

Development of an indirect ELISA for detection of serum antibodies to *leporid herpesvirus-4*



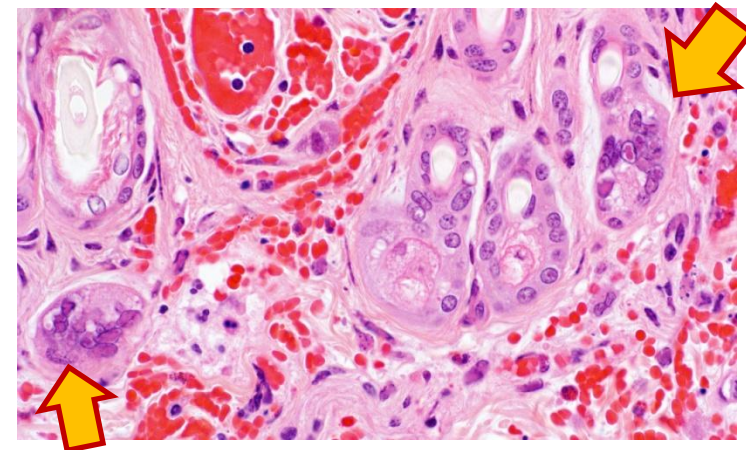
Janet Sunohara-Neilson¹, Éva Nagy¹, Susy Carman²,
Patricia V Turner¹

¹Department of Pathobiology and ²Animal Health
Laboratory, Laboratory Services Division, University of
Guelph, Guelph, ON

CAHLN-RCTLSA Meeting, May 28, 2013

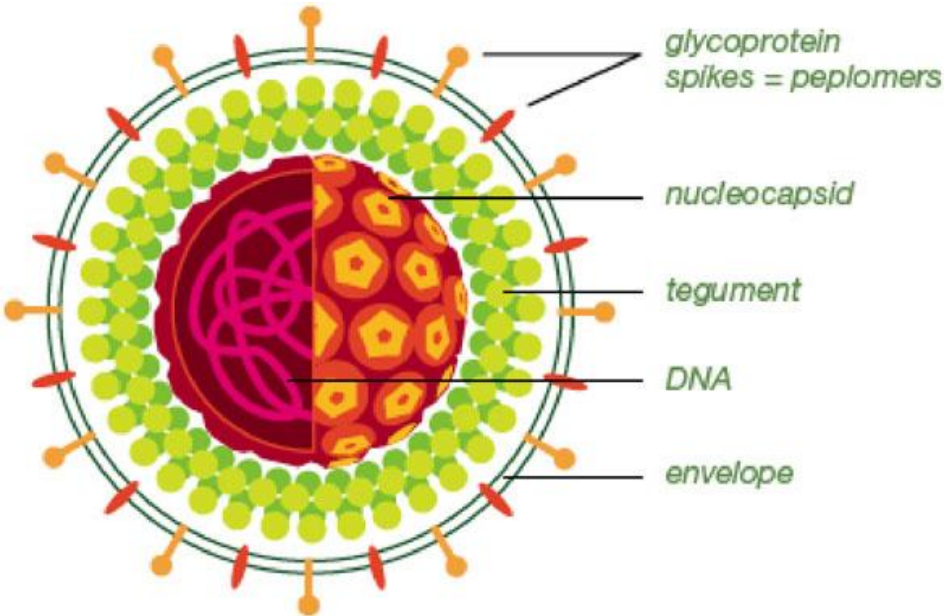
Leporid herpesvirus-4

- Novel alphaherpesvirus
- 55% morbidity, 20% mortality
- Commercial rabbits (Alaska)
 - Jin et al. (2008) Vet Path. 45(3): 369
- Pet rabbit (N. Ontario)
 - Brash et al. (2010) CVJ. 51(12): 1383
- **Clinical signs:** lethargy, anorexia, conjunctivitis, fever, abortions
- **Pathology:** multifocal pulmonary hemorrhage and edema, hemorrhagic dermatitis, splenic necrosis, hepatic necrosis



Syncytia with intranuclear inclusion bodies

Leporid herpesvirus-4



Family **Herpesviridae**

Subfamily **Alphaherpesvirinae**

Genus **Simplexvirus**

Enveloped, Tegument

Linear dsDNA – 125 kb

Aerosol droplet transmission or
direct contact

Rapid growth and cytopathic
effect in cell culture

Objective of the study

- To develop a **safe, rapid** and **sensitive** test for screening and diagnosis of LHV4
- Indirect ELISA – Antibody detection
 - **Whole virus antigen** preparation
 - **Sub-unit protein** expression in Baculovirus system

