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Abstract: The recent availability of serologic tests has changed the practice of veterinary medicine. These assays not only assist with the diagnosis and treatment of recognized syndromes, but they permit identification of new, poorly defined disease entities. Assays kits are available for FeLV, dirofilariasis, rheumatoid arthritis, enterotoxigenic colibacillosis, and failure of passive transfer. The purpose of this report is to discuss the theory behind diagnostic serologic testing, rebut selected misconceptions, and suggest strategies for interpreting the results of diagnostic serologic tests. Also, the authors have provided adaptations of standard statistical methods, sample calculations, general references, and a glossary of terms.
Titers, tests, and truisms: Rational interpretation of diagnostic serologic testing

Jeff W. Tyler, DVM, MPVM, PhD, and James S. Cullor, DVM, PhD

The recent availability of serologic tests has changed the practice of veterinary medicine. These assays not only assist with the diagnosis and treatment of recognized syndromes, but they permit identification of new, poorly defined disease entities. Once devoted solely to the detection of antigen-specific immunoglobulins (lg), diagnostic serology has expanded to include assays detecting antigens, toxins of environmental or clinical interest, and products of host homeostasis or metabolism. Additionally, diagnostic serologic testing is shifting from central reference laboratories to the stall side or examination table. Assay kits are available for FeLV, dirofilarialis, rheumatoid arthritis, enterotoxigenic colibacillosis, and failure of passive transfer. Bovine milk progesterone assays have been placed in the client's hands. Neither diagnostic immunologists nor practitioners participate in the performance of the assays or their interpretation.

Although immunodiagnostic aids are rapidly becoming available to practitioners, their use may not guarantee improved or cost-effective practice. Any diagnostic procedure carries an inherent risk of false-positive results. As the number of tests increases, so does the potential for erroneous results. Traditional, low, or appropriate technology procedures, including physical examinations and case histories, remain the cornerstone of disease diagnosis.

Immunoperoxidase development must mature beyond historically accepted conventions. Immunoperoxidase design and optimization, although important, are not the end of assay development. The information provided by each additional test will have minimal merit unless attempts are made to provide the practitioner with meaningful and readily interpretable reports. Ideally, test results should include diagnostic probabilities, not titer or binomial (positive/negative) test results. This information requires test performance data (sensitivity and specificity) drawn from large populations of known disease status and an accurate assessment of actual disease prevalence in representative reference populations.

Optimal use of serologic testing requires working knowledge of disease pathogenesis, immunodiagnostic techniques, and statistical inference. Many veterinarians, although adept in disease diagnosis and treatment, are less comfortable with immunology and statistics. Lacking these skills, they may base decisions on historic truisms, and extrapolate test results beyond their established context. The purpose of this report is to discuss the theory behind diagnostic serologic testing, restate selected misconceptions, and suggest strategies for interpreting the results of diagnostic serologic tests. Also, we have provided adaptations of standard statistical methods, sample calculations, general references, and a glossary of terms.

Serology—a measure of antigen–antibody complex concentration

The primary occurrence in any immunosassay is the binding of Ig to an antigen. The measured end point relates directly to the detection of these antigen–antibody complexes. Differences in amino acid sequence in the variable portions of the Ig molecule give antibodies diverse specificities, accounting for the widespread application of immunosassays. The lock and key analogy is often used to describe antigen binding. Factors affecting binding specificity include structural and spatial conformity of antigen and antibody, hydrogen bonding, electrostatic interactions, Van der Waals forces, and hydrophobic interactions. Expanding this analogy, antigen binding might be better described as the behavior of worn master keys (antigens) in aged locks (antibodies). Some combinations fit better than others and some do not fit at all.

Antigen-antibody binding may be viewed as a reversible chemical reaction, governed by the laws of mass action. Two adaptations of this equation, specifically dealing with antigen-antibody complex formation, are as follows:

From the Department of Clinical Pathology, School of Veterinary Medicine, University of California, Davis, CA 95616. Dr. Tyler's present address is Department of Large Animal Surgery and Medicine, Auburn University, AL 36849.

