

# Detection of Porcine Deltacoronavirus via a Luminescent Immunoprecipitation System

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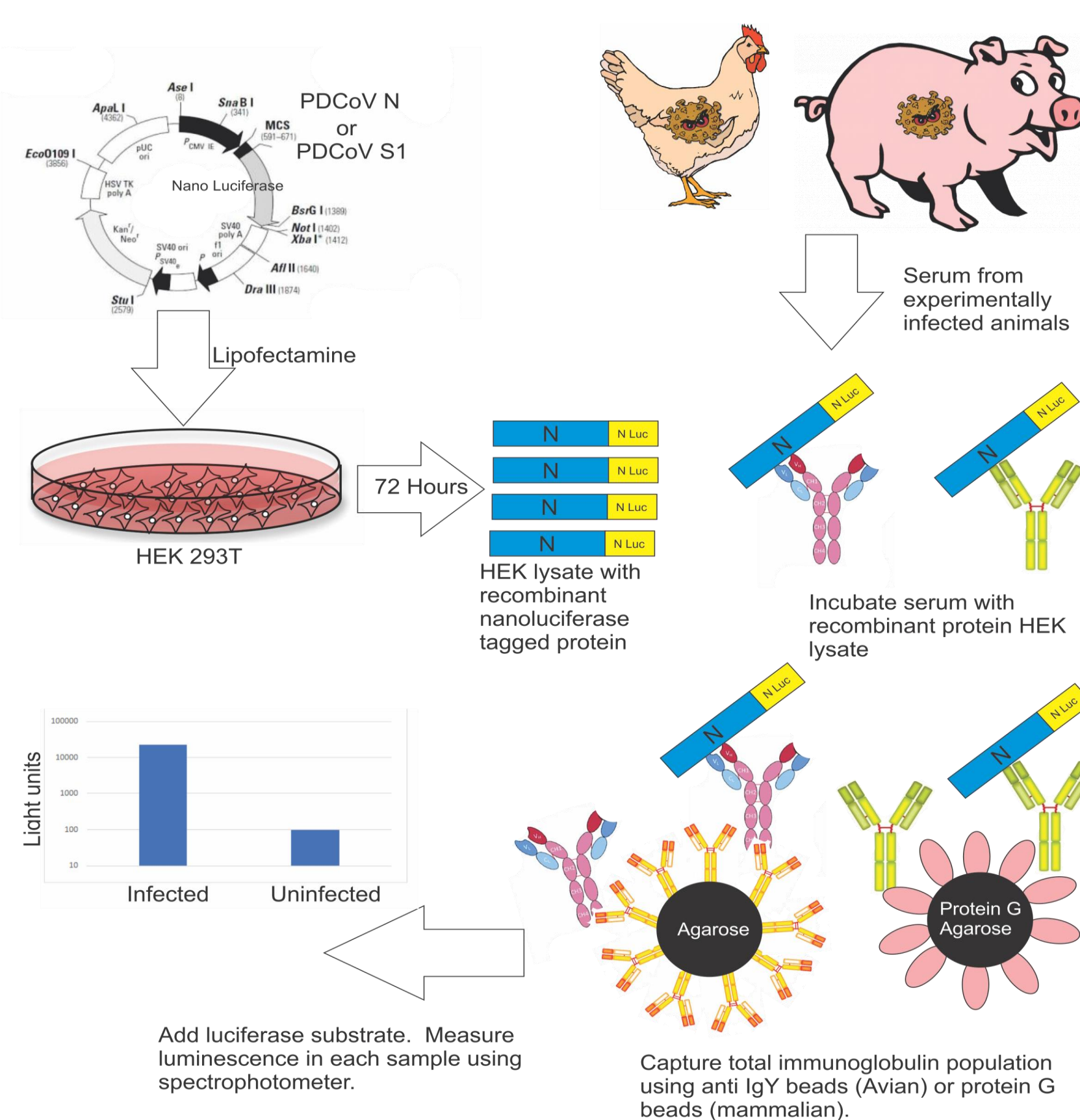
## INTRODUCTION

