

Reverse transcription-polymerase chain reaction (RT-PCR) is a laboratory technique that allows the sensitive detection of genetic material following repeated rounds of amplification.

It provides a cost-effective way of screening large number of animals for the presence of BVD virus. It can identify acutely or persistently infected animals as part of a disease reduction or eradication programme and can detect BVDV in new born and young calves without interference from maternally derived antibodies.

This assay has been validated at VSD for detection of BVDV type 1, BVDV type 2 and Border disease virus (BDV) in sera and milk (individual and bulk tank samples).

Uses of this test:

### **Testing of bulk tank milk (BTM) samples**

Purpose: a cost effective method to screen for the presence of persistently infected (PI) cattle among the milking cows.

Validation: dilution studies have shown with this test milk from a single PI animal will still test positive even when diluted 1/1,000 in antibody-positive milk. Field studies have shown that the test has successfully detected one virus positive animal out of 265 contributing to the bulk milk sample.

Sample submission: 20-40 ml of bulk milk sample comprising milk from all lactating cows should be sent to the laboratory in a sterile container containing a preservative tablet. Note should be taken of the cows contributing to the tank milk, as dry cows and bulls will have to be individually sampled.

Interpretation: a negative BVDV RT-PCR result strongly indicates that none of the contributing cows is persistently infected with BVDV and individual testing is not required. A positive result confirms the presence of one or more BVD virus positive cows (PI or acute) among those contributing to the sample. Such an animal is highly likely to be persistently infected. Note that residual RNA from acute infections may be detected and this can produce low positive or inconclusive results. When a positive result is obtained, animals should be individually sampled and tested, typically by ELISA.

**Note:** for preliminary investigation of a herd for BVDV infection status, the following test package (**BVD Herd Check**) is recommended: **BTM antibody + BTM BVDV RT-PCR + BVDV antibody on 5 unvaccinated, home reared cattle from each separately managed group of cattle in the age range 9 to 18 months (preferably non-vaccinated).**

### **Pooled serum samples**

Purpose: a cost effective method to screen for the presence of persistently infected cattle.

Validation: Validation studies at VSD showed serum from one persistently infected animal still gave a positive result when diluted 1/1,000 in antibody-positive serum.

Sample submission: submit individually identified clotted blood samples to the laboratory where they will be pooled and tested. Unless otherwise instructed, samples will be pooled in sets of 25. Note that samples should be collected using a fresh needle for each animal.

Interpretation: if the result is negative, none of the cattle making up the pool are persistently or acutely infected and no further testing is required. Pools giving positive results must be retested as individual samples to identify the virus-positive cattle.

### **Individual serum samples**

RT-PCR is not generally used for testing individual serum samples since the ELISA test is a more cost-effective option. However it is available on request e.g. to confirm ELISA results, to detect transiently infected cattle, to test young animals where false negative results due to the presence of maternally derived antibodies are a concern.

### **Ear notch samples**

Purpose: ear notch (skin) samples can also be used to screen for persistently infected cattle, either by ELISA (single samples) or real time RT-PCR samples. This sample type has the benefit of overcoming concerns about false negative results in young calves due to maternally derived antibody. When applied to pooled samples, it is a cost effective screening method.

Sample submission: samples should be submitted individually in dry sealed tubes marked with the ear tag numbers of the sampled animals. Ideally the sample should be collected as part of the ear tagging process using specially designed tags and applicators. Otherwise, samples can be collected using a secondary tag specially designed for this purpose. VSD will shortly be evaluating both management and identification tags for this purpose.

Interpretation: a negative result on a pooled sample is consistent with none of the animals present being persistently infected and consequently no further testing is required. A positive result requires testing of the individual samples to identify the virus positive cattle present.