OneBAT Training on Bat Sanitary Surveillance

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The Development of Pseudotyped Viruses for Cell Tropism and Serosurveillance Studies of Bat Viruses

Simon D Scott

s.d.scott@kent.ac.uk

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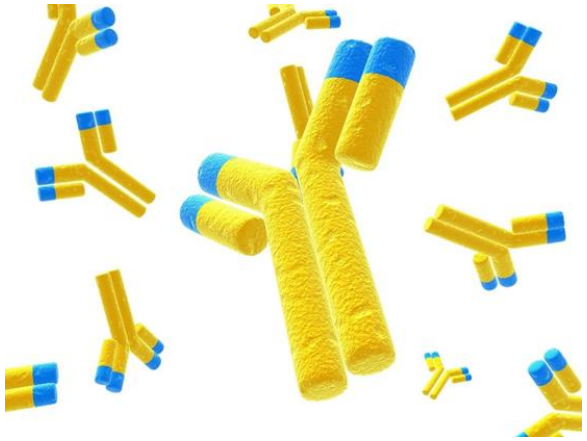
Bat Virus Detection

- The identification of virus infection in animals (including bats) and man is usually via detecting viral genomic material (DNA, RNA) or proteins
- A positive test result tells us that the bat either has a current infection or is recovering from it
- The 'window of opportunity' to detect these molecules is relatively narrow – days or weeks usually
- For wild animals this would mean sampling would have to take place during this period
- An alternative detection method is to identify **virus-specific antibodies**, which have been produced during the immune response to the infection
- Antibody production begins during the infection and gradually builds
- These antibodies circulate for months or years ('memory')
- This gives a wider 'window of opportunity' for detection



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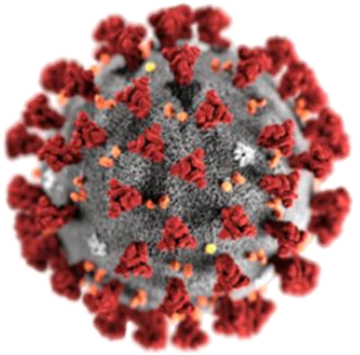
Antibody (Serology) Tests

- There are a number of methods of detecting specific antibodies – serological tests
- Some measure the **binding** of antibodies to virus-specific proteins, often those on the surface of the virus, which are the target of immunological responses in the host
- Others measure anti-virus biological activity – antibody **neutralisation** of viruses
- Neutralisation assays can be conducted using native, replicative virus
- However if investigating pathogenic or potentially pathogenic viruses, these assays have to be carried out in high biological containment facilities for safety reasons
- Such facilities are only found in a relatively few institutes/laboratories, which can act as a 'bottleneck' for research
- However, there is an alternative approach to perform pathogenic virus antibody neutralisation assays – using so-called **pseudotyped viruses** as surrogates



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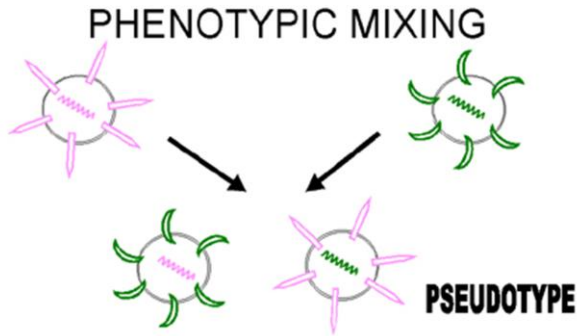
Pseudotyped Viruses (PVs) & Utilisation