Porcine deltacoronavirus (PDCoV) is an enteric viral pathogen circulating in swine herds in the U.S. and worldwide, causing acute diarrhea in nursing piglets(1). How this disease spreads among swine farms remains unknown. Recently we have shown that layer chickens and turkeys are susceptible to the disease, resulting in diarrhea, reduced weight gain and bird-to-bird spread of the disease (2). This suggests that PDCoV may be a threat to the poultry industry and that poultry may be contributing to the spread of the disease. We hypothesize that PDCoV is already present in the commercial poultry industry, to some extent, and may serve as a reservoir for transmission to swine and potentially humans. To test our hypothesis, we developed a serological assay called luminescent immunoprecipitation system (LIPS) (3) to specifically detect PDCoV antibodies. LIPS functions by incubating serum samples with recombinant PDCoV antigen linked to luciferase, followed by immunoprecipitation of total immunoglobulins present in the test serum utilizing sepharose beads. Detection of immunoprecipitating luminescent PDCoV antigen indicates antibody positivity within the sample. Validation of the assay will lead to a new commercializable diagnostic tool for confirming PDCoV infections.

## AIMS

- Develop a PDCoV-specific LIPS, allowing assessment of seroprevalence of PDCoV antigens.
- Evaluate the exposure levels of commercial poultry flocks to PDCoV infection utilizing LIPS.

