Lyophilisation of lentiviral pseudotypes for the development and distribution of virus neutralisation assay kits for rabies, Marburg and influenza viruses

Stuart Mather[1], **Edward Wright**[2]*, Simon Scott[1] & Nigel Temperton[1]

[1]Viral Pseudotype Unit (Medway), Medway School of Pharmacy, University of Kent, Chatham Maritime, UK
[2]Viral Pseudotype Unit (Fitzrovia), Faculty of Science and Technology, University of Westminster, London, UK

INTRODUCTION

Virus neutralisation assays detect and quantify neutralising antibody responses raised against the envelope glycoproteins of many pathogenic viruses, enabling vaccine evaluation and serosurveillance studies to take place. However, the necessity to perform these assays in biosafety level (BSL-) 3 or 4 laboratories, and the expensive associated personnel and equipment costs of such precautions, inhibits the broad application of such assays, especially for laboratories with poor funding or resources.

Pseudotype viruses (PVs) are a potential solution to this dilemma. PVs are chimeric, replication-deficient virions, often comprised of a retroviral core harbouring a reporter gene, combined with heterologous envelope glycoproteins, which mimic the binding and entry mechanisms of their wild-type counterparts. Successful transduction of a permissible cell line results in reporter gene integration and expression, which can be quantified as a measure of PV infectivity. They can therefore be safely employed in pseudotype virus neutralisation assays (PVNAs), bypassing high biosafety requirements and performing comparably with established serological assays[1-2].

Selection of 'cheap' reporter genes such as β-galactosidase or secreted alkaline phosphatase (SEAP)[3], low serum sample requirements (<5µl) and multiplexing all reduce the cost-per-assay burden of the PVNA platform[2]. Despite this, the current necessity to maintain dry-ice transportation and cold-chain storage of PV supernatant presents a serious financial obstacle for global distribution of PVNA-based kits.

AIM

To assess the viability of PV lyophilisation with a view to producing, transporting and using a PVNA-based serological kit. Pseudotype infectivity was calculated after lyophilisation[4] and immediate reconstitution, as well as incubating freeze-dried pellets at a variety of temperatures and humidities before use. Integrity of glycoprotein structure was also examined by employing reconstituted PVs in downstream neutralisation assays.

MATERIALS AND METHODS

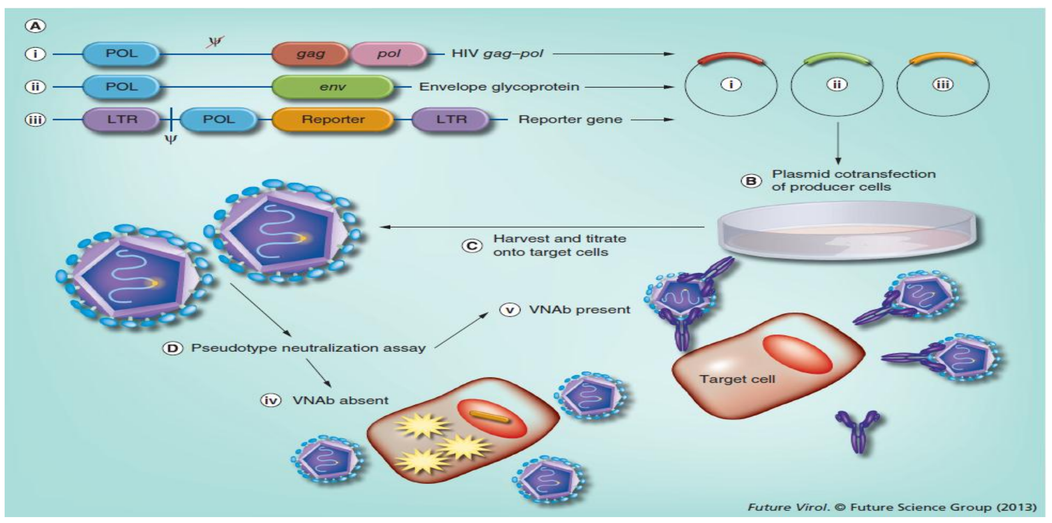


Figure 1: Schematic of the 3-plasmid co-transfection system of PV production for use in neutralisation assays[5]

Generation of pseudotype viruses

Influenza H5 A/Vietnam/1194/2004, rabies (RABV) Evelyn Rokitniki Abseleth (ERA) and Marburg (MARV) Lake Victoria strains were produced by transfection (Fugene6, Promega) of plasmids encoding HIV *gag-pol* (p8.91), firefly luciferase (pCSFLW) and either influenza HA, rabies G or Marburg GP proteins (pI.18-HA, pI.18-G or pCAGGS-GP) into HEK293T/17 producer cells. For H5 PV production, exogenous recombinant neuraminidase from *Clostridium perfringens* (Sigma) was added 24 hours after transfection. Viral supernatants were harvested at 48 hours post-transfection, passed through a 0.45µm-pore filter and stored at -80°C[1-2].