Collection of serum samples



<http://www.ahwla.org.uk>



<http://www.ontariorabbit.ca>

Infection trial

Live virus inoculated
via intranasal
infection

Sunohara-Neilson et al. Comp Med
(accepted for publication, 2013)

Polyclonal antibody

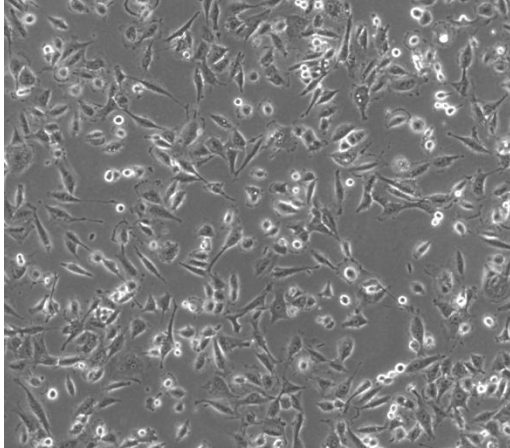
Inactivated virus
injected with adjuvant

Field samples

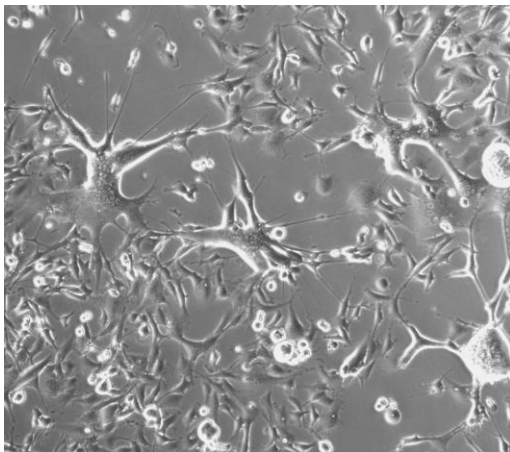
Collected from 250
commercial and 20
pet rabbits