Figure 1. LIPS Assay Principle



## METHODS

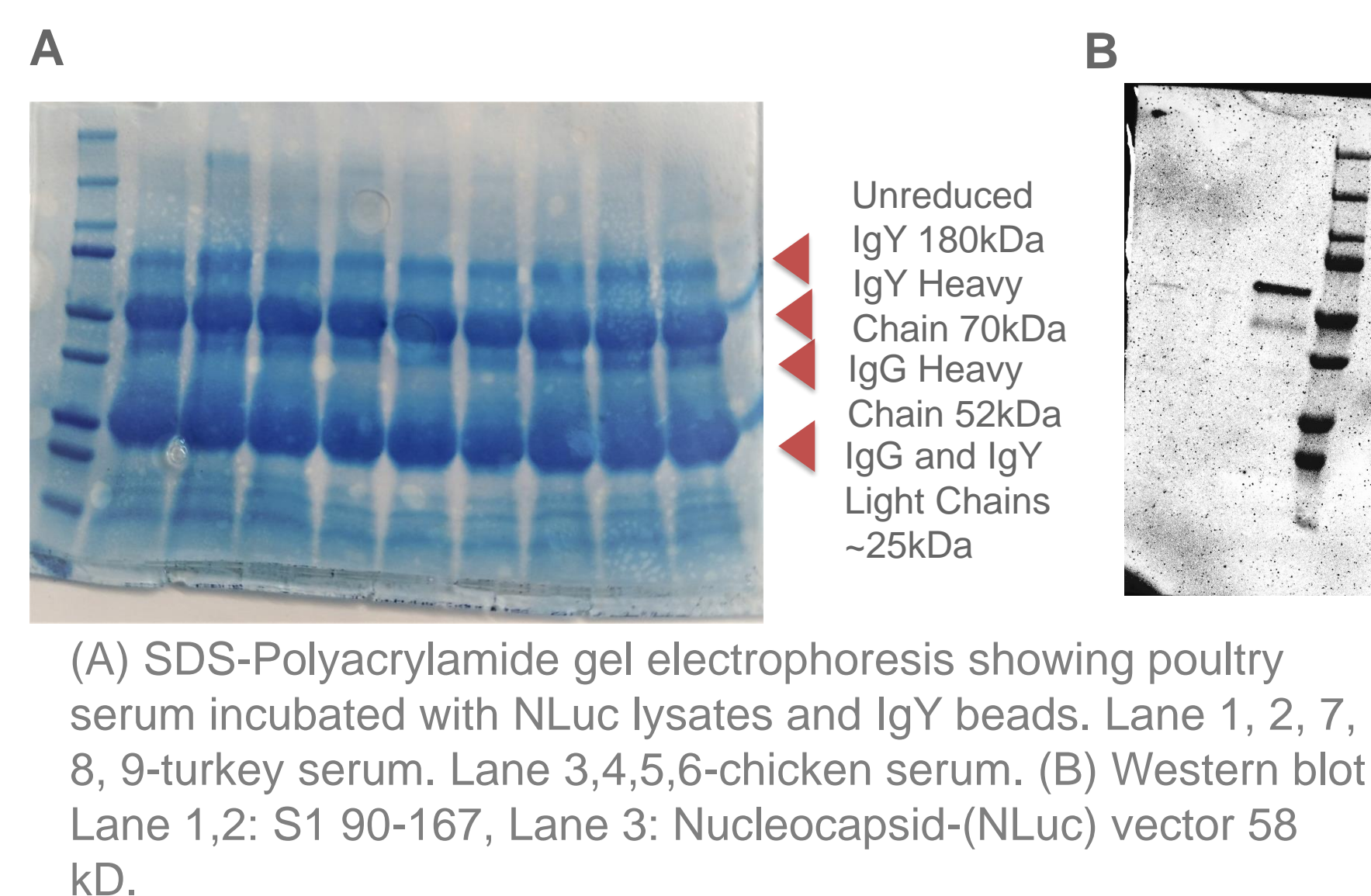
- ❖ Clone Nucleocapsid (N) and Spike (S1) of PDCoV into luciferase-tagging plasmid.
- ❖ Select clonal bacterial colonies with correct insert, grow on plate, culture, extract DNA, and sequence.
- ❖ Transfect plasmid into HEK293T human kidney cells using Lipofectamine LTX transfection kit.
- ❖ 2 days post transfection, lyse cells and harvest recombinant luciferase-tagged protein.
- ❖ Confirmed protein expression using western blot and normalize based on luciferase light units.
- ❖ Incubate experimental serum samples with recombinant luciferase-tagged bait protein. Precipitate total immunoglobulins with protein A or anti-IgY beads. Measure recombinant luciferase protein Co-precipitating with immunoglobulins.
- ❖ Validate assay with infected pig serum. Cross check against porcine epidemic diarrheal virus (PEDV) and transmissible gastroenteric virus (TGEV) positive pig host serum.
- ❖ Optimize in poultry samples using anti-IgY beads.

Figure 2. PDCoV DNA Inserts



(DNA from three PDCoV inserts cloned into vector. Lane 1: N gene 1028 bp, Lane 2: S1 gene, 1353 bp and Lane 3: S1 aa 90-167, 390 bp.

