Testing for bovine viral diarrhoea virus: What are the options?

F Hill
Gribbles Veterinary Pathology, PO Box 536, Palmerston North

Three bovine viral diarrhoea virus (BVDV) tests are commercially available but a bewildering array of possibilities exists 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 methods 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 antibody formed by the immune system in response to virus. Diagnostic tests for BVDV have been recently reviewed, and I contributed significantly to the published paper (Lanyon et al. 2013) and have summarised much of the information here.

**Virus or virus-specific antigen detection**

Whether investigating individual disease cases, endeavouring to eradicate BVDV from a herd or region, or identifying infected animals posing an epidemiological threat, it is vital to accurately detect BVDV virus or specific antigen (Ag). The use of virus isolation, Ag detection ELISA, immunohistochemistry (IHC), nucleic acid probe hybridisation and reverse transcriptase polymerase chain reaction (PCR) have all been used for the diagnosis of BVDV. In New Zealand only Ag ELISA and PCR are in commercial use.

**PCR**

Virus isolation is the ‘gold standard’ for BVDV diagnosis but PCR has replaced it as the practical standard, as it is less time consuming, less expensive, not restricted to laboratories with cell culture facilities and highly sensitive. A variety of samples, including blood, milk, follicular fluid, saliva and tissue, can be tested successfully by PCR, prolonged storage has minimal effect, and both transient and persistent infections can be detected.

PCR can be used on bulk tank milk (BTM) samples to detect persistently infected (PI) cows contributing to the milk tank. The maximum theorised herd size has been estimated to be as high as 5,000, while practically, herd sizes a lot lower than this are sampled. A positive BTM PCR result may also indicate transient infections in the milking herd, as opposed to the presence of PI cows. While a positive BTM PCR result indicates BVDV infection, a negative BTM PCR result does not necessarily indicate the herd is not infected – only the infected individual was not contributing to the BTM when the sample was taken. Animals not contributing to the BTM tank should be tested separately.

The same principle can be applied to pooled serum samples. PCR detects infected individuals contributing to the pool. The least-cost pooling strategy is an initial pool size of 20, with pools returning a positive test result prompting testing of individual samples.
Antigen ELISA

The Ag ELISA is a simple, rapid method for detection of PI animals and a variety of samples can be tested, such as serum, milk and ear notches. The Ag ELISA is a very robust, simple, cost-efficient diagnostic method, with results being minimally affected by prolonged storage. Cow-side detection of PI animals with rapid ELISA technology is possible, although the sensitivity of these methods is lacking, and not currently recommended.

Ag ELISAs cannot be used to test pooled serum samples, with reported sensitivity of <15% in pools of only two serum samples. In addition, colostral antibodies may affect the sensitivity of the Ag assay on samples from suckling calves. Cross-reactivity with Border Disease Virus can also occur (McFadden et al. 2012) but the addition of primers to the PCR test now avoids this issue with PCR testing at Gribbles Veterinary, and low values on Ag ELISA would alert the laboratory to this infection.

Immunohistochemistry

Immunohistochemistry is one of the most popular methods of BVDV Ag detection in the USA. It is perceived as robust and suitable for large numbers of samples but it faces disadvantages as it is restricted to tissue samples, is labour intensive, prone to technical error, relies on a subjective scoring system by experienced personnel, and is unreliable for use on samples stored in formalin for >15 days. This method is not used in New Zealand.

BVDV specific antibody detection

Detection of antibody (Ab) in cattle is a valuable way of determining an individual animal’s immune status and any previous exposure to BVDV. A positive antibody test in an unvaccinated individual indicates it has been previously exposed to BVDV, and is also not PI. However, a negative antibody result in an individual does not confirm the animal as BVDV naïve. Further virus or Ag testing is required to confirm the animal is not PI. At a herd or region level, high antibody prevalence indicates a high likelihood of the population currently containing a PI animal, while negative test results indicate that the population is unlikely to contain a PI. Also, low antibody seroprevalence provides evidence supporting the need for careful protection of the population. Conversely, high seroprevalence suggests that little benefit will be gained from vaccination against BVDV.

Several Ab detection methods are available including: a rapid dot-blot enzyme immunoassay, an agarose gel immunodiffusion (AGID) test, and a microsphere based immunoassay. However, the only methods for detecting specific Abs to BVDV in New Zealand are the serum neutralisation test (SNT) and the Ab ELISA.

The SNT is a highly specific test, however, it is expensive and time consuming due to a need for tissue culture. An SNT titre in a given animal will rise for at least three months following transient infection. This test can be undertaken in New Zealand at the Ministry for Primary Industry’s animal health laboratory.

