New Diagnostic Advances: Syndromic Approach to Diagnostic Testing

Jessie Monday, DVM, MS
Texas A&M Veterinary Medical Diagnostic Laboratory
Amarillo, TX, USA

Introduction

Understanding of laboratory testing and the interpretation of diagnostic results in light of clinical suspicion of disease and test characteristics is important in the efficient use of the diagnostic lab as a tool in disease investigation. When ideal pretest clinical suspicion cannot be developed due to lack of historical or patient information, there are a few strategies that can be utilized to increase the utility of diagnostic testing.

Laboratory testing

There are four reasons for ordering a laboratory test:

1. Diagnosis: to rule in or rule out a specific disease based on pathogen presence, exposure, or physiological effect
2. Monitoring: to check response to therapy or the efficacy of preventative, vaccination, or biosecurity programs
3. Screening: for genetic diseases, infectious disease carriers, or persistently infected animals
4. Research: to understand the pathophysiology of a particular disease process

The first step in diagnostic investigation utilizing diagnostic testing is differential list development based on patient or herd history and clinical findings. When the differential list is limited to a small number of diseases or pathogens, a pre-test probability can be established and testing selected and applied. When the differential list only includes regional syndromes, testing can be utilized to further characterize the disease process and then pre-test probability applied to the interpretation of specific diagnostic testing. With this method the results of selected diagnostic testing are combined with clinical suspicion based on history and examination of the patient to confirm clinical suspicion, adjust probability of disease, narrow the window of uncertainty, or possibly change the mind of the clinician depending on test characteristics. Selection of appropriate tests depends on the diagnostic question that best serves each investigation. Different testing methodologies answer different diagnostic questions.

Testing methods

Tests that diagnose exposure to pathogen of interest include AGID, ELISA, agglutination tests, microagglutination tests (MAT), and virus neutralization. Paired titers will increase the confidence of interpretation of titer results but two-week delays in testing are not always practical in disease outbreak situations. Tests that identify antigen or genetic material (PCR) or rely on pathogen recovery via bacterial culture of virus isolation on cell culture will identify pathogens associated with disease processes at various sensitivities and specificities. The recovery of potential pathogens or evidence of their genetic material must be interpreted with information from history, clinical assessment, and pathogen pathophysiology. Tests that investigate disease processes rather than exposure or presence of particular pathogens are useful for specific differential list development, investigating the effect of disease on organ function and systemic health, and to speak to therapy choice or efficacy or prognosis. These include clinical pathologic tests (CBC, Chemistry Profile, Urinalysis) and histopathology. These tests provide valuable information during the initial clinical assessment as well as when monitoring the efficacy of therapeutic plans or herd disease prevention protocols.

How to interpret results depending on the test strength

The decision of which diagnostic test or tests to select is based on many factors. Certain diseases that can be associated with the identified syndrome are tested for because of the likelihood of that disease based on population risk and clinical signs. Some pathogens are tested for, regardless of low likelihood of disease based on risk or consequence of that disease to humans. Similarly, some tests are run to evaluate the possible risk of further disease in the exposed population rather than to change the outcome of disease in the animal tested. Testing can also be selected to investigate the potential benefit of a chosen therapy or change in management or prevention protocols. Some testing is done to provide information to owners about expectations for recovery, treatment success, or duration of disease outbreak. The strength of evidence that testing can provide to these various diagnostic questions depends on sample quality, testing methodology, and test accuracy.

The most familiar estimates of test accuracy are test sensitivity and specificity. In general terms sensitivity is a tests ability to detect diseased animals and specificity is a tests ability to detect non-diseased animals. Ideally the test selected should have perfect sensitivity and specificity. Tests of this caliber are rare and even if they are applied to samples of poor quality, they will not provide ideal answers. It is important to remember that sensitivity and specificity are reciprocal. If changes made to make an assay more sensitive based on the diagnostic cut-off value, the specificity will suffer. In general, highly sensitive tests are used to rule out a diagnosis with a good degree of confidence because they have fewer false negatives. It follows that highly specific tests are utilized to rule in a diagnosis because they have fewer false positives. Consequence of result interpretation should also be considered when selecting tests with less ideal accuracy. If the consequence of failing to diagnose a disease in an affected animal/population is unacceptable (false negatives), then a test with higher sensitivity is needed. If the consequence of an incorrectly diagnosing a disease (leading to high consequence/high risk therapy or inappropriate removal from the herd) is unacceptable (false positives), then a test with higher specificity is needed. Tests with combined sensitivity and specificity totals ≥ 170 are likely to prove clinically useful when properly interpreted in relation to suspicion of disease. These are generalizations and should be used as loose guidelines when selecting tests and weighing the results of the tests.

