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.

Special Commentary

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 (Ig), 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, dirofilariasis, 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.

Immunoassay development must mature beyond historically accepted conventions. Immunoassay 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 (sensi-

tivity 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, rebut 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 immunoassay is the binding of Ig to antigen. The measured end point relates directly to the detection of these antigen-antibody complexes.[1-3] Differences in amino acid sequence in the variable portions of the Ig molecule give antibodies diverse specificities, accounting for the widespread application of immunoassays.[4,5] 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 binding, electrostatic interactions, Van der Waals forces, and hydrophobic interactions.[4-6] 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 law of mass action.[1,2,4,6] Two adaptations of this equation, specifically dealing with antigen-antibody complex formation, are as follows:

$$K = \text{[antigen-antibody complexes]}/ \text{[antigen]} \cdot \text{[antibody]}$$

or,

$$\text{[antigen-antibody complexes]} = K \cdot \text{[antigen]} \cdot \text{[antibody]}$$

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.[6] Additionally, affinity often increases during the course of a humoral immune response.[7] 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.[4,5] Multiple bonds may increase the strength of antigen-antibody binding a much as 10^7-fold.[4] The total strength of antigen-antibody binding is termed avidity.[4] The constant in the mass action equation represents the effects of both affinity and avidity. The concentration of antigen-antibody complexes formed in an immunoassay, and hence, titer, is a function of antigen concentration, antibody concentration, epitope density and valence on the test antigen, antibody valence, and affinity.[1,2,4,6]

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.[7] Altered physical properties include agglutination, precipitation, and nephelometry (light scatter).[7-9] Biological activities include complement fixation, virus neutralization, and in vivo prophylaxis (eg, mouse protection against clostridial toxins).[5,7] Assays in which labeled immunoreactants are used have greatly lowered the limits of detectable immunoreactant. Commonly used markers include enzymes (in ELISA),[10] fluorochromes (in quantitative immunofluorescence),[9,11] and radioactive isotopes (in radioimmunoassay).[5]

Immunoglobulin isotypes vary markedly in the strength of assay signals that they will produce. Complement fixation is restricted to IgM and sub-classes of IgG. Pentameric IgM generally produces more graphic changes in physical properties as a result of immune complex formation. The larger span of the Ig 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 (eg, 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; SD, 100) and disease-positive (mean titer, 700; SD, 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
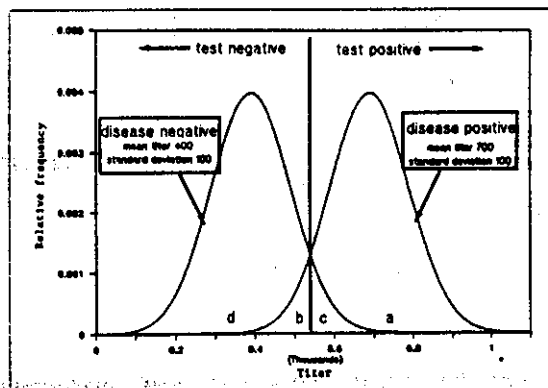

Figure 1—Relative frequency of measured assay signals in hypothetic normal and diseased populations, illustrating those portions of the graph corresponding to (a) correctly identified true-positives; (b) false-negatives; (c) false-positives; and (d) correctly identified true-negatives.
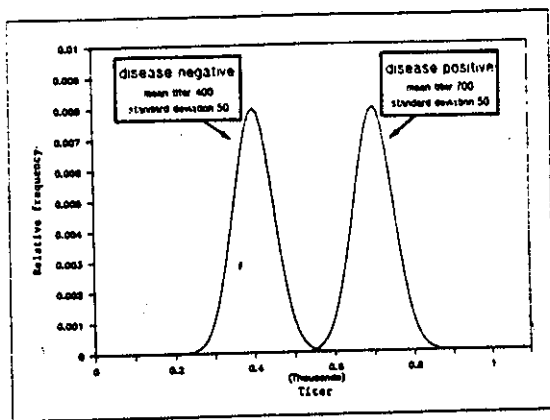
Figure 2—Relative frequency of measured assay signals in hypothetic normal and diseased populations, illustrating the effect of test result variability on diagnostic utility.