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K = [antigen-antibody complexes]/
[antigen] · [antibody]

or,

[antigen-antibody complexes] =
K · [antigen] · [antibody]

where K represents the association constant of the reaction, and | | represents the concentration of the immunoreactant. Although the concentration of antigen-antibody complexes varies directly with that of either immunoreactant, the concentration of one immunoreactant, typically antigen, is fixed in any given assay. In this ideal reaction, antigen-antibody complex concentration varies directly with antibody concentration. However, the affinity of immune complex formation is not a constant property. Affinity varies, not only between Ig preparations, but also between the idiotypes in any polyclonal preparation. Additionally, affinity often increases during the course of a humoral immune response. Consequently, serum samples with identical Ig concentrations can and will generate varying concentrations of antigen-antibody complexes.

Antigen-antibody binding is further complicated by the valence of antigen and Ig. Microbial antigens are polyvalent, and an Ig molecule will have 2 (IgG, IgE), 4 (secretory IgA), or 10 (IgM) antigen-combining sites. Multiple bonds may increase the strength of antigen-antibody binding a much as 10^2-fold. The total strength of antigen-antibody binding is termed avidity. The constant in the mass action equation represents the effects of both affinity and avidity. The concentration of antigen-antibody complexes formed in an immunassay, and hence, titer, is a function of antigen concentration, antibody concentration, epitope density and valence on the test antigen, antibody valence, and affinity.

Detection systems equate antigen-antibody complex concentration, and therefore, indirectly, antigen-specific Ig concentrations, with a conveniently measured signal. Commonly used signals include altered physical properties, altered biological activity, and detection of a label or marker in one of the immunoreactants. Altered physical properties include agglutination, precipitation, and nephelometry (light scatter). Biological activities include complement fixation, virus neutralization, and in vivo prophylaxis (e.g., mouse protection against clostridial toxins). Assays in which labeled immunoreactants are used have greatly lowered the limits of detectable immunoreactant. Commonly used markers include enzymes (in ELSA), fluorochromes (in quantitative immunofluorescence), and radioactive isotopes (in radioimmunoassay).

Immunoglobulin isotypes vary markedly in the strength of assay signals that they will produce. Complement fixation is restricted to IgM and subclasses of IgG. Pentameric IgM generally produces more graphic changes in physical properties as a result of immune complex formation. The larger span of the IgM molecule permits it to cross-link antigens that are usually held apart by repulsive forces. Consequently, IgM tends to produce stronger agglutination and precipitation reactions. These isotypical variations in antibody activity should be considered when selecting or choosing a serologic test.

Introduction to Test Interpretation

The premise underlying immunoassays is detection of a signal permitting the differentiation of normal and diseased animals. The measured signal may be reported directly (e.g., optical density, light scatter, plaque reduction) or as the highest sample dilution at which the measured signal exceeds a chosen end point (titer). If hypothetic distributions of a conveniently reported signal are examined in disease-negative (mean titer, 400; 20, 100) and disease-positive (mean titer, 700; 50, 100) populations (Fig 1), it will be noticed that although normal and diseased populations have different means, there is an overlapping area in which a titer could be consistent with either normal or disease status. One solution to overlapping signal distributions is to optimize the test system and more clearly delineate normal and diseased populations (Fig 2). If the variation is reduced within positive and negative populations with the same mean responses, the overlap is decreased. However, not only measured signal distributions, but the underlying distributions of antibody concentrations often overlap, limiting the ability of immunoassay procedures to discriminate between normal and diseased animals. Given this area of overlap, interpretation of the results of diagnostic serologic tests centers on predicting disease status.

