# Rapid Barn-side Detection of Swine Pathogens

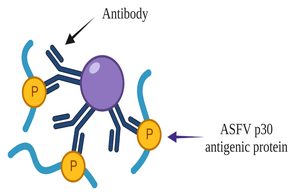

**Favour Akinfemi Ajibade[1], Anthony Jide Ogunbadewa[2], Solomon O. Odemuyiwa[2]**

[1]Faculty of Veterinary Medicine, University of Ibadan, Nigeria, [2]Veterinary Medical Diagnostic Laboratory, Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Missouri, Colombia, MO


Veterinary Research Scholars Program
University of Missouri

## BACKGROUND

- African swine fever virus (ASFV) and porcine reproductive and respiratory syndrome (PRRSV) cause financial losses to farmers in affected regions, with PRRSV causing over $660 million in annual losses in the United States. ASF is endemic in Nigeria. PRRS has not been previously reported in Nigeria.
- Acute ASF causes nearly 100% mortality. It is spreading rapidly to different parts of Europe and Asia. There is no effective vaccine against ASFV. Farm biosecurity programs and rapid barn-side assays to detect ASFV and PRRSV are crucial to preventing the spread of these diseases.
- Using the principle of antigen-antibody interaction, in which specific antibodies bind to their target antigen, we will develop an assay to screen samples for antibodies to the antigenic protein p30 of ASFV.


## AIM

- Compare the nucleic acid extraction efficiency of a manual magnetic rack system versus that of an automated KingFisher MagMax from a PRRSV-positive lung sample using real-time PCR.
- Express antigenic ASFV p30 protein in Cos-1 cells
- Develop rapid barn-side assays for monitoring ASFV and PRRSV in resource-constrained countries.

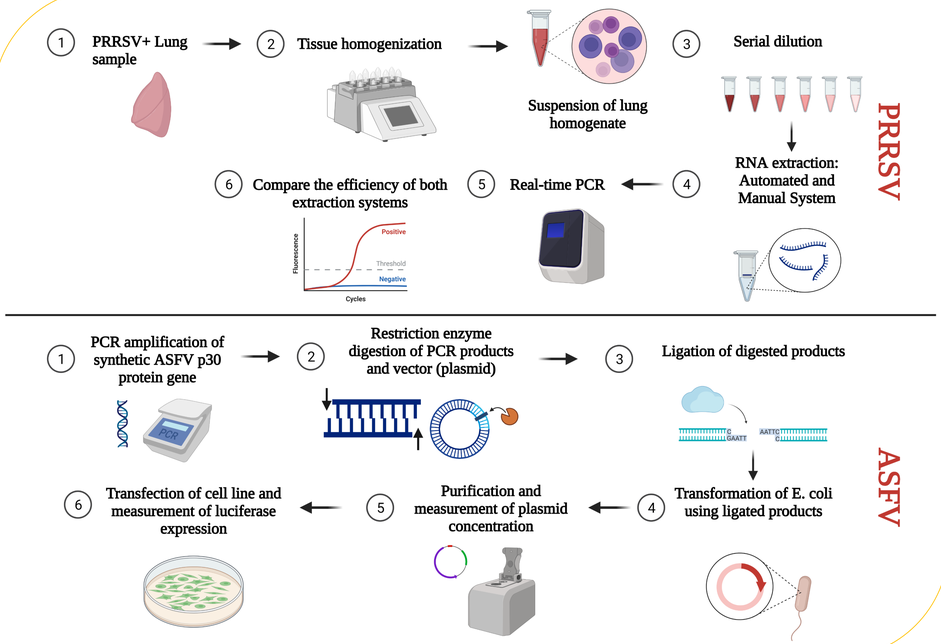
## HYPOTHESIS

The nucleic acid (NA) manual magnetic rack extraction system can be used to produce an amount of nucleic acid comparable to the automated KingFisgher MagMax System.