Virus Neutralization Assay

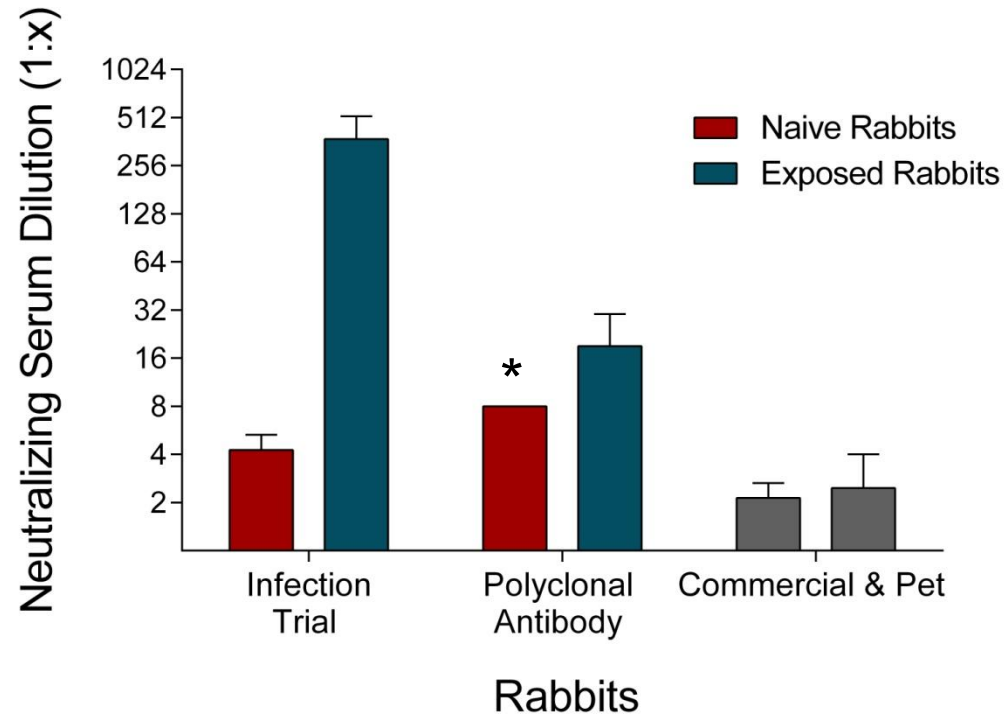
Serology Gold Standard



Uninfected CRFK cells

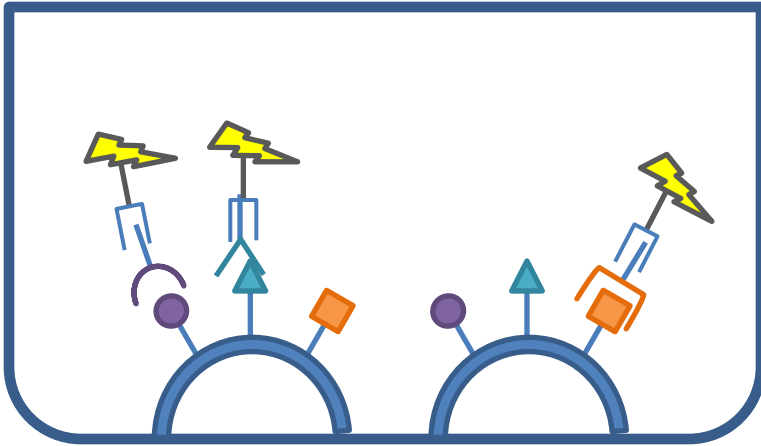