Assay signals exceeding an arbitrary end point are termed positive (Fig 1) and those less than end point are termed negative. Some proportion of diseased animals will have assay signals less than the

Veer 1.1—Relative frequency of measured assay signals in hypothetic normal and diseased populations, illustrating those portions of the graph corresponding to (a) correctly identified true-positives; (b) false-positives; (c) false-positives; and (d) correctly identified true-positives.
chosen end point. Those animals will be erroneously identified as test-negative and the assay results will be termed false-negative.1,21,12 The proportion of disease-positive animals correctly identified is termed test sensitivity (see Glossary).1,2,3,12 Likewise, some finite proportion of disease-negative animals will have assay signals exceeding the chosen end point (false-positives). The proportion of disease-negative animals correctly identified is termed the test specificity.1,2,11 End-point selection can have a marked effect on test performance (Fig 1), such that if the end point is lowered (shifted to the left), sensitivity is enhanced and the proportion of false-positives is reduced. Unfortunately, specificity is reduced and the proportion of false-positives increases. Once an immunoassay design is chosen, any end-point change made to increase either sensitivity or specificity can only be achieved at the expense of other measures of assay performance.1,2

Titters do not directly measure antibody concentration

Titter is, at best, an ordinal measure of antibody concentration. The relative nature of titters can be appreciated by comparing the lower limit of Ig detected, using a variety of immunoassay procedures (Table 1).1,21 An undiluted sample producing a positive test result (titer = 1) by use of immuno-electrophoresis could be diluted 10 billion-fold and still produce a positive test result (titer = 10^10) in a radioimmunoassay. Titter is as much a function of our ability to detect antibody-antigen complexes as it is a measure of complex concentration. Relative sensitivity factors (Table 1) are only theoretical approximations and cannot be used to compare the results of different assay systems.

Titter measurements are extremely variable, even within general classifications of immunoassay procedures. Because antigen-antibody bond is the summation of several weak forces, minute changes in assay conditions can greatly affect titer. Antigen-antibody bonds tend to dissociate at either low pH or high ionic strength.1,2,17 Conditions favoring binding will generate high titers and restrictive conditions will result in low titers, but select for high affinity antibodies.

Antibody concentration is not a direct measure of disease resistance

Direct correlations between disease resistance and serologic recognition are, at best, tenuous. Immunoglobulin is not evenly distributed among host tissues and fluid spaces. Immunoglobulins of the IgM isotype are largely restricted to the blood vascular system and only IgA is actively secreted at mucosal surfaces.1,2,5,7 Consequently, serum titers may not reflect the humoral immune status of tissues or mucosal surfaces.1,2 Although Ig is produced after antigenic exposure, the Ig produced need not confer protection. Immunoglobulin binding has minimal inherent microbial activity. Immunoglobulins serve principally as a surveillance mechanism, focusing direct effector mechanisms against foreign antigen.4,5,7 The absence of complement cascade,1,9,10 T-lymphocytes,11 or phagocytic cells (macrophages, neutrophils)12,23 may all result in life-threatening disease, regardless of humoral immune status.

Salmonella dublin-carrier cattle have high specific antibody titers and, although it is believed that these antibodies promote phagocytosis, the bacteria withstand the microbial environment of the phagolysosome.1,9,17 High antibody titers indicate antigenic exposure rather than protection. Microorganisms capable of surviving within the phagolysosome (eg, Salmonella spp, Brucella abortus, Listeria monocytogenes)1,21 establishing a protected latent niche within the host cell cytoplasm (eg, infectious bovine rhinotracheitis virus)18,19 or genome (eg, bovine leukemia virus)10,21 spreading directly cell to cell without an extracellular bacte remia or viremic phase (eg, respiratory syncytial virus)15,25 or resisting cytoplastic cell complement activity.17 may evade immune mechanisms involving antigen-specific Ig.