Freeze-drying treatment of pseudotype viruses (Figures 2 & 3)

Lyophilisation of pseudotype viruses: PV supernatant in a range of sucrose-PBS[4] concentrations was lyophilised (Labconco Freezone 2.5) overnight at final conditions of -50°C and <0.133mBar before being reconstituted and PV infectivity in relative luminescent units per ml (RLU/ml) calculated.

Durability of lyophilised pseudotypes: Lyophilised PVs in the presence of 1M, 0.5M or no sucrose-PBS cryoprotectant were stored for 4 weeks at -80°C, -20°C, +4°C, +20°C and 37°C (70% and 95% relative humidities) prior to reconstitution and RLU/ml calculation.

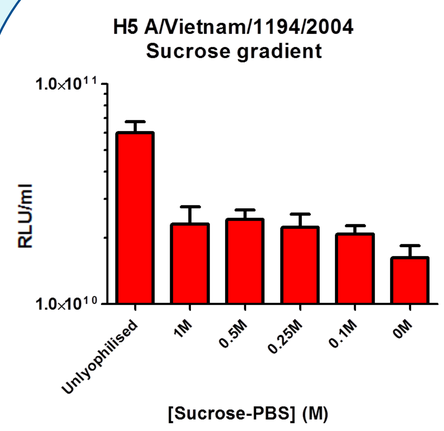
Neutralisation of reconstituted pseudotypes (Figure 4)

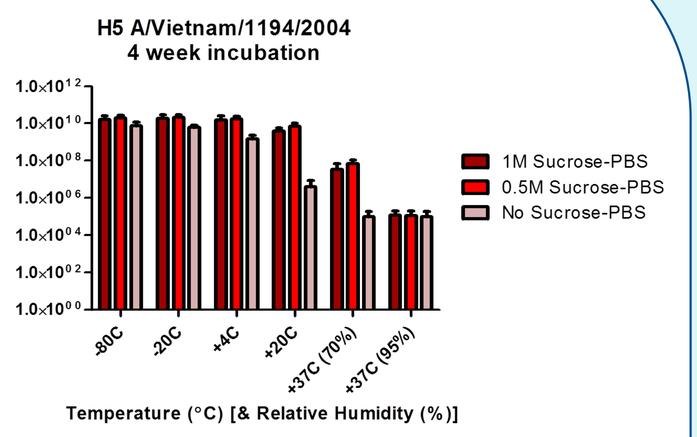
Reconstituted H5 and rabies PVs were compared to unlyophilised PV supernatant when employed in PVNAs against confirmed virus neutralising antibody (VNAb)-positive sera:

- H5 – chicken sera post Volvac® AI KV H5N2 vaccination (Boehringer Ingelheim)
- RABV – human sera post Rabipur® vaccination (Novartis)

Serum serial dilutions were incubated with $1x10^6$ RLUs of PV for 1hr at 37 °C before addition of $1x10^4$ target cells – HEK293T/17 for H5 PVNAs or BHK-21 for RABV PVNAs - to each well of a 96-well culture plate, incubation for 48hrs and RLU measurement (Bright Glo reagent/Glomax 96 luminometer, Promega). IC_{50} and IC_{90} values (reciprocal of highest serum dilution that confers 50% and 90% neutralisation respectively) were calculated using GraphPad Prism 5 software.

RESULTS





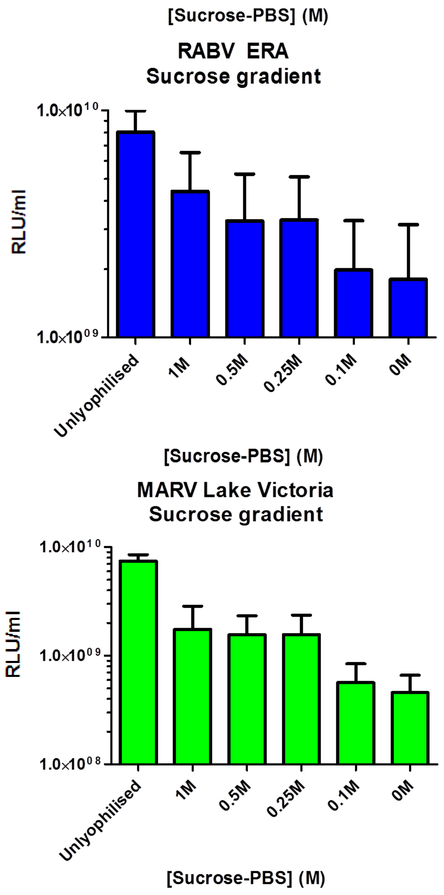


Figure 2: Lyophilisation of lentiviral pseudotypes[6]