LHV-4 infected CRFK cells



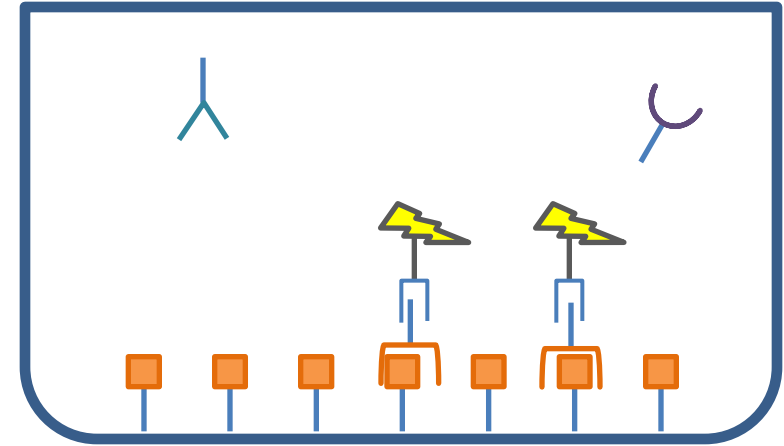
* <1:8 Toxic effect of serum

Indirect ELISA



Whole virus antigen

- decrease time to diagnosis

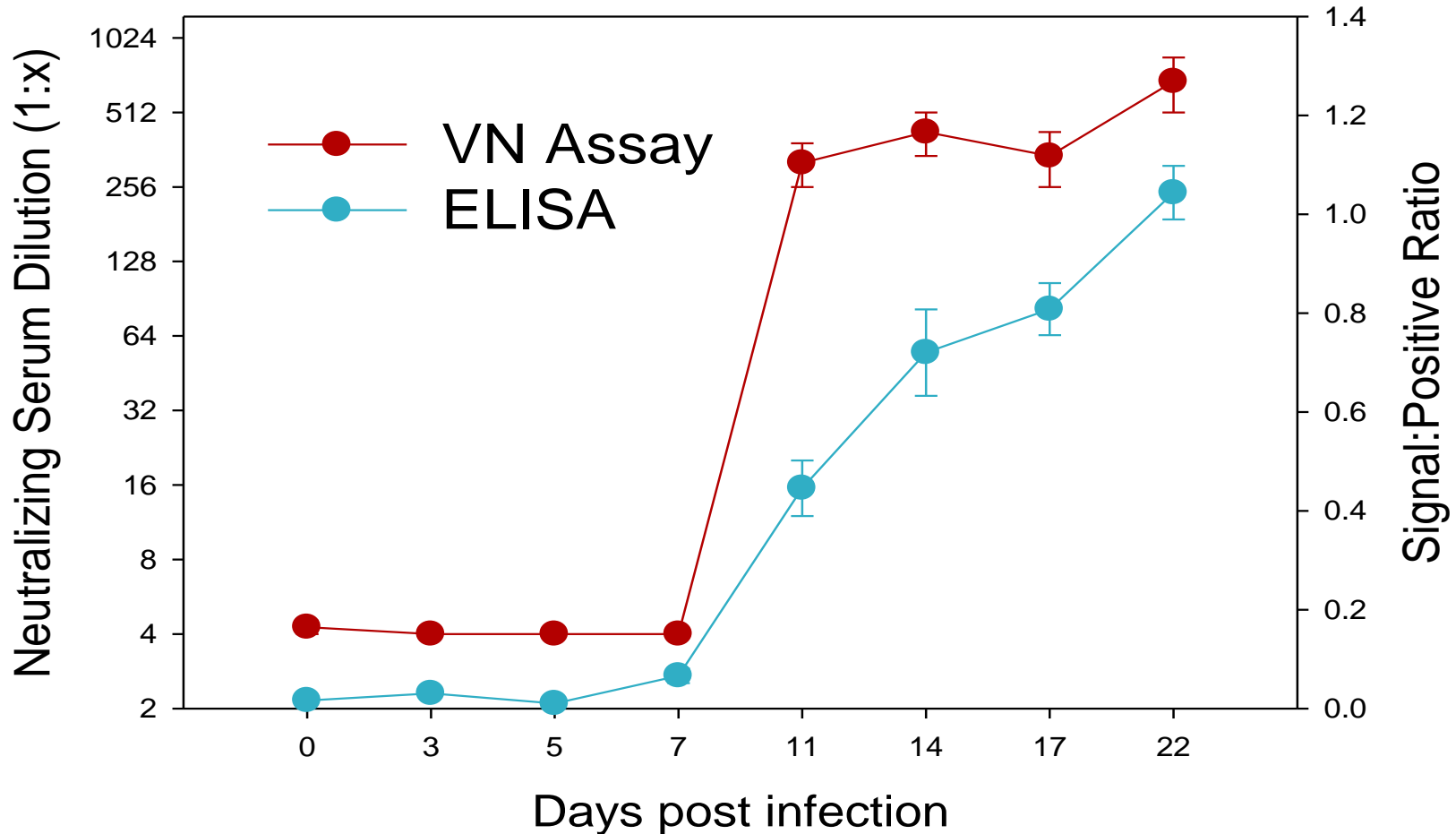


Sub-unit protein expression

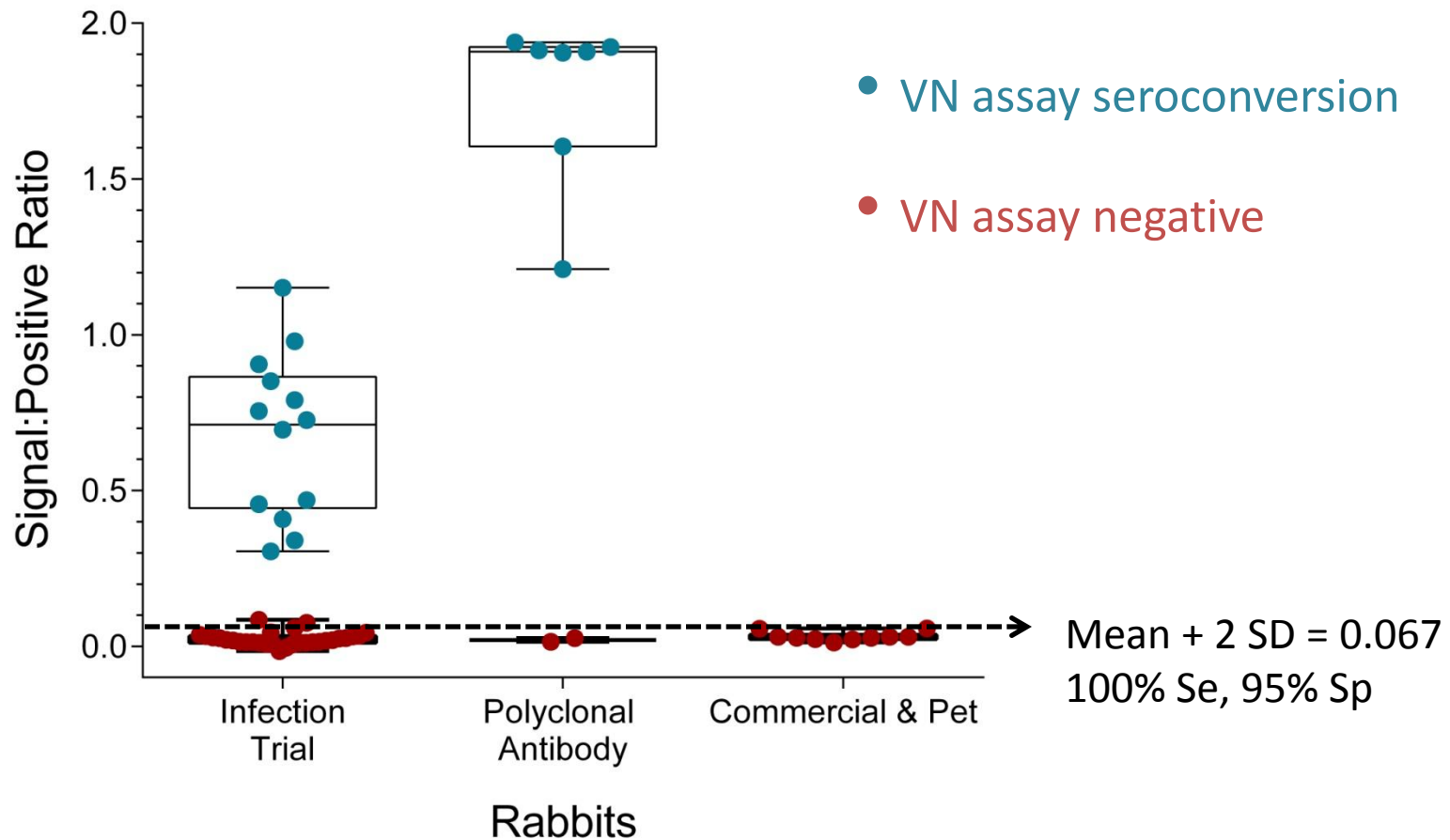
- increased efficiency
- increased biosafety