In some cases, in vivo antigen-antibody recognition is not only ineffective, but detrimental to host health. The 3 forms of immediate hypersens-
Positive tests are not positive diagnoses

Positive test results can arise from the correct identification of a diseased animal (true positive) or the incorrect identification of a normal animal (false positive). False-positive results may occur because of laboratory errors, exposure to an antigen similar to the test antigen, or true exposures unrelated to disease status. From an intuitive perspective, it can be appreciated that tests with low specificity and populations with low prevalence tend to produce large proportions of false-positives. The predictive value of a positive test result is the probability that a test-positive animal truly has the disease condition of interest. The following formulae illustrate calculation of selected measures of test performance.12,13

\[
\begin{align*}
\text{Test status} & \quad + \quad - \\
\text{Animal status} & \quad a \quad b \\
& \quad c \quad d
\end{align*}
\]

where \(a = \) No. of animals both test-positive and disease-positive; \(b = \) No. of animals test-positive and disease-negative; \(c = \) No. of animals test-negative and disease-positive; \(d = \) No. of animals both test-negative and disease-negative; sensitivity = \(a/(a+c)\); specificity = \(d/(b+d)\); prevalence = \((a+b)/(a+b+c+d)\); predictive value (positive test) = \(a/(a+b)\); predictive value (negative test) = \(d/(c+d)\); odds ratio = \((a\times d)/(b\times c)\); and \(x^2 = ((a\times d) - (b\times c))^2 / ((a+b) \times (a+c) \times (b+d) \times (c+d)).\)

After a \(x^2\) value is calculated, the \(P\) value is then determined by use of a standard statistical \(x^2\) table with 1 df. Techniques are available that permit the analysis of matched pairs, small samples, and discontinuous data.

Let us assume that we are going to eradicate parakeratosis from a herd of 10,000 cattle, using diagnostic serologic testing. Sample calculations of sensitivity, specificity, and predictive value, premised on a prevalence of 1%, and test sensitivity and specificity of 0.99 are as follows:

\[
\begin{align*}
\text{Test status} & \quad + \quad - \\
\text{Animal status} & \quad 99 \quad 991 \\
& \quad 1 \quad 9,801
\end{align*}
\]

sensitivity = \(99/(99 + 1) = 0.99\); specificity = \(9,801/(9,801 + 99) = 0.99\); prevalence = \((99 + 1)/(99 + 991 + 1 + 9,801) = 0.01\); predictive value (positive test) = \(99/(99 + 991) = 0.05\); and predictive value (negative test) = \(9,801/(9,801 + 1) = 1.0000\).
of disease. Seroconversion could logically follow either vaccinal or natural exposure (with or without disease) to the test antigen or to another antigen with similar binding properties. Cross-reactive antigenicity is common and has been used to develop broad-spectrum diagnostic reagents (e.g., antisera used to group Salmonella spp and Streptococcus spp).39,42 and vaccines for use in passively immune neonates.43 Consequently, seroconversion only suggests exposure and is not diagnostic of disease. A recent development is the use of specific deletion mutants and highly purified antigens as vaccinal or immunodiagnostic reagents, permitting differentiation of vaccinal and natural exposures.34,45

Paired acute and convalescent serum samples from cattle are often submitted in an effort to diagnose enzootic reproductive failure. It is assumed that the initial sampling is performed shortly after antigenic challenge and that the 10 to 14 days between sample collections permits the detection of an increasing titer to the etiologic agent. This assumption warrants close scrutiny. If antigenic exposure precedes clinical disease by a sufficient time span, the acute serum sample may in fact reflect the maximal humoral response. This problem is accentuated if the host has already undergone a primary exposure. If natural exposure or vaccination is commonplace, a primary response will have occurred long before the clinical disease episode, and an anamnestic response should develop within 3 days.3 If the lag between exposure and the clinical event exceeds 3 days, acute serum samples will be obtained after the anamnestic response (Fig 3). The varying intervals between challenge exposure and clinical disease, between disease and clinical events, and between exposure and serologic response suggest that the detection of seroconversion may be as much the result of luck as of a planned diagnostic approach.

