

SEROLOGY



BASICS

OVERVIEW

- Serology is narrowly defined as the study of serum derived from whole blood, and in particular, its immunologic components. These include antibodies produced by the host organism, and any antigen that may be present in the circulation. More broadly, serology may refer to the application of serologic assays to other body fluids such as cerebrospinal fluid or milk.
- Serologic assays are diagnostic tools premised upon the known interactions of antigens and antibodies. They are valuable for the diagnosis of disease in individual animals and for disease surveillance in herds or larger population groups.
- The effective use of serologic testing in clinical practice or production medicine requires an understanding of 1) general immunology and antibody-antigen interactions; 2) the type, technical specifications, and performance characteristics of the test to be used; 3) the biology and epidemiology of the disease in question; and 4) how best to interpret the serologic test in the context of clinical observations and the results of other diagnostics.

TYPES OF ASSAYS

- Effective clinical use of serologic tests requires an understanding of the basic types of serologic assays, the advantages and disadvantages of each, and whether the test to be used is targeting serum antibodies or circulating antigen.
- The **complement fixation** test is one of the earliest serologic assays. It utilizes the ability of antigen-antibody complexes to activate complement via the classical pathway. Complement fixation assays can identify either antibody or antigen.
- **Agglutination** assays are based upon the ability of a single antibody molecule to bind more than one antigen particle. This crosslinking results in visible clumping of the bound particles when antibody is present.
- **Precipitation** assays also utilize the crosslinking property, but are performed in solution or agar (radial or double immunodiffusion). Precipitation occurs when antigen and antibody are present in ideal amounts for crosslinking to occur.
- **Immunofluorescence** assays use antibody labeled with fluorescent dyes that allow visualization of the bound complex.
- **Radioimmunoassays** use isotope labeling and typically have very high sensitivity. IgE can be measured using this method.
- **Enzyme-linked immunosorbent assays** (ELISAs) have been used for the detection of antibody and antigen since the 1970's. In an ELISA assay, antigen or antibody is bound to an enzyme, and the action of the enzyme upon added substrate indicates the presence of the target molecule. There are several types of ELISAs, including direct, indirect, sandwich and competitive assays.

- Serologic assays vary in their ability to detect target antigen and antibody. Agglutination, precipitation, and immunodiffusion assays are generally less sensitive than techniques such as ELISAs or indirect immunofluorescence, which incorporate signal amplification strategies.

BASIC PRINCIPLES OF SEROLOGY

- The fundamental principle underlying all serological assays is the molecular interaction of antibody and antigen.
- Antibodies (immunoglobulins) are soluble antigen receptors produced by B-cells following co-stimulation.
- There are five classes of antibodies: IgG, IgM, IgA, IgD, IgE.
- IgG is the predominant immunoglobulin class in serum.
- IgM is the second most abundant immunoglobulin class in serum, and is the key immunoglobulin secreted during a primary immune response.
- IgA and IgE are involved in local immune responses and are present only in very small quantities in serum.
- IgD exists primarily in its B-cell surface receptor form.
- Each immunoglobulin class may have several subclasses, such as IgG1 or IgG2, with the number of subclasses varying across species.
- Antibodies and antigens interact via non-covalent (primarily hydrophobic) bonding.
- The species, class and subclass of antibody targeted by, or used as a reagent in a serologic assay influences the suitability, technical success and interpretation of the test. Certain assays may target specific classes or subclasses of antibodies. For example, IgM is more efficient than IgG in the induction of agglutination, precipitation, or fixation of complement, but the IgG1 subclass fixes complement whereas the IgG2 subclass does not.
- Although the generation of antibodies is a function of the adaptive immune system, and therefore involves a degree of specificity, cross-reactions can occur that may also influence the results and interpretation of an assay.
- Serological test results are often reported as a **titer**. This is the highest dilution of a serum sample that yields a positive test result.
- For a given assay, higher titers equate with higher concentrations of antibody.
- Titers are only relative measures of antibody concentration, therefore a titer from one assay system cannot be compared directly to a titer produced by a different assay system.
- Antibodies identified in serological assays, and therefore titers, may not translate to immunity.
- The clinical performance of a serologic assay is important to consider when interpreting results. There are several standard characteristics used to describe the performance of a given test.
 - The **sensitivity** of an assay is the probability that it will correctly identify true positives.
 - The **specificity** of an assay is the probability that it will correctly identify true negatives.
 - The **positive predictive value** of an assay is the probability that a test-positive animal is disease-positive.
 - The **negative predictive value** of an assay is the probability that a test-negative animal is disease-negative.
- The clinical performance of a serologic assay is dependent on the prevalence of disease.
- The predictive value of a positive test generally increases as prevalence increases.
- The predictive value of a negative test generally increases as prevalence decreases.
- The validity of an assay's specified performance characteristics will depend upon the standard against which that test was evaluated.

