



Methods for Detection of BVDV

Virus isolation has been the backbone of virology, but has been losing favor recently because of the expense involved and the perceived slowness of the process. It will remain an integral part of the diagnostic process because it is the only way to discover new viruses or old viruses in new animal species. Also, it provides new isolates for comparison with current vaccines or old field strains (BVDV type 1 vs. BVDV type 2). Viruses are not static entities; changes in them need to be tracked.

Antigen detection tests are a second way of detecting viruses in clinical samples. The most common of these is the fluorescent antibody test done on frozen tissues. A similar test is the immunoperoxidase test usually done on formalin-fixed tissue. Other antigen detection tests are those that capture antigen on some type of solid support (antigen-capture ELISA or ACE tests). These processes are quick, specific, and thus preferred when sufficient viral protein is present such as in BVDV persistently infected animals. A lack of sensitivity in the ACE assay can, however, be a problem and should never be used to detect acutely infected animals

Antibody detection tests are tests that identify antibody as an indirect means of inferring the presence of a viral infection. There is a whole host of antibody detection tests, and the type of test selected will be determined by the type of virus that one wishes to detect and the types of tests available. For BVDV, the standard antibody detection test is the serum (virus) neutralization assay. For non-vaccinated animals, both a type 1 and type 2 SN should be requested.

Nucleic acid detection is the newest technique for finding viruses. Of the various methods used for this purpose, the polymerase chain reaction or PCR is the one most favored. While this test is very sensitive, it is fraught with technical difficulties and one must be very careful in the selection of the laboratory doing the testing. It is very critical to question the type and number of controls run with the test sample. If the test result is reported as negative, did the lab run the appropriate positive controls? Likewise, have false positive results been ruled out by use of appropriate controls. The strength of PCR (its exquisite sensitivity) is also its downfall because tiny amounts of contaminating nucleic acid can be amplified to produce a false positive test result.

Tests Offered by Cornell Diagnostic Laboratory

- 1 Bovine Viral Diarrhea Immune Peroxidase test (IP)
- 2 Bovine Viral Diarrhea Serum Isolation (with IP detection)
- 3 Bovine Viral Diarrhea Fluorescent Antibody test (FA)
- 4 Bovine Viral Diarrhea Virus Isolation
- 5 Bovine Viral Diarrhea Virus Serum Neutralization test (SN)
- 6 Bovine Viral Diarrhea Serum Isolation
- 7 Bovine Viral Diarrhea Whole Blood Isolation
- 8 PCR Detection Using (Bulk) Milk Samples
- 9 Bovine Viral Diarrhea Antigen Capture ELISA test [ACE] (serum or milk)
- 10 Bovine Viral Diarrhea Antigen Capture ELISA test [ACE] (skin)
- 11 Pooled PCR testing for herd screening
- 12 BVDV PCR test

Tests 2, 4, 6 and 7 are all variations on virus isolation tests. Test 5 is the only one that detects antibody to BVDV. Tests 1, 3, 9 and 10 are antigen detection tests while test 8, 11 and 12 are nucleic acid detection tests.

The choice of test depends on the current clinical problem and on the past test data from the herd or animal. Examples of how these tests should be used to detect and identify a BVDV problem are given along with a few details about each test.