Immunodiagnostics—experiments in search of controls

Let us assume that we detect a positive seroconversion event, either a fourfold titer increase or a single titer of sufficient magnitude to suggest recent exposure in 5 of 7 cattle with documented reproductive failure. The coincidental nature of seroconversion and the observed clinical events does not imply causality or even an association. To determine whether the positive test was, in fact, related to the clinical event, some reference or comparison group is required. The most reasonable comparison group would be those cattle in a similar stage of gestation that did not experience reproductive failure. Statistical techniques are available to compare the risk factors in case (reproductive failures) and control populations. The most commonly used measure of association is odds ratio. Odds ratio approximates the risk of a disease outcome, given the presence of a postulated risk factor. The risk factor of immediate interest will be seroconversions with respect to the suspected cause of reproductive failure.

In the following hypothetic population, the calculated odds ratio, 1.00, suggests that cattle that seroconverted are at no greater risk of reproductive failure than are those whose titers either remained constant or decreased.

Reproductive failure

<table>
<thead>
<tr>
<th>+</th>
<th>-</th>
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<tbody>
<tr>
<td>25</td>
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</table>

Seroconversion + 5 25

Odds ratio = (5 / 10) / (25 / 2) = 1.00.

In a second hypothetic population, the odds ratio of 6.25 means that cattle that seroconverted are 6.25 times as likely to experience reproductive failure as are those that did not seroconvert.

Reproductive failure

<table>
<thead>
<tr>
<th>+</th>
<th>-</th>
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</thead>
<tbody>
<tr>
<td>10</td>
<td></td>
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</tbody>
</table>

Seroconversion + 2 25

Odds ratio = (3 / 25) / (10 / 2) = 6.25; x² = [(3 / 25) - (10 / 2)]² / (10 / 25 - 15 / 15 - 27) = 4.67; P value < 0.05.

Odds ratios < 1 suggest that the postulated risk factor is associated with a decreased risk of disease. Odds ratio then measures the magnitude of the relationship between the measured risk factor and the disease. The statistical significance of these associations can be assessed by use of x² test. The observed P value (< 0.05) suggests that there is only a small probability that the observed association was attributable to chance.

Often, the small numbers of cases (disease events) and controls preclude meaningful analysis. To assist diagnosis of reproductive failure in the preceding example, samples were collected from 42 cows. Practitioners rarely embark on such extensive diagnostic efforts, for obvious reasons; however, such extensive serologic testing may be necessary if trustworthy information is to be obtained. Alternative techniques for calculation of
odds ratios and significance of associations are available for matched cases and controls. When control or comparison groups are not readily available, test results are often compared to historical data drawn from populations with known disease status. Calculations of predictive value are not possible unless prevalence data are available from a representative reference population. These problems are accentuated when serologic assays are used to diagnose disease syndromes with unknown or poorly documented causes.

Some positives are more positive than others

Underlying traditional cross-classified analysis of serologic test results is the hypothesis that disease and immunity behave as threshold phenomena. This assumption is roundly rejected by practitioners. Most observers would ascribe greater importance to a tier of 1-4,000 than to one of 1:400, even if both fall comfortably within a positive range. Conversely, if an ELISA end point is set at 40% positive control optical density (PCOD), the skeptic questions designating a 59% PCOD sample as negative, and a 64% PCOD as positive. The arbitrary choice of an end point ignores the possibility that measured assay signals might follow other than a binomial distribution.

For many of the newer assay kits, results are read by visual appraisal. If the distribution of responses from positive and negative animals are clearly different and have minimal overlap, if the substrate-indicator system has a steep dose response curve, and if changes in the measured response occur only at those concentrations of Ig greater than those in disease-negative animals and less than those in disease-positive animals, then continuous outcome of measured assay signals may begin to behave in a binomial fashion. When we use tests that give positive or negative results, the problem of overlapping measured end-point distributions still exists, but we have consciously abdicated the responsibility for making the diagnostic decision.

In Figure 1, notice that on the far left side of the graph (low measured assay signal) there is virtually a 100% probability of disease-negative status. In the middle of the x axis, there are increasing probabilities of disease, and on the far right side, there is essentially a 100% disease probability. Disease probability does not suddenly shift from 0 to 100%. Alternative statistical techniques, including logistic regression and discriminant analysis, are available to permit disease probability estimates at varying assay signals.