Sensitivity and specificity cannot be directly translated to everyday practice because they are population calculations and do not answer the correct diagnostic inquiry. What veterinarians want to know is: "What is the percent of test positive (or test negative) animals that are truly diseased (or free of disease)?" Sensitivity and specificity calculations answer the question: "What is the percentage of diseased animals (or disease-free animals) that are correctly classified by the test?" Positive and negative predictive values will tell clinicians the probability of a test positive (or negative) animal actually having disease (or being disease-free). Unfortunately, these statistical estimates are strongly dependent on prevalence of disease (or vaccine exposure) that are not always available for external application to individual cases. Also, predictive values are population estimates that do not translate the interpretation of individual animal test results. When considering the predictive value of a test it is important to remember that testing an uncommon disease, even with a highly accurate (highly sensitive and specific) tests can give an inaccurate result when used on a healthy population as a whole. Veterinarians can maximize the predictive value of an assay by utilizing it on a population of animals that are likely to have the disease (maximizing the prevalence of disease in the population sampled).

In order to interpret individual test results, clinicians can utilize likelihood ratios. Likelihood ratios (LR) quantitate the frequency of positive and negative test results in diseased and disease-free cases. LR do not depend on disease prevalence as so can be applied to cases regardless of population risks and characteristics. Positive LR (LR+) = Sensitivity / (1-Specificity). Negative LR (LR-) = (1- Sensitivity) / Specificity. The likelihood ratios are translated into clinical practices by using Bayes theorem. This will link the pre-test suspicion of disease (pre-test odds/pre-test probability/pre-test priori) with post-test disease suspicion using the likelihood ratios. In other words, clinicians will use information about the test (sensitivity and specificity translated into the likelihood ratio) to adjust the probability of disease in their patient based on the test result. The simplified version of the mathematical formula is:

Post-test probability of disease = Pre-test suspicion * LR / (1-Pre-test probability + Pre-test probability * LR)

The pre-test probability is a numerical number that represents the clinician's suspicion that the patient has the disease. This number is based on known risk of disease in the population, knowledge of pathophysiology of the disease in relation to physical exam findings, pattern recognition, and clinical experience. Final test interpretation is straightforward when test results agree with clinical suspicion based on history and patient assessment.

Uncertainty enters the picture when test results do not agree with clinical suspicion or when there is insufficient evidence before testing to pinpoint pre-test probability to anything more specific than a narrow range. The chances of uncertainty due to inaccurate pretest probability are high when veterinarians are investigating syndromes in which they have little prior experience. Graphical methods have been developed to incorporate uncertainty into pretest probability estimates and final diagnostic interpretations. These nomograms provide visual tools that can be considered when deciding how much "weight" to give a diagnostic test result that contradicts with clinical suspicion. The next step in the diagnostic investigation can then be considered: diagnosis and treatment, more testing to investigate same suspicion, or more diagnostic testing to investigate other differentials.

How to improve evidence provided by diagnostic testing

The pre-test probability of disease or pre-test clinical suspicion has a large impact on reliability of test result interpretation. Doing a good physical exam and having enough information of patient history, risk of disease, and herd management will increase the utility of diagnostic testing greatly. When that is not possible, panels of tests designed to inform the pre-test clinical suspicion of specific pathogen tests can be utilized to help interpret specific tests results. It is important when interpreting test results to remember that tests are not infallible. If a result dose not agree with the pre-test clinical suspicion, the issue should be further analyzed rather than believed despite the information provided by the history and clinical assessment of the patient. To prevent test results that further confuse the diagnostic investigation, rather than provide good information to further in the investigation or diagnose the case, “shotgun” diagnostics should be avoided. Tests should only be used if the result will influence the course of action.

References

1. Bianchi, Matt T., Brian M. Alexander, and Sydney S. Cash. "Incorporating uncertainty into medical decision making: an approach to unexpected test results." *Medical Decision Making* 29.1 (2009): 116-124.
2. Brigden, Malcolm L., and John C. Heathcote. "Problems in interpreting laboratory tests." *Postgraduate Medicine* 107.7 (2000): 145-158.
3. Gallagher, E. John. "Clinical utility of likelihood ratios." *Annals of emergency medicine* 31.3 (1998): 391-397.
4. Guyatt, Gordon H., et al. "A framework for clinical evaluation of diagnostic technologies." *CMAJ: Canadian Medical Association Journal* 134.6 (1986): 587.
5. Halkin, A., et al. "Likelihood ratios: getting diagnostic testing into perspective." *QJM: monthly journal of the Association of Physicians* 91.4 (1998): 247-258.
6. Lamb, C. R. "Statistical briefing: estimating the probability of disease." *Veterinary Radiology & Ultrasound* 48.3 (2007): 297-298.
7. McKenna, Shawn LB, and Ian R. Dohoo. "Using and interpreting diagnostic tests." *Veterinary Clinics: Food Animal Practice* 22.1 (2006): 195-205.
8. Phelps, Molly A., and M. Andrew Levitt. "Pretest Probability Estimates: A Pitfall to the Clinical Utility of Evidence-based Medicine?." *Academic Emergency Medicine* 11.6 (2004): 692-694.
9. Wians, Frank H. "Clinical laboratory tests: which, why, and what do the results mean?." *Laboratory Medicine* 40.2 (2015): 105-113.