- 1. Bovine Viral Diarrhea IP** This immunoperoxidase (IP) method provides an *antigen* detection test that uses formalin-fixed tissue. It is generally done in conjunction with a necropsy or histopathology submission. It is a very reliable antigen detection test and it can be used to detect BVDV in acute infections as well as animals persistently infected with BVDV. This test is the basis of the skin biopsy IHC/IP detection test for detecting PI calves. As a skin biopsy test, it does not reliably detect acutely infected animals.
- 2. Bovine Viral Diarrhea Serum Isolation and Bovine Viral Diarrhea Serum Isolation (with IP detection)** These tests are virtually identical virus isolation tests with the exception of the method used to detect the presence of the virus. Normally, the virus is detected using fluorescent antibody staining of the test cells. The test "with IP detection" uses an immunoperoxidase system to detect the presence of BVDV. These tests are used to detect persistently infected animals when a small number of animals are to be tested or when the level of certainty of a negative status is of paramount importance, i.e. exports or animals qualifying for AI centers.
- 3. Bovine Viral Diarrhea FA** This is an antigen detection test run on fresh frozen tissue as contrasted with the IP test which uses formalin-fixed tissues. This test can be done more rapidly than the IP test, but the reliability of the FA test is less than the IP test. It is most useful in detecting acute infections.
- 4. Bovine Viral Diarrhea Virus Isolation** Virus isolation has been the "gold standard" for BVDV detection and will continue to be an important diagnostic test. This test is done primarily on *tissue samples* or swabs from acutely infected animals as well as from persistently infected animals. For animals suspected of being acutely infected, a whole blood sample should be submitted if possible (see item 7). For BVDV isolation, feces are *never* the sample of choice.
- 5. Bovine Viral Diarrhea Virus SN** This is the only test routinely used to detect and quantify *antibodies* specific for BVDV. In the classical use of this test, acute and convalescent sera are tested to determine whether a recent infection has occurred. This test is also valuable using single serum samples from a group of animals to determine the infection status of a herd. It can also be used to evaluate the vaccination program. For non-vaccinated animals, both a type 1 and type 2 SN should be requested.
- 6. Bovine Viral Diarrhea Serum Isolation** See item 2 above.
- 7. Bovine Viral Diarrhea Whole Blood Isolation** This test is a virus isolation which uses the mononuclear cells in unclotted whole blood as the test inoculum for cell culture. This test is reliable even in animals with both virus and antibodies, as the buffy coat is separated from the serum and the mononuclear cells are cultured live on cells.
- 8. PCR Detection Using Bulk Milk Samples** This test uses the sensitivity of the PCR test to screen several hundred lactating animals for PI

status with a single sample. A 200 ml sample of bulk tank milk is sent cold but not frozen to the lab for testing. The number of animals represented in the sample should ideally be less than 400 even though dilution tests suggest that positive results can be obtained with 1 animal in 600. If the number of animals is large, we suggest taking several samples representing different groups of animals. Positive results will be reported by phone to the submitting veterinarian. The fee for the test is \$60 per sample. This test should **not** be used to assess PI status of the entire herd because most PI animals do not survive to produce milk. A negative bulk tank test tells you nothing about the non-lactating animals. A complete description of this test is available at the end of this section.

9. Bovine Viral Diarrhea Antigen Capture ELISA(serum or milk) This test detects BVDV antigen in serum from persistently infected animals. Antibody will interfere with the test. As a result, it should not be used with animals less than about three months of age.

10. Bovine Viral Diarrhea Antigen Capture ELISA(skin) This test detects BVDV antigen from skin biopsy samples. It is a reliable antigen detection test for the detection of persistently infected animals. Because it occasionally detects acute infections, animals with positive test results should be retested with viral or antigen detection tests 3 weeks later, to confirm PI status prior to culling. This test performs well in animals with colostral antibodies, so it can be applied to samples from any age animals.

11. Pooled PCR Testing for Herd Screening. For herd screening purposes, pooling of whole blood/serum samples is an economical way to detect PI animals. Testing can be done on the whole herd including calves with colostral antibodies (whole blood). Individual samples are submitted to the laboratory which does the pooling. Testing strategies will identify the individual animals that are persistently infected.

12. BVDV PCR Test PCR testing can be used in any situation where virus isolation would be appropriate. Because of the sensitivity of the test, interpretation of positive test results can be problematic when MLV vaccines have been administered close to sample collection. In addition, a positive PCR test on a single sample cannot distinguish between an acutely infected animal and a PI animal.

Testing Strategies

The test strategies discussed here, are herd level strategies, they are designed to answer questions on the herd level infection status. These strategies may not be optimal to detect an individual animal, or to diagnose BVDV in for example aborted fetuses. The choice of test depends on the current clinical problem and on the past test data from the herd or animal. Examples of how these tests should be used to detect and identify a BVDV problem are given along with a few details about each test. Below we have identified three scenarios; the first is identification of persistently infected cattle. These are the carriers of the BVD virus, and the main source of infection. The second scenario is the ongoing surveillance of infection status in herds that wish to maintain a low BVDV risk status. The third scenario is the herd evaluation of the vaccination program.

