MRC PPU
Reagents
and Services

ANTIBODY TESTING RESULTS
Standard Reporting Template

Name: Sarah Cole **Date:** 15/05/2024
Group Leader: Edward Hutchinson

Antibody Name: Anti-IDV-NS1

Full Antigen Name: NS1 (non-structural protein 1) protein of influenza D virus (IDV) strain D/bovine/Oklahoma/660/2013

Antigen Species: Virus

Sheep Number: DA252

Bleeds Tested: 1st, 2nd, 3rd, 4th, 5th
Recommended Bleed: All bleeds suitable for applications listed.

Immunoblotting:

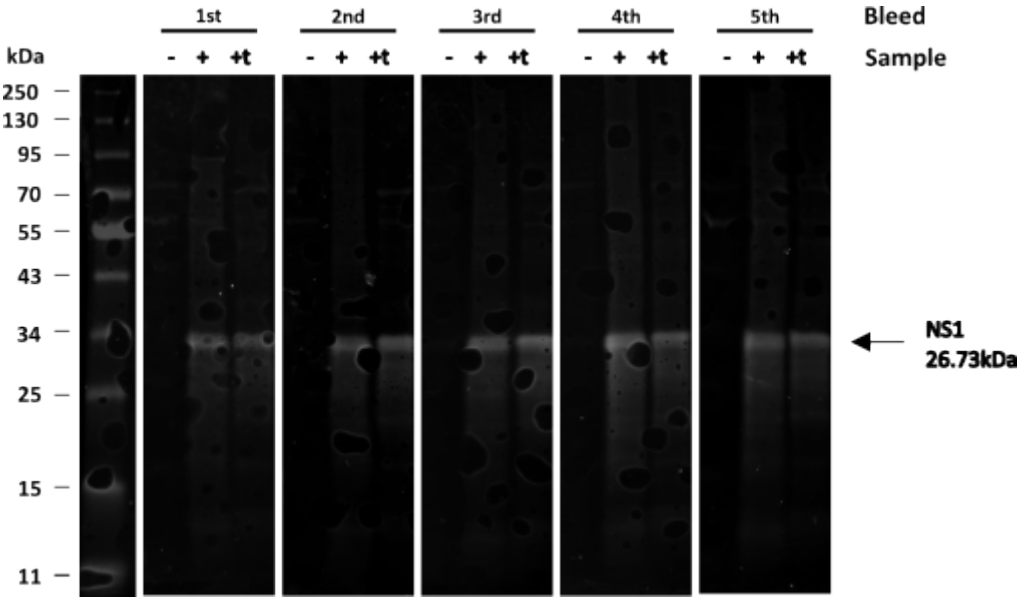
Method

MDCK cells were infected with IDV strain D/bovine/France/5920/2014 at an MOI of 2.5 × 10^{-4} PFU/cell, or mock infected.
293T cells were transfected with IDV pDUAL:segment 7 (a bidirectional reverse genetics plasmid that encodes the NS1 protein of D/bovine/Oklahoma/660/2013) or mock transfected, using Lipofectamine 2000 (Thermo Fisher) using cell density and plasmid mass as recommended in the manufacturer's protocol.
Cells were lysed in Laemmli buffer at 7 days post-infection or 48 h post-transfection, separated by electrophoresis on precast Any kD™ polyacrylamide gels (Bio-Rad) and transferred to nitrocellulose membranes.
Membranes were blocked with 5 % milk in PBS-T (phosphate buffered saline with 0.1 % Tween 20) overnight at 4 °C, rinsed in PBS-T then probed with primary antibodies at 5 µg/ml in PBS-T for 1 h at room temperature. Membranes were washed ×3 in PBS-T followed by further incubation with anti-sheep IR680 or IR800 (Thermo Fisher) at 1 in 10,000 in PBS-T, for 1 h at room temperature.
Membranes were scanned with a Licor Odyssey CLx Infrared imaging system.

Results

IDV NS1 = ~27 kDa. Ladder is the Page Ruler Prestained NIR Protein Ladder (Thermo Fisher).