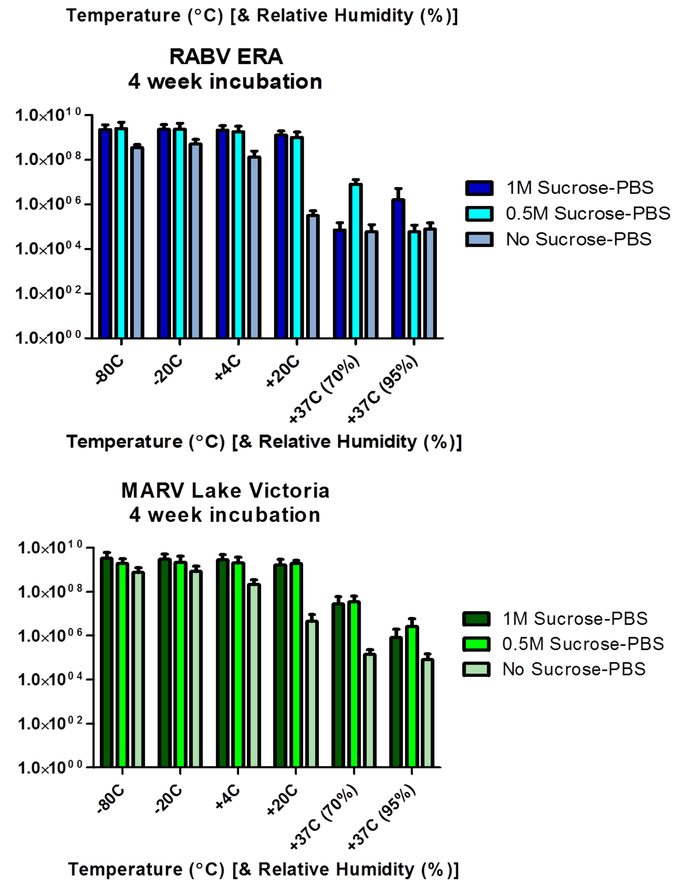


Figure 3: Durability of lyophilised pseudotypes[6]

Pseudotype virus (PV)	IC_{50}	IC_{90}
H5 A/Vietnam/1194/2004 - lyophilised	2560-5120 (4679)	1280-2560 (1390)
H5 A/Vietnam/1194/2004 - unlyophilised	2560-5120 (3919)	1280-2560 (1345)
RABV ERA – lyophilised	40960-81920 (44072)	5120-10240 (6381)
RABV ERA – unlyophilised	20480-40960 (27324)	5120-10240 (6222)

Figure 4: Neutralisation of reconstituted pseudotypes – IC_{50} and IC_{90} VNAb titres[6]

CONCLUSIONS & FURTHER CONSIDERATIONS

- Lyophilised PVs (in 0.5M Sucrose-PBS) are stable after 4weeks' incubation at up to 37°C, retain infectivity following reconstitution and can be as efficiently neutralised as fresh PV supernatant, confirming the viability of a PVNA-based kit for global distribution
- Further considerations:
  - Extrapolate data by storage-testing of PV pellets for longer durations
  - Assess sensitivity of other pseudotype cores e.g. MLV, VSV, EIAV
  - Calculate cryoprotective efficiency of other suspending media

REFERENCES

[1] Temperton *et al.* (2007) *Influenza and Other Resp Viruses* 1:105–12
[2] Wright *et al.* (2008) *Journal of General Virology* 89:2204-13
[3] Kaku *et al.* (2012) *J. Virol. Methods* 179(1):226-32
[4] Shin *et al.* (2010) *J Biomed Mater Res A* 93(4):1252-9
[5] Mather *et al.* (2013) *Future Virology* 8(8):745-55
[6] Mather *et al.* (2014) *J Virol Methods* 210:51-8

CORRESPONDENCE

Stuart Mather sm751@kent.ac.uk
Simon Scott s.d.scott@kent.ac.uk **Nigel Temperton** n.temperton@kent.ac.uk
Web: www.viralpseudotypeunit.info Twitter: @ViralPseudotype

Viral Pseudotype Unit

[6]