Purpose	Situation	Test	Description
Identify persistently infected cattle	Herd Screening Pre-purchase, Recent purchase	Pooled PCR Test	For herd screening purposes, pooling of whole blood/serum samples is an economical way to detect PI animals. Testing can be done on the whole herd including calves with colostrum (whole blood). Individual samples are submitted to the Laboratory, which then does the pooling. Testing strategies will identify the individual animals that are persistently infected.
		ACE Test (Serum or milk)	This test detects BVDV antigen in serum from persistently infected animals. Antibody will interfere with the test. As a result, it should not be used with animals less than three months of age.
		ACE Test (skin)	This test detects BVDV antigen from skin biopsy samples. It is a reliable antigen detection test for the detection of persistently infected animals. Because it occasionally detects acute infections, animals with positive test results should be retested with viral or antigen detection tests 3 weeks later to confirm PI status prior to culling. This test performs well in animals with colostral antibodies, so can be applied to samples from any age animals.
		Bovine Viral Diarrhea Whole Blood Isolation	This test is a virus isolation test which uses the mononuclear cells in blood as the test sample. For acute infections, this is the most reliable sample for a BVDV diagnosis. It can also be used to detect persistently infected animals of any age. It is particularly useful for animals under 3 months of age when the serum isolation test is unreliable.
Ongoing herd surveillance	Maintain long term BVD low risk status.	Bovine Viral Diarrhea Virus SN	This is the only test routinely used to detect and quantify <i>antibodies</i> specific for BVDV. In the classical use of this test, acute and convalescent sera are tested to determine whether a recent infection has occurred. This test is also valuable using single serum samples from a group of animals to determine the infection status of a herd. The test should be used in unvaccinated animals greater than three months of age.

		Sentinel Animal Antibody Surveillance	One innovative method of monitoring BVD circulation within a group of animals is to introduce a sentinel animal to that group and monitor its antibody status utilizing the BVD SN test above. The sentinel must be evaluated as a demonstrated non-PI animal and must remain unvaccinated throughout its life within the herd. One example of the use of this animal would be to introduce him to a calf cohort and test him at times critical to the transmission of BVD virus within the cohort. One strategic testing strategy could include three weeks post introduction, at 3 months of age for the cohort, cohort prefreshening, and annually once introduced to the milking herd. Utilize the BVD SN test for this evaluation.
	Continued PI calf surveillance	Pooled PCR Test	For herd screening purposes, pooling of whole blood/serum samples is an economical way to detect PI animals. Testing can be done on the whole herd including calves with colostrum(whole blood). Individual samples are submitted to the laboratory which does the pooling. Testing strategies will identify the individual animals that are persistently infected.
		ACE Test (serum or milk)	This test detects BVDV antigen in serum from persistently infected animals. It has the same use as the micro-plate herd screen and has the same restrictions ...antibody will interfere with the test. As a result, it should not be used with animals less than three months of age. This test is at least as sensitive as the Microplate isolation test, and has a shorter lag time.

		ACE test (skin)	This test detects BVDV antigen from skin biopsy samples. It is a reliable antigen detection test for the detection of persistently infected animals. Because it occasionally detects acute infections, animals with positive test results should be retested with viral or antigen detection tests 3 weeks later to confirm PI status prior to culling. This test performs well in animals with colostral antibodies, so can be applied to samples from any age animals.
		Bovine Viral Diarrhea Whole Blood Isolation	This test is a virus isolation which uses the mononuclear cells in blood as the test sample. For acute infections, this is the most reliable sample for a BVDV diagnosis. It can also be used to detect persistently infected animals of any age. It is particularly useful for animals under 3 months of age when the serum isolation test is unreliable.
Evaluation of Vaccination Program	Surveillance at Breeding time or Pregnancy Check	Bovine Viral Diarrhea Virus SN	Evaluation of the level of protection in the herd consequent to a comprehensive vaccination program may be necessary to assess the efficacy of the vaccination strategy. The SN test can be employed for this purpose. Testing a random percentage of the herd or defined risk groups are two strategies that may be employed. The detection of adequate antibody level is the goal of this vaccination strategy evaluation process. The BVD SN test described above is appropriate for this purpose. The test should be used in vaccinated animals greater than three months of age.
Regulatory Testing For Sale, Export or entry into an Artificial Insemination Center	Testing prior to movement, sale or at routine intervals	Bovine Viral Diarrhea Serum Isolation and Bovine Viral Diarrhea Serum Isolation (with IP detection)	These tests are virtually identical virus isolation tests with the exception of the method used to detect the presence of the virus. Normally, the virus is detected using fluorescent antibody staining of the test cells. The test 'with IP detection' uses an immunoperoxidase system to detect the presence of BVDV. These tests are used to detect persistently infected animals when a small number of animals are to be tested or when the level of certainty of a negative status is of paramount importance, i.e. exports of animals or animals qualifying for AI centers.