Ab ELISAs also return quantitative results with optical densities (ODs) expected to rise for 10 to 12 weeks post-infection. Multiple commercial ELISAs are available for the detection of BVDV-specific Abs, and have been validated for use in serum, milk and bulk milk and will detect colostral antibodies in suckling calves. Testing BTM can be a valuable, efficient and cost-effective method for determining milking cow herd immunity levels. Antibody concentrations in BTM indicate the prevalence of immune cows in the milking herd, and the likelihood of the herd being infected. This method of identifying infected herds will, unlike BTM PCR, return a positive result even when the PI animal maintaining the infection is not contributing to the BTM, for example, a cow being treated for mastitis, a bull, or a heifer. In addition, as Abs persist for several years at least, BTM Ab testing is more valuable when used for regular surveillance so changes in seroprevalence can be observed.

Testing pooled serum samples by Ab ELISA can estimate seroprevalence amongst those individuals contributing to the pool. This is particularly valuable in non-lactating stock, including young or dry dairy stock, beef cattle and bulls.
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**Diagnosis of transient (acute) infection**

Clinical signs associated with transient BVDV infection are often mild, so the aim of diagnosing transient infection is to determine if; a pregnant female is at risk of delivering a PI calf, an infection is secondary to BVDV-associated immunosuppression, or if reproductive loss is a result of transient BVDV infection.

PCR is the most sensitive method of BVDV detection, capable of detecting relatively low levels of virus shed during transient infections. However, detection of virus on a single occasion presents an ambiguous result as it could signify either transient or a persistent infection. If a quantitative PCR protocol for BVDV detection was developed it would offer the ability to distinguish between transient and persistent infections based on the amount of virus present. Currently, absence of virus on a subsequent sample collected at least 19 days later will confirm transient infection.

A transiently infected (TI) animal should become seropositive for BVDV-specific Ab within two to three weeks post-infection. Hence, testing for antibody several weeks after initial testing can be used to distinguish between TI and PI infection in animals with positive PCR results. Alternatively, paired Ab tests pre- and post-infection showing a rise in Ab concentration may be sufficient to confirm the occurrence of a TI. However, pre-infection samples or samples collected during the period of transient viraemia are often difficult to obtain.

If you suspect an animal is TI 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 TI status 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 TI is a positive virus (PCR or antigen ELISA) test followed by a negative virus test 28 days later. On the ELISA test, a numerical optical density value, called S-N (sample value minus negative control value) is created. Experience in our laboratory indicates TIs have S-N values of <1.2 and PIs have S-N values >2. Recently we changed our reporting to show weak positive (S-N <1.2), positive (S-N 1.2-2), and high positive (S-N >2) results against individual animal results. A weak positive result can be taken to indicate an animal is TI while a high positive result indicates the animal is PI. A positive result could be TI or PI so retesting is indicated.

**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 and virus will still be present 28 days later. Mucosal disease is a PI with a transformed variant of BVDV 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.

**Calves <35 days of age**

When attempting to diagnose PI in young, colostrum-fed calves, the efficacy of Ag ELISAs is questionable (Fux and Wolf 2012). Therefore, PCR is the preferable diagnostic method for this purpose. Laboratory testing systems have been developed to overcome this problem. Testing all the calves by pooled PCR is a valid starting point. If no BVDV is detected in the pool, then none of the calves in the pool are PI. If virus is detected the pool then there are two options. If an urgent result is required, each calf can be tested individually by PCR for an additional cost. Alternatively, wait until the calves are older than 35 days, and test again by Ag ELISA.

**Diagnosis of mucosal disease**

To make a diagnosis of mucosal disease, PI status must first be confirmed and virologically both cytopathogenic and non-cytopathogenic BVDV must be present in the affected animal. However, identification of PI combined with pathological lesions of mucosal disease is sufficient to confirm the diagnosis.
Testing options
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.

Foetus and calf testing options
Trying to decide if BVDV infection is involved in reproductive problems or foetal loss is one of the most challenging aspects of BVDV diagnosis. Only two BVDV tests can be used in foetuses, depending on the age of the foetus. To decide how old a bovine foetus is, measure the length from the top of the head to the rump just above the tail (crown rump (CR) length).

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

40 - 120 days gestation (CR length 2.5-27cm)
BVDV PCR on any foetal fluid to detect virus is a possibility. Check the thorax for fluid, and only sample if there is a liquid without any chunks or solids. Store the fluid in a plain vacutainer for transport to the laboratory.

150 days – birth (CR length 38-105cm)
At this stage, both PCR testing and antibody testing are possible, as the foetus is now immunologically competent. Aseptically collect a clean, fluid sample from the foetus (e.g. fluid in the thorax, abdomen or heart blood), for testing.

Foetal antibody testing
Detecting BVDV antibody in the foetus indicates there has been viraemia of both the dam and foetus after 150 days gestation, when the foetus is immuno-competent and antibody has been formed. However antibody positive foetuses have not necessarily died because of BVDV infection. Nevertheless, antibody is a significant finding as it shows there was virus circulating in the herd.