## ACKNOWLEDGMENTS


- **Student Support:** stipend was provided by the University of Missouri College of Veterinary Medicine Office of Research.
- Special thanks to the University of Missouri Veterinary Medical Diagnostic Laboratory staff for welcoming me to their workplace.


## METHODS




## METHODS

**PRRSV**: RNA was extracted from a PRRSV-positive lung sample using the automated MagMax and the magnetic rack system with the same reagents. The amount of nucleic acid obtained from both systems was then quantified using real-time PCR.

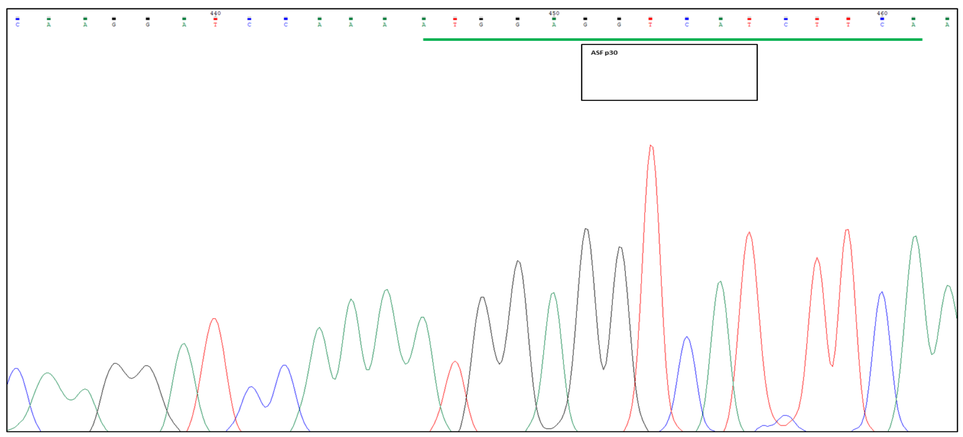
**ASFV:** Recombinant DNA containing the ASFV antigenic protein gene p30 was used to transfect Cos-1 cell line. Transfected cells were then stained with monoclonal antibodies to detect expressed p30 protein.

## FUTURE DIRECTIONS

- Validation and optimization of the luciferase assay for the rapid detection of antibodies against the antigenic p30 protein.
- Screening of collected pig samples in Nigeria using the luciferase assay and comparing results with that obtained from performing ELISA.
- Development and validation of an isothermal transcription-mediated amplification assay for PRRSV detection.

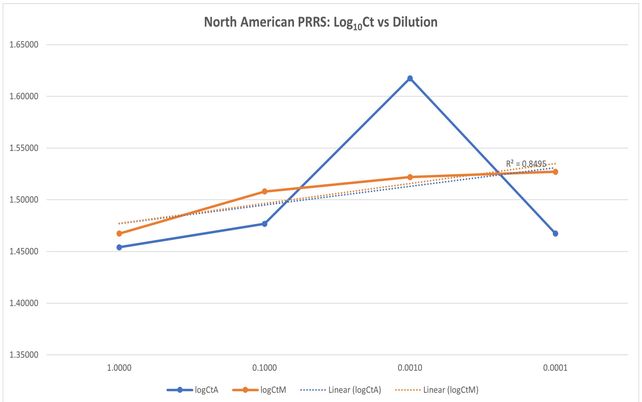
## RESULTS

- P30 was successfully ligated into pREN2 and confirmed by sequencing.




- The cycle threshold (CT) in real-time PCR is the number of amplification cycles required for the fluorescence signal to cross the threshold, i.e., exceeds the background level.
- Line plots of real-time PCR cycle thresholds of automated and manual magnetic rack system extracted RNA were plotted on the y-axis vs the dilutions on the x-axis. With a positive correlation of 0.85, the manual extraction system produced a comparable amount of nucleic acid as the automated extraction system.

## RESULTS




## CONCLUSION

- The manual magnetic rack system produces a comparable amount of nucleic acid to the automated system and can be effectively used for NA extraction in resource-constrained countries.
- ASFV antigenic p30 protein was successfully expressed in Cos -1 cell line.