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CLINICAL APPLICATIONS OF NEXT-GENERATION SEQUENCING AND MICROBIOME ANALYSIS:

The Solution to No-Growth Results

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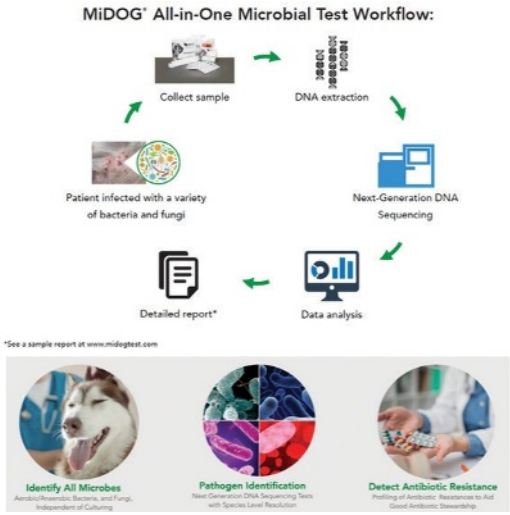
Effective treatment of a disease requires an accurate diagnosis. Infectious diseases are no exception to this rule. However, more than 90 million Americans are suffering from chronic infectious diseases each year. By 2050, an estimated 10 million people will die from bacterial infections that are caused by multi-resistant bacteria worldwide.

A striking 75% of emerging pathogens are zoonotic[1]. Today, for every antibiotic that has been developed, pathogens have been isolated that are resistant to the treatment[2]. Culture testing remains the standard diagnostic tool for detection, diagnosis, and treatment decisions of these pathogens and their antibiotic resistances, especially in veterinary medicine. This approach is centuries old and inadequate to give a complete picture of what is causing the infections. Based on novel molecular-based DNA technologies, we now know more than 10^{20} microbes from three different domains of life inhabit this world together with us. They affect our metabolism, health, and can cause infectious diseases. About 1% of all these microbes are in fact culturable in the lab[3]. The other 99% go unrecognized by culture methods and are often missed upon analysis of infectious disease cases. The end result is recurring and chronic infections, for us and our animals. Molecular diagnostics tools, such as DNA sequencing, are available to aid this urgent medical need to detect and identify all culprits that are causing infections. Specifically, next-generation DNA sequencing (NGS) offers the diagnosis of infections using microbial DNA as the analyte and bypasses culture testing along with its deficiencies. The 'no growth' result often associated with culturing is no longer an issue, as no clinical sample is sterile, even urine harbors several bacteria and fungi[4]. For NGS diagnostics, microbial, genomic DNA is extracted from clinical samples (i.e. urine, feces, or skin/ear/nose/throat swabs), then purified and tagged. The resulting DNA molecules are copied thousands of times and massively sequenced in parallel. Thus, millions of DNA sequence reads from all microbes comprising a sample are being analyzed at once. These DNA reads are then filtered, quality controlled, and compared to genomic databases to assign the microbial species identity and



pathogenicity. This process provides a complete picture of which microbes are present and allows the quantitation of each species at the time and sight of sample collection from the patient. Opposite to targeted PCR panels, NGS is an untargeted and de-novo approach to identifying microbial pathogens, so no prior knowledge about the sample is needed. NGS technology has been widely adapted to study the 'microbiome', or the 'microbial profile', in all kinds of different sample types from humans and animals. The utility of this technology to not only detect all microbes present, but also the ability to identify novel pathogens and biofilms has warranted its application in human clinical diagnostics to achieve better health outcomes for patients.

Today, NGS-based diagnostic tools are finding their way into veterinary practices as well: MiDOG, LLC offers NGS to veterinarians to further improve infection diagnostics while also aiding good antibiotic stewardship. A new collaboration between Western University of Health Sciences, College of Veterinary Medicine and MiDOG (Irvine, CA) has helped introduce student scholars to NGS microbial sequencing-based diagnostics. Pilot studies[5] from this collaboration highlighted the utility and need for NGS diagnostics in veterinary medicine for different sample types (i.e. urine, skin swabs, oral swabs for periodontitis cases, feces) and species continued →

(i.e. cats, dogs, frogs, geese), while other studies remain ongoing. The MiDOG® All-in-One Test used for NGS diagnostics allows the detection of all bacteria and fungi comprising a sample, including antibiotic resistance information. Specifically, for urinary tract infections (UTI) culture testing can be insufficient as several pathogens are anaerobic or particularly difficult to culture (case study example available online, https://www.midogtest.com/urinary-tract-infection-case-study).

A recent study reported that indeed urine samples from dogs suspected of having UTIs demonstrate 64% negative culture results[6]. Using an NGS approach instead, this study showed that dogs with clinical signs of UTI frequently presented with bacteria (i.e. *Mycoplasma canis*, *Mycoplasma cynos, Proteus mirabilis, Klebsiella oxytoca*, and *Escherichia coli*) as well as fungi (i.e. P*enicillium sp., Ascomycota sp.*), confirming the UTI. Several of these pathogenic microbes were previously undetectable in the individual patients based on culture testing. Consequently, NGS diagnostics allow for actionable clinical application through targeted use of antibiotics to improve patient outcomes.

MiDOG's NGS microbial sequencing-technology optimizes patient care and can easily be incorporated into the veterinary clinic (Figure 1). The world of microbes impacts animal health dramatically and knowing exactly which microbes are present, and how they respond to a treatment, will undoubtedly assist in saving lives. MiDOG enables better diagnosis for guided treatment decisions. **P**

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