Table 1—Relative sensitivity of immunoassay techniques

Technique	Approximate sensitivity (mg/dl)	Sensitivity factor[a]
Immunoelectrophoresis	5 to 10	1 to 2
Single radial immunodiffusion	< 1 to 2	5 to 10
Double immunodiffusion	< 1	10
Electroimmunodiffusion	< 0.5	20
Nephelometry	0.1	100
Complement fixation	0.001	10^4
Agglutination	0.001	10^4
ELISA	< 0.001	10^4
Quantitative immunofluorescence	< 10^{-9}	10^{10}
Radioimmunoassay	< 10^{-9}	10^{10}

[a] Sensitivity factors were calculated by dividing the lower detection limit of immunoelectrophoresis by that of the test in question.

chosen end point. Those animals will be erroneously identified as test-negative and the assay results will be termed false-negative.[12,13] The proportion of disease-positive animals correctly identified is termed test sensitivity (see Glossary).[12,13] 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.[12,13] 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-negatives 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.[12]

Titers do not directly measure antibody concentration

Titer is, at best, an ordinal measure of antibody concentration. The relative nature of titers can be appreciated by comparing the lower limit of Ig detected, using a variety of immunoassay procedures (Table 1).[7,14] An undiluted sample producing a positive test result (titer = 1) by use of immunoelectrophoresis could be diluted 10 billion-fold and still produce a positive test result (titer = 10^{10}) in a radioimmunoassay. Titer 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.

Titer 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 disassociate at either low

pH or high ionic strength.[15-17] 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.[4,5,7] Consequently, serum titers may not reflect the humoral immune status of tissues or mucosal surfaces.[18] Although Ig is produced after antigenic exposure, the Ig produced need not confer protection. Immunoglobulin binding has minimal inherent microbicidal activity. Immunoglobulins serve principally as a surveillance mechanism, focusing direct effector mechanisms against foreign antigen.[4,5,7] The absence of complement cascades,[19,20] T-lymphocytes,[21] or phagocytic cells (macrophages, neutrophils)[22,23] 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 microbicidal environment of the phagolysozome.[24,25] High antibody titers indicate antigenic exposure rather than protection. Microorganisms capable of surviving within the phagolysozome (eg, Salmonella spp, Brucella abortus, Listeria monocytogenes),[26,27] establishing a protected latent niche within the host cell cytoplasm (eg, infectious bovine rhinotracheitis virus)[28,29] or genome (eg, bovine leukosis virus),[30,31] spreading directly cell to cell without an extracellular bacteremia or viremic phase (eg, respiratory syncytial virus),[32-35] or resisting cytolytic serum complement activity,[27] 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 hypersensi-

tivity—anaphylaxis, arthus reactions, and serum sickness—are set in motion by antigen-antibody binding.[36] Early attempts to prevent pneumonic pasteurellosis in cattle used killed whole-cell bacterins. High specific antibody titers were often associated with an unaltered or a more severe disease process.[37-39]

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]:

Disease status

Test status		+	−
	+	a	b
	−	c	d

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 = $(a + c)/(a + b + c + d)$; predictive value | positive test = $a/(a + b)$; predictive value | negative test = $d/(c + d)$; odds ratio = $(a \cdot d)/(b \cdot c)$; and $\chi^2 = [(a \cdot d) - (b \cdot c)]^2 \cdot (a + b + c + d)/[(a + b) \cdot (a + c) \cdot (b + d) \cdot (c + d)]$. After a χ^2 value is calculated, the P value is then determined by use of a standard statistical χ^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 paratuberculosis 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:

Disease status

Test status		+	−
	+	99	99
	−	1	9,801

sensitivity = $99/(99 + 1) = 0.99$; specificity = $9,801/(9,801 + 99) = 0.99$; prevalence = $(99 + 1)/(99 + 99 + 1 + 9,801) = 0.01$; predictive value | positive test = $99/(99 + 99) = 0.50$; and predictive value | negative test = $9,801/(9,801 + 1) = 1.0000$

Table 2—Predictive value (positive and negative) assuming a test sensitivity and specificity of 0.99 at varying disease prevalences

	Disease prevalence			
	0.001	0.010	0.100	0.250
Predictive value	positive test	0.008	0.500	0.917	0.971
Predictive value	negative test	1.000	1.000	0.999	0.997

The test would correctly identify 99% (99/100) of infected cattle. However, the same assay would incorrectly identify 1% (99/9,900) of the disease-negative cattle as test-positive. Only 50% (99/198) of test-positive cattle would actually have paratuberculosis. This example illustrates the dilemma faced by regulatory agencies in their attempts to control infectious diseases. Eradication reduces prevalence, inexorably reducing the predictive value of positive tests. The success of test and eradication programs makes the assay system obsolete.[40] Improved specificity becomes necessary to continue eradication while avoiding losses associated with culling false-positive animals.

Serologic testing cannot compensate for inadequate examination or history

Although the aforementioned test might not be suitable for eradication purposes, it might be a useful tool in the hands of an astute clinician. Mass screening bears little resemblance to diagnostic serologic testing in clinical practice. A sample selected for serologic testing might be restricted to only those adult cattle with chronic diarrhea and weight loss. Judicious case selection narrows the population of interest to cattle with a higher disease prevalence. Prevalence affects predictive value and, consequently, the diagnostic utility of any assay system (Table 2). The formula previously presented permits calculation of predictive value for all possible combinations of sensitivity and specificity and disease prevalence. Although these calculations are straightforward, they are often not possible. In many cases, the diagnostic immunologist cannot determine true disease status. Lacking a diagnostic gold standard, specificity and sensitivity are difficult to obtain. Disease prevalence may also be illusive and restricted to diseases of regulatory interest, and available figures may not reflect local conditions. The experienced clinician often develops an intuitive algorithm, whereby he or she assesses the predictive value of laboratory procedures on the basis of an index of suspicion created by signalment, history, clinical signs, and suspected prevalence.

Increasing titers—response rather than disease

Seroconversion is the production of increased concentrations of antibodies capable of binding the test antigen in a defined assay system. Seroconversion, whether defined as a twofold or fourfold titer increase, is often considered presumptive evidence

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 (eg, antisera used to group *Salmonella* spp and *Streptococcus* spp)[41,42] 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.[44,45]

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.[7] 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 event, 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
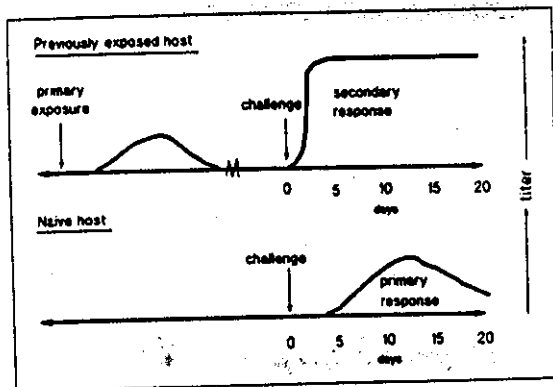

Figure 3—*Lag interval preceding humoral response and magnitude of response in previously exposed and naive hosts.*

factor. The risk factor of immediate interest will be seroconversion 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	
		+	−
Seroconversion	+	5	25
	−	2	10

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

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	
		+	−
Seroconversion	+	5	10
	−	2	25

Odds ratio $= (5 \cdot 25)/(10 \cdot 2) = 6.25$; $x^2 = [(5 \cdot 25) - (10 \cdot 2)]^2 \cdot 42/(7 \cdot 15 \cdot 35 \cdot 27) = 4.67$; 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 event. The statistical significance of these associations can be assessed by use of a x^2 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 also available for matched cases and controls.[13] 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 titer 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 39% PCOD sample as negative, and a 41% 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.[46]