- Pseudotyped viruses (PVs) are hybrid particles with a protein **'core' (capsid)** encasing a virus genetic material (genome), encapsulated in an **'envelope'** derived from host cell outer membrane
- 'Pseudotyping' occasionally occurs naturally after co-infection of cells by 2 different virus strains, releasing offspring (progeny) with mixed components derived from the 2 parents
- Importantly, the envelope displays surface proteins from the other (heterologous) virus
- These surface proteins are **essential for virus infection** (attachment & entry) and **stimulate host immune responses (e.g. antibodies)**
- PVs can be artificially engineered, and are usually non-replicative – thus need low biosafety containment
- PVs can be utilised to study virus entry, cell tropism, receptors,

  immune responses (**antibody neutralisation**) and antiviral screening



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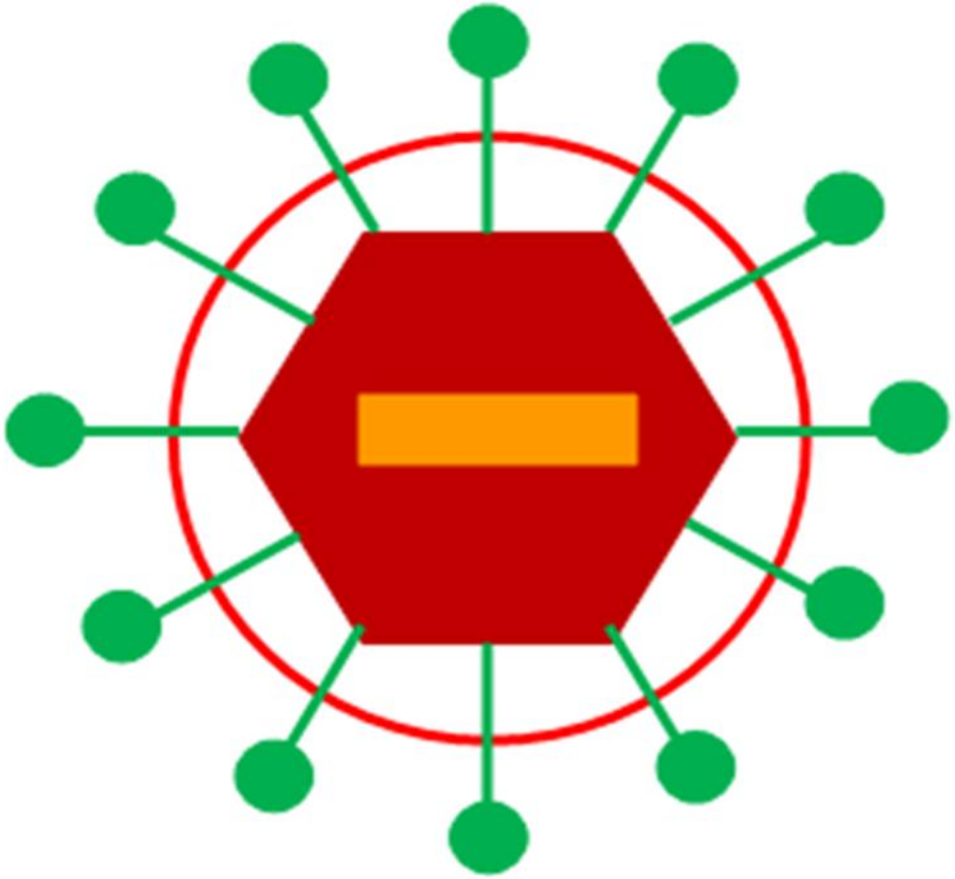
Pseudotyped Virus Structure



PVs have 3 main components:

- **Envelope** glycoprotein(s)
- **Core** (e.g. derived from a lentivirus)
- **Transgene** (e.g. ‘reporter gene’ for quantifying infection or inhibition of infection (i.e. immunity)

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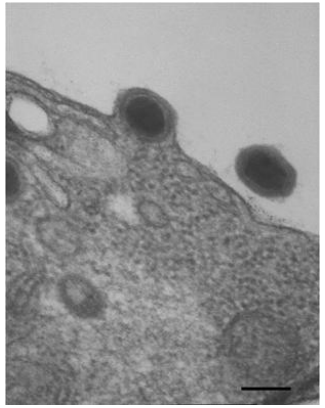
Pseudotyped Virus Generation