- Although serologic testing has been in use for a very long time, advances in test methodology and interpretation are continually being made.
- One example is the use of synthetic antigens to improve performance characteristics of existing assays as investigated by McGiven and colleagues (2015).
- Efforts have also been made to evaluate the use of molecular testing as an adjunct to serologic testing with varied results. Polymerase chain reaction (PCR) assays have differed in correlation with serological methods for detecting Q-fever (*Coxiella burnetii* infection) in small ruminants. PCR has shown promise as an adjunct diagnostic assay for lentivirus infections in small ruminants. Molecular methods have their own advantages, disadvantages and nuances of interpretation. It is important to understand the molecular methodology used to determine if results support serologic assay results.

INTERPRETATION OF SEROLOGIC TESTS

Interpreting Positive Titers

- The presence of antibodies to a specific pathogen rarely provides a de facto diagnosis of disease.
- Bovine leukosis virus (BLV) provides an example. Virtually all seropositive cattle are infected, and the vast majority of infected cattle have positive serologic test results. Assays for antibodies to BLV have excellent diagnostic value as only those cattle sufficiently exposed to the pathogen to become infected will mount immune responses. Infected cattle remain infected for life and infection causes a consistent, reproducible humoral immune response.
- Positive titers on BLV assays equate with viral infection and not neoplasia.
- Although this principle is common to other retroviral diseases such as caprine arthritis encephalitis (CAE) and ovine progressive pneumonia (OPP), it unfortunately does not apply to the majority of livestock diseases making test interpretation more difficult.
- In these circumstances, a positive titer may indicate only a history of exposure. It is not unusual for cattle to be seropositive for diseases such as IBR, BVD, BRSV, rotavirus and other diseases that are ubiquitous or are included in routine vaccination protocols. Therefore a positive titer may or may not relate to the current episode of disease, and other information about the animal must be considered. For example, extremely high titers to BVD virus in late term pregnant cows may be an indication of a persistently infected fetus.
- One approach to interpretation is to use paired titers. Samples are drawn during the acute and convalescent phases of disease and titers are compared. A fourfold rise in titer is considered supportive of a recent, active infection.
- Timing must be considered when interpreting a fourfold rise in titer. Antibodies generally are present in serum about one week after primary exposure and a few days after secondary exposure. Peak antibodies will generally be lower following primary exposure compared to secondary exposure. Therefore, the timing of the patient's presentation to the veterinarian and the immune status will influence the relative rise in titer.
- The biology of the disease in question must be considered. For example, IBR is a recognized cause of abortion in cattle. However, the observed clinical event, abortion, can occur a considerable length of time after exposure. Cattle often will have achieved maximum humoral response by the time they abort and a rise in convalescent titer may not be present.
- A thorough history can also assist with the interpretation of paired titers, including previous disease episodes, incidental exposures, and the vaccination record. Circulating antibodies from previous disease episodes may persist for an extended length of time, and a recent history of vaccination could account for the presence of antibodies or a rise in titer.

- One disadvantage to the paired titer approach is that it is not diagnostically relevant until the convalescent titer can be processed, and therefore may not be helpful in determining how best to treat an animal in the acute phase of an unknown disease.
- A positive pre-suckle titer in a neonate suggests gestational exposure to the disease in question. Calves may respond to viral proteins as early as midway through gestation. Since they receive negligible transplacental transfer of antibodies, positive serologic test results on samples collected before the ingestion of colostrum suggest exposure to the specific pathogen of interest during gestation.
- This approach has been used extensively in the diagnosis of BVD-associated disease, particularly birth defects caused by gestational exposure. Exposure of the bovine fetus to BVD in the last trimester usually causes no ill effects. Under these circumstances the importance of positive precolostral serology is often overemphasized.

Interpreting Negative Titers

- A negative titer may or may not indicate the absence of disease. As with paired titers, the timing of the serologic test relative to exposure will influence serologic test results.
- Serologic tests for BVD present a special case. Cattle persistently infected with BVD tend to be immunotolerant with respect to closely related strains of BVDV. Given that BVD is ubiquitous and vaccination programs are common, the observation of negative titers with respect to BVD is suggestive, but not diagnostic, of persistent BVD infection.
- Inappropriate negative titers may suggest a lack of response to vaccination because of vaccine handling, timing of sampling, or abnormal host responses. This outcome may warrant further investigation as repercussions for herd health may occur.

