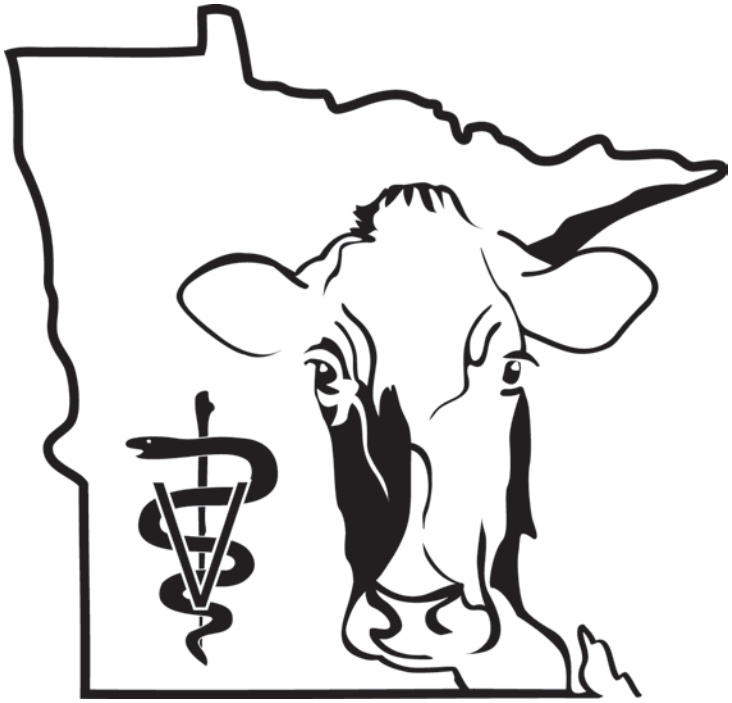
THIS ARTICLE IS SPONSORED BY THE
MINNESOTA DAIRY HEALTH CONFERENCE.

UNIVERSITY OF MINNESOTA

College of Veterinary Medicine

VETERINARY CONTINUING EDUCATION



ST. PAUL, MINNESOTA
UNITED STATES OF MINNESOTA

Use of Diagnostic Laboratory Services

Donald C. Sockett DVM, MS, PhD
Diplomate ACVIM
Wisconsin Veterinary Diagnostic Laboratories
University of Wisconsin-Madison

Introduction

The type of diagnostic services offered by veterinary laboratories is changing rapidly. Twenty years ago, laboratories offered primarily necropsy services supported by virology, bacteriology, serology and chemistry/toxicology sections. Many labs were required by state mandates to do export and regulatory animal surveillance testing as well. Veterinary pathologists were the dominant force in the laboratories and the majority of laboratory directors were veterinary pathologists. In the 1970's the first laboratories were accredited by the American Association of Veterinary Laboratory Diagnosticians (AAVLD). The requirements for accreditation were poorly defined and not of tremendous significance except for prestige.

Today the situation is quite different. Veterinary accreditation is very important because only AAVLD accredited or "equivalent" laboratories are recognized to test animals, semen and embryos for international export. Testing procedures and protocols are written by the World Trade Organization's Animal Health Organization which is known as the Office for International Epizootics (O.I.E.) which is located in Paris, France. In the near future (2-5 years) all AAVLD accredited laboratories must be ISO 9000 compliant and implement good laboratory practices (GLP) procedures. These means that laboratories must have written and documented quality control and assurance procedures and protocols for every test and procedure done in the laboratory from making reagents, to calibrating equipment etc. The standardization of diagnostic tests is a good thing and will reduce the number of discrepant results obtained between laboratories for identical samples. This is a well recognized problem for virus neutralization tests.

There are other significant changes happening in diagnostic laboratories as well. First the number of necropsies performed on food producing animals and poultry is declining. Typically laboratories are performing up to 50% fewer necropsies than they did 20-30 years ago but the ones they do are subjected to much more sophisticated testing procedures such as PCR, IHC (immuno-histochemistry), in-situ hybridization etc. The net result of these changes are there will be fewer pathologists but those that remain will have expertise in scientific procedures such as IHC. However, there will be more veterinarians employed in other sections of the laboratory such as immunology, molecular diagnostics and endocrinology. For the non-veterinarians in the laboratory, there will be an increased demand for individuals with advanced scientific training (masters and PhD. There will always be the need for technical staff to perform diagnostic tests using kits manufactured by private companies.

Diagnostic laboratories will be required to do more disease surveillance testing so that out trading partners will have confidence for example that Minnesota and Wisconsin are low incidence states for Bluetongue virus. This is the concept of regionalization of the country

according to disease risk. This means that laboratories will probably have a veterinarian on staff to do epidemiology and report findings to practitioners, USDA etc. In the long term, laboratories will continue to do an adequate job for companion animals but will be recognized for a particular area of expertise such as catfish in Mississippi, poultry in Georgia, swine in Iowa, Minnesota and South Dakota, and dairy in New York, Pennsylvania, Minnesota and Wisconsin.