- PVs can be generated by the introduction of essential genes/components on small circular DNA molecules called **plasmids** (via genetic engineering) into a **'producer' cell line**
- Once the plasmids enter the cells; expression of genes into proteins, synthesis of new genomes then assembly of virus 'core' particles within cell
- Finally, the PV particles escape from the producer cells by **'budding'** (pushing through outer cell membrane) and being wrapped in the host-derived **envelope** (containing specific virus glycoproteins - **GP**s)
- The success of the process (*which is by no mean guaranteed!)* is measured via a **'titration' assay** – determines concentration of biologically active viral particles
- If concentration or **'titre'** is sufficient the PVs can be used for antibody detection

- **Pseudotyped Virus Neutralisation Tests/Assays (PVNT/PVNA)**



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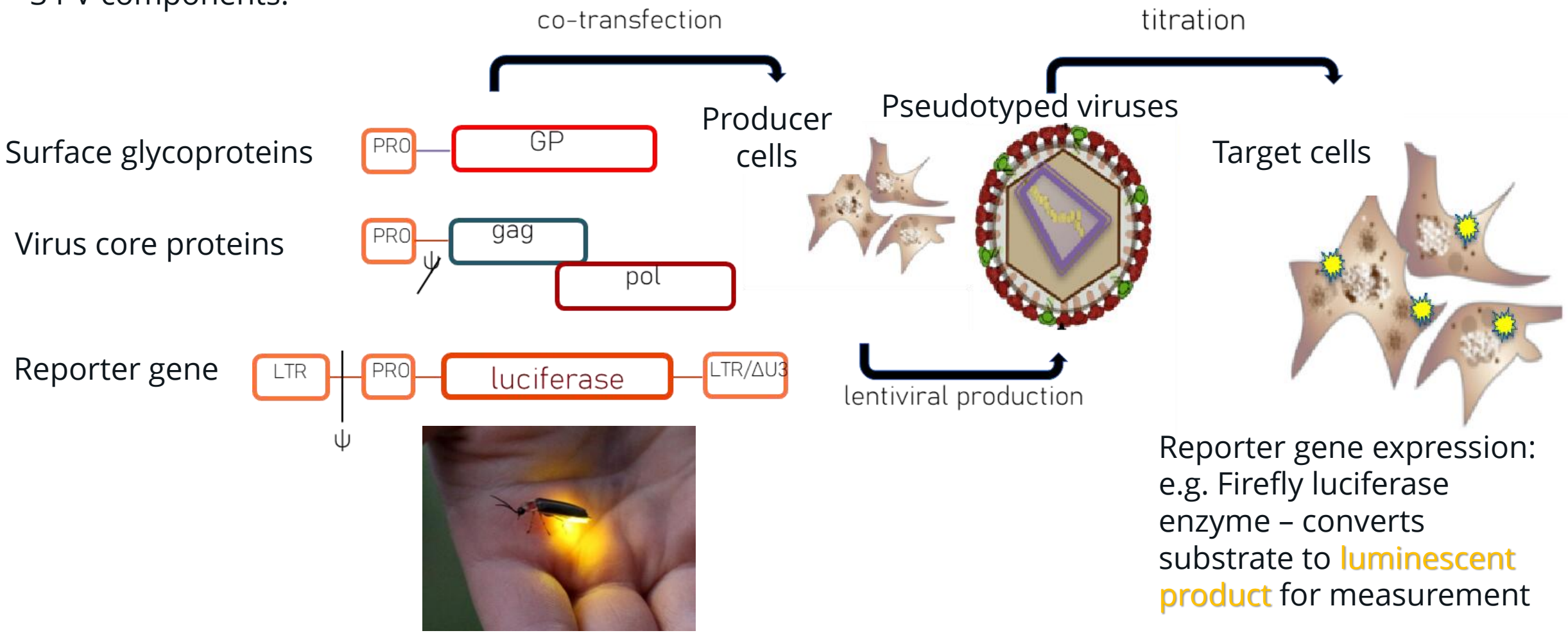
Pseudotyped Virus (PV) Generation & Titration

