No

1. Summary of the impact (indicative maximum 100 words)

Pseudotype viruses (PV) developed by Dr Nigel Temperton at Kent are innovative, biosafe virus mimics. PV have been brought to two international companies (VisMederi, Blue Water Vaccines), two national companies (MAP Diagnostics, DIOSynVax), and a UK Government agency (NIBSC), with cost savings, time savings, and increased safety reported as key benefits. This technology has: 1) enabled the rapid development of reference standards for influenza and WHO Blueprint Pathogens (NIBSC-Virology); 2) facilitated the development of a SARS-CoV-2 clinical diagnostic test (MAP Diagnostics); 3) accelerated vaccine R&D (VisMederi, DioSynVax, Blue Water Vaccines); and 4) enabled screening of antibodies with potential therapeutic applications (NIBSC-Biotherapeutics). Specifically, NIBSC-Virology produced reference standards for Ebola, MERS-CoV, and SARS-CoV-2. SARS-CoV-2 pseudotypes were successfully employed as direct assay standards by MAP Diagnostics. A new PV-based assay (PV-ELLA) for the measurement of response to neuraminidase in influenza vaccines was co-developed with VisMederi. A SARS-CoV-2 vaccine (DIOSCoVax) and a trivalent Ebola/Marburg/Lassa Fever vaccine were developed at DIOSynVax, and have moved to Phase 1 clinical trials, and PV technology has sped up Blue Water Vaccines' and DIOSynVax's universal influenza programs.

2. Underpinning research (indicative maximum 500 words)

Since 2009, Temperton's team at Kent have pioneered the design, construction, and deployment of pseudotype viruses (PV) **[R1-R6; G1-G3]**. These are biosafe, replication-defective viruses (retroviruses and lentiviruses) with foreign viral glycoproteins derived from the viral envelope of the pathogen under investigation on their surface. These glycoproteins are important for viral entry into the host cell, and thus PV mimic the pathogenic virus with respect to cell entry, but are safer to work with, as they cannot replicate and make progeny viruses once inside the cell. PV can thus be handled by end users in biosafety level 1/2 (BSL-1/2) instead of BSL-3/4 laboratories. In research published in 2014, Temperton and co-workers demonstrated that PV are stable over a range of temperatures and can be freeze-dried, enabling safe transport to end users and facilitating application in resource-limited laboratories lacking cold-storage facilities **[R2]**.

Pseudotypes **[R1-R6]** developed by the Kent team also carry a quantifiable reporter that is integrated into the target cell genome on viral entry. The product of the reporter gene (commonly a fluorescent or luminescent protein) is readily detectable, enabling sensitive, high-throughput cell entry-based assays to be performed. Additionally, PV-based assays are simple to upgrade, as only the nucleotide sequence of the surface glycoprotein(s) from the pathogenic virus is required. This makes them ideal for emerging RNA viruses (avian influenza, rabies, SARS/MERS/SARS-CoV-2, ebola, and other WHO Blueprint priority pathogens), which commonly evolve through mutation(s) arising in these sequences.

In 2011, Temperton's expertise in pseudotype technology led to the creation of the Viral Pseudotype Unit (VPU) at Kent. Since then, the VPU has acted as an interface between academia, industry and animal and public health laboratories, with the purpose of translating

Kent's basic virus research into *in vitro* cell culture PV-based assays that can be readily employed for the characterisation of vaccines, antivirals, and therapeutic antibodies **[R1-R6]**.

Between 2011 and 2020, the Kent team used the VPU portfolio of pseudotypes to develop PV-based neutralization assays, which provide a measure of how efficiently antibodies neutralise viral infection and demonstrated their use in immunogenicity testing of vaccines and the characterisation of broadly neutralising antibodies **[R1, R3, R4]**. Temperton's research showed that PV assays are serum and antigen sparing requiring relatively fewer reagents than standard assays, and functional antibody responses correlate strongly with live pathogenic virus assays **[R1, R5, R6]**, resulting in a significant safety benefit and cost saving for the end-user given the exorbitant costs and health risk of undertaking serological (serum-based) assays with high-containment viruses **[R1-R6]**.

