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New diagnostic technologies: What do they mean for the veterinary practitioner?

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In the last three issues of *Swine Health and Production*, we have featured a series of "Diagnostic Notes" that have described the methodology and technology of some relatively new diagnostic tools, including polymerase chain reaction (PCR),[1] immunohistochemistry (IHC),[2] and in situ hybridization (ISH).[3] What will these new technologies mean to you? Will they make your life as a clinical diagnostician easier or more complicated? Will these techniques give you a better idea of what is happening in your clients' herds or merely give you more data?

While these new techniques promise dramatic advances for veterinary diagnostics, it is important to realize that each of these new tests, just like the older techniques, has intrinsic advantages and disadvantages. There is no one perfect test. Selecting the "best test' is a question of considering how these multiple factors interrelate to answer the specific question you're asking. Below I discuss some of the key issues from the perspective of a veterinary diagnostic pathologist.

Antemortem versus postmortem

Of the three tests described, only the polymerase chain reaction (PCR) test has great potential to become a widely used antemortem diagnostic tool because it can be readily applied to antemortem samples such as blood, serum, semen, feces, biopsies, and swabs. The extreme sensitivity of the PCR test will likely make it the preferred test in the future whenever a high degree of sensitivity is required. It is commonly used on boar semen to detect porcine reproductive and respiratory syndrome virus (PRRSV) shedding, providing valuable information to semen companies. However, PCR's promise of high sensitivity is not always realized. For example, the cattle industry is very interested in detecting low-level fecal shedders for Johne's disease, but interfering substances in the fecal matter have not allowed the PCR test to perform to its high theoretical potential. Swine diagnosticians may find similar frustrations when applying the PCR test to swine feces to detect animals shedding *Lawsonia*, *Salmonella*, or *Serpulina*.

As mentioned above, the PCR test can be readily used as an antemortem tool. The technology should adapt to almost any infectious agent. Clinicians should tell diagnosticians their immediate needs, as well as their hopes and dreams, for antemortem monitoring. Clear dialogue and communication will allow diagnostic labs to direct research and development toward the most useful areas.

Once a new antemortem test is perfected and in place, it may be used to keep a negative herd negative by testing all herd additions. Under this scenario, using the most sensitive antemortem test available is obviously the key to success.

Antemortem testing may not be solely limited to the PCR test. Recently, researchers from the University of Nebraska have demonstrated that the immunohistochemistry (IHC) test applied to skin biopsies can also be used to detect cattle persistently infected with BVD virus, and similar uses may be discovered in swine.

Although not covered in our series, improvements and additional test offerings are continually being made in the field of serology. Serology will continue to be an important antemortem monitoring tool in the future.

Speed is relative

Speed is frequently mentioned as a major advantage of the new diagnostic technologies. However, speed is relative, and we must know exactly what is being compared to make a fair judgment. It may be true that the PCR is faster than a standard cell-culture virus isolation test that can take days to weeks to grow a virus. However at this time in most labs in the United States, the fluorescent antibody test (FA) would routinely be completed much sooner than the PCR test. The FA test is quick and simple compared to the many steps involved with PCR testing, but may be less sensitive. Furthermore, if PCR testing becomes a popular test for pathogen identification, then batching of numerous samples will become necessary, which will create lag periods while samples wait for their turn in the next test cycle.

Immunohistochemistry (IHC) and in situ hybridization (ISH) both require that the tissues be fixed in formalin for several hours, processed in a tissue processor for several more hours, individually stained for each suspect disease for several minutes, and then individually examined under the microscope for several more minutes. The FA test is performed on quality fresh tissue that is quick frozen (minutes), individually stained for each disease (minutes), and individually examined under the microscope (several minutes). If tissue quality is high, the results can be quite comparable for FA, IHC, and ISH. If the fresh tissue has decomposed in transit (a common problem), then the FA test will not perform well. The formalin-preserved

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samples travel better in transit and give IHC and ISH a large advantage in that regard. However, over-fixation can also be a potential problem for IHC and ISH, possibly decreasing sensitivity.

Cost is relative

To fairly judge the cost issue, we must know exactly what we are comparing. To be fair to any new test, we cannot merely look up the fee for the traditional test (for example a culture test) in the state diagnostic lab fee schedule and compare it to the new test. Most state labs are subsidized as key components of their state's agriculture industry. If true cost accounting is done for the new test, the same must be done for the traditional test to make a fair comparison. Any fantastic new test that would be highly useful to the industry will usually be offered at a reasonable fee.