3 PV components:



Reporter gene expression: e.g. Firefly luciferase enzyme – converts substrate to luminescent product for measurement

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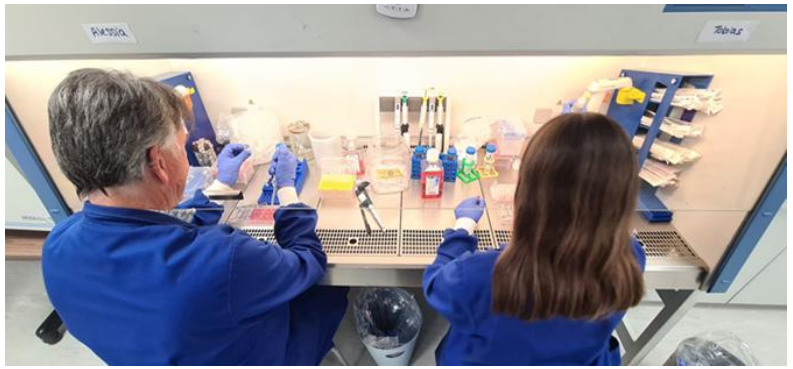
Use of PVs to investigate cell 'tropism'

- PVs can be utilised to examine which cells can be infected by study viruses – i.e. cells from different animals and humans (some mammal lines developed in OneBAT)
- This enables a wide range of cells to be analysed without need to handle the native pathogen
- For OneBAT we investigated the 'tropism' of 3 different bat viruses in a number of cell types: Lloviu filovirus (**LLOV**) & 2 lyssaviruses **-** Lleida & West Caucasian Bat Viruses (**LLEBV** & **WCBV**)
- All 3 viruses could infect the hamster, dog and human cells we tested, with varying efficiency
- Interestingly, only cells from certain bat species could be infected
- Included cells from *Miniopterus schreibersii* (known host of the 3)
- Most permissive cells were human kidney (HEK293)
- Thus HEK293 utilised as target cells in future assays