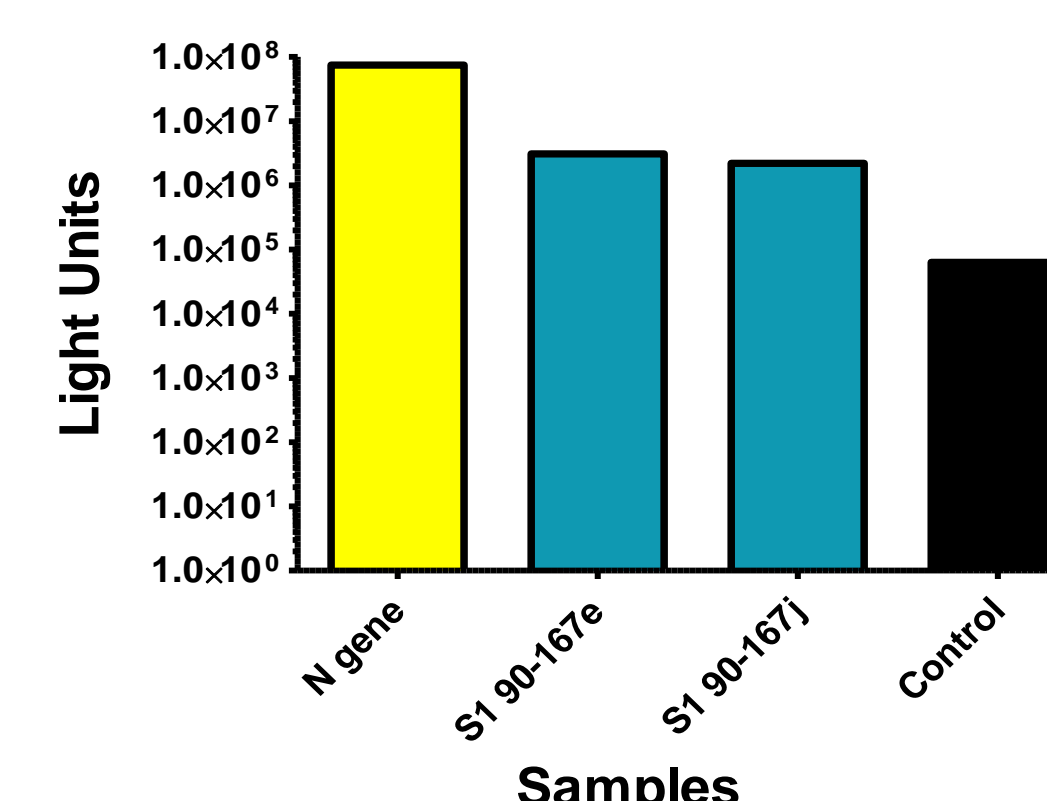
Figure 3. Verification of Protein Expression



(A) SDS-Polyacrylamide gel electrophoresis showing poultry serum incubated with NLuc lysates and IgY beads. Lane 1, 2, 7, 8, 9-turkey serum. Lane 3,4,5,6-chicken serum. (B) Western blot Lane 1,2: S1 90-167, Lane 3: Nucleocapsid-(NLuc) vector 58 kD.