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
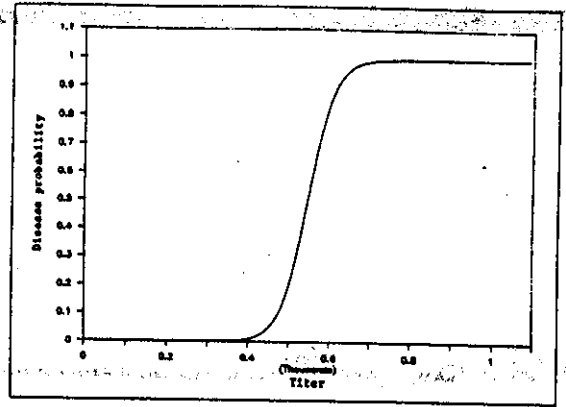
Figure 4—Hypothetic probability of disease at various measured signals. Reported probabilities were calculated by use of the method reported in the appendix and premised on a mean titer of 400 in disease-negative animals, a mean titer of 700 in disease-positive animals, and a titer SD of 100 in both groups.

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 presumption that measured assay signals are normally distributed. Biological phenomena notoriously resist description by Gaussian distributions.[47] 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 a normal animal 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

No. of tests performed	Test specificity				
	0.900	0.950	0.975	0.990	0.999
1	10.00	5.00	2.50	1.00	0.10
2	19.00	9.75	4.94	1.99	0.20
3	27.10	14.26	7.31	2.97	0.30
4	34.39	18.55	9.63	3.94	0.40
5	40.95	22.62	11.89	4.90	0.50
6	46.86	26.49	14.09	5.85	0.60
7	52.17	30.17	16.24	6.79	0.70
8	56.95	33.66	18.33	7.73	0.80
9	61.25	36.98	20.38	8.65	0.90
10	65.13	40.13	22.37	9.56	1.00

to bovine practice, infectious infertility, or even diagnostic serologic testing. A serum biochemical panel often contains 12 separate tests, and a single CBC 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. Indiscriminant 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. Indiscriminant use of test panels increases the likelihood of false-positive results.

Glossary of terms[4,5,7,12,13]

Affinity—The binding strength between isolated antigenic determinant and antigen combining sites.

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 (eg, 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 immunoassay 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[47,48]:

$$f = \frac{1}{\sqrt{2\pi\delta^2}} \cdot e^{-(x - \mu p)2/2\delta2}$$

where f = relative frequency of a test outcome in a population of defined disease status; x = observed test outcome; μp = mean test outcome in a population of defined disease status; δ = 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:

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

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.

References

1. Butler JE, Peterman JH, Suter M, et al. The immunochemistry of solid-phase sandwich immunosorbent assays. *Fed Proc Fed Am Soc Exp Biol* 1987;46:2548–2556.

2. Butler JE, Feldbush TL, McGivern PL, et al. The enzyme linked immunosorbent assay (ELISA): a measure of antibody concentration or affinity? *Immunochemistry* 1978;15:131–146.

3. Kennel S. Binding of monoclonal antibody to protein antigen in fluid phase or bound to solid supports. *J Immunol Methods* 1982;55:1–12.

4. Roitt IM, Brostoff J, Male DK. *Immunology.* St Louis: CV Mosby Co, 1986:25.1–25.10.

5. Stites DP, Stobo JD, Fudenberg HH, et al. *Basic and clinical immunology.* 4th ed. Los Altos, Calif: Lange Medical Publications, 1982;366–383, 735–745.

6. Herron JN. Equilibrium and kinetic methodologies for the measurement of binding properties in monoclonal and polyclonal populations of anti-fluoresyl-IgG antibodies. In: Voss EW Jr, ed. *Fluorescein hapten: an immunological probe.* Boca Raton, Fla: CRC Press Inc, 1984;49–76.