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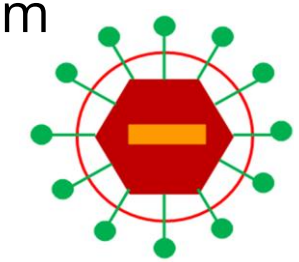
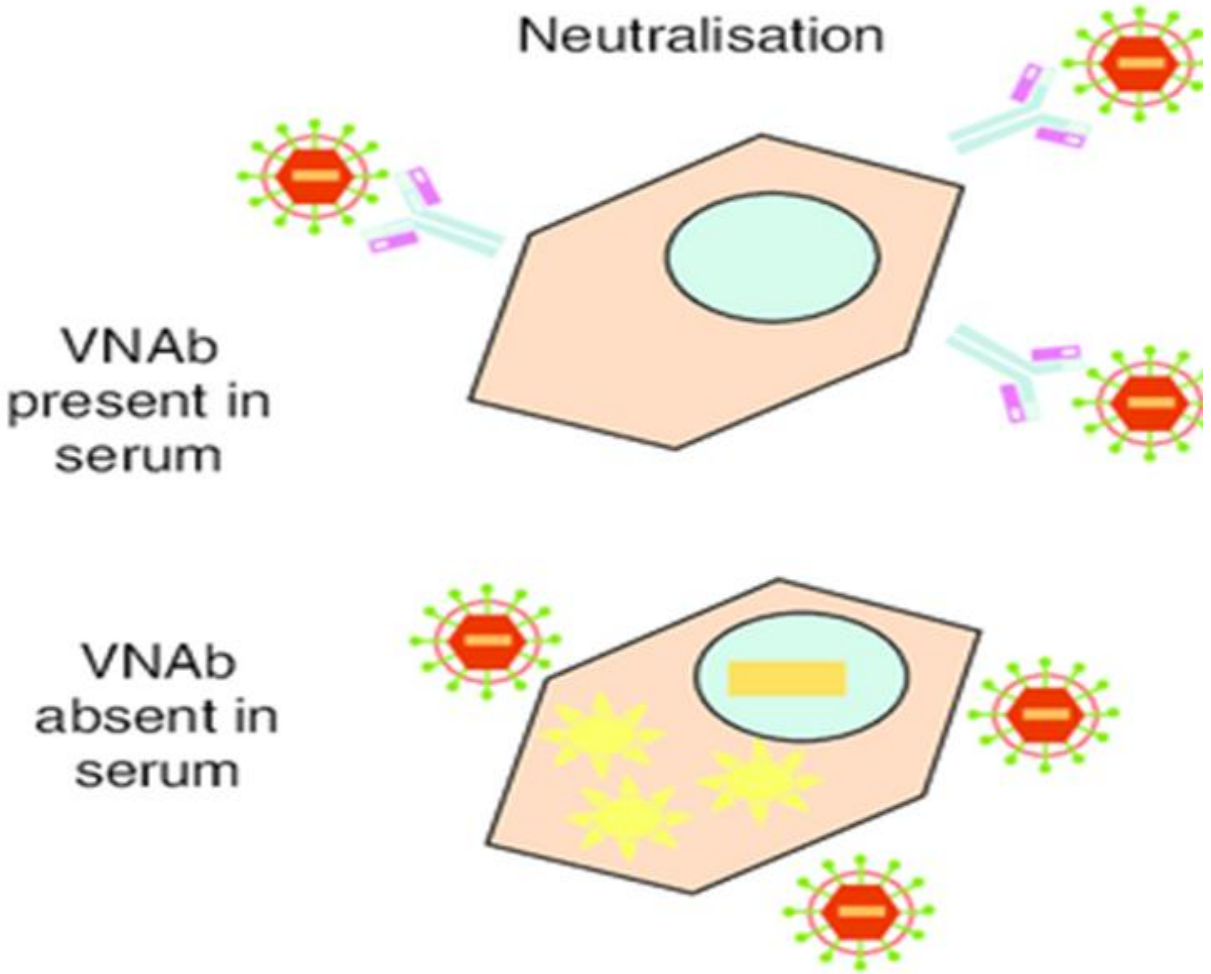
Pseudotyped Virus Neutralisation Test/Assay (PVNT/PVNA)

- Without presence of antibodies PVs can er
  display the matching **'receptor'** on their su
  luminescence
- If PVs are incubated with a serum sample
  the PV surface these antibodies interfere v
  target cells → luminescence reduced ↓
- Thus if a bat has had a prior infection with
  serum. These antibodies will 'neutralise' th
  reduced ↓
- Amount of luminescence inversely propor
  antibodies (VNAbs) in bat serum





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Screening bat serum samples for viruses

- The **Viral Pseudotype Unit (VPU)**, University of Kent, UK has over a decade of experience in generating and utilising many different PVs for research
- VPU collaborations with some members of the OneBAT consortium before grant initiated – i.e. working on the Lloviu filovirus (LLOV) with Dr Kemenesi @ University of Pécs, Hungary
- As part of the OneBAT project ~1400 bats (from multiple roosts in 5 countries, over several years) have had small volumes of blood taken and sent to VPU
- Screening of sera via PVNA for antibodies to LLOV, LLEBV & WCBV – thus each sample analysed for VNAbs from 3 different viruses
- For this **'Triplex'** system we need 3 separate, independent **reporter** read-outs
- Thus, we generated 3 different PVs & 3 separate assays – then combined