EPIDEMIOLOGIC APPROACHES IN THE INTERPRETATION OF SEROLOGY

- Epidemiologic approaches can add value to herd diagnostics and disease prevention programs, but tend to be underutilized in practice settings.
- The following example illustrates the strength of an epidemiologic approach, and how an understanding of the above concepts can improve clinical practice.

A client raising registered Polled Herefords calls a veterinarian because his cows are not calving within the constraints of a defined calving season. The herd consists of 20 mature cows, 5 replacement heifers, and 1 mature herd bull. At the time of the initial contact, the herd was 60 days into the 90-day calving season and a total of 8 cows and heifers had calved. Rectal palpation and pregnancy diagnosis were performed on all cows that had not yet calved (17). Ten of the cows and heifers were open and 7 were pregnant. The client expressed concern that the apparent reproductive failure may be related to an infectious disease.

Serology was performed on samples of convenience, 4 of 10 open cows. Three of 4 cows were positive for *Neospora*. All other results were within normal limits. This presents a challenge for interpretation. Neosporosis can cause dramatically impaired fertility, but many herds with high prevalence will have near normal reproductive performance. The positive titers substantiate exposure, but not causality. Serologic testing for *Neospora* then was performed on the entire herd. The following results were obtained:

Result	Open	Pregnant	Total	Incidence
Serology positive	6	2	8	$6/8 = 0.750$
Serology negative	1	16	17	$1/17 = 0.059$
Total	7	18	25	$7/25 = 0.280$

- Relative risk (RR): Quantifies the risk of an event (reproductive failure) in individuals exposed to a risk factor (serology positive) relative to the risk of the event in non-exposed individuals (serology negative)

$$\text{RR} = \text{incidence in exposed} / \text{incidence in nonexposed}$$

$$= 0.750 / 0.059$$

$$= 12.75$$
- Attributable risk (AR): An assessment of the risk of an event (reproductive failure) attributable to exposure to risk factor (serology positive).

$$\text{AR} = \text{incidence in exposed} - \text{incidence in nonexposed}$$

$$= 0.750 - 0.059$$

$$= 0.691$$
- Population attributable risk (ARp): The incidence of an event in a population associated with the occurrence of a risk factor.

$$\text{ARp} = \text{AR} \times \text{prevalence of exposure to risk factor}$$

$$= 0.691 \times 0.32$$

$$= 0.221$$
- Population attributable fraction (AF): The proportion of a disease in a population associated with exposure to a risk factor.

$$\text{AF} = \text{ARp} / \text{population incidence}$$

$$= 0.221 / 0.280$$

$$= 0.790$$

In this herd, serology positive cows were 12.75 times more likely to be classified as reproductive failures. The additional incidence of reproductive failure expected in serology positive cows is 0.69. Twenty-two percent (0.221) of cattle can be expected to experience reproductive failure associated with serology positive status. Nearly 80% of the reproductive failure was associated with positive serology status. This analysis supports a conclusion that recent introduction of *Neospora* into the herd caused dramatically impaired fertility.

SUMMARY

- Serological assays have been a fundamental component of veterinary diagnostics for many years.
- Interpretation of serologic test results is often complex.
- Knowledge of the testing methodology, test performance characteristics, biology of the disease in question and epidemiologic principles enables the most valid and clinically useful interpretation of serologic test results.
- Serologic methods are continually being developed and improved.

- Molecular methods may be a valuable adjunct to serologic testing, but must be interpreted with consideration for their unique technical and performance characteristics.



MISCELLANEOUS

ABBREVIATIONS

- AF = population attributable fraction CAE = caprine arthritis encephalitis
- AR = attributable risk
- ARp = population attributable risk
- BRSV = bovine respiratory syncytial virus
- BVD = bovine viral diarrhea
- BVDV = bovine viral diarrhea virus
- ELISA = enzyme-linked immunosorbent assay
- IBR = infectious bovine rhinotracheitis
- OPP = ovine progressive pneumonia
- RR = relative risk

SEE ALSO

- Blood Chemistry (www.fiveminutevet.com/ruminant)

Suggested Reading

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Acknowledgment The author and Book Editors acknowledge the prior contribution of Jeff W. Tyler