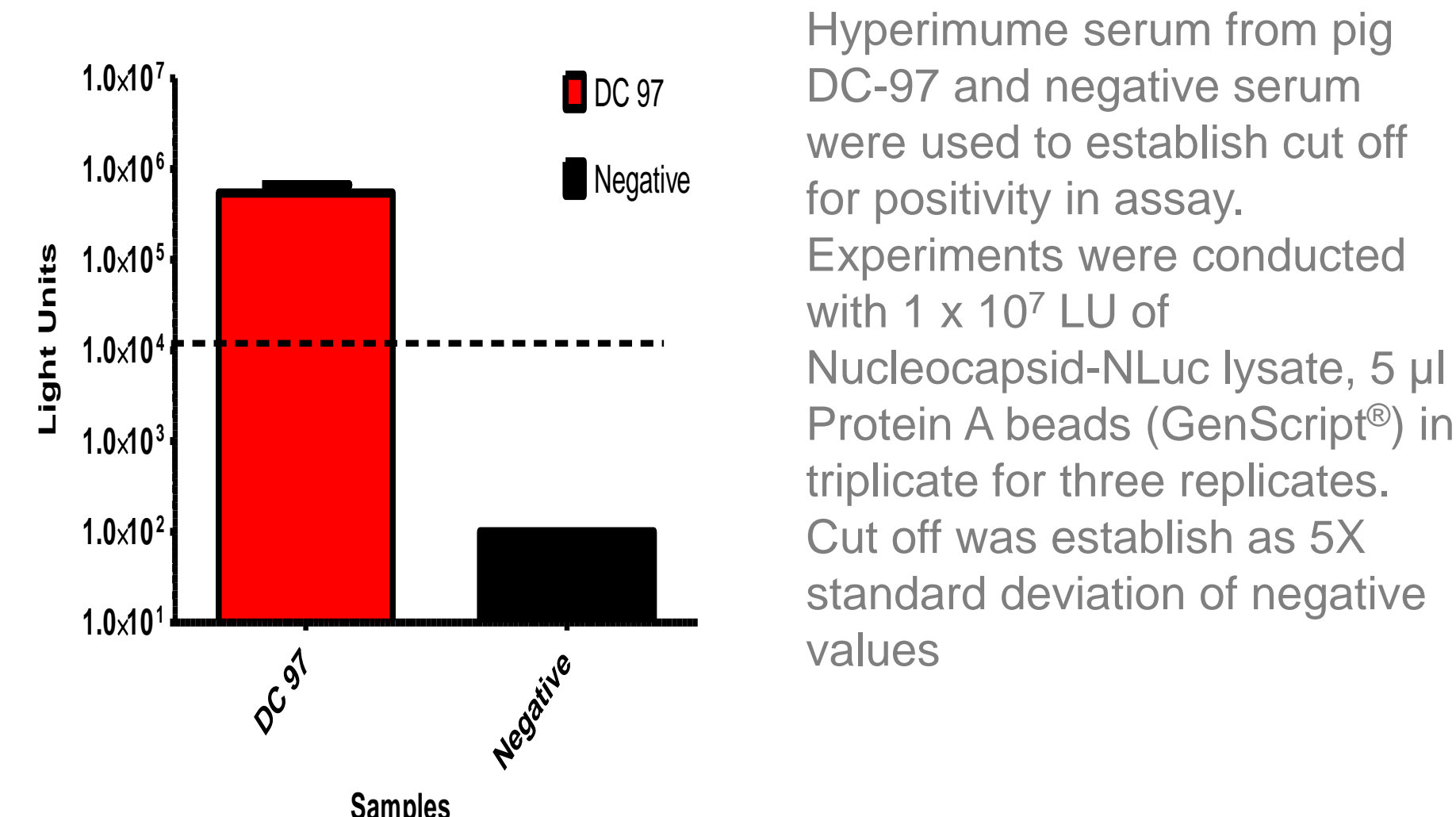
## RESULTS

Figure 4A. Expression of N Luc-Tagged Bait Proteins



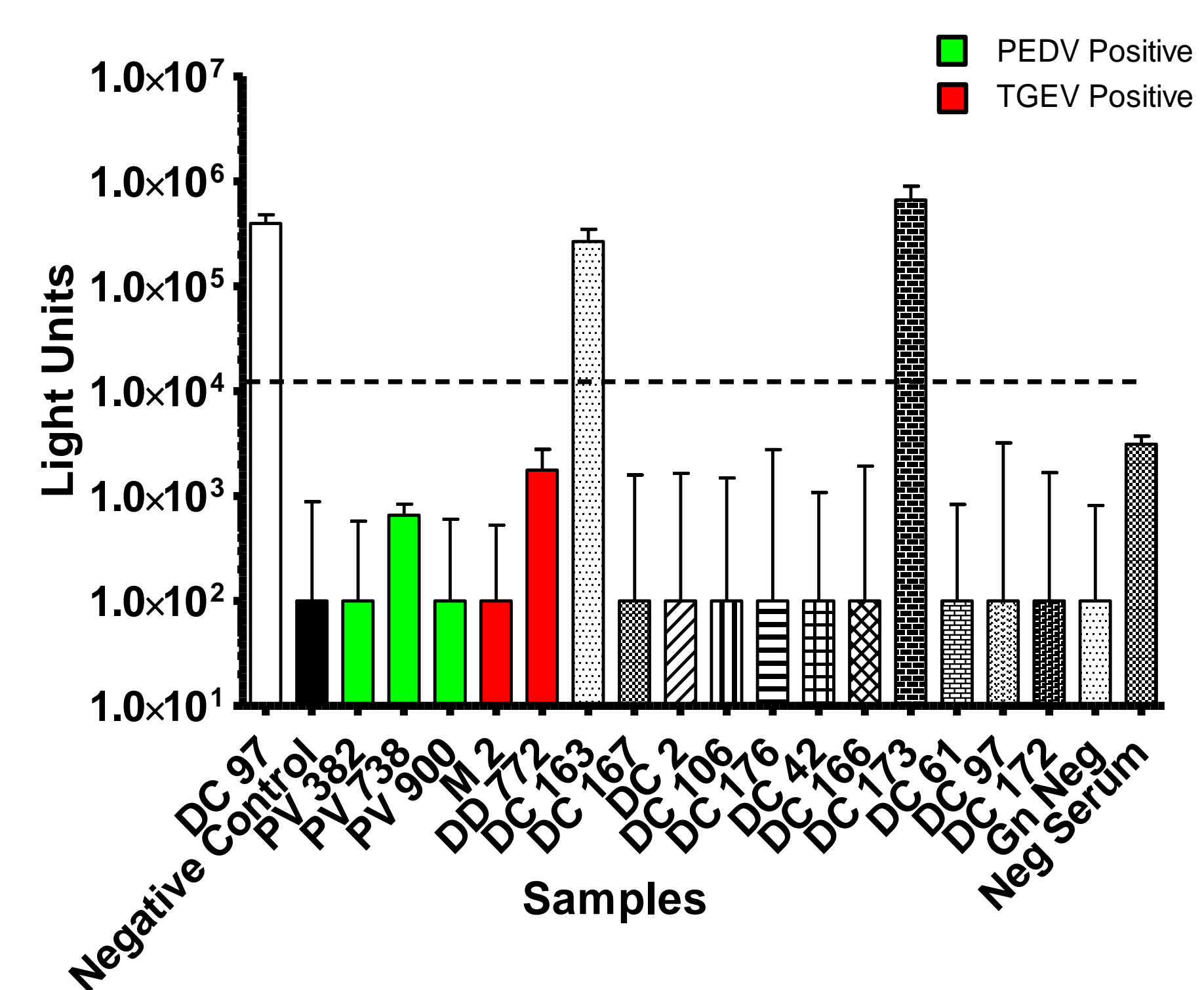
Nucleocapsid-NLuc lysate and two S1 90-167-NLuc lysate clones were tested to determine light units per  $\mu$ l of lysate using Promega Nano-glo<sup>®</sup> Luciferase Assay System.

Figure 4B. Precipitation of N-NLuc with hyperimmune serum



Hyperimmune serum from pig DC-97 and negative serum were used to establish cut off for positivity in assay. Experiments were conducted with  $1 \times 10^7$  LU of Nucleocapsid-NLuc lysate, 5  $\mu$ l Protein A beads (GenScript<sup>®</sup>) in triplicate for three replicates. Cut off was established as 5X standard deviation of negative values

Figure 4C. Testing Unknown Serum Samples



Validation of assay using pig samples previously tested for PDCoV, PEDV and TGEV. Samples testing positive in assay were DC 163, inoculated with PDCoV, serum collected 3 DPI, DC 173, inoculated with PDCoV, serum collected 28 DPI. Identity of samples was masked during the experiment.

## CONCLUSIONS & DISCUSSION

- ❖ Created N Luc expression tagging vector plasmid.
- ❖ Inserted PDCoV N and truncated S1 90-167 (Fig. 2) into N Luc expression plasmid.
- ❖ Nucleocapsid and S1 90-167 were able to pull down IgY on protein gel (Fig 3A) however, S1 90-167 was not detected in western blot.
- ❖ Assay was validated for recombinant protein expression (Fig 4A) and to establish cut off (Fig 4B). Using a pool of unknown porcine samples including several previously testing positive for PDCoV (Fig 4C). Samples previously testing positive for other coronaviruses (PDEV and TGEV) were also included. There was no evidence of cross reactivity.
- ❖ We have successfully established the PDCoV LIPS assay for pig serum utilizing recombinant Nucleocapsid protein as bait.
- ❖ The assay was validated in porcine samples with no cross reactivity detected towards other porcine coronaviruses.
- ❖ LIPS assay is currently being optimized using Spike 1 protein and in poultry samples.

## BIBLIOGRAPHY

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