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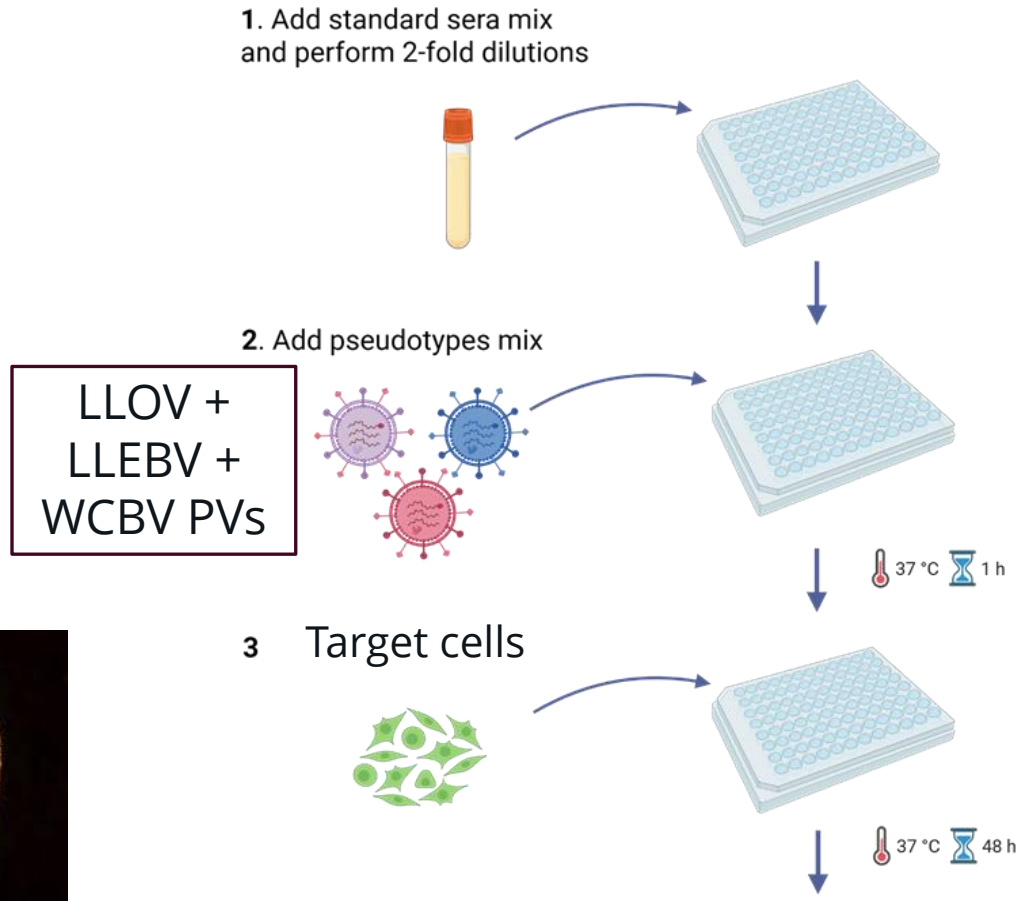
PV Neutralisation Assays – reporter genes

- PVNA carried out in 96 well assay plates
- Serial dilution of bat sera + standard PV amount
- Initial approach was using Green Fluorescent protein (GFP) gene in LLOV PVs, Firefly Luciferase (Fluc) for WCBV & Renilla Luciferase (Rluc) for LLEBV
- These reporters can all be detected independently without interference – 3 different readouts for the 3 virus/antibody combinations

Aequorea victoria

Lampyridae spp.

