

The “best” diagnostic test

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As a diagnostic veterinary pathologist, practitioners occasionally ask me “what is the best diagnostic test for . . . ?” for a variety of disease situations. The practitioner hopes for a simple bottom-line answer, which is often difficult to produce without several additional questions. There are a number of criteria that determine the “best test” for any given situation, including cost, sensitivity, specificity, speed, and availability. All of these criteria must be correlated to the specific situation. This paper will discuss some of the issues that go into determining “the best diagnostic test.”

What is the specific question you are attempting to answer?

This question is critical. Are we attempting to identify clinical disease? Are we looking for proven carriers? Are we looking for exposed animals that are possibly carriers? Are we looking for subclinical disease? Are we looking for immune or naive animals? Are there regulatory rules that apply? Are we . . . ? Our diagnostic training and diagnostic laboratory infrastructure in the United States largely has been focused on two major goals:

- Identifying the precise cause of acute disease outbreaks, which enables precise control methods to be quickly implemented, while collecting disease data to enable management strategy changes that will prevent the same problem in the next production cycle.
- Identifying and eliminating known exposed animals (largely by serology) to eradicate certain regulated diseases (e.g., brucellosis).

My golden rule of clinical disease diagnosis is relatively straightforward:

- first, identify pathological lesions that explain the clinical signs, and
- then, identify pathogens or factors (trauma, toxins, environment, etc.) that are known to produce those lesions.

Lesion and pathogen (or factor) identification are both critical and work synergistically to produce a definitive diagnosis. For infectious disease investigations, observing lesions without microbiological support considerably narrows the diagnostic possibilities, but a definitive diagnosis may not be possible with lesions alone. For example, purulent meningitis in a 2-week-old pig indicates bacterial meningitis. However, for a definitive diagnosis, cultures would be needed to determine whether it was caused by *Streptococcus suis*, *Haemophilus parasuis*, *Escherichia coli*, or others. Conversely, identifying microbes without corresponding pathological lesions is merely identifying potential pathogens since many of the common disease-producing agents are ubiquitous in the animal population and the environment.

Diagnostic notes are not peer-reviewed.

Matching lesions to pathogens prevents us from mistakenly blaming incidentally identified organisms during the course of an outbreak investigation.

If the goal of the diagnostic test is something other than to diagnose clinical disease, then it becomes even more important to clarify the precise goal of the test. For example, if you are trying to determine whether a herd is clean of a particular disease, factors to consider include sensitivity and specificity of the test, expected prevalence level, and a satisfactory confidence level. It may be necessary to consult an epidemiologist to determine how many animals optimally to test under various conditions.

What level of sensitivity and specificity is acceptable?

Simply defined, sensitivity is the ability of a test to detect all true positives, whereas specificity is the ability of a test to detect only true positives. If a test is 100% sensitive, there will be no false negatives (no missed true positives). If a test is 100% specific, there will be no false positives (no missed true negatives). Discussion of sensitivity and specificity should occur together, since they frequently affect each other. Efforts to enhance sensitivity may decrease specificity, and efforts to enhance specificity may decrease sensitivity. If a test was 100% sensitive and 100% positive, it would be a perfect test. Unfortunately, there are very few perfect diagnostic tests in the world, and we have to settle for a certain level of acceptability.

The acceptability level should be related to the specific question we are attempting to answer. For example, once a decision is made to eradicate a particular disease, regulatory officials frequently choose a highly sensitive test to identify infected individuals. The most sensitive test possible would be preferred, even if specificity was slightly compromised, because during eradication a rare false positive (truly negative) would be better for the eradication process than a rare false negative (truly positive). The false-negative animal could be passed or shipped and continue to spread the disease to other populations, thus seriously setting back the eradication process.

In general, sensitivity is paramount when testing animals that might bring in a disease to a clean group and cause an outbreak. Specificity becomes paramount when there is concern that a false positive might end the usefulness of a valuable animal (such as valuable breeding stock).

Is test speed an issue?