Neutralisation assays carried out using bespoke panels of Temperton's influenza PV have been shown to be exquisitely sensitive for the measurement of responses to the HA stalk (a conserved region of the hemagglutinin (HA) viral coat protein), one of the primary targets of many big pharma 'universal' vaccine approaches **[R1]**. The availability of these PV influenza panels for use in neutralisation assays is advantageous to end users as the traditional hemagglutinin inhibition assay (for which a correlate of immunity exists) used by the regulators only measures responses against the globular HA head and is not fit for purpose for the licensing of many of these new 'universal' vaccines.

Isolating single domain antibodies (sdAbs) from camelids is a key therapeutic avenue for many viral diseases. Due to their exquisite sensitivity, Temperton's influenza PV panels have been instrumental in the characterization of broadly neutralizing sdAbs isolated for influenza A and B **[R1, R3]**. Using PV-based neutralisation assays, Temperton with NIBSC-Biotherapeutics showed that one of these antibodies, R1a-B6 , which targets the HA stalk, can protect against multiple subtypes of influenza when delivered by an adeno-associated viral (AAV) vector, making it critical for informing universal influenza vaccine design **[R1, G1, G2]**.

Temperton's research has also highlighted the use of PV neutralisation assays in the production of accredited reference standards for WHO Blueprint list priority viruses **[R6]**.

3. References to the research (indicative maximum of six references)

[R1] Del Rosario, J. M. M., Smith, M., Zaki, K., Risley, P., **Temperton, N. J.**, Engelhardt, O. G., Collins, M., Takeuchi, Y., and Hufton, S. E. (**May 2020**). 'Protection from Influenza by Intramuscular Gene Vector Delivery of a Broadly Neutralizing Nanobody Does Not Depend on Antibody Dependent Cellular Cytotoxicity'. *Frontiers in Immunology* 11:627. doi:

10.3389/fimmu.2020.00627

[R2] Mather, S. T., Wright, E., Scott, S. D., and **Temperton, N. J**. (**December 2014**). 'Lyophilisation of influenza, rabies and Marburg lentiviral pseudotype viruses for the development and distribution of a neutralisation-assay-based diagnostic kit'. *Journal of Virological Methods*. 210: 51-58. doi: 10.1016/j.jviromet.2014.09.021

[R3] Ramage, W., Gaiotto, T., Ball, C., Risley, P., Carnell, G. W., **Temperton, N. J.**, Cheung, C. Y., Engelhardt, O. G., and Hufton, S. E.(February 2019). 'Cross-Reactive and Lineage-Specific Single Domain Antibodies against Influenza B Hemagglutinin'. *Antibodies* (Basel) 10:8(1): 14. doi: 10.3390/antib8010014

[R4] Thompson, C. P., Lourenço, J., Walters, A. A., Obolski, U., Edmans, M., Palmer, D. S., Kooblall, K., Carnell, G. W., O'Connor, D., Bowden, T. A., Pybus, O. G., Pollard, A. J., **Temperton, N. J.**, Lambe, T., Gilbert, S. C., and Gupta, S. (**September 2018**). 'A naturally protective epitope of limited variability as an influenza vaccine target'. *Nature Communications* 9(1): 3859-3868. doi: 10.1038/s41467-018-06228-8

[R5] Hyseni, I., Molesti, E., Benincasa, L., Piu, P., Casa, E., **Temperton, N. J.** Manenti, A., and Montomoli, E. (**September 2020**). 'Characterisation of SARS-CoV-2 Lentiviral Pseudotypes and Correlation between Pseudotype-Based Neutralisation Assays and Live Virus-Based Micro Neutralisation Assays'. *Viruses* 12(9): 1011-1028. doi: 10.3390/v12091011

[R6] Mattiuzzo, G., Bentley, Sang Hwan Seo, E., Hyuk Cho, N., Kim, J.-O., Richardson, S., Hassall, M., Atkinson, E., Hockley, J., Kim, Y.-S., Gurry, C., Gómez Román, R., Holst, J., Kristiansen, P., Grehan, K., **Temperton, N. J.**, Harvey, R., Song, M., Page, M., and the collaborative study participants (**2020**). Establishment of 1st WHO International Standard for anti-MERS-CoV antibody. Expert Committee on Biological Standardization. WHO/BS/2020.2398 and for SARS-CoV-2 WHO/BS.2020.2403