- Optimization determined using checkerboard design
- Results compared to VN assay as gold standard

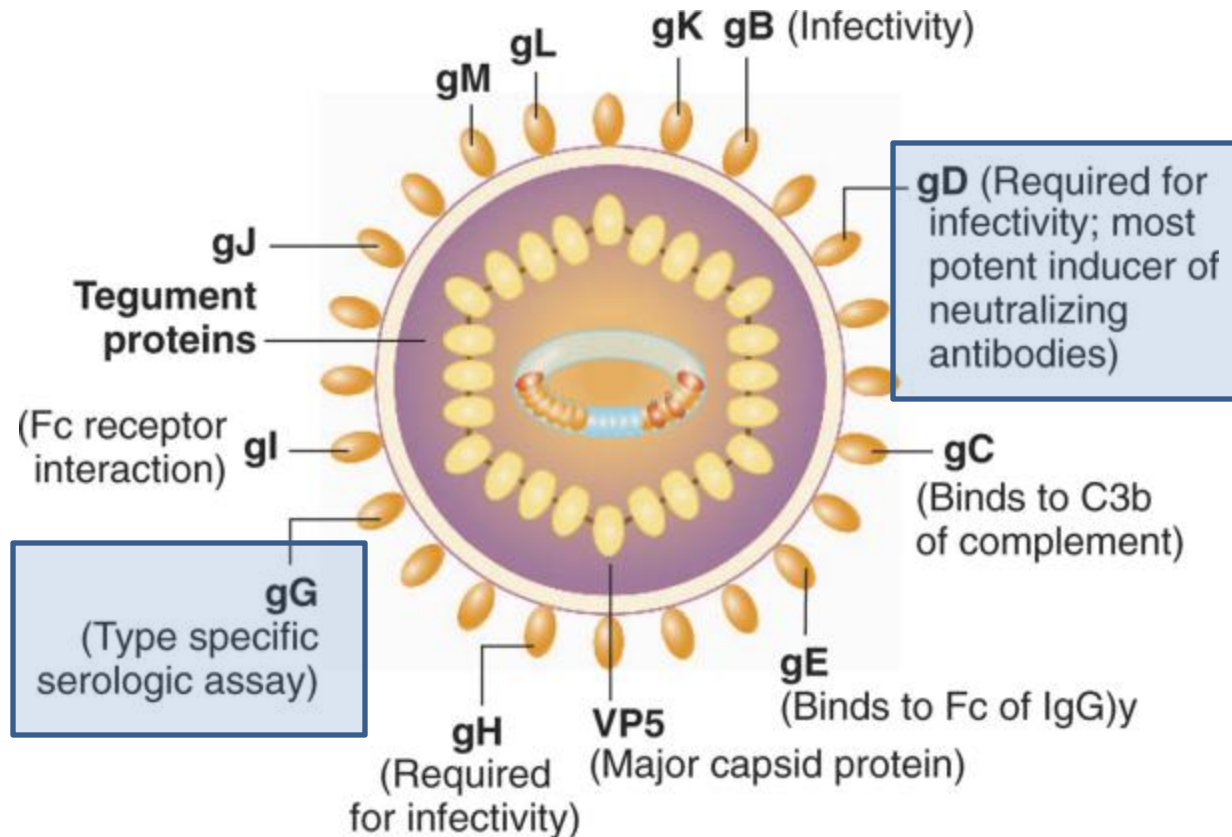
Neutralizing antibodies produced from 11 dpi (VN assay and ELISA)



