The Use and Abuse of Serologic Testing

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Introduction

Serology, the measurement of specific antibody in serum or body fluids, has become widely used in routine veterinary diagnostics. Detection of specific antibody in serum provides a valuable means of determining exposure of a herd or flock to a particular bacteria, virus, or other antigen. More commonly, serologic detection of specific antibody is used to make or confirm a clinical diagnosis. Unfortunately, it is equally possible to misdiagnose on the basis of serology by interpreting serologic information incorrectly or relying on serology results when inappropriate. Common misuses of serology include evaluation of serologic data without supporting clinical information or history, relying on serologic results in the face of contradictory clinical information, and the more common errors of inappropriate sampling (including inappropriate specimen type, sampling time, or number of samples). Technical information, including the sensitivity and specificity of the assay procedure, is often ignored or unavailable, but is critical to the meaningful interpretation of serologic results. The valid use and interpretation of serology relies on both an understanding of the methodology employed and on the pathogenesis of the disease in question. It is the purpose of this report to briefly review serology as a diagnostic tool.

Methodology

The procedures for detecting and measuring antibody in body fluids fall into two basic categories: the primary binding assays and the secondary binding assays. The more sensitive primary binding tests include direct and indirect immunofluorescent assays, ELISA, and other enzyme immunoassays that directly measure the binding of antibody to antigen. Secondary binding tests are generally easier to perform but less sensitive and measure results of antibody-antigen interactions such as precipitation, agglutination, neutralization, and complement fixation or activation. The ability of primary tests to detect antibody may approach the 10^{-8} milligrams of protein per milliliter, while that of secondary tests range from 10^{-3} to 10^{-7} milligrams of protein per milliliter.

For both primary and secondary binding tests it is important to remember that immunologic assays are affected not only by the quantity of specific antibody, but also by the subclass and properties of specific and non-specific antibodies in the serum. The IgM class of antibody is relatively non-specific, has low affinity (ability to bind to specific antigen), is efficient at agglutination, neutralization and complement fixation, but less efficient in precipitation assays. The large size of the IgM molecule is frequently responsible for interference (steric hinderance) of other specific immunoglobulins in serologic assays. The IgM response appears within 2 to 5 days and peaks at 1 to 2 weeks following antigenic challenge.

The properties of the IgG class of immunoglobulin vary between species, but in general tends to be strong in precipitation assays, although less efficient than IgM in agglutination and neutralization assays. The IgG response appears 4 to 7 days following antigenic stimulation and peaks between 1 and 3 weeks post-exposure. The classes of immunoglobulins further contain subclasses (i.e. IgG^2, equine IgT and IgB) that vary in their properties. Examples of subclass variation affecting serologic testing include the predominant IgG subclass produced by pregnant cows or ewes that is significantly more efficient at complement activation than the subclass produced by the non-pregnant animal, or the non-precipitating nature of equine IgT as opposed to IgG.

As the immune response of an animal to a particular antigen develops over time, the affinity of specific IgG for the antigen increases, far exceeding the affinity of IgM or early IgG responses. Early in the IgG response, numerous "clones" of antibody with varying degrees of affinity are produced against the antigen in question. As the immune response progresses, antibodies that bind more tightly continue to be produced, while those with less affinity cease to be expressed. The net effect is that the IgG response becomes progressively more focused, recognizing fewer and fewer parts (epitopes) of the antigen, but binding progressively more tightly to those parts. Even though the total amount of IgG may remain similar or decrease, in more chronic infections primary binding tests will tend to have a stronger response due to increased antibody affinity, while secondary binding assays that depend on function tend to become less reactive. Assays employing subunit or highly specific antigens are more sensitive to changes in affinity due to maturation of the immune response.

Immunoglobulin class and subclass properties affect both serologic methods and interpretation of results. Agglutination responses, because they readily detect IgM, are more likely to detect early infection; however, for the same reasons are also prone to "false positives" due to non-specific and cross-reacting IgM antibodies. This problem is recognized in serologic screening for avian mycoplasmosis. Though mycoplasma plate agglutination assays detect infection earlier than ELISA or hemagglutination inhibition (HI) methods, they are also more

prone to cross-reactions and false positive reactions than are either ELISA or HI methodologies. In general, recent exposure to oil-based vaccines and adjuvants non-specifically stimulate IgM production thus provide common sources of "interference" in most agglutination-based assays.

