

Spotlight on Science

Emerging Disease Surveillance and Diagnostic Method Development for Veterinary Medicine



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What is the main focus of your facility?

The University of Minnesota Veterinary Diagnostic Laboratory (VDL) is accredited by the American Association of Veterinary Laboratory Diagnosticians (AAVLD) and serves veterinarians, scientists, educators, companion animal owners, and the livestock and poultry industry by identifying and monitoring emerging diseases and developing new diagnostic methods. We utilize a variety of methods for pathogen identification, including bacteriology, serology, viral isolation, histopathology, and a wide array of molecular diagnostic tests. These include viral and bacterial qPCR tests, Sanger sequencing for molecular subtyping, and next-generation sequencing (NGS) for unknown pathogen identification and complete viral/bacterial genome sequencing. I work in the Molecular Development section

of the VDL where our team participates in collaborative research projects to monitor new pathogenic strains infecting animals from agricultural sources or the wild. We update and create new qPCR tests to respond to evolving pathogenic strains and utilize shotgun metagenomic NGS for samples without any other reasonable diagnosis.

What are the implications of viral exposure in an animal colony?

Rapid identification of viral pathogens among agricultural sites is extremely important to minimize dissemination. For example, the pork production industry relies on multiple geographical sites across the country for animal development, which can minimize viral infection between sows and piglets. However, this method can also allow for greater spread of viral pathogens among different farms. PRRS and PCV2 viruses have had detrimental animal and economic effects on the industry. We are constantly monitoring these and other viruses for substantial molecular changes and work with industry partners to update vaccines when necessary.

What tools are you currently using to evaluate these animals and what are the limitations associated with these tools?

Currently, qPCR testing of animal samples is our fastest and cheapest method for accurate viral and bacterial identification. However, the major limitation of these tests is they require previous knowledge of the viral pathogen DNA/RNA sequence. Rapidly evolving pathogens can escape sequence-specific qPCR testing, leading to false negative results and greater potential for pathogen spread. In cases that are suspected to be pathogenic, but are qPCR-negative, we often employ shotgun metagenomic short-read NGS. This method has the unique ability to sequence any nucleic acid in the sample without any previous sequence knowledge. Using bioinformatic tools, these data are compared against databases of known sequences to help identify possible pathogens. Even if we cannot find exact matches, we can often identify sequences that appear to have viral or bacterial origin which help us characterize potentially new microbes.

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How has Trio RNA-Seq enabled your work?

A major limitation of shotgun metagenomic NGS is the presence of host sequences in our samples. Host rRNA is a major component of all host cells and can negatively affect the detection of low-abundance microbes/viruses. Thus, the NuGEN Trio RNA-Seq library prep kit has substantially improved our results, primarily due to their AnyDeplete methodology. We provided NuGEN with the most abundant rRNA sequences found in our samples and they designed oligos to specifically deplete these sequences from our Illumina sequencing libraries. This method has greatly increased the percentage of viral/bacterial sequences in our final sequencing data. This has allowed for easier pathogen identification and genome assembly.

What are the advantages of upfront SPIA amplification for viral detection?

We get lots of samples from lots of different organisms with varying levels of RNA quality and viral loads. Pre-amplification using SPIA, allows us to have a single workflow for all these sample types. Additionally the amplification increases the

chances of detecting rare or low abundant infectious transcripts.

How do you see NGS providing value to your services?

In many cases, a pathologist has exhausted common diagnostic methods and has turned to NGS for possible diagnostic clarification. In other cases, if a sample was qPCR-positive for a particular virus, we use NGS to obtain the complete genome sequence, only to discover that a different virus was much more abundant. Thus, using NGS will allow for identification and genome assembly of pathogens, but will also allow us to study the entire virome of important production animals — which simply cannot be done with traditional technologies.

What are the ultimate goals of this work?

The VDL Molecular Development lab is committed to offering NGS services for diagnostic testing purposes in a variety of samples types. We believe there are many important symbiotic relationships between hosts and microbes, whether mutualistic, commensalistic, or parasitic. In addition, many subtyping methods currently depend on sequencing only a very small region of a microbe's genome,

which can leave us blind to other molecular changes in the organism. However, complete de novo genome assembly via NGS will greatly increase our understanding of molecular evolution in important pathogens and may help determine appropriate treatment or containment responses.

For more information on Trio RNA-Seq, please visit our website @nugen.com/products/trio-rna-seq or contact your local account executive.