Alternatively, the predictive value of a measured assay signal can be estimated as a function of the normal density of measured end points in disease-negative and disease-positive populations and disease prevalence (see Appendix). Figure 4 illustrates calculated probability of disease at varying assay signals. It is apparent that predictive values, positive or negative, are not static properties and vary with measured assay signal. The previous example, although descriptive of an idealized setting, illustrates the benefits of alternative analyses. Such a calculation requires knowledge of disease prevalence and of test performance in normal and diseased populations (also requirements in the more traditional categorical analysis of test results). A further requirement of this technique is the assumption that measured assay signals are normally distributed. Biological phenomena notoriously resist description by Gaussian distributions. Alternatively, locally weighted regression could be used to provide estimates of the relative frequency of measured signals in normal and disease populations.

Test batteries—diagnostic booby traps

Any diagnostic procedure entails a probability of incorrectly identifying normal animals as disease-positive (false-positive). If a test with a specificity of 0.90 were applied in a population of 100 normal animals, 10 would be incorrectly identified as test-positive and 90 would be correctly identified as test-negative. If the 90 test-negative animals were subjected to a second examination with a specificity of 0.90, an additional 8.1 false-positive animals would be identified. By performing sufficient numbers of tests, the clinician can be assured of a diagnosis; it may be an incorrect diagnosis (false-positive), but it is still a diagnosis (Table 3). The preceding example is not as odd as it might appear. Faced with a potential infectious infertility problem, a bovine practitioner might request diagnostic serologic testing for infectious bovine rhinotracheitis virus, bovine viral diarrhea virus, leptospirosis (at least 5 serovars), brucellosis; and Hemophilus somnus infection. Analysis for a serum or dietary micronutrient (selenium) is also often performed. The problem is not unique
Table 3—Expected percentages of false-positive diagnoses at various combinations of test specificity and numbers of unrelated tests performed

<table>
<thead>
<tr>
<th>No. of tests performed</th>
<th>0.025</th>
<th>0.050</th>
<th>0.075</th>
<th>0.090</th>
<th>0.099</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10.00</td>
<td>5.00</td>
<td>2.50</td>
<td>1.00</td>
<td>0.10</td>
</tr>
<tr>
<td>2</td>
<td>10.00</td>
<td>5.00</td>
<td>2.50</td>
<td>1.00</td>
<td>0.10</td>
</tr>
<tr>
<td>3</td>
<td>27.10</td>
<td>13.50</td>
<td>6.75</td>
<td>3.37</td>
<td>1.35</td>
</tr>
<tr>
<td>4</td>
<td>34.29</td>
<td>17.15</td>
<td>8.57</td>
<td>4.29</td>
<td>1.71</td>
</tr>
<tr>
<td>5</td>
<td>40.96</td>
<td>20.48</td>
<td>10.24</td>
<td>5.12</td>
<td>2.05</td>
</tr>
<tr>
<td>6</td>
<td>46.80</td>
<td>23.40</td>
<td>11.70</td>
<td>5.85</td>
<td>2.34</td>
</tr>
<tr>
<td>7</td>
<td>52.17</td>
<td>26.09</td>
<td>13.05</td>
<td>6.52</td>
<td>2.60</td>
</tr>
<tr>
<td>8</td>
<td>56.95</td>
<td>28.98</td>
<td>14.48</td>
<td>7.33</td>
<td>2.90</td>
</tr>
<tr>
<td>9</td>
<td>61.25</td>
<td>31.62</td>
<td>15.81</td>
<td>8.15</td>
<td>3.21</td>
</tr>
<tr>
<td>10</td>
<td>65.13</td>
<td>34.06</td>
<td>17.20</td>
<td>8.95</td>
<td>3.51</td>
</tr>
</tbody>
</table>

To bovine practice, infectious infertility, or even diagnostic serologic testing. A serum biochemical panel often contains 12 separate tests, and a single case may entail 20 measurements. Test specificity is rarely reported and predictive value is never mentioned. Some laboratories report a normal range, but it is usually not identified in a statistically interpretable manner. As the number of tests increases, so does the probability that at least one test will have results that fall outside the normal range. Indiscriminate use of diagnostic aids expands the population tested, lowers the de facto prevalence, and reduces the predictive value of any positive test.