Renilla reniformis



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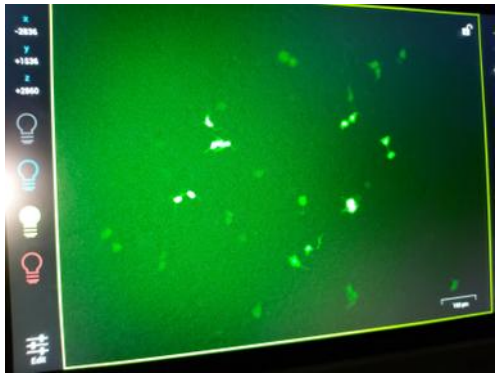
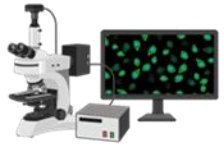
PVNA - data readouts

- GFP read via microscopy or image analyser (clear plate)
- In VPU done by manually counting PV infected, green cells
- Less green cells if antibodies present



4. Read the plate fluorescence



5. Add Dual-Glo® Luciferase Assay Reagent to the plate.

20-25 °C 10 minutes

6. Transfer into a white plate and measure firefly luminescence

7. Add Dual-Glo® Stop & Glo® Reagent to the plate.

20-25 °C 10 minutes

8. Measure *Renilla* luminescence

Luciferase read using a Microplate Reader
- Fluc then Rluc (different substrates to produce luminescence)

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OneBAT serum sample ‘triple’ screening – ongoing…

- Green (GFP) cell counting proved too time consuming manually
- Would need expensive image analyser to automate
- For practical reasons now testing LLOV separately (using Fluc), with lyssaviruses still Fluc & Rluc
- Readout appear as a **‘heat map’** with numerical values to analyse
- Experiments include positive & negative control sera
- **Screening of the ~1400 bat sera ~50% complete**

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Serum screening data analysis – ongoing…

- Transfer data to spreadsheets & analysis of all the data being produced
- Comparison of test sample data to controls
- Statistical analysis
- Compiling study data to gain overall picture of virus distribution across Europe

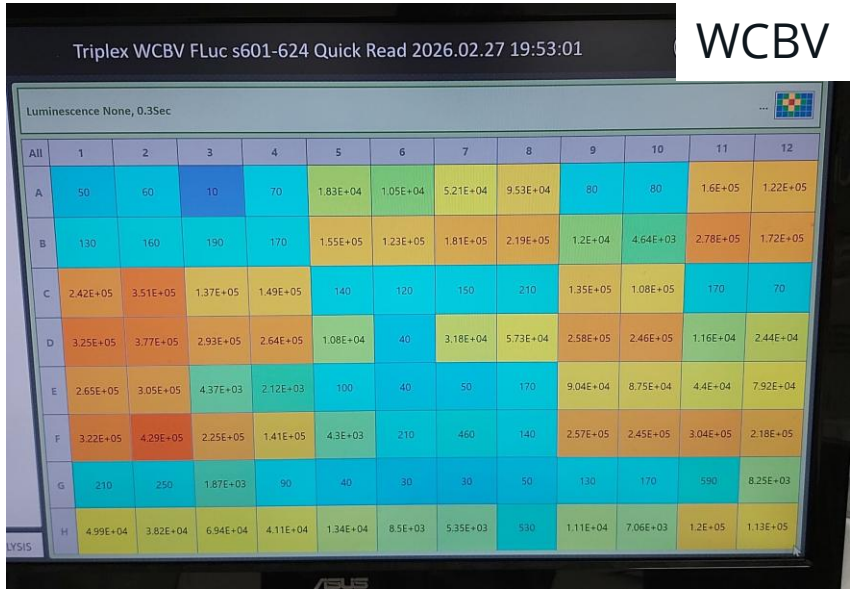
Controls:

Cell only

PV only

Negative serum

Positive serum



WCBV

LLEBV

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Contacts

Simon Scott

University of Kent

s.d.scott@kent.ac.uk

Maria Francesca Priore

IZSVE

MFPriore@izsvenezie.it

Edward Wright

University of Sussex

e.wright@sussex.ac.uk

Thanks to all OneBAT samplers and cell line developers!

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Thanks for your attention!

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