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Serologic monitoring: Uses and implications

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Types of tests

ELISA, IFA, HI, SN, PCR, and CF are just a few of the most popular. Talk to your diagnostic laboratory to see which tests are offered and what they recommend for interpretation of results. Serology can either test for the antigen itself or an animal's response to the presence of antigen (antibody production). There tend to be gold standards for testing of certain diseases and the practitioner is cautioned to make sure that, when discussing results, the audience is familiar with the test, its strengths and weaknesses. What may seem obvious to a member of the veterinary profession can be ambiguous and misleading to someone else. For example, a SIV HI titer of 160 in a vaccinated animal is expected, but a layperson sees the laboratory interpretation of positive and assumes that the animal is infected with SIV.

For more information about specific tests, please refer to your diagnostic laboratory of choice.

Numbers to test

There are numerous tables available to determine the number of animals that need to be tested in order to detect disease occurring at a certain level within a given population. The most important factors when determining the number of animals to test are sensitivity, specificity, prevalence, and confidence level.

Sensitivity

Defined as the number of positives divided into the number of true positives and false negatives. For example, a sensitivity of 80% would identify the true positives 80% of the time, with the remaining 20% being identified as false negatives. The other definition of sensitivity relates to the minimum amount of disease required to cause a positive result. Although 0.4 is recommended as a cut-off in the PRRS ELISA test for negative versus positive results, many practitioners use 0.2 instead. The rationalization is that on any given day the ELISA can vary up to 0.2 and in a negative population a practitioner would rather have false positives than false negatives. By adjusting the cut-off value the sensitivity and specificity of the test have changed. The practitioner will expect to work up a case of a false positives every so often, owing to the heightened sensitivity of the test.

Specificity

Defined as the number of negatives divided into the total of true negatives and false positives. So a test with 85% specificity would correctly identify a negative animal as negative 85% of the time and would identify that animal wrongly 15% of the time. A test with high diagnostic sensitivity will most likely be of lower specificity—if a test only takes 4 virus particles to register positive, it will probably have more false positives than a test that requires 400 virus particles.

Prevalence

Defined as the percentage of the population infected with the disease in question. This value is most often used prior to testing based upon the practitioner's best estimate of the disease level in a given population. Tables usually use values of 1, 20, 50, and 80 when determining the number of animals to test.

Confidence level

Used in many tables at 90% and 95%. The relative certainty that in a population with a given prevalence, a test will be able to pick up a truly positive animal and identify a herd as positive. This becomes more important within negative populations when detection of a new disease is critical.

Animals to test

Isolation

Isolation animals are tested to confirm disease status, detect exposure, or check vaccine compliance. Since isolation animals are generally the producer's greatest risk of disease introduction or continuation on a farm, larger sample sizes are more appropriate to get a greater confidence level. PRRS-positive gilts entering a positive sow farm may only require a minimum number of animals be tested to be sure of consistent exposure prior to arrival. This differs greatly from PRRS-negative animals entering a negative sow farm, where the prevalence of disease is considerably less and mistakes are more costly if an infected gilt slips through the testing.

Stage of gestation
Since exposure to many diseases or vaccinations occurs at a specific time during gestation, this may also be an area to monitor. For example, if the sow herd were routinely vaccinated for SIV prior to farrowing, then testing at weaning in the sows or pigs would give a good estimate of the quality of vaccination.

Parity
The parity of the sow will give the practitioner an idea of the relative stability of disease within the herd, given a few assumptions. One assumption would be: in a stable herd gilts are adequately exposed to all pathogens from the sow herd and become immune. Following that logic, gilt titers should be significantly higher than older sows. That being said, SIV doesn't always follow that pattern. Many times, gilt titers will be lower due to lack of natural exposure, whereas sow titers will be high owing to repeated vaccination after natural infection.

Piglets
Piglet testing is a great screening tool for sow stability and exposure. High maternal antibodies compared to lower maternal antibodies for the same pathogens indicate exposure (either naturally or by vaccine) during gestation. Suckling pigs also provide a source for isolation or sequencing of PRRS.

