

METAGENOMICS

TECHNICAL PAPER

Cambridge Technologies

KEY POINTS:

- 1** METAGENOMICS, A NEXT GENERATION SEQUENCING TECHNIQUE, IDENTIFIES ALL PATHOGENS IN A SAMPLE, KNOWN OR UNKNOWN, INCREASING DIAGNOSTIC CAPABILITY.
- 2** PROACTIVE USE OF METAGENOMICS CAN HELP DETECT AND MANAGE DISEASE WITHIN LARGE PRODUCTION SYSTEMS.
- 3** VERY USEFUL TOOL IN CASES WITH UNCLEAR ETIOLOGY AND IN COMPLEX DISEASES.
- 4** THE ABILITY OF METAGENOMICS TO IDENTIFY NEW AND EMERGING PATHOGENS MAKES IT AN INVALUABLE TOOL IN THE DEVELOPMENT OF AUTOGENOUS VACCINES.

INTRODUCTION

The next generation in molecular sequencing for the detection and identification of viruses and bacteria includes a technique known as metagenomic sequencing. The development and adoption of metagenomic methods in the diagnostic laboratory has greatly elevated the diagnostic capabilities in challenging cases. The process involves a direct genetic analysis of the genomes contained within an environmental sample such as a nasal swab or a fecal sample, allowing for simultaneous detection of multiple infectious agents within that one sample⁴.

Today, forward thinking diagnostic laboratories such as Cambridge Technologies utilize metagenomic diagnostics to take their pathogen detection and tracking techniques to the next level. Not only does it enhance the

capabilities for diagnosis of complex disease cases, it can also be used to proactively detect the presence of infectious agents within a production system which can help focus health management efforts. In addition, by using metagenomics to identify all potential pathogens involved in a disease process, autogenous vaccine formulations can be developed that best fit the need.

ADVANTAGES OF METAGENOMIC DIAGNOSTICS

Traditional diagnostic platforms such as PCR are somewhat limited by process, amount of data produced, and – perhaps most importantly – the tendency to only identify known pathogens already linked to particular clinical syndromes². When faced with a unique case, historically diagnostic labs would have to run multiple tests trying to

identify a single pathogen (e.g. Sanger sequencing at \$80 per gene). However, with metagenomic sequencing, a single \$250 test can produce a large number of genetic reads as it sequences all viruses and bacteria present in the sample.



Metagenomic technology allows diagnostic scientists to gather large amounts of data within a relatively quick turn-around time. One of the most exciting uses for metagenomic diagnostics is the ability for detection of novel viruses and/or viral variants¹. Often, a virus for which little information is known can be identified, such as was the case recently with PCV3 in swine and Influenza D in cattle.

APPLICATIONS

Metagenomic sequencing is very useful when confronted with a clinical disease where the cause is complex or unclear. In some cases the clinical signs are unusual and not associated with a known pathogen and in other cases the clinical signs point to a known pathogen but that pathogen cannot be found through other diagnostic methods.

In 2015, the Kansas State VDL was presented with a case of 700 five to 14-week-old pigs with an unknown

neurological disease. The piglets exhibited intention tremors, increased respiratory rates, and eventually an inability to swallow. The diagnosticians turned to metagenomics when conventional diagnostics failed to identify any pathogens. A brain homogenate prepared from a number of affected pigs was sequenced, and atypical porcine pestivirus (APPV) was identified.

In another case, PCV2 was suspected based on clinical presentation, however, tissues were negative for PCV2 by both PCR and IHC. When metagenomic sequencing was run, the results revealed a novel circovirus – PCV3.

In a case of respiratory disease in cattle, Mitra, et al., detected twenty-one different viruses via metagenomic sequencing of nasal swabs from feedlot cattle suffering from acute BRD. In addition to already-known viruses, two more genotypes were identified, a newly-proposed species of bocaparvovirus was characterized, and the first North American appearance of ungulate tetraparvovirus 1 was detected³.

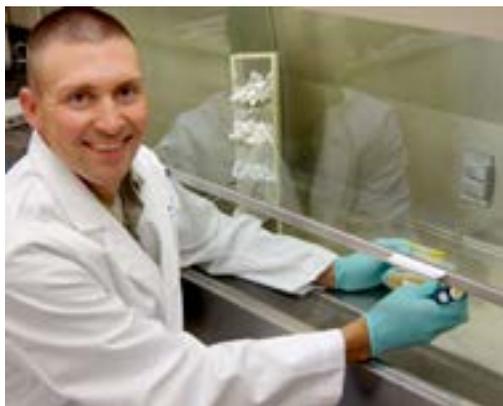
Metagenomics can be used proactively to help answer questions about potential disease transmission by offering the ability to profile animals coming from multiple sources, points of comingling and concentration, and transport vehicles moving in and out. Longitudinal metagenomic profiling can be used to identify virus and bacteria changes taking place in a herd over time. This information can help veterinarians and

producers identify times of transmission and exposure to infectious agents and develop improved health management and biosecurity plans.

Another proactive use of metagenomics is in feedback exposure programs, a common method of introducing a virus to a sow herd in order to produce maternal antibodies for that particular virus. While effective, it can be risky as there may be other agents in a sample that are unidentified by PCR. For example, in the case of APPV, there is the potential for infecting pigs in utero and producing persistently infected animals. Metagenomic sequencing gives veterinarians the opportunity to screen feedback material before introducing it into a general sow population.

CAMBRIDGE TECHNOLOGIES

Cambridge Technologies has embraced metagenomics as a cornerstone of their diagnostic offerings. The ability of metagenomic sequencing to reveal the existence of new and/or emerging pathogens makes it a perfect tool in the development of autogenous vaccines, as products can be formulated to combat these unique threats before they become



widespread. Utilizing techniques such as metagenomics and next-generation sequencing, diagnosticians can identify the specific threats and antigens that need to be included in the product. Armed with that information, the experienced Production team can then formulate and manufacture a vaccine customized to the needs of each individual customer, including antigen concentration, multiple adjuvant choices, dose sizes, and the option of SoliDose implants in cattle products. Should a new strain or agent be discovered in the future, upcoming manufacturing runs of the autogenous product can be revised to include the new threat.

CONCLUSION

Metagenomics have revolutionized the diagnostic process in not only speed and amount of data mined, but perhaps most importantly in detection of new and emerging pathogens. Laboratories such as Cambridge Technologies utilize metagenomic technology to not only present a clear picture of the current disease threat, but also to direct what, if any, further work is needed.

Early detection and classification of disease threats is a critical aspect of any successful herd health protocol. The metagenomic approach allows Cambridge Technologies diagnostic scientists to stay on the leading edge of tracking the spread and evolution of pathogens, while simultaneously giving them the information needed to build custom vaccine solutions targeting these developing challenges.

SOURCES

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