The economic benefit to the swine industry for a highly accurate test will also effect its future availability. If the information saves major dollars for the industry, it would be willing to pay for a more expensive test. At the same time, efficiency of volume, more suppliers of reagents and equipment, as well as the desire to enhance diagnostic success will gradually reduce fees for key tests.

Focused identification versus unknown pathogen identification

Routinely, a bacterial culture takes 24–48 hours and a PCR test can be done in a few hours. However, you must perform a separate PCR test for each organism you want to detect. When we streak a blood agar plate, our potential to isolate a large variety of bacteria is great (nonselective). However, when we run a PCR test, we must run an "individual" assay for each suspect organism (absolutely selective). In general, traditional culture tests (viral or bacterial) have the ability to simultaneously detect a broad spectrum of organisms, and this may be the most desirable approach when dealing with many unknown potential pathogens. The typical scenario practitioners and diagnosticians deal with during acute disease investigations is that of many unknown potential pathogens.

Focused identification may be the preferred method when dealing with specific regulated diseases, or diseases caused by organisms difficult to grow or identify, or when screening replacement animals for specific diseases. The potential for enhanced sensitivity and specificity of the new tests would be advantageous under such circumstances.

The best degree of sensitivity

New ultrasensitive tests (such as the PCR test) have raised a new issue in diagnostic medicine: can a test be too sensitive? There is still much to learn in this area. For example, the PCR test may readily pick up vaccine virus, requiring additional tests or more specific tests to determine whether an organism is of wild or vaccine origin. The PCR test might pick up more normal flora than other tests (i.e., more readily identify "potential" pathogens). Will this information help or hinder interpretation? The PCR test might pick up the etiological agents of silent resolving infections weeks or months beyond onset. This phenomenon may vary by pathogen. Will this cloud the question of what is causing the current outbreak? All of these issues make it necessary to ask what the right degree of sensitivity is for the specific question being asked. Research and additional experience will hopefully guide us through this maze of unknowns.

What can enhanced sensitivity do for disease investigation?

On the other hand, enhanced sensitivity opens up many new exciting opportunities in disease investigation. Some old disease syndromes may be clarified. We may have to rethink current dogma about some old disease syndromes. For example, the role of different types of *Clostridium perfringens* isolated from cases of baby pig enteritis is being rethought in light of PCR, which can precisely type *Clostridium* isolates.

There may also be new syndromes waiting to be discovered. Diseases that are caused by organisms difficult to grow or identify by traditional methods may be readily evaluated for their disease potential with any of the new tools described in our series. An example from bovine medicine is *Neospora*. This protozoan is a major cause of abortion in cattle, but cannot be easily grown in culture and is difficult to identify in tissue sections. However, IHC staining easily demonstrates this organism and precisely confirms *Neospora* infection. There may be other pathogens that are currently under-diagnosed because of difficulties in routinely confirming their involvement in specific disease syndromes. Alternative new methods to identify such infections should clarify difficult disease syndromes.

Lastly, the evaluation of pathogens at the molecular level (such as with the PCR) should allow for more precise sorting into different strains. Molecular differentiation of strains will also be a valuable aid in epidemiology studies.

The best test reviewed

The usefulness of new test technology will evolve over time and by trial and error. Not all labs will approach diagnostic investigations in an identical manner, nor do they need to if they achieve the same results using different diagnostic tools. A new test is not automatically the best test just because it is new. Your evaluation of a diagnostic workup should be based on whether it answers the questions you needed answered, not on what type of test was used. In such an evaluation you must factor in the specific choices you and the animal owner made in determining the testing protocol. Were the proper tests requested to answer your specific questions? Complicated epidemiological questions may require consultation with an epidemiologist to develop a specific test strategy to answer your questions with an acceptable degree of confidence.

Diagnostic labs continually evaluate new technology in light of current technology, cost, speed, consistency, and accuracy. New tests that perform well under those parameters will eventually become a routine test offering in all labs. New tests that struggle under the same parameters may find specialty niches, be dropped, or be replaced by an even

better test in the future.

How, then, do we determine what is the best diagnostic test?[4] The answer still depends on the following questions:

- What is the specific question you are attempting to answer?
- What degree of sensitivity and specificity is acceptable?
- Is test speed an issue?
- Is cost an issue?
- Is the test readily available?

I am convinced that the new diagnostic technologies we've described in our Diagnostic Notes series this year will positively enhance our overall understanding of animal disease syndromes, increase our diagnostic success rates, and enhance our abilities to strategically monitor diseases circulating through large populations of animals.

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