Nursery
Nursery pigs are another indication of sow farm stability. Evaluation of seroconversion to disease in the nursery compared with maternal antibody decline is a good indicator of sow and nursery stability. Viruses can be isolated, and titers measured and compared at entry and exit.

Finishing
Less testing tends to be completed during the finishing phase of production. However, it is beneficial in diagnosing disease (SIV) and monitoring vaccine compliance and efficacy (Mycoplasma) of vaccines and treatment strategies. Finishing serology is used more often as a tool with tissue diagnostics and slaughter checks, rather than alone.

Farms to test

Multiplication
This area is the most valuable in financial and production considerations. Serology can be a helpful tool in ensuring that this area has the desired disease status. The number of animals tested is greater in order to increase confidence, positives are proved false, and animals are tested throughout their growing phase so that a history is developed for each group of animals.

Commercial
Because of the large number of animals produced in commercial systems, diagnostic testing of animals is expensive. Evaluating the quality of vaccines and altering disease status can help the producer be more profitable.

Boar studs
This is perhaps the most intensively tested area within swine production, owing to the value of the animals and their importance to the whole production system. There ought to be no assumptions when evaluating serology results.

Test for which diseases?

PRRS
For detecting PRRS in a negative population, ELISA is used as the primary test to identify negative animals. It has an extremely high sensitivity and specificity and identifies infection to US and European strains of PRRS. However, false positives are found on occasion and these animals need to be proved negative. ELISA measures antibodies as early as 7–10 days after infection.

IFA also measures antibodies, takes as many as 10–14 days after infection, and is a subjective test. The results of the IFA test are an interpretation, so there are differences among laboratories related to both technical expertise and the PRRS strain used by the lab. IFA is useful in detecting infection in animals that tested positive to the ELISA (either falsely or truly).

Another PRRS diagnostic test is PCR. PCR is able to identify antigen within the pig to detect exposure. It is also very sensitive in finding PRRS in pigs. Because serum-neutralizing antibody can reduce the amount of PRRS found circulating in the blood, this test is used more often for detection of PRRS within 10 days of exposure. Virus isolation is another test for the PRRS virus but results are variable owing to sample stability.

When developing a guide for testing, it becomes very important to consider the weaknesses and strengths of these tests.

SIV
ELISA and HI are both used for determining exposure to SIV. HI has been the gold standard for many diagnostic laboratories for the detection of SIV antibody in serum. Titers greater than 1:40 are considered positive. Antibodies can be detected as early as 5–7 days after infection. Natural exposure tends to create different titers than vaccination, which allows for some interpretation by the practitioner. It should also be noted that there is some difference among test strains at each diagnostic laboratory—as well as differences among technicians conducting the tests—that might create variation among results. ELISA

has recently become available through diagnostic laboratories, but has grown in popularity owing to the rapid turnaround time for results and more consistent results from laboratory to laboratory. Again 0.4 is the cutoff for positive and negative values. Less is known as to whether levels of titers can be interpreted as vaccine titer versus one resulting from natural exposure.

MHYO

ELISA is used predominately, both comp and IDEXX. The practitioner should become familiar with which test is being used by a laboratory and stay with a consistent test to reduce opportunities for misinterpretation. Positives are greater than 0.4, after a suspect range of 0.5–0.6. Positive titers after exposure or vaccination take around 14–28 days to develop, so identifying a specific time of exposure is more difficult.

PRV

The gene-deleted ELISA is used to detect field exposure, whereas the screening ELISA indicates exposure to vaccine or field virus. The development of the G1 ELISA has enabled an eradication program to be successful. It would be a great help if there were other serologic tests that allowed for the differentiation between vaccine and natural exposure.