How can I get better service from my laboratory?

1) Develop a good relationship with the laboratory. It is important that the laboratory receives feedback from its clients both good and bad. If for example, the laboratories submission forms irritate you contact the laboratory and let them know what the problem is.

2) Visit the laboratory and meet the staff. Offer suggestions for improvement.

3) Invite representatives from the laboratory to visit your practice and talk to some of your clients. I really enjoy giving talks and visiting producers because it gives me a pulse of what the problems you are seeing and what the laboratory can do to help you.

4) Don't hesitate to ask the veterinarians what type of problems they are seeing. Please remember you get a really good idea what the infectious disease problems are at the laboratory.

5) If you have any questions about proper sample submission, difficult cases etc. please don't hesitate to call and discuss the case particularly if you are submitting samples.

6) If there is some type of diagnostic service you would like the laboratory to add, please call and discuss it and if it is really important keep the pressure on.

7) Never hesitate to call and ask for preliminary results or complain about a bill etc. Keep the lines of communication open.

The following is an example of an article I wrote in the lab's newsletter is response to questions about BVDV and herd expansions. I believe laboratories should provide more of this type of information.

Control of BVDV in Dairy Herd Expansion Herds

BVDV control during and after dairy expansion is based on four concepts.

1) Isolation

2) Vaccination

3) Testing for PI animals

4) Control of farm traffic

All new arrivals (herd replacements) entering a dairy herd should be isolated for a minimum of 30 days. When possible, non-lactating cows and/or springing heifers should be purchased since they are easier to isolate on dairy farms. Isolated animals should not share feed, water, air and equipment with the resident herd. During the isolation period, the replacement animals should be vaccinated for BVDV and other infectious diseases such as Leptospirosis and IBR virus as well. It is important that the resident herd be properly vaccinated for BVDV before the introduction of the replacement animals. The topic of which vaccine to use (MLV or killed) is a subject of considerable debate. Most experts recommend that calves be vaccinated twice with MLV-BVDV vaccine with the last immunization occurring no later than 30 days prior to first breeding. The issue of what is the most appropriate vaccination schedule for open cows and first-calf-heifers has not been resolved but many dairy practitioners are now using MLV-BVDV vaccines.

All new herd additions and their offspring (if raised on the farm) should be tested for persistent infection during the isolation period. This recommendation is based on the knowledge that BVDV vaccination provides protection from disease (illness and death) but is not 100% effective in preventing fetal exposure particularly if PI animals reside in the herd. Therefore to maintain herd reproductive efficiency and to protect animals that do not respond with a protective immune response to BVDV vaccines; replacement animals should be tested for PI infection. The PI test is a virus isolation test that is done in microtiter plates with results available with 4-5 days after the test is set up. The test is extremely accurate to detect PI animals. The sensitivity is approximately 99% and the specificity is 100%. The reason the test is very accurate because PI animals typically have from 1000 to 1 million virus particles per milliliter of blood. Animals cannot be sampled for 2 weeks following vaccination with MLV-BVDV vaccines and whole blood (not serum) should be submitted for animals under 3 months of age. Serum cannot be used from young animals because maternal antibody can neutralize the virus and lead to a false negative test. Any animal that tests positive for persistent infection (viremic) should be retested in 2 to 3 weeks to rule out acute vs. persistent infection. PI animals will remain test positive whereas acutely infected animals will test negative. Serum samples cannot be pooled for PI testing because antibodies from immune animals will neutralize the virus from PI animals and lead to a false negative test.

BVDV can also be introduced by people and/or equipment contaminated with virus. Whenever possible people's access should be limited to the dairy barn which may mean locking the barn door and posting a warning sign to keep visitors out. It is extremely important that when visitors do enter the barn they should wear clean boots and clothing. Visitors should use a foot bath and clean their boots with a brush and disinfectant before entering the barn. It is a good idea to have bull calves and other sale animals picked up without allowing the dealer or transporter to enter the barn. The same policy should be used for renderers picking up dead animals as well.

Studies have shown that in some herds BVDV can cycle for 2 years following the removal of PI animals from the herd. In Wisconsin, we recommend testing for persistent infection if any of the 4 situations applies.

1. All herd replacements including their offspring should be tested. This is especially important for bulls.

2. If a PI animal dies on the farm, animals of the same age group should be tested since PI animals tend to cluster in groups on affected farms. If more PI animals are found, consideration should be given to testing the entire herd. If the dairyman has good records, only animals less than 2 years of age need to be tested as well as older animals that have no offspring in the herd. This recommendation is based on the knowledge that if a calf tests negative for persistent infection, the dam is not a PI. On the other hand, if the calf tests positive its dam must be tested to see if she is persistently infected,

3. Calves born in a herd after a known acute BVDV outbreak. Calves should be tested for at least 12 months beginning with fetuses that were in the first trimester of pregnancy at the beginning of the outbreak.