7. Tizzard I. *Veterinary immunology: an introduction.* Philadelphia: WB Saunders Co, 1987;xiii–xviii, 33–45, 129–153.

8. Sternberg JC. Rate nephelometry. In: Rose NR, Friedman H, Faley JL, eds: *Manual of clinical laboratory immunology.* 3rd ed. Washington, DC: American Society for Microbiology, 1986;33–37.

9. Sittampalam G, Wilson GS. Theory of light scattering measurements as applied to immunopreciptin reactions. *Anal Chem* 1984;56:2176–2180.

10. Charan S, Gautam OP. Applications of enzyme-linked immunosorbent assay in veterinary medicine: a bibliography. *Vet Res Commun* 1984;8:255–267.

11. Confer AW, Wright JC, Cummins JM, et al. Use of a fluorometric immunoassay to determine antibody response to *Pasteurella haemolytica* in vaccinated and nonvaccinated feedlot cattle. *J Clin Microbiol* 1983;18:866–871.

12. Schwabe CW, Riemann HP, Franti CE. *Epidemiology in veterinary practice.* Philadelphia: Lea & Febiger, 1977;66–100.

13. Fleiss JL. *Statistical methods for rates and proportions.* New York: John Wiley & Sons, 1981;56–82, 112–137.

14. Ritzmann SE. Introduction. In: Ritzmann SE, ed. *Physiology of immunoglobulins.* New York: AR Liss Inc, 1982;1–11.

15. Anderson KK, Benyamin Y, Douzou P, et al. The effects of organic solvents and temperature on desorption of yeast 3-phosphoglycerate kinase from immunoadsorbent. *J Immunol Methods* 1979;25:375–381.

16. Abercrombie DM, Chaiken IM. Zonal elution quantitative affinity chromatography and analysis of molecular interactions. In: Dean PDG, Johnson WS, Middle FA, eds. *Affinity chromatography.* Washington, DC: IRL Press, 1978;191–206.

17. Zoller M, Matzku S. Antigen and antibody purification by immunoadsorption of non-biospecifically bound proteins. *J Immunol Methods* 1976;11:287–295.

18. Wilkie BN. Respiratory tract immune response to microbial pathogens. *J Am Vet Med Assoc* 1982;181:1074–1079.

19. Winkelstein J. Genetically determined deficiency of the third component of complement in the dog. *Science* 1981;212:1169-1170.

20. Perryman LE. Primary and secondary immune deficiencies of domestic animals. *Adv Vet Sci Comp Med* 1979;23:23–52.

21. Brummerstedt E, Flagstad T, Basse A, et al. The effect of zinc on calves with hereditary thymus hypoplasia (lethal trait A46). *Acta Pathol Microbiol Immunol Scand [A]* 1971;79:686–687.

22. Smith GS, Lumsden JH. Review of neutrophil adherence, chemotaxis, phagocytosis, and killing. *Vet Immunol Immunopathol* 1983;4:177–236.

23. Gobig TG. Leukocyte abnormalities. *Med Clin North Am* 1980;64:647–666.

24. Smith BP, Habasha FG, Reina-Guerra M, et al. Immunization of calves against salmonellosis. *Am J Vet Res* 1980;41:1947–1951.

25. Merritt FF, Smith BP, Reina-Guerra M, et al. Relationship of cutaneous delayed hypersensitivity to protection from challenge exposure with *Salmonella typhimurium* in calves. *Am J Vet Res* 1984;45:1081–1085.

26. Winter AJ. Mechanisms of immunity in bacterial infections. *Adv Vet Sci Comp Med* 1979;23:53-69.

27. Woolcock JB. Pathogenesis of bacterial infections: some determinants of virulence of gram negative bacteria. *Aust Vet J* 1985;62:177–181.

28. Thiry E, Saliki J, Bublot M, et al. Reactivation of bovine rhinotracheitis virus by transport. *Comp Immunol Microbiol Infect Dis* 1987;10:59–63.