Research Grants

[G1] Innovate UK (Technology Strategy Board): Business Only Subcontract from Cambridge DIOSynVax SME 'Digital Immune Optimized and Selected Pan-Influenza Vaccine Antigens (DIOS pan-IVa)'. **2020-21**. Grant Ref: 105078. PI: Jonathan Heeney, Cambridge. Value: £150,176 (Kent).

[G2] Bill and Melinda Gates Foundation: Grand Challenges Universal Influenza Vaccines 'Digital Immune Optimized and Selected Universal Influenza Vaccine Antigens (DIOS-UIVA)'. **2019-21**. Grant Ref: G101404. PI: Jonathan Heeney, Cambridge. Value: £89,306 (Kent).

[G3] UKRI 'Humoral Immune Correlates for COVID-19: Defining protective Ab Responses and Critical Readouts for Clinical Trials of Vaccines and Therapeutics'. **2020-21**. Grant Ref: MC_PC_20016. PI: Wilhelm Schwaeble, Cambridge. Value: £160,351 (Kent).

[G4] UKRI-MRC/NIHR COV0170: Humoral Immune Correlates of COVID-19 (HICC) consortium. **2020-21**. Value: £1,522,681 (total award.

4. Details of the impact (indicative maximum 750 words)

1) Rapid development of reference standards for Ebola, Lassa Fever, MERS-CoV and SARS-CoV-2 using pseudotype technology

The National Institute for Biological Standards and Control (NIBSC) has identified pseudotypes as a technology that can facilitate the development of accredited reference standards in a significantly shorter timeframe **[a]**, which is particularly important for emerging viruses. Collaboration between Temperton and NIBSC, initiated in 2015, resulted in the development of WHO-accredited nucleic acid and antibody reference reagents for Ebola **[b]**. Mark Page (Head of the Emerging Viruses Group at NIBSC) states that 'most of the WHO R&D Blueprint are pathogens in hazard group 3 or 4', and that Temperton's PV have been identified by NIBSC 'an essential tool for the characterisation of candidate reference reagents, alleviating the need to work at a high containment level, which for Ebola would not have been possible as NIBSC does not possess a CL4 laboratory' **[a]**. Temperton's PV-based neutralisation assays were critical for the evaluation of the Ebola plasma standard and PV were essential for production of the nucleic acid test (NAT) standards that are made by packaging the Ebola genome inside Temperton-designed retroviral pseudotype particles. Without access to Temperton's pseudotype technology, these standards would have taken significantly longer to develop and release. Page confirms that 'The use of pseudotype viruses for the development of reference material has now been adopted by default at NIBSC and permits the production of standards to a responsive and fluent timeframe for the priority pathogens listed in the WHO R&D Blueprint', and further states that 'The impact of this is that NIBSC has been made an implementing partner with Coalition for Epidemic Preparedness Innovations (CEPI) […] to produce antibody standards to enable vaccine development against priority pathogens' **[a]**. In February 2019, NIBSC, enabled by Temperton's pseudotype technology, leveraged contracts from CEPI for 1m USD to produce standards for Lassa fever virus **[a]**, and, in collaboration with Temperton, NIBSC have also produced reference reagents for MERS-CoV and SARS-CoV-2 'with an accelerated time frame' [R6] **[a]**. Page concludes that 'There will always be the potential for […] outbreaks that could have global impact and the VPU and NIBSC alliance is ideally suited to respond to the next emergency' **[a]**.