Reasons for testing

Vaccine compliance

No matter which stage of production, the cost of vaccines is looked at critically. In order to evaluate whether a vaccine is efficacious for a certain disease condition, it is imperative that the vaccine is given in the manner that it is recommended by the veterinarian. Far too often, vaccines are discontinued because of "failing;" such failure have often turned out to be the result of animals that were vaccinated at different times or in different ways than what was assumed. Getting the farm to document when vaccines are given and who administered them gives the practitioner an idea of when to test for compliance and with whom to discuss the results, whether the results come back positive or negative.

Disease introduction/diagnosis

For a negative population, positive results allow for a presumptive diagnosis of a particular disease. The question arises as to whether the disease caused the present problem, or whether seroconversion preceded a new problem. Diagnosing disease in a positive herd is much more difficult. Tissue diagnostics need to confirm any serology. Sometimes neither serology nor tissues will yield a diagnosis and clinical signs offer the only way to diagnose a disease.

Herd stabilization

This type of serologic monitoring is done after a disease break or as a continual project to monitor change over time. Without continual exposure, a herd should have declining titers. Vaccination can make interpretation even more difficult, causing a rise in titers without "field" exposure. There is also a question of what the rate of decline in titer ought to be for each disease. There are published values for SPF animals after initial exposure, but extrapolating that information to a commercial sow herd in a pig-dense area may be misleading. Continued circulation of disease tends to occur over a longer period of time, especially in large herds with multiple buildings, or positive herds with subpopulations.

Proof of herd status

Proof of herd status may be required for animal movements, selling of breeding stock, semen, or pigs for further growing. Numbers to test are determined by the disease for which you are testing, the history of the farm, and location of the farm.

Monitoring versus detection

Herd monitoring is handled differently than detecting disease in a population. Monitoring suggests that the disease status is known, and therefore provides a good estimate of the prevalence of a disease within a group of animals. It looks for changes over time for a group of animals. Less re-testing and re-sampling are conducted because the expected results are positive. On the other hand, detection of a disease or negative herd testing requires a different mindset. In addition to monitoring a herd, the practitioner is also proving that the herd is negative. A negative herd may be needed to sell, move, prove regulatory compliance, or for the owner's documentation. This requires testing with a greater confidence, using tests with higher sensitivity and specificity, and ruling out false positives.

Serologic monitoring within a large production system

These questions need to be answered and discussed by the production group to determine what farms to test, how many to test, what diseases to test for, and what to do with the information gained.

What is the information worth to the company? If herds are negative for a disease and the production team wishes the herds to remain negative, then determine what this "insurance policy" is worth in time and effort. The group needs to understand what they are testing for with each test and the tests' limitations so that appropriate decisions can be made.

What will you do with the information? Do not wait for results to come back prior to making a deci-

sion. Although this statement seems backward in its logic, it *does* make sense. Create an algorithm beginning with the first test and determine what your actions will be if the result is negative or positive (or if the result is expected or unexpected). If the result is positive, arrange follow-up tests for the original sample and plan a retest of the animal in 7 days. Include people to contact regarding the results, production guidelines (e.g., whether movements on or off the farm are suspended), and identification of other farms to test. If the result is negative for the original test, then follow your scheduled monitoring.

If you do not know what you will do if the results are unexpected, then this type of monitoring is worthless, because you will tend to have different reactions with each positive, leading to misleading interpretations. On the same level, if the results do not affect the reactions—that is, your reaction will be the same whether the results are negative or positive—then this type of monitoring is a waste of time and money.

How much does it cost? As with any testing program it costs money and time for both the people involved in collecting the blood and those participating in roundtable discussions concerning the results. The program is a large investment not to be taken lightly. Farms should be added gradually to the program, beginning with the most important and working downward. That way, no one is overwhelmed with the data collected and the process can be perfected for the system.

Many herds need to be tested routinely for regulatory reasons. It works well to coordinate serologic monitoring with this regulatory testing so that the only additional cost is for the test itself, not for labor or veterinary assistance.

Summary

In summary, to use serologic monitoring effectively you need to have (and you need to teach others) a basic knowledge of statistics and an understanding of the strengths and limitations of serologic tests You will also require a management plan for when the results return.

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The author will be starting a new position with ELANCO September 9, 2002.