29. Pastoret PP, Thiry E. Diagnosis and prophylaxis of infectious bovine rhinotracheitis: the role of virus latency. *Comp Immunol Microbiol Infect Dis* 1985;8:35–42.

30. Bendixen HJ. Bovine enzootic leukosis. *Adv Vet Sci Comp Med* 1965;10:129–204.

31. Miller JM. A review of bovine leukosis. *Proc Annu Meet Am Assoc Bovine Pract* 1983;15:30–34.

32. Kimman TG, Westenbrink F, Schreuder BEC, et al. Local and systemic antibody response to bovine respiratory syncitial virus infection and reinfection in calves with and without maternal antibodies. *J Clin Microbiol* 1987;25:1097–1106.

33. Henderson FW, Collier AM, Clyde WA, et al. Respiratory syncitial virus infections, reinfections, and immunity. *N Engl J Med* 1979;300:530–534.

34. McNulty MS, Bryson DG, Allan GM. Experimental respiratory syncitial virus pneumonia in young calves: microbiologic and immunofluorescent findings. *Am J Vet Res* 1983;44:1656–1659.

35. Prince GA, Horswood RL, Camargo E, et al. Mechanisms of immunity to respiratory syncitial virus in cotton rats. *Infect Immun* 1983;42:81–87.

36. Robbins SL, Cotran RS, Kumar V. *Pathologic basis of disease.* Philadelphia: WB Saunders Co, 1984;163–176.

37. Martin SW. Vaccination: is it effective in preventing respiratory disease or influencing weight gains in feedlot calves? *Can Vet J* 1983;24:10–19.

38. Friend SCE, Wilkie BN, Thomson RG, et al. Bovine pneumonic pasteurellosis: experimental induction in vaccinated and nonvaccinated calves. *Can J Comp Med* 1977;41:77–83.

39. Wilkie BN, Markham RJF, Shewen PE. Response of calves to lung challenge exposure with *Pasteurella haemolytica* after parenteral or pulmonary exposure. *Am J Vet Res* 1980; 41:1773–1778.

40. Nicoletti P. Further evaluation of serologic test procedures used to diagnose brucellosis. *Am J Vet Res* 1969; 30:1811–1816.

41. Luderitz O, Staub AM, Westphal O. Immunochemistry of O and R antigens of *Salmonella* and related Enterobacteriaceae. *Bacteriol Rev* 1966;30:192–225.

42. Farmer SG. Immunology of bacterial infection. In: Lenette EH, Balows A, Hausler WJ Jr, et al, eds. *Manual of clin-

ical microbiology*. Washington, DC: American Society for Microbiology, 1985:898–904.

43. Carmichael LE. Immunization strategies in puppies—why failures? *Compend Contin Educ Pract Vet* 1983; 5:1043–1052.

44. Lerner RA. Synthetic vaccines. *Sci Am* 1983;248:66–74.

45. McGregor S, Easterday B, Kaplan AS, et al. Vaccination of swine with thymidine kinase-deficient mutants of pseudorabies virus. *Am J Vet Res* 1985;46:1494–1497.

46. Afifi AA, Clark V. *Computer aided multivariate analysis*. Belmont, Calif: Lifetime Learning Publications, 1984; 74,246–308.
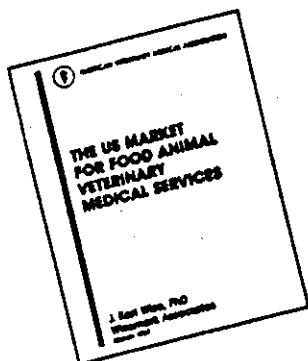
47. Elveback LR, Gulliver CL, Deating ER Jr. Health, normality, and the ghost of Gauss. *JAMA* 1970;211:69–75.

48. Daniel WW. *Biostatistics: a foundation for analysis in the health sciences*. 3rd ed. New York: John Wiley & Sons, 1983;79–83.