2) Employing SARS-CoV-2 pseudotypes as direct assay standards for clinical diagnostic tests

In 2020, UKRI funded the Humoral Immune Correlates of COVID-19 (HICC) consortium, which is comprised of a core of founder members in receipt of direct funding (University of Cambridge, Kent Viral Pseudotype Unit. MAP Diagnostics, Royal Papwoth and Addenbrookes NHS Trusts) and a large network of collaborators **[G3]**, to carry out SARS-CoV-2 research into immune

correlates. In 2020, a collaboration between Temperton and the diagnostics company MAP Diagnostics (Bedford, UK), which develops novel diagnostic tests based around MALDI-ToF mass spectrometry, was initiated via HICC **[c].** This collaboration involves the use of Temperton's SARS-CoV-2 pseudotypes directly as assay standards. MAP Diagnostics is developing SARS-CoV-2 clinical diagnostic tests based on direct MALDI-ToF mass spectral analysis of gargle/saliva samples, and Temperton's pseudotypes have been incorporated into the company's protocols, enabling safer and rapid methods development at reduced cost **[c]**. Professor Ray Iles (CEO of MAP Diagnostics) states: 'The COVID-19 pandemic prompted the development of a mass spectral technique for identification of viral envelope proteins direct from gargle/saliva samples. This would not have been possible without the use of SARS-2 pseudo-type, and other corona viral pseudo-types, developed by Dr Temperton's research' **[c]**. The timely availability of 'safe to manipulate mock [pseudotype] virus; with the same biochemical, conformational and physical biology of the target proteins in situ within a viral-like envelope exosome, allowed not only the spectral but pre-processing biochemistry to be developed in a non CAT3 restrictive laboratory setting' **[c]**. Isles further states that their protocol is now 'being adopted by other mass spectral analysis centres', and the company is sending Temperton's pseudotypes 'to global collaborators as a quality and positive control to be run with every diagnostic test' **[c]**. In addition to using Temperton's SARS-CoV-2 pseudotypes, Isles states that, via collaboration with Temperton, 'Other viral pseudo-types are being tested in order to discover the […] "spectral fingerprint" of virus infections', and highlights that 'the cost, speed and utility of the technique is going to have major impacts on global health care and disease diagnosis beyond the COVID-19 pandemic' **[c]**. The assays used by HICC and MAP Diagnostics are all calibrated using the NIBSC SARS-CoV-2 reference reagents **[R6]**.

3) Applying the pseudotype virus platform to vaccine research and development for influenza, SARS-CoV-2, Marburg and Lassa Fever

Temperton has a longstanding collaboration with VisMederi (Siena, Italy), which undertakes serological assays for pharma and towards licensure of influenza, coronavirus, and other vaccines **[d]**. Temperton's influenza and SARS-CoV-2 neutralisation assays and know-how have been successfully translated to VisMederi via this collaboration, and in 2016 a new PV-based enzyme-linked lectin assay (PV-ELLA) was co-developed that enables the measurement of responses to neuraminidase (NA) in influenza vaccines **[d]**. This new assay is highlighted by CEO Professor Emanuele Montomoli: 'In 2016, a VisMederi researcher, Fabrizio Biuso, joined Nigel Temperton's laboratory for refining the […] pseudotypes platform. In particular the project […] aimed to study the development of [an] ELLA assay […] to evaluate human anti-NA antibodies' **[d]**. According to Montomoli, the transfer of Temperton's PV technology 'allowed VisMederi to execute several clinical and preclinical studies and it was […] applied to Flu, Rabies and now [in 2020] it will be useful […] in SARS CoV-2 studies in order to accelerate the development of SARS-CoV-2 assays' **[d]**. Montomoli states that working with PV will 'eliminate the need [… for] wild-type virus, meaning […] this assay can be performed at biosafety level II (BSL2)', and that 'R&D of this nature would be laborious and expensive to perform with the native virus', confirming that 'the collaboration with Nigel Temperton […] saved costs to the company and increased the staff safety level' **[d]**.