4. Closed herds that are experiencing reproductive problems (early embryonic death, sporadic abortions) and calfhood death losses (>2 weeks of age) and BVDV is isolated from either the calves or aborted fetuses. If BVDV virus is not isolated from aborting fetuses consider doing SN testing on aborting cows and gestationally matched animals that did not abort. There should be a minimum of 5 cows in each group for a total of 10 animals. If there is a statistically significant difference in titers among the 2 groups of animals then it is reasonable to conclude that BVDV is associated with the abortions. In small herds, serum can be collected from calves before ingestion of colostrum (collect calves for 2 to 6 months) to see if they have serum neutralizing antibodies to BVDV. The presence of antibodies indicates in-utero exposure to BVDV. Freeze the sera in a non-self defrosting freezer during the collection period.

Proper Sample Submission

Detection of PI animals

- Submit 5 ml of serum for animals 3 months of age and older.
- Submit 10 ml of whole blood [heparin (preferred) or EDTA from animals under 3 months of age.

Serum should be removed from the clot prior to shipping or the blood collected in serum separator tubes. All samples should be sent chilled to diagnostic laboratory for testing.

Detection of Acute Disease

Submit samples from several animals early in the course of the disease. It is important to remember that in some herds; type 2 BVD can cause sudden death with no gross lesions suggestive of BVDV infection.

Submit at least 10 ml of whole blood heparin (preferred) or EDTA. Samples should be sent chilled promptly to a diagnostic laboratory for virus isolation. In addition to whole blood, submit nasal swabs in viral transport medium if the animal(s) have signs of respiratory disease. If the animal dies, send both fresh and formalin-fixed tissues to the laboratory. If you cannot send the samples promptly, freeze the fresh tissue samples and place the formalin-fixed tissues in 70% ethanol after 24 hours. Extended time in formalin adversely affects (false negative) the immunohistochemical (IHC) test for BVDV. The IHC test detects BVDV specific proteins in formalin-fixed tissues. Note: If you freeze the tissue samples, they cannot be used for bacteriological examination. If you want bacteriological culture, keep a separate set of tissue samples for submission to the laboratory. Collect the following tissues for virus isolation and IHC. If you observe gross lesions suggestive of BVDV, submit those tissues as well.

- lung
- kidney
- spleen
- intestine (Peyer's patch region)
- mesenteric lymph node

At the time of the acute outbreak collect serum from acutely affected animals and freeze the sera in a non-self defrosting freezer. If virus isolation and/or IHC testing is negative, collect convalescent serum 2-4 weeks after the acute outbreak. Submit both the acute and convalescent sera as a pair and request SN testing for both types and type 2 BVDV. A four fold or greater rise in titer indicates exposure to BVDV.

Abortion testing

A whole refrigerated fetus sent promptly to the laboratory is the sample of choice for first and second trimester fetuses. For third trimester fetuses, submit both fresh and formalin-fixed tissues. Collect the following tissues for virus isolation and IHC:

- lung
- kidney
- spleen
- heart
- brain
- placenta

If you cannot send the samples promptly, freeze the fresh tissue samples and place the formalin-fixed tissues in 70% ethanol after 24 hours. Do not submit samples from aborting cows for virus isolation. Cows that abort are rarely viremic.

Abortion serology

Generally, abortions caused by BVDV virus occur within 1-2 months after exposure; however, abortions can continue as long as 8 months after an acute outbreak. This 1-8 month delay in abortions makes testing of paired serum samples of little value in diagnosing BVDV abortion. It is important to obtain serum from more than one animal for BVDV serum neutralization testing. A single serum sample has little diagnostic value. Collect serum from all animals that have aborted within the previous 30 days and also bled an equal number of gestationally matched animals to see if there is a difference in titers to type 1 and 2 BVDV between the two groups.

Selected References

1. Baker JC: The clinical manifestations of bovine viral diarrhea virus infection. The Veterinary Clinics of North America: Food Animal Practice. pp 425-445, W.B. Saunders, Philadelphia, PA November 1995.

2. Bolin SR, Littledike ET et al: Serologic detection and practical consequences of antigenic diversity among bovine viral diarrhea viruses in a vaccinated herd. Am J Vet Res 52:1033, 1991.

3. Pellerin C, Van Den Hurk et at: Identification of a new group of bovine viral diarrhea virus strains associated with severe outbreaks and mortalities. Virol 203:260-268, 1994.

4. Ridpath JF, Bolin SR, Dubovi EJ: segregation of bovine viral diarrhea virus into genotypes. Virology 205:6674, 1994.

5. Sockett DC et al: Fetal exposure to bovine viral diarrhea virus despite vaccination in a Wisconsin Herd: Proceedings First International Vaccines and Diagnostics Conference. Madison, WI 1999.