- \- Mock treated cell lysate
- \+ infected cell lysate
- +t transfected cell lysate



Strong specific signal was detected for all bleeds at 5 µg/ml.

Recommendation

All antisera can be used at 5 µg/ml for western blot with infected and transfected cell lysates.

Immunofluorescence:

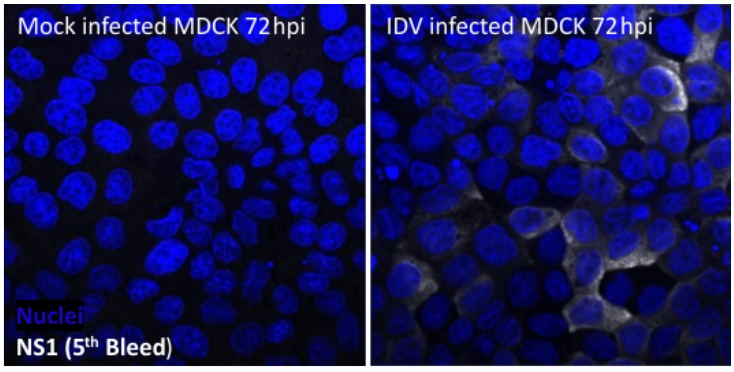
Method

MDCK and 293T cells were seeded at 2×10^5 cells/well in 24 well plates containing 13 mm coverslips. For 293T cells, coverslips were pre-coated with poly-D-lysine to promote adhesion.
The day after seeding, MDCK cells were infected with IDV at an MOI of 2 PFU/cell and 293T cells were transfected with IDV pDUAL:segment 7 as above using Lipofectamine 2000, following the manufacturer's protocol.
Cells were fixed at 72 h post-infection or 48 h post-transfection in 4 % formaldehyde in PBS, then permeabilised using 0.2 % Triton-X100 in PBS/2 % fetal bovine serum (FBS) before blocking for up to 1 h in PBS/2 % FBS.
All antibody dilutions were carried out in PBS/2 % FBS. Cells were incubated with primary antibodies at 1:50 for 1 h at room temperature, washed 3 times in PBS/2 % FBS then probed with donkey anti-sheep IgG (H+L) Cross-Adsorbed Secondary antibody, AlexaFluor 647(Invitrogen) at 1:500 and DAPI at 1:1000 for 45 minutes at room temperature.
Cells were washed 3 times in PBS/2 % FBS and once in PBS, excess moisture was removed before mounting coverslips on slides using ProLong Gold Antifade mounting agent (Thermo Fisher). Mounting agent was allowed to harden overnight before observation using a Zeiss 710 confocal microscope.

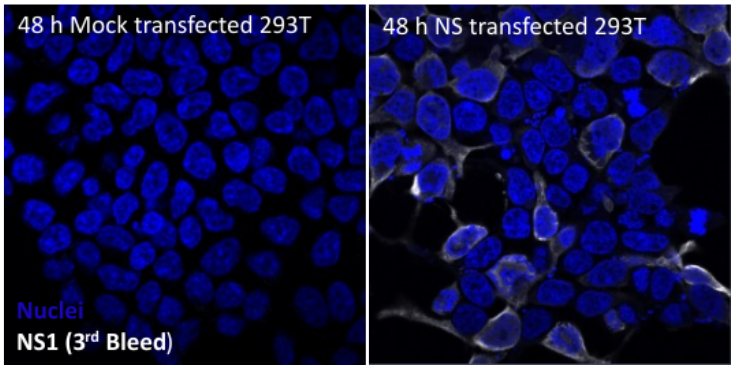
Results

A specific signal was detected for all bleeds in both infection and transfection. Images show 5th bleed antibody at 72 h post-infection and 3rd bleed antibody at 48 h post-transfection; similar results were observed for the other bleeds.

a)



b)



a) Signal detected using all bleeds at 1 in 50 dilution in IDV infected cells.
b) Signal detected using all bleeds at 1 in 50 dilution in cells transfected with. *IDV pDUAL:segment 7*

Recommendation

All 5 bleeds are suitable for use in IF of both infected and transfected cells at a 1 in 50 dilution.

PUBLICATIONS:

None to date.