		Bovine Viral Diarrhea Whole Blood Isolation	This test is a virus isolation test that uses the mononuclear cells in blood as the test sample. For acute infections, this is the most reliable sample for a BVDV diagnosis. It can also be used to detect persistently infected animals of any age. It is particularly useful for animals under 3 months of age when the serum isolation test is unreliable.
		ACE Test	Depending on age (presence of colostral antibodies) and convenience, either serum or skin samples can be submitted for pre-purchase or pre-sale screening to detect persistently infected individuals.
		Pooled PCR Test	Pooling of whole blood/serum samples is an economical way to detect PI animals. Testing can be done on any age animal, including calves with colostral antibodies (whole blood, only). Individual samples are submitted to the laboratory, which does the pooling. Testing strategies will identify the individual animals that are persistently infected.

Clinical Scenarios and BVD Tests of Choice

At least for the foreseeable future, BVDV will continue to be a problem for the cattle industry. The selection of the proper tests for the correct reasons is paramount for success in diagnosing a BVDV problem. One should never rely on a single test especially when confronted with an acute episode of clinical disease. If there is any doubt about the testing strategy for BVDV or any disease situation, feel free to contact the laboratory for assistance. Veterinarians should work with producers to design a testing scheme appropriate to the specific needs of a farm.

Live animal with clinical signs of an acute infection

Virus isolation (test no. 7) is the preferred test and an unclotted whole blood sample is the specimen of choice. Serum should not be submitted for virus isolation when an acute infection is suspected, regardless of the test format used. A PCR test (no.12) would also be appropriate.

Serum should be collected to determine the antibody status (test no. 5) of the animal at the time of the clinical signs and several weeks later. The four-fold rule applies, i.e. the change in antibody titer must be at least a factor of four before one can consider BVDV (or any infectious agent) as a potential instigator of the clinical episode.

Animal found dead with gross lesions suggestive of BVDV

In these cases, it is always best to submit the entire animal for a necropsy. If this is not possible, then tissues should be collected for histopathology and immunoperoxidase testing for BVDV (formalin fixation) (test no. 1); fresh tissue should be collected and shipped overnight on ice for virus isolation (test no. 4), fluorescent antibody testing (test no. 3), or PCR (test no 12)

Sporadic abortions, calf pneumonias, poor reproductive performance

These types of problems are suggestive of a chronic BVDV problem, but no convincing evidence is available. In this situation, the BVDV SN (test no. 5) is the test of choice to determine whether the virus is in the herd. A BVDV SN test on a single sample from 6-10 animals, which have not been vaccinated for BVD (request type 1 and type 2 SN), or on animals with defined vaccination histories can be used to detect the presence of BVDV in a herd. In addition, appropriate diagnostic testing on sick animals and fetuses should be done. Once evidence is obtained for the presence of BVDV in the herd, then other tests can be done to manage and monitor the problem, such as bulk milk tests and individual animal tests.

Identifying persistently infected animals

Six month old poor doing calf with no acute signs

A serum virus isolation test (tests no. 2 and no. 6) or an Ace test (test no 9) are the best tests for the determination of a PI animal of this age. A whole blood virus isolation (no. 7), PCR (no 12) or IP test on skin(no. 1) are also acceptable in this circumstance.

Eight month old bull going to an AI center

The serum virus isolation test (no. 2 and no. 6) is adequate to detect PI animals in this age group. It should always be used when the consequences of a false negative test result are substantial. Whole blood virus isolation (test no 8) or PCR (test no 12) can also be used but at higher cost.

Firm evidence of BVDV in herd with possible persistently infected animals

While one would not go wrong using a serum virus isolation test, the economics of the situation dictate that the serum or skin ACE tests (tests no 9 & 10) or pooled PCR test (no 11) be used. These tests are priced and formatted as herd screening tests. If animals are under 3 months of age, then the serum ACE test is inappropriate because of potential interference by colostral antibodies.

