

Bovine Viral Diarrhoea Virus: what test can I use?

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Three bovine viral diarrhoea virus (BVDV) tests are available but a bewildering array of possibilities exist for using them depending on the age, physiological status, and infection status of the animal of interest, as well as the sample collected. For economical investigation, pooled testing options have been developed, further extending the testing options. Polymerase chain reaction (PCR) and antigen enzyme linked immunosorbent assay (ELISA) are both detecting virus, while antibody ELISA detects proteins formed by the immune system in response to virus.

A series of ready reckoner tables have been prepared as an option for quick test selection depending on whether you are investigating the BVDV status of calves (table 1), individuals (table 2) or groups (table 3). The tables have been created so you can quickly tell whether a test is possible in the animal you are interested in and what test you can undertake.

Fetus and calf test options

Table 1 – Fetus and calf BVDV tests

Age	PCR	Antigen ELISA	Antibody ELISA	Sample
Conception – 40 days	No	No	No	N/a
40 -120 days gestation	Yes	No	No	Foetal fluid
150 days – birth	Yes	No	Yes	Foetal fluid
Birth -35 days	Yes	No	No	serum
35 days -10 months	Yes	Yes	No	serum/skin
10 months and older	Yes	Yes	Yes	Serum/skin

Trying to decide if BVDV infection is involved in reproductive problems or fetal loss is one of the most challenging aspects of BVDV diagnosis.

No fetal products are likely to be available for testing in the first 40 days of pregnancy, so no testing options exist.

If an aborted fetus aged between 40 and 120 days gestation can be found, a BVDV PCR on any fetal fluid to detect virus is a possibility. To decide how old a bovine fetus is, measure the length from the top of the head (crown) to the rump just above the tail ie crown rump (CR) length. This ranges from 25-270 mm in 40-120 day old fetuses.

Once fetuses are older than 150 days until term (CR length 387-1050 mm), both PCR testing and antibody testing are possible, as the fetus is now immunologically competent. Collect a fluid sample from the fetus eg fluid in the thorax or heart blood for testing. I believe BVDV antigen ELISA testing is contraindicated in fetuses due to autolysis induced false positive results, and only recommend PCR testing.

After the calf is born, things get more complicated once the calf has suckled.

Colostrum interference (the colostrum gap) plays havoc with BVDV antigen ELISA tests in young calves less than 35 days of age. Only PCR testing is possible. Once they get over this age, the problems disappear and the usual range of virus tests can be used. It is thought maternal colostrum BVDV antibody binds to BVDV virus in the calf preventing detection of virus by the ELISA test. PCR testing is not interfered with by antibody.

PCR testing is undertaken on serum only, while skin and serum samples can be tested by the antigen ELISA.

Until calves are 10 months of age, BVDV antibody from colostrum can be detected in serum, hence consider the age of the animals before screening for antibody. Pooled antibody testing is only recommended in cattle older than 10 months.

Individual test options

Table 2 – Individual BVDV tests

Disease or physiological state	PCR	Antigen ELISA	Antibody ELISA	Sample
Acute infection (TI)	Yes	Yes	Yes	serum
Conceptus loss	No	No	Yes	serum
Pregnant (Trojan)	No	No	No	n/a
Persistent infection (PI)	Yes	Yes	No	serum/skin
Mucosal disease	Yes	Yes	No	serum/skin

Once cattle are older than 10 months-of-age, all BVDV tests are possible options, but then it depends on the disease form you suspect, and the physiological state of the animal for test selection.

Acute (transient) infection

If you suspect acute (transient) infection you can use either PCR, antigen ELISA or antibody ELISA testing but the timing of their use relative to the stage of infection is critical. Once infected, cattle will be viraemic for 10-14 days and either PCR or antigen ELISA will detect the virus, albeit as weak reactions. About 2-4 weeks after infection is cleared, antibodies to BVDV will be produced and can be detected. To confirm acute infection would require a positive PCR or antigen ELISA test, with a negative test 28 days later, or seroconversion from negative BVDV antibody status to positive. The standard protocol to confirm an animal is transiently infected (TI); is a positive virus (PCR or antigen ELISA) test followed by a negative virus test 28 days later.

Conceptus loss

Trying to show BVDV infection was responsible for conceptus loss would require demonstration of seroconversion over the period concerned. This would require a pre-pregnancy serum without evidence of seroconversion, followed by a post-fetal loss serum, with seroconversion. As this is impractical on farm, it is unlikely to be useful.

Finding trojans

Detecting non-PI pregnant cows carrying PI fetuses (ie a Trojan cow) is a testing area eluding diagnosticians at present. No testing option exists in the cow pre-term. Wait till the calf is born and test it for virus as soon as possible. Experimentally, demonstrating very high antibody titres in the dam or virus testing of amniotic fluid have been tried but poor success rates and technical difficulties have meant they are not recommended.

Confirming persistent infection

Finding individual PI is a relatively simple procedure. PCR or antigen ELISA tests on serum, or antigen ELISA tests on skin will detect large amounts of virus. Virus will still be present 28 days later. Mucosal disease is a PI with a transformed variant of BVD virus from the non-cytopathogenic form to the cytopathogenic form. Virus mutation

within a PI creates the lethal cytopathogenic form of BVDV, leading to immune cell destruction and the clinical presentation of mucosal disease. As well as the characteristic lesions, these animals will be virus positive.

Group test options

Table 3 Group BVDV tests

Physiological state	PCR	Antigen ELISA	Antibody ELISA	Sample
Milking	Yes	No	Yes	Bulk milk
Non milking	Yes	No	Yes	Pooled serum

Testing of pools of milk or serum provide cost effective options for testing groups of cattle quickly. PCR tests can detect BVDV in milk or serum pools. BVDV antibody tests in milk or serum pools derive a number used to predict the likelihood of virus exposure within the group. BVDV antigen testing is ineffective in pooled samples and cannot be used.

A testing option utilising PCR, antigen ELISA, or antibody ELISA exists for most ages and stages of BVDV infection. In some situations, delaying testing till later may be the only option and a few disease states (eg Trojans, 0-40 day fetuses) defy diagnosis. If an animal is already dead the best test is BVD antigen ELISA on skin. Spleen is no longer recommended as a test sample.

If only virus negative bulls are used, no virus is present in the herd and biosecurity is robust, you have **Defined**, **Assessed** and **Acted** upon the first three steps of the BVD steering committees guideline for BVDV control. Then year-on-year **Monitoring** allows you to ensure reinfection hasn't occurred.