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## TESTING

### Can you test other animals on your farm and include them in the eradication programme?

It remains open to herd owners to conduct additional testing over and above the required tagging of calves, even when all of the tag results are negative. This may be done to accelerate the programme of herd screening to confirm that the herd is free from infection as quickly as possible, for example, through testing cattle born before the start of the compulsory programme. These additional samples should be sent to designated labs for BVD testing, which can then transfer the results to the AHWNI database in the same way as that for tissue tag results. A list of designated labs and the samples (tissues, blood, milk) for which they are designated is available at [www.animalhealthni.com](http://www.animalhealthni.com). The laboratory will require the necessary data sharing permissions to be given by the herdowner before the transfer of such results can proceed to the AHWNI database.

### BVD testing apart from tissue samples?

It is envisaged that the primary means of sampling of calves will be the tissue tag. However, this may be supplemented by blood sampling. For example, herd owners may use their veterinary practitioners to re-sample animals where there has been a problem with sample collection initially or, more commonly, to carry out re-testing of calves with positive or inconclusive results and the dams of these calves. Veterinary practitioners may also use blood sampling and bulk tank milk RT-PCR testing as part of wider herd-level investigations. The results of such testing, provided it is fully traceable, has been carried out by designated laboratories and the required data sharing permissions have been given, will also be held on the AHWNI database.

### Can the blood samples taken for Brucellosis be used for BVD testing?

PI cattle are most likely to be found among calves. Screening older animals will only confirm that they are not themselves PI, giving a snapshot of the herd at that time. Because the majority of PI calves occur following a transient infection of the mother, a negative test result for heifers and cows is no guarantee that they will not produce a PI calf from the current or later pregnancies. In contrast, testing the calf crop gives an on-going screen for the birth of PI calves, allowing these to be identified and removed as quickly as possible. In addition, if a given calf is virus negative, its dam cannot be PI. In this way, tagging of calves gives a “two for-one” result, so that in addition to the direct virus result for each calf, indirect results also accumulate for all cows that have produced a calf.

### Do you test an aborted foetus?

Yes. Under The Bovine Viral Diarrhoea Eradication Scheme Order (NI) 2016 all calves born on or after 1<sup>st</sup> March 2016 must be tagged and tested for BVD. BVD virus can be a cause of abortion, and this may be the first sign noticed following introduction of infection. Make sure the ear of the aborted foetus is dry before taking the sample to minimise the chance of the tag slipping and no tissue being collected.

### Do you test stillborn calves?

Yes. Under The Bovine Viral Diarrhoea Eradication Scheme Order (NI) 2016 all calves born on or after 1<sup>st</sup> March 2016 must be tagged and tested for BVD. The screening of all calves born (including abortions and stillbirths) each year provides intensive surveillance of each herd. Dry the ear of the still born calf before taking the sample to minimise the chance of the tag slipping and no tissue being collected.

### Is it necessary to test both animals when twins are born?

A negative virus result for one of a pair of twins generally confirms that neither animal is PI with BVD virus and that the mother of the twins is also not PI. However, there are occasional descriptions of one twin being PI and the other one not, presumably due to infection of calves that are at the upper age limit at which unborn calves can become PI (approximately 120 days of pregnancy). In any event, the Bovine Viral Diarrhoea Order requires that all calves born on or after 1<sup>st</sup> March 2016 are tagged and tested.

### When should you test the cow if you receive an initial positive result for its calf?

You have the option to re-test the calf to confirm it is a PI. The dam should be blood tested in order to obtain a direct blood sample result. In practice, if the calf is being re-tested it is most straightforward to test the cow at the same time. If you do not intend to re-test the calf, then you should test the dam as soon as possible. It is crucial that at least 21 days are left between first sample collection and the second (blood) sampling of the calf. An interval of at least three weeks will allow time for virus to be cleared from a transiently infected (TI) animal.