Selected References:

- 1) Sweeker WS, Allison MN, Bolin SR, Cole RM. Type II Bovine Virus Diarrhea Virus Infection in a Closed Herd of Simmental Cattle. *The Compendium*, February 1997: s79-s73.
- 2) Bezek DM. Bovine Virus Diarrhea Virus Infection: Individual and Herd Diagnosis. *The Compendium*, August 1995: s57-s62.
- 3) Tremblay, R. Transmission of bovine viral diarrhea virus. *Veterinary Medicine*, September 1996: 858-866.
- 4) Bolin SR, Ridpath JF. The clinical significance of genetic variation among bovine viral diarrhea viruses. *Veterinary Medicine*: October 1996: 958-961.
- 5) Dubovi EJ. Laboratory diagnosis of bovine viral diarrhea virus infections. *Veterinary Medicine*, October 1996: 867-872.
- 6) Cortese VS. No cause for Alarm: Current BVD vaccines appear to cross-protect against virulent type 2 virus strains. *Topics In Veterinary Medicine*, Vol 5 No 2 1994: 10-14.
- 7). Kelling, CL. Planning bovine viral diarrhea virus vaccination programs. *Veterinary Medicine*, September 1996: 873-877
- 8) Saunders, WB. *The Veterinary Clinics of North America*: Vol 11, No 3, November 1995.
- 9) Communications / Review: Dubovi, EJ and Brunner, MA: NYS Diagnostic Laboratory, Cornell University, Ithaca, NY.

Addendum:
BULK MILK TANK TESTING
FOR THE DETECTION OF
BVDV PERSISTENTLY INFECTED LACTATING COWS

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The Animal Health Diagnostic Center at Cornell University runs both the polymerase chain reaction test (PCR) and virus isolation (VI) on all bulk tank milk samples submitted for BVD testing.

Both tests are run on the sample because each is about 95% sensitivity, but running both picks up close to 100% of the tanks with BVD. Different conditions may tend to cause negative results with one test or the other if BVD is present. For example, freezing a sample would probably eliminate the possibility of getting a positive VI, but PCR would probably remain positive. Bacterial overgrowth on a sample not

kept adequately chilled may interfere with PCR, but VI may still be positive.

The lab feels that a bulk tank milk sample representing milk from up to 400 cows is an appropriate sample for testing. While they have detected one positive cow in a 600-cow sample, they recommend a lower number cut-off.

It is best to take samples after one milking. This not only reduces the chance of proportional dilution, but an infected cow might be in the tank for only one out of 4-milkings, while other cows will be in the tank for all 4-milkings. It complicates record-keeping and may contribute to errors, especially if lists are not made until the end of each milking, and fail to account for changes in the population tested over the time period in which the sample was collected. This probably is more likely to be a practical issue when the herd is comprised of larger numbers of animals.

A serious consideration to be made in advance of testing, is to accurately record all of the cow ID's associated with a bulk tank sample, as well as for any whole blood or skin notch testing.

There have been a few instances in which bulk tank tests have been positive for virus, and then when individuals in the herd are tested, a PI animal has not been found. The lab feels that the most likely explanation in such cases is that cull animals or deaths, subsequent to bulk tank sampling, account for the removal of PI's from the herd prior to further investigation. In the aforementioned instances, some cows were no longer available for individual testing after the positive bulk tank test was reported.

Successful string sampling strategies would require total confidence that samples are actually complete and distinguishable. It is ideal if milk filters are changed between string samples, and all transfer tanks emptied. It is critical that the population within the string sample is accurately identified over the time of sampling.

Sampling a bulk tank for only the first milking after tank sanitation has the added advantage of simplifying record keeping, just to cow ID's associated with a single milking. Following tank sampling, and until a negative tank result is obtained, a diagnostic sample, such as a skin notch or serum sample, should be saved from any lactating cow leaving the herd, including dead cows (skin sample). In the event of a bulk tank positive, it is recommended to test these saved samples and any "soon-to-be-culled" animals first. If one or more are positive, then retest the bulk tank, without their milk contribution, prior to individual testing of the remainder of the lactating cows. Otherwise, move on to individual testing of the remainder of the lactating animals.

Submit approximately 200 ml of milk, shipped in a leak proof, screw-cap plastic bottle (an empty clean bottled water or soda/pop bottle, sterility not required) overnight with freezer packs, to arrive on Tuesday, Wed or Thursday. Agitate tank for 5 minutes prior to collection.

Ship samples to: Diagnostic Laboratory, College of
Veterinary Medicine, Cornell University, Upper Tower Road
Ithaca, NY 14853