Diagnosis of foetal malformation
If the lesions induced by BVDV infection are severe, the foetus will die and be aborted. Some foetuses may survive and be born with a variety of malformations depending on the tissue affected. Demonstration of BVDV in any tissues of affected foetuses or calves would confirm foetal BVDV infection. When infection is acquired after 150-180 days of gestation, the foetus is able to mount an effective immune response, clear the virus, and will be born Ab positive and virus or Ag negative. For example, if you are investigating dummy calves, and suspect or confirm cerebellar hypoplasia, don’t be put off if the calf is virus negative. Check the antibody status as well as you would expect the calf to be antibody positive and antigen negative.

Use of antibody tests in calves
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.
Table 1. BVDV tests to use in calves from conception to 10 months of age.

<table>
<thead>
<tr>
<th>Age</th>
<th>PCR</th>
<th>Antigen ELISA</th>
<th>Antibody ELISA</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conception-40 days</td>
<td>No - X</td>
<td>No – X</td>
<td>No - X</td>
<td>N/A</td>
</tr>
<tr>
<td>40-120 days gestation</td>
<td>Yes - √</td>
<td>No – X</td>
<td>No - X</td>
<td>Foetal fluid</td>
</tr>
<tr>
<td>150 days – birth</td>
<td>Yes - √</td>
<td>No – X</td>
<td>Yes - √</td>
<td>Foetal fluid</td>
</tr>
<tr>
<td>Birth-35 days</td>
<td>Yes - √</td>
<td>No – X</td>
<td>No - X</td>
<td>serum</td>
</tr>
<tr>
<td>35 days -10 months</td>
<td>Yes - √</td>
<td>Yes - √</td>
<td>No - X</td>
<td>serum/skin</td>
</tr>
<tr>
<td>10 months and older</td>
<td>Yes - √</td>
<td>Yes - √</td>
<td>Yes - √</td>
<td>Serum/skin</td>
</tr>
</tbody>
</table>

Diagnosis of abortion or reproductive failure

To investigate if reproductive failure was due to BVDV infection would require demonstration of seroconversion of the dam over the period of early pregnancy. Serial blood samples from the dam 4-6 weeks apart would indicate transient infection, however abortion of a PI foetus leads to a decline in antibody concentration, making titres difficult to interpret. Vaccination history would also be needed to interpret the titres. While it is difficult to conclusively diagnose BVDV as the direct cause of reproductive failure, BVDV is a significant contributor to reproductive disease. Herd-level evaluation of BVDV status is warranted when BVDV is suspected in individual cases.

Maternal antibody testing

Collecting a serum sample for BVDV antibody testing from the dam of the aborted foetus is a useful adjunct to foetal testing. If the dam is BVDV Ab negative, BVDV infection has not been a factor in the abortion. If she is BVDV Ab positive, BVDV infection may or may not have been involved. Ideally, you should also investigate the BVDV status of a herd with the other tests available eg pooled milk and serum samples for antibody and virus detection.

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-foetal-loss serum, with seroconversion. As this is impractical on farm, it is unlikely to be useful.

Diagnosis of the trojan cow

Current diagnostic tests will identify a Trojan cow (defined as a non-PI cow carrying a PI foetus) as virus negative and Ab positive, i.e. the same as an immune animal carrying a normal calf. While Trojan cows are known to have very high Ab concentration, using Ab concentration to distinguish between Trojan cows and cows carrying normal calves has met with only moderate success (Lindberg et al. 2001).

An alternate method of detecting Trojan cows is to test amniotic or allantoic fluids collected via intrauterine puncture for viral Ag (Lindberg et al. 2002). However, this method requires sedation and local anaesthesia and there are risks associated with such a sampling procedure. These factors make the test impractical for widespread field application. At present, virus testing of calves soon after birth remains the most practical way of assessing neonate BVDV status.
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### Table 2. Individual tests for BVDV depending on disease or physiological state

<table>
<thead>
<tr>
<th>Disease or physiological state</th>
<th>PCR</th>
<th>Antigen ELISA</th>
<th>Antibody ELISA</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transient infection (TI)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Serum/skin</td>
</tr>
<tr>
<td>Conceptus loss</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>serum</td>
</tr>
<tr>
<td>Pregnant (Trojan)</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>N/A</td>
</tr>
<tr>
<td>Persistent infection (PI)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Serum/skin</td>
</tr>
<tr>
<td>Mucosal disease</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Serum/skin</td>
</tr>
</tbody>
</table>

### Group test options

<table>
<thead>
<tr>
<th>Physiological state</th>
<th>PCR</th>
<th>Antigen ELISA</th>
<th>Antibody ELISA</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milking</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Bulk milk</td>
</tr>
<tr>
<td>Non milking</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Pooled serum</td>
</tr>
</tbody>
</table>

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 (e.g. Trojans, 0-40 day foetuses) defy diagnosis. If an animal is already dead the best test is BVDV 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 BVDV steering committees guideline for BVDV control. Then year-on-year Monitoring allows you to ensure reinfection hasn’t occurred.

### References