### How soon should you test a newborn calf?

It is recommended that calves are tested as soon as possible after birth. In practice this means once the calf is dry.

### Why wait until a calf is dry to tag it?

This reduces the chance of a mistake in the tagging process due to the wetness of the ear.

### If a re-test is required following an empty sample, how long will it take to receive a button tag?

The delivery time for button tags is approximately 3 – 4 days. Contact your tag supplier for further information. You can collect the second sample from the calf at the earliest possible convenience.

### If a re-test is required following an initial positive result, how long should be left between the two tests?

It is recommended that at least 21 days are left between first and second sampling. An interval of at least three weeks will allow time for virus to be cleared from a transiently infected animal. The animals should be strictly isolated until the retest result is known. The second sample must be a blood sample collected by a veterinary surgeon and submitted with the appropriate paperwork to the designated laboratory.

### What is the turnaround time for a blood sample on a retest?

Designated laboratories undertake to report 95% of results within 7 working days with a median turnaround of 5 days or less. In practice, many results are reported more rapidly.

### How stable are samples after collection?

It is recommended that samples are sent to the laboratory as soon as possible after collection but in any event within seven days. In the interim, it is recommended that samples are stored in a cool, dark place, ideally in a non-domestic fridge. Stability studies have shown that using current test methods, samples are stable beyond this period, even when held at higher temperatures.

### Can a button tag be used to test a cow?

Yes. A matching supplementary button tag bearing the official ID number of the animal can be used. It can be ordered from your tag supplier, and should take approximately 4 days to be delivered. Please contact your tag supplier for further information. The dams of positive calves should be tested using a blood sample taken by your PVP.

### If a calf has a negative result when first tested, can it turn positive in the future?

A negative result is consistent with the calf not being PI. However, it may still become transiently infected (TI) for a short period. If sampled during this period, it is likely to test positive for virus. A negative repeat test 3-4 weeks later would confirm this as a transient infection.

### Should you test stock bulls for BVD virus?

Pedigree stock animals may have been tested for BVD virus, to confirm that they are not PI, prior to sale at certain registered breed society sales. However, it is strongly advisable and good practice to ask for confirmation of this prior to purchase. Where the status of the bull is not known, it would be wise to have his BVD status confirmed as soon as possible.

### Is there a provision to use existing BVD blood test results obtained for individual animals in the voluntary phase of the programme?

Blood tests carried out in herds that took part in the voluntary phase of the programme may be captured in the database and made available in the compulsory phase subject to certain conditions.

Firstly, the samples need to have been tested to an accredited standard in a designated laboratory; the samples must be identified with the full national ID number for each animal; the laboratory must be willing to effect the transfer and the farmer or vet must provide a written instruction to transfer the results.

### Does the age of the calf affect the accuracy of the test?

Older calves may have come in contact with a BVD virus and thus may have a transient infection (TI) which will show up as a positive result. Therefore, it is important to collect the sample by tagging as early as possible after birth. Note that blood samples from young calves may NOT be reliably tested by ELISA (typically below 75 days of age) due to the possibility of false negative results caused by interference from maternally derived antibodies (MDA). This period is often referred to as the "diagnostic gap". The ELISA currently in use for testing ear punch samples is marketed as not having a diagnostic gap, although it is possible that false negative results may occur occasionally. Should a false negative result be suspected, you should isolate the animal in question and contact the testing laboratory with a view to arranging a re-test. There is considered to be no diagnostic gap when testing samples by RT-PCR.

### If in-calf animals are blood tested with negative results does this mean the herd is free of infection?

Not necessarily; this only indicates that there are no PI breeding females. It does not give the status of the calves that they are carrying. Approximately 90% of PI calves are born to mothers that are not themselves PI, but instead have undergone a transient infection in the early part of pregnancy.

Testing of successive generations of calves provides ongoing assurance that the herd has not been exposed and further PI calves created.