Temperton has been collaborating with DIOSynVax, a Cambridge University spin-out company, since 2017. Professor Jonathan Heeney, CEO of DIOSynVax, explains that DIOSynVax is focused on vaccine R&D using a technology platform that encompasses a computational approach 'for the rapid selection of vaccine antigens that are able to elicit cross protective immune responses to an array of different viruses' **[e]**. Temperton acted as a scientific advisor from September 2016, and sought to develop a trivalent vaccine to protect against Ebola, Marburg and Lassa Fever. Heeney states that 'The project included successful animal trials and the vaccine has now moved to Phase 1 clinical trials' **[e]**. Temperton's PV-based serological assays form a key component of the company's new technology platform, and scientists at DIOSynVax are routinely trained in PV development, production, and deployment with Temperton's support **[e]**. Heeney states that 'The PV assays are integral to the vaccine development pipeline as the data is fed back into machine learning processes to improve vaccine antigen selection', and that 'R&D of this nature would usually be laborious and expensive to perform with the native virus, however, our process design enables adaptability to new virus

strains and is […] safer for staff to work with' **[e]**. According to Heeney, results obtained using Temperton's PV assays have underpinned 'successful grant applications' that have enabled the company to 'move into new areas of vaccine R&D'. Current collaborative projects include vaccine development for influenza **[G1, G2]** and coronavirus, with the DIOS-CoVax vaccine having recently received funding to enter a Phase 1 clinical trial **[e]**. Protective Ab responses and critical readouts for clinical trials of COVID-19 vaccines including DIOS-CoVax are being addressed in a joint-funded NIHR project [G3].

In 2018, Temperton's collaboration with Oxford University on the discovery of a new 'universal' influenza vaccine epitope, which involved the use of the Kent team's PV-based neutralisation assays **[R4]**, resulted in a US-based spin-out company, Blue Water Vaccines. The company's vaccine program aims to commercialise a vaccine that could protect against all influenza strains and provide life-long immunity. Dr Craig Thompson (CEO at Blue Water Vaccines) states that 'our relationship with Dr Temperton has centred around the pseudotyped virus assay technology his lab has developed […]. This technology has become central to the discovery of novel antigen targets as part of our influenza vaccine program' **[f]**. Thompson confirms that the use of Temperton's PV-based neutralisation assay has enabled the assessment of 'protective neutralising antibody responses […] under CL2 conditions', and that 'Without this assay we would need to work under more restrictive CL3 or SAPO4 conditions using live influenza virus, which would be impossible to undertake at the scale we require. Consequently, our use of the […] assay has sped up the development of our influenza vaccine' **[f]**. Thompson also states that the provision of laboratory assistance, constructs, and techniques developed by the Kent team 'has been vital in expanding the range of influenza glycoproteins analysed' by the company **[f]**.

4) Enabling isolation and characterisation of antibodies with potential immune prophylaxis and vaccine potency application through the use of pseudotype neutralisation assays

Since 2017, Temperton has built a strong relationship with NIBSC Biotherapeutics, which discovers and develops single domain antibodies (sdAbs). They have been using Temperton's influenza pseudotypes in neutralisation assays for first-line screening to identify broadly neutralising sdAbs with potential therapeutic, diagnostic, and vaccine potency application **[R1, R3]**. Live pathogenic viruses could not have been used in such assays owing to the exorbitant costs and safety constraints associated with the running of their R&D screening pipeline under high containment **[g]**. Through this partnership, Dr Hufton has additionally leveraged funding from the Biomedical Advanced Research and Development Authority (BARDA) in the US to 'develop an influenza vaccine potency assay based on broad neutralising and lineage specific single domain antibodies. This "universal" assay will ultimately reduce the need for the seasonal generation of strain specific reagents and so speed up the influenza vaccine development pipeline.' **[g]**.

5. Sources to corroborate the impact (indicative maximum of 10 references)

[a] Letter of support from the Head of the Emerging Viruses Research Group, NIBSC (UK).

[b] Press release from the UK Government reporting the endorsement of NIBSC's Ebola reference reagents as global standards by the World Health Organization (WHO).

[c] Letter of support from the Chief Executive Officer, MAP Diagnostics (UK).

[d] Letter of support from the Chief Scientific Officer, VisMederi (Italy).

[e] Letter of support from the Chief Executive Officer, DIOSynVax Ltd (UK).

[f] Letter of support from the Chief Scientific Officer, Blue Water Vaccines (USA).

[g] Letter of support from the Section Head, Molecular Immunology, Biotherapeutics Division, NIBSC (UK).