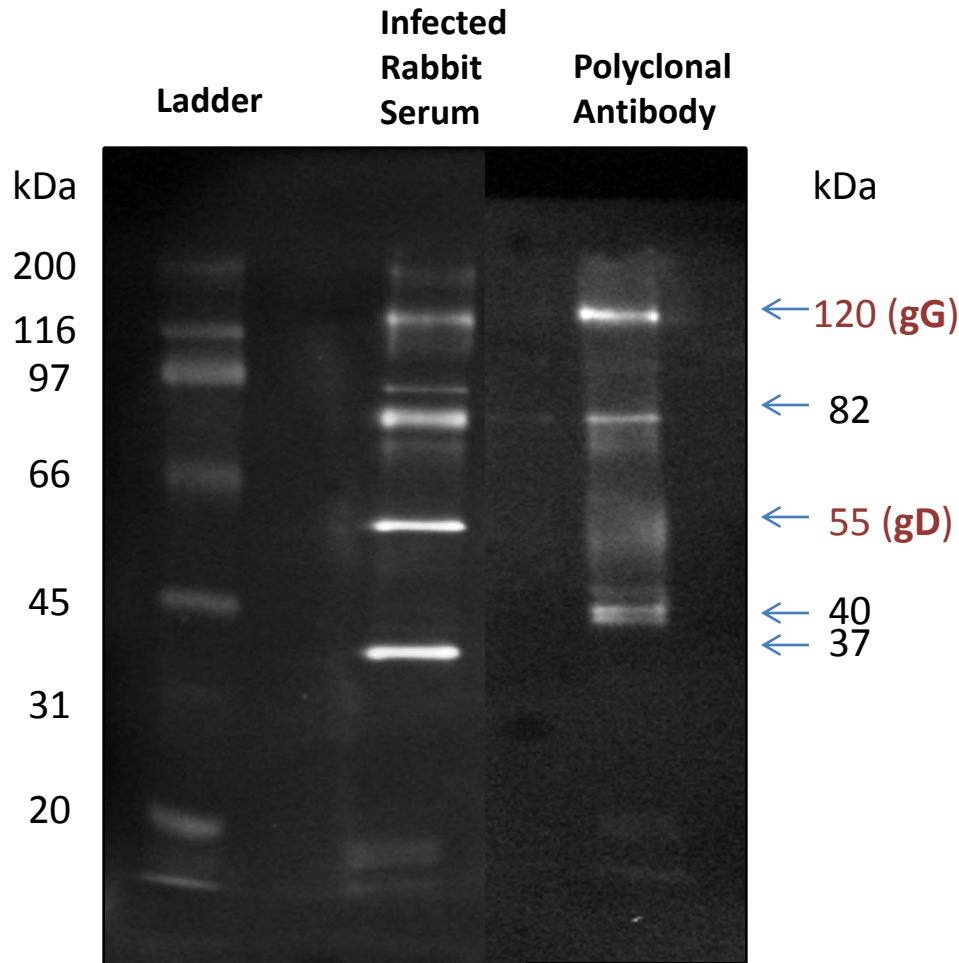
Whole Virus ELISA



Herpesvirus glycoproteins



Immunoreactive protein profiles



A. Infected Rabbit Serum (live)
Representative of test samples

B. Polyclonal Antibody (inactive)
Positive control

120 kDa protein

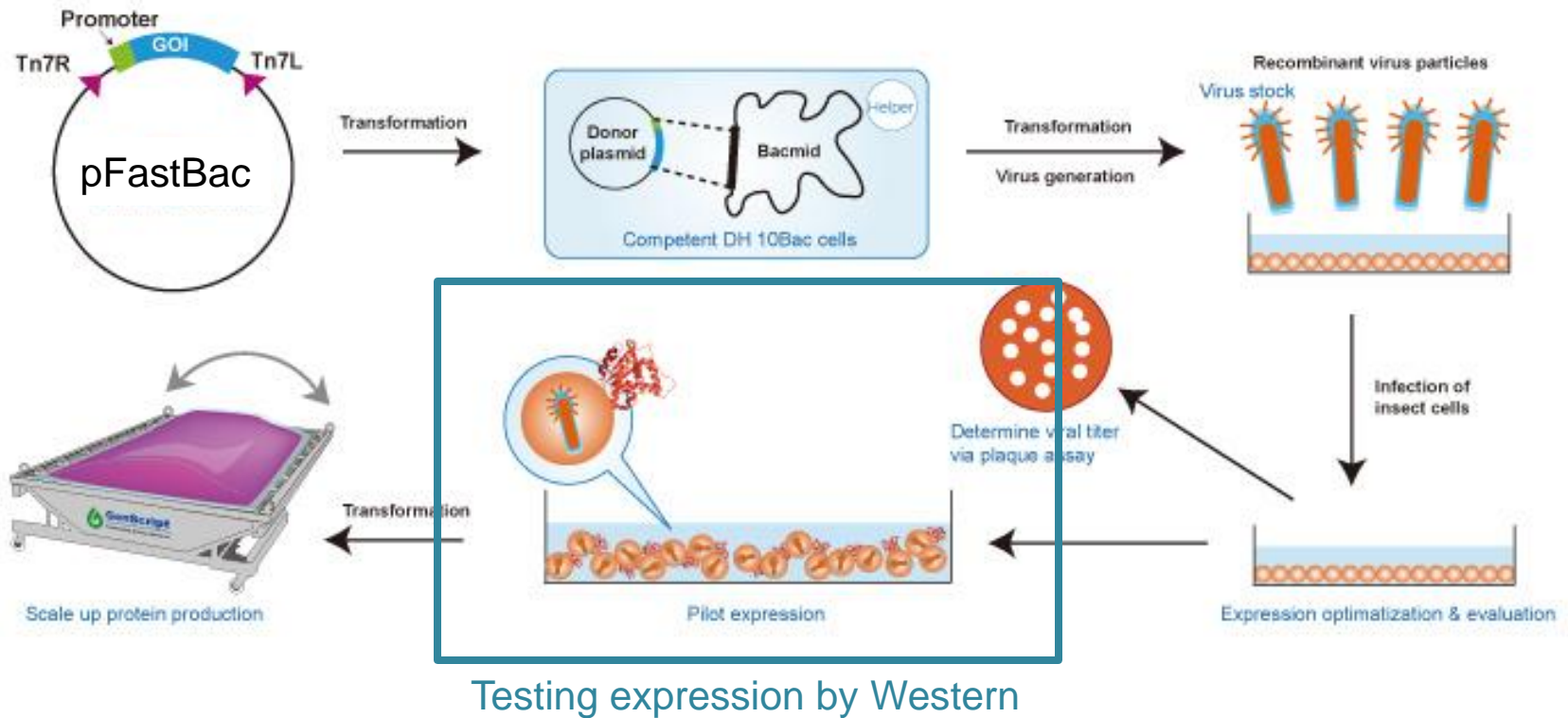
- Glycoprotein G (suspected)
- Antibodies present in both sera

55 kDa protein

- Glycoprotein D (suspected)
- Antibodies absent in polyclonal antibody preparation

Baculovirus expression system

Glycoprotein G
from LHV4



Summary

- *Leporid herpesvirus-4*
 - Novel alphaherpesvirus of rabbits
 - Health concern for the rabbit industry
- Serology – Indirect ELISA
 - Whole virus – sensitive and specific
 - Sub-unit – glycoprotein G and D
- Direct virus detection
 - qPCR assay in development

Acknowledgements

- CAHLN Graduate Travel Award
- Advisory Committee
 - Patricia V. Turner
 - Éva Nagy
 - Susy Carman
 - Lela Riley
- Technical Assistance
 - Jutta Hammermueller
 - James Ackford (Peter Krell baculovirus lab)
 - Brianne Davis