### Define 'isolation', in terms of purchased animals.

On purchase, animals should ideally be quarantined for at least 4 weeks by physical separation i.e. they should be in separate airspaces, sheds and paddocks from home-bred animals, with separate drainage. The use of shared equipment or utensils should be avoided.

### Can the tissue sample be used to identify other diseases?

These samples are not suitable for testing for other diseases.

### What costs are associated with blood samples taken for confirmatory testing?

Required confirmatory testing will be conducted on blood samples collected by a veterinary surgeon. The laboratory charge for blood tests submitted for confirmatory testing of calves and testing of DAMPI (mother of positive calf) and OFFPI (offspring of positive mothers) animals when these samples are submitted to an approved laboratory is included in the fee that you pay to your vet. A submission form for these samples is included with the letter that you will be sent identifying animals to be tested. This form must be returned with the blood samples. You will be liable for any costs incurred with sample collection by your vet.

### When retesting using a supplementary button after an empty sample, is this free?

No. You will have to order and pay for the supplementary button tag in the usual way. Please contact your tag supplier for further advice. In most cases you will only pay for the button tag and not the test as this will already have been paid for.

### Is there a set price for testing in designated laboratories?

No, the price for testing is incorporated when purchasing a tag with a designated tag supplier. Please contact your tag supplier for further advice.

### How reliable are the results of the testing?

No biological test is 100% accurate.

All designated laboratories are independently accredited for BVD testing and the test methods (ELISA or RT-PCR) used will, in the great majority of cases, accurately detect the presence or absence of virus in tissue and blood samples when submitted and tested correctly. However, results relate only to the sample as received by the laboratory and, whilst laboratories warrant that their tests will meet their applicable declared specifications, the laboratories make no other warranty and accept no responsibility or liability in respect of false results which are within the limits of the declared specifications of the tests offered. Estimates from programmes elsewhere suggest that false negative results may occur in between 0-3% of PI calves while false positive results occur at a lower level still (less than 1%). Should false negative or positive results be suspected, the animal should be isolated and re-tested. Any query relating to the reliability of tests or test results should be addressed directly to the laboratory concerned.

### If a calf gets colostrum, will the antibodies from the mother interfere with the test?

The tissue tag test is the most suitable sample for testing young calves. Blood samples from very young calves may not be reliably tested by ELISA due to the possibility of false negative results caused by interference from maternally derived antibodies (MDA) and for this reason negative results from such animals are considered valid only if produced by a RT-PCR test. Typically, this applies to calves up to 75 days of age, although the lower age limit for ELISA testing should be confirmed with individual laboratories before submitting samples to them. This period is often referred to as the “diagnostic gap”. Samples submitted for confirmatory testing are processed using protocols that take this diagnostic gap into account. The ELISA currently in use for testing ear punch samples is marketed as not having a diagnostic gap, although it is possible that these may occur occasionally. Should a false negative result be suspected, you should isolate the animal in question and contact the testing laboratory with a view to arranging a re-test.

## Why do all laboratories not use the same test?

Each designated laboratory has been independently accredited for the provision of testing. As part of this accreditation process each laboratory is able to choose the test method and kit that they will use, being required to select a method that meets the needs of the customer and which is appropriate for the situation in which it is being used (in this case primarily the detection of BVD virus in ear notch samples). Should a false negative or positive result be suspected, the animal should be isolated and re-tested using a blood sample or supplementary tag.

Each designated laboratory should be able to provide customers with further details of the test methods used and the claimed performance of those methods.

## What non-laboratory factors may affect the accuracy of test results?

A variety of factors may influence the accuracy of the test result reported for a given animal. These include errors in tagging, storage and submission of samples. It is recommended that samples are submitted as soon as possible after collection, and certainly within 7 days. In the meantime they should be stored in a cool, dark location, preferably a non-domestic refrigerator.