The speed of a test is always an issue when investigating outbreaks of acute clinical disease. Veterinarians are waiting for test results to make

or modify treatment and management strategies to alleviate the affects of the outbreak. In large production units, delays in test results can add to the economic losses of the producer. Usually there are numerous tests being conducted simultaneously during an infectious disease outbreak, such as bacterial cultures, antibiotic sensitivity tests, fluorescent antibody tests for viruses, electron microscopy for viruses, cell culture for viruses, immunohistochemistry tests, parasitology tests, serology tests, toxicology tests, and histopathology. Some test results are completed and available before others. It is tempting to run with the results of the quicker tests, but it is best if those early results are viewed and shared with the producer as “preliminary results” until all testing is completed. For example, isolating *S. suis* from the brain on the second day of testing would be insignificant if on the fourth day pseudorabies virus was isolated with histological evidence of viral encephalitis. The pathologists role as case coordinator is to be sure the history, clinical signs, lesions, and test results all correlate to render a definitive diagnosis.

When testing does not involve acute clinical disease outbreaks, then the speed of the test becomes less of a “test issue” and more of a “time management issue.” If the animals are being tested for exportation, the following must be coordinated with the anticipated animal shipping date:

- bleeding time,
- specimen shipping time,
- testing schedule of the lab,
- time needed to complete the test once set up, and
- time needed to receive the paperwork.

The laboratory generally cannot choose what type of test to perform for export or other regulatory purposes, since it is usually mandated by regulatory officials.

Is cost an issue?

In veterinary medicine, cost is always an issue. The animal populations that we deal with all have an economic value. The cost of controlling a particular disease has to be correlated to the cost of the disease in terms of production loss. Diagnostic testing is just one item on the total bill for disease control. If the combined cost of disease control pays for itself by production savings, then it's an easy sell to our customers. In one situation, a \$10 test might be useful but a \$25 test might not satisfy the criterion of economic usefulness. In another situation, a \$50 test might be very useful in preventing economic loss. Again, we should clarify the specific question we are attempting to answer with the test and then assess how economically useful that answer will be. What the client ultimately is willing to pay for diagnostic testing always will be determined by long-term and/or short-term economic advantages.

The popularity of a test often affects the price. Once demand for a test becomes heavy, the necessary equipment and reagents tend to become more commonplace and less expensive. People working with the test tend to find better ways of doing it or ways to automate it. Efficiency of volume also comes into play. Diagnostic labs must consider how the

costs of new tests compare to the costs of older tests, as well as their various other advantages and disadvantages when adding to or deleting from their test offerings.

Is the test available?

Some tests might be useful for research purposes, but not as useful or available for routine diagnostics. Even a perfect test, unless readily available, can't help the industry. Factors frequently involved include the lack of reagents, specific equipment, or specific skills. Shipment regulations across national borders can also be complicating factors. Patent issues also can create road blocks to availability. Environmental and safety concerns are important. For example, an excellent test that uses radioactive isotopes might be fine for limited research purposes, but not conducive to large-scale use for routine diagnostics.

What tests a particular laboratory chooses to place on line often depends on the demand in its area. For example, a state diagnostic lab in a state that has very few pigs may choose to cooperate with another state lab for some of the uncommon submission requests. This type of cooperative effort has been fostered by the American Association of Veterinary Laboratory Diagnosticians (AAVLD), a national organization dedicated to promote and improve veterinary diagnostics.

Summary

The first step in selecting the “best test” always should be defining the testing goals. In everyday language, the best test will be the quickest, cheapest, and most reliable test available to meet those goals.

These are exciting times in the diagnostic industry. There has been an explosion of new testing technology in the laboratory that is finding practical applications. Diagnostic labs are working to compare the advantages and disadvantages of the older tests to the new tests. All of the factors discussed above come into play when a lab decides to change or not to change to a new test. Newer isn't automatically better and sometimes brings its own set of new problems. However, there are currently great hopes for creating many improved tests using the latest technology, especially molecular technology.

Cooperation among practitioners groups such as the AASP and diagnostic groups such as the AAVLD can greatly enhance the development of new useful diagnostic tests. Diagnosticians need swine practitioners to tell them what their future diagnostic strategies will be. The established diagnostic strategy in the midst of high morbidity/mortality acute outbreaks will remain about the same. However, the routine diagnostic strategies in larger production units likely will shift towards more ante-mortem monitoring diagnostics as well as more routine necropsies for monitoring purposes even during periods with low mortality/morbidity. A dialogue between practitioners of the AASP and diagnosticians of the AAVLD should enable the diagnostic lab research and development efforts to be focused and on target with the demands of the practitioners.