Conclusions

1. Titers are, at best, relative measures of Ig concentration, and are interpretable only within the narrow context of a single, defined assay system.
2. Immunoglobulin concentration may or may not correlate with disease resistance.
3. Humoral response suggests exposure; immunity or disease status may be another matter entirely.
4. An appreciation of test sensitivity and specificity, disease prevalence, and predictive value is central to rational test interpretation.
5. The terms positive and negative imply the choice of an arbitrary end point.
6. The probability of immunity or disease is not a static property and may vary with measured assay signals.
7. Conscientious attention to physical examination and history permits the clinician to target high-risk populations and improves the predictive value of laboratory procedures.
8. Positive test results are often coincidental with unrelated disease processes. Use of appropriate control groups will minimize spurious results.
9. Indiscriminate use of test panels increases the likelihood of false-positive results.

Glossary of terms

**Avidity**—The total strength of binding between an antigen and antibody. This property is a function of affinity and antigen and Ig valence.

**Binomial**—A categorical designation having 2 possible, mutually exclusive outcomes (e.g., diseased vs normal).

**Class**—Isotype; examples of Ig class are IgA, IgG, IgM, etc.

**Disease**—An arbitrarily defined state of health deviating from normality.

**False-positive**—Identification of a normal animal as test-positive.

**False-negative**—Identification of a diseased animal as test-negative.

**Humoral immunity**—Immunity associated with noncellular elements of body fluids.

**Idiotype**—The antigen specificity of the variable region of an Ig molecule.

**Isotype**—Immunoglobulin class, determined by variations in the constant (nonantigen-binding) portions of the heavy chain.

**Normal** (disease status)—The absence of the disease of interest.

**Normal** (statistical)—Following a distribution pattern originally described by Gauss.

**Odds ratio**—An approximation of the relative probability of a disease outcome, given an observed risk factor.

**Ordinal**—An outcome approximated by categories of increasing magnitude.

**Predictive value**—Positive test—The probability that a test-positive animal is diseased.

**Predictive value**—Negative test—The probability that a test-negative animal is normal.

**Prevalence**—The proportion of a population actually having a disease.

**Sensitivity (statistical)**—The probability of correctly identifying true-positive (diseased) animals.

**Specificity (statistical)**—The probability of correctly identifying true-negative (normal) animals.

**Titer**—The highest dilution of a sample capable of producing a positive test result.

**Test**—A procedure used to approximate disease status.

**Valence**—Having multiple active sites.

Appendix

The following formulas estimate the probability of a disease status with a binomial distribution, given an observed immunosassay result, based on normally distributed, continuous, known test out-
come distributions in defined normal and diseased populations.

The relative frequency of a particular test outcome in a population of defined disease status may be estimated as a function following the normal distribution using the formula:

$$ f = \frac{1}{\sqrt{2\pi}} e^{-\frac{(x - \mu)^2}{2\sigma^2}} $$

where $f =$ relative frequency of a test outcome in a population of defined disease status; $x =$ observed test outcome; $\mu =$ mean test outcome in a population of defined disease status; $\sigma =$ SD of a test outcome in a population of defined disease outcome. Therefore, the probability of disease may be estimated for any measured test outcome as follows:

$$ D = \frac{fp - pd}{(fp - pd) + fn - (1 - pd)} $$

where $D =$ probability of disease-positive status, given a measured test outcome; $fp =$ relative frequency of a test outcome in a disease-positive population; $fn =$ relative frequency of a test outcome in a disease-negative population; $pd =$ prevalence of the disease in the general population; $1 - pd =$ prevalence of disease-negative status in the general population.

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