The detection of antibodies in serum indicates exposure of an animal to a specific organism or antigen, though not clinical disease. In some instances, the information provided by serologic testing is straightforward and requires no interpretation. In any instance where exposure without subsequent disease would not be expected, positive serology on a single sample is diagnostic. For instance, detection of specific-antibody in fetal fluids or pre-colostral sera is indicative of infection, a positive Coggins test is indicative of equine infectious anemia, or a positive latex assay indicates pseudorabies in non-vaccinated swine. Though there are cases where exposure is indicative of disease, in most diagnostic situations the detection of antibody alone does not prove that the concurrent disease is due to the organism in question. Vaccination, passive transfer, environmental exposure, and cross-reacting antigens account for specific antibody responses in the absence of infection. In the majority of viral and bacterial infections, the presence of specific antibody in a single serum sample has no diagnostic significance. A four-fold rise in antibody titer between a serum sample collected in the acute stage of infection and a convalescent sample collected 2 to 4 weeks later does, however, provide significant support of concurrent infection in the face of clinical observations. Bovine infectious rhinotracheitis, PI-3, equine rhinopneumonitis, leptospirosis, and Newcastle disease are a few among the list of serologies that require paired (acute and convalescent) sera for accurate interpretation.

Pathology/Clinical Information

In interpreting serologic test results it is critical to understand the pathogenesis of the organism and disease. The source and route of infection, the type of immune response induced, disease history, and epidemiology are crucial in the appropriate use and interpretation of serologic assays.

The site of infection and type of immune response should be considered when evaluating serology. Antibody responses are notoriously low or absent in walled-off abscesses, as seen with chronic *Corynebacterium pseudotuberculosis* infection, or when the organism localizes in sites removed from the circulatory system, as is possible with chronic *Brucella ovis* carrier rams. In such instances serologic testing may provide "false negative" results. Immunotolerance induced by fetal exposure to bovine viral diarrhea or border disease virus may also result in negative serology in the face of border disease and BVD infections in affected sheep and cattle, respectively. At the opposite extreme, infections due to *Chlamydia spp.* result in polyclonal activation, or non-specific stimulation of antibody in the animal, thus elevating the level of all circulating antibodies.

The epidemiology of the pathogen additionally provides valuable information for serologic interpretation. With highly contagious diseases such as pseudorabies in swine, *Brucella ovis* in sheep, or Johne's in a closed dairy herd, positive serology in a limited number of animals from the herd can generally be used to predict large-scale herd infection.

The incubation period and onset of clinical signs should also be considered in evaluating serologic results. In the case of abortions due to equine herpes virus type-I, as well as other abortogenic agents, the infectious process occurs for a period prior to the actual abortion. During this period the animal may appear clinically normal, though serologic evaluation would demonstrate increasing antibody titers. By the time the fetus is aborted, high specific antibodies are already detectable, making acute and convalescent sampling less reliable.

Predictive Value

The most important consideration in interpreting serologic results is the reliability or predictive value of an assay. For any serologic test, the factors necessary for meaningful interpretation are the sensitivity of the assay, the specificity of the assay, and the prevalence of the disease or measurable serologic response in the population.

The sensitivity of the test is the probability that an animal which has the specific disease will be detected; i.e. "called positive" by the assay. Specificity is a test's probability of recognizing those animals free of disease as negative. A test with perfect sensitivity has no false negative results, and a test with perfect specificity will have no false positive results. There are no serologic tests with perfect specificity and sensitivity, and at a particular point, any increase in sensitivity of an assay results in decreased specificity, and visa versa. Screening tests, such as the brucellosis or mycoplasmosis plate tests, are designed based on sensitivity, they detect a high percentage of the true positive animals at the expense of accepting an increased percentage of "false positives" (decreased specificity). Confirmatory tests, on the other hand emphasize specificity, accepting "false negatives" rather than "false positives."

Though sensitivity and specificity measure assay accuracy given the disease status of the animal or herd is known, they do not indicate the reliability of the assay when the disease status is unknown. For instance, in a herd known to be free of a particular disease, using sensitivity and specificity of the assay, the probability of getting a negative serology result from that herd can be determined. However, in a clinical situation, when obtaining a negative serologic result, what is the probability that the negative test actually indicates a lack of disease? A statistical calculation, termed predictive

value, can be used to provide an estimate of test accuracy. Predictive values are based on the sensitivity and specificity of the serologic assay as well as on the prevalence of the disease in the population. In situations of high prevalence, the predictive value of a positive serologic result increases, while in areas of low prevalence the predicative value of a negative result increases. The positive predictive value for *Borrelia burgdorferi* (Lyme disease) serology may approach 100% in endemic areas, where the same assay and result will have a considerably lower predictive value in areas where the disease is not known to occur.

The prevalence of disease should also be considered when evaluating herd or flock health by serology. The probability that an individual result is representative of a population for any serologic test is dependent on an accurate and representative sampling of the flock or herd. In diseases of high prevalence, the number of samples required to accurately represent the flock or herd decreases dramatically, whereas in diseases with very low prevalence, the appropriate number of samples may exceed 50% of the herd or flock.

The field of serology is changing at an incredible pace with the development and utilization of new technologies such as DNA/RNA probes, *in situ* hybridization, restriction enzymes, and monoclonal technology. The value of the information gained from any serologic test however remains dependent on appropriate use and interpretation of the test. Serology when used correctly as a diagnostic tool in combination with other diagnostic information and procedures provides a valuable and reliable means of confirming or eliminating diagnoses, monitoring flock or herd health, and evaluating management practices.

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