## RETESTING

### What age does the calf have to be for retesting with blood ELISA tests?

The tissue tag test is the most suitable for testing young calves. Blood samples from very young calves may not be reliably tested by ELISA due to the possibility of false negative results caused by interference from maternally derived antibodies (MDA) and for this reason negative results from such animals are considered valid only if produced by a RT-PCR test. Typically, this applies to calves up to 75 days of age, although the lower age limit for ELISA testing should be confirmed with individual laboratories before submitting samples to them. This period is often referred to as the “diagnostic gap. Samples submitted for confirmatory testing are processed appropriately using protocols that take this diagnostic gap into account. The ELISA currently in use for testing ear punch samples is marketed as not having a diagnostic gap, although it is possible that these may occur occasionally. Should a false negative result be suspected, you should isolate the animal in question and contact the testing laboratory with a view to arranging a re-test.

### What is required for confirmatory blood testing?

Blood samples must be submitted to an approved lab for confirmatory testing. All labs approved to carry out confirmatory testing are listed on the AHWNI website. Confirmatory testing will only be conducted on blood samples collected by a veterinary surgeon. A submission form for these samples is included with the letter that you will be sent identifying animals to be tested. This form must be returned with the blood samples. You will be liable for any testing and sample collection costs incurred. Blood samples from young calves must be tested using specified protocols to ensure accuracy of testing. These protocols are typically more expensive than those used for ear punch samples or blood samples from older animals.

### How long does confirmatory testing take?

Designated laboratories undertake to report 95% of results within 7 working days with a median turnaround of 5 days or less. In practice, many results are reported more rapidly.

## RESULTS

### How quickly will results be available?

Designated laboratories undertake to report 95% of results within 7 working days with an average turnaround of 5 days or less. In practice, many results are reported more rapidly. The results will then be transmitted to AHWNI, linked to NIFAIS and sent by text directly to the farmer where AHWNI has their mobile number.

### Why are the results going to AHWNI and not the farmer in the first instance?

One of the challenges of a national eradication programme is the handling and coordination of all the test data that will be generated. Experience in other countries has shown that the use of a central database for handling results is highly beneficial for the efficient delivery of the programme.

In addition, the use of a central database reduces the workload for the diagnostic labs and therefore the cost associated with testing. The AHWNI database is ideally placed to provide this, with interactions with DAERA's systems allowing results to be transferred, the movement of PI animals to be controlled and the facility to identify the mothers of virus positive calves and to monitor the progress of the programme over time.

### I have received a positive result for a strong, apparently healthy calf- is it definitely a PI?


Many PI calves can be deceptively healthy looking in early life but will generally deteriorate as they grow older. You have an option to do a re-test three to four weeks after the initial sample was taken to clarify the calf's status, with a second positive result confirming it as PI. A negative result on a re-test is typically due to the calf having been transiently infected (TI) with BVD virus when the first sample was collected. Even when apparently healthy, PI calves represent a significant risk of infection to other animals in your and neighbouring herds and you are advised to isolate them prior to confirmatory testing and to cull them as soon as possible after confirmation of PI status.

### Are the results of all BVD testing transferred to the AHWNI database?

The criteria by which laboratories are designated for provision of test results for the BVD eradication programme requires them to forward without delay the results of all BVD tests relating to animals born after 1st March 2016 to the AHWNI database. BVD test results from other animals can be uploaded to the database provided they have been tested by an approved lab using an approved method, that the sample is identified with the full tag number of the animal and that the herdowner has given the necessary data sharing permission.

### How do I access my BVD results via AHWNI Database?

In order for a herd owner to access their BVD results online, go to <http://www.animalhealthni.com>

and click on the  button. Enter your Government Gateway ID (issued by DAERA) and password in the appropriate box to log into the AHWNI results database. If a herdowner requires assistance with finding these log in details, they should contact the DAERA helpline on 03002007852. It is possible for a herdowner to view all of their testing results and print negative results declarations from within the AHWNI database.