Qualification of a Serum Neutralization Assay for Bovine Respiratory

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Abstract

Introduction

Bovine Respiratory Disease (BRD) is a significant cause of respiratory illnesses leading to death in cattle. It is commonly referred to as "shipping fever". BRD is a combination of viral infection, environmental factors, and bacterial infection. Viral infection is primarily caused by five respiratory viruses, including: bovine respiratory syncytial virus, bovine parainfluenza-3 virus, infectious bovine rhinotracheitis (bovine herpesvirus type-1), and bovine viral diarrhea virus types 1 and 2. These diseases currently require neutralization assays.

My research focuses on the qualification of a neutralization assay for Bovine Respiratory Syncytial Virus (BRSV) specifically. . The history of epidemics related to BRSV include an initial outbreak in the mid-80s, the disease is still likely to be found in isolated herds. As the disease becomes more endemic, viral-bacterial synergism in BRD has taken the focus. Synergy occurs in part due to bronchial epithelial cell necrosis and inhibition of pulmonary alveolar macrophage functions. Proper treatment and control of BRSV rely upon recognition, diagnosis, and treatment of cattle when initially infected with one of the viruses mentioned above.

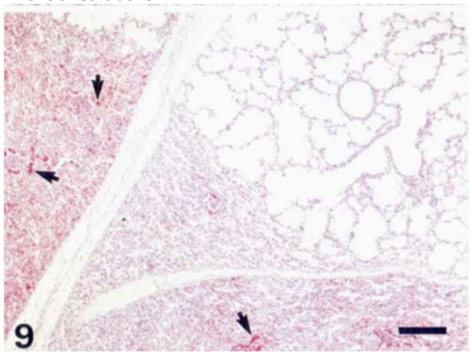

Figure 1: Example BRSV infected bovine cells.

Objective

The objective of this project is qualification of a neutralization assay for BRSV. We qualify and validate the virus neutralization assay protocol for diagnostic use for BRSV. As we qualify the protocols, we optimize the assay to decrease the variability in test results. We evaluate factors such as cell line, virus concentration, and incubation time. The qualified assays will allow for identification of cattle that have been exposed to BRSV.

Methods

Preparing Assay

When creating an assay of this style there are two typical methods that can be used. Working with 96 well plates will require either a constant virus and varying serum, or varying virus with a constant serum.
We will be using a constant virus with varying serum due to virus being more readily available than serum.
-Prepare 96 well plate with a constant virus concentration of 100-150
-Apply a varying dilution of serum to each row of wells
-Incubate
-Observe plates to view the affect that has occurred based on the serum dilutions.

Figure 2: Assay Qualification design steps

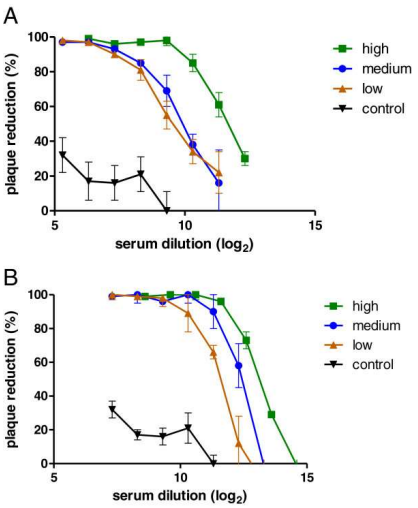

Figure 3: Example of a standard Qualification Assay

Conclusions

Our initial results on testing different Bovine Serums to identify the best serum to use have been inconclusive. Result numbers have been low and further testing has begun to try and isolate variables and give consistent outcomes with B-HV. Additional serums can also be studied. However, results with B-PIV3 have shown a more reliable process to be developed into a Qualification Assay.

Future Studies

One of the steps for creating a Qualification Assay is the optimizing of the assay. After results have been collected for our current methods, we will attempt to optimize the assay by selecting different incubation times or infection times. Additional serum testing will be required.

Other variables can be discussed and adjusted as they arise. The final steps for the study will be to verify the assay with several repetitions of the methods to show consistency in neutralization assay for BRSV.

Acknowledgements

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