BVDV (NS3)

ELISA kit for serodiagnosis of
Bovine Virus Diarrhoea (BVDV)
Blocking test for blood sera and plasma
Diagnostic test for cattle
Monowell

I - INTRODUCTION

BVD--bovine virus diarrhoea--and mucosal disease (MD) are two different clinical disorders caused by the same virus. BVD is the result of an acute infection in susceptible animals. Onset may occur at any time after birth. BVD has a brief course and low mortality. Mucosal disease, in contrast, is a deadly disease of low morbidity. It develops in viraemic animals that have been contaminated *in utero*. The characteristic of this *in utero* infection is the existence of specific immunotolerance that prevents the animals from producing antibody against the infective strain but not against another, antigenically different BVD strain. These persistent carriers which can live for years without developing clinical signs of the disease can only be detected by laboratory screening tests. While the only valid method for detecting animals with persistent viral infections remains identification of the BVD virus itself, it is possible to use a serotest in order to avoid to subject all animals of a farm to cumbersome testings as the detection of BVD virus in leucocytes. Indeed, one has the greatest chance of finding animals with persistent infections in a herd of perfectly seronegative animals. However, this group can also include animals that have never come in contact with the virus. Serotests also enable to monitor the serological status of a vaccinated herd and identify animals that have been contaminated by monitoring increases of their serum titres (seroconversions).
As it is a blocking test, it can be used in all animal species.

II – PRINCIPLE OF THE TEST

The test uses 96-well microtitration plates sensitised by monoclonal antibody specific to NS3 protein from BVD virus (P80). This antibody is used to purify the protein from lysate of the cells in which the virus was grown. The whole plate is coated with the viral protein. The operator deposits the previously diluted test sera and plasma in the microplate's wells. After 2 hours' incubation and a rinse step, the operator adds the conjugate, which is a specific monoclonal antibody against BVDV NS3 protein coupled to peroxidase. After incubating and washing the preparation, the operator adds the chromogen tetramethylbenzidine (TMB). This chromogen has the advantages of being more sensitive than the other peroxidase chromogens and not being carcinogenic. The intensity of the colour is inversely proportionate to the sample's serum titre. Positive and negative control sera are provided with the kit to be able to validate the test results

III - COMPOSITION OF THE KIT

- **Microplates**: 96-well microtitration plate (12 x 8). The entire surface of each microplate has been sensitised with NS3 BVDV protein.
- **Washing solution**: One bottle of 20x concentrated washing solution. The solution crystallises spontaneously when cold. If only part of the solution is to be used, bring the bottle to 21°C +/- 3°C until disappearance of all crystals. Mix the solution well and remove the necessary volume. Dilute the buffer 1:20 with distilled or demineralised water.
- **Dilution buffer**: One bottle of 5X colored, concentrated buffer for diluting samples and conjuguate. The bottle's content is to be diluted with distilled or demineralised water. If a deposit forms at the bottom of the container filter the solution on Whatman filter paper.
- **Conjugate**: One bottle of anti-NS3 BVDV protein peroxidase conjugate (horseradish peroxidase-labelled anti-NS3 BVDV protein monoclonal antibody).
- **Positive reference**: Bottle of positive serum. Store this reagent between +2°C and +8°C.
- **Negative reference**: Bottle of negative serum. Store this reagent between +2°C and +8°C.
- **Single component TMB** One bottle of the chromogen tetramethylbenzidine (TMB). Store between +2°C and +8°C protected from light. This solution is ready to use.
- **Stopping solution**: One bottle of the 1 M phosphoric acid stop solution.

	BIO K 230/2	BIO K230/5
Microplates	2	5
Washing solution	1 X 100 ml (20 X)	1 X 250 ml (20 X)
Colored Dilution buffer	1 X 30 ml (5 X)	1 X 30 ml (5 X)
Conjugate	1 X 0,5 ml (50 X)	1 X 1,4 ml (50X)
Positive serum	1 X 0,5 ml (1 X)	2 X 0,5 ml (1 X)
Negative serum	1 X 0,5 ml (1 X)	2 X 0,5 ml (1 X)
Mono-component TMB	1 X 25 ml (1 X)	1 X 55 ml (1 X)
Stop solution	1 X 15 ml (1 X)	1 X 30 ml (1 X)

IV - ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

Distilled water, graduated cylinders, beakers, plastic tubes, tube rack, dispenser tips, reagent reservoir for multichannel pipettes, lid, adhesive for microplates, graduated automatic (mono- and multichannel) pipettes, microplate reader, and microplate washer and shaker (optional)

V - PRECAUTIONS FOR USE

- This test may be used for "in vitro" diagnosis only. It is strictly for veterinary use.
- The reagents must be kept between +2°C and +8°C. The reagents cannot be guaranteed if the shelf-life dates have expired or if they have not been kept under the conditions described in this insert.
- The concentrated wash solution and dilution buffer may be stored at room temperature. Once diluted, these solutions remain stable for six weeks if kept between +2°C and +8°C.
- Unused strips must be stored immediately in the aluminium envelope, taking care to keep the desiccant dry and the envelope's seal airtight. If these precautions are taken, the strips' activity can be conserved up to the kit's shelf-life date.
- Do not use reagents from other kits.
- The quality of the water used to prepare the various solutions is of the utmost importance. Do not use water that may contain oxidants (e.g., sodium hypochlorite) or heavy metal salts, as these substances can react with the chromogen.
- Discard all solutions contaminated with bacteria or fungi.
- The stop solution contains 1 M phosphoric acid. Handle it carefully.
- All materials and disposable equipment that come in contact with the samples must be considered potentially infectious and be disposed of in compliance with the legislation in force in the country.
- To guarantee the reliability of the results, one must follow the protocol to the letter. Special care must be taken in observing the incubation times and temperatures, as well as measuring the volumes and dilutions accurately.

VI – PROCEDURE

1- Bring all components to 21°C +/- 3°C before use. Remove the microplate from its wrapper.

2- PREPARATION AND DILUTION OF SAMPLES
The blood serum or plasma samples must be diluted twofold. Avoid using haemolysed samples or samples that contain coagulum.
Deposit 50 µl of buffer dilution directly into the wells of the kit's microplate. Add 50 µl aliquots of each sample to each well. Do the same for the reference sera (postive and negative controls). Cover with a lid and incubate the plate at ± 37°C for 2 hours.

3- Rinse the plate with the washing solution prepared as instructed in the section "Composition of the Kit". To do this, dispose of the microplate's contents by flipping it sharply over a container filled with an inactivating agent. Let the microplate drain upside-down on a sheet of clean absorbent paper so as to eliminate all liquid. Add 300 µl of the washing solution, and then empty the plate once again by flipping it over above the containment vessel. Repeat the entire operation two more times, taking care to avoid the formation of bubbles in the microwells. After the plate has been washed three times proceed to the next step.

4- Dilute the conjugate 1:50 in the dilution buffer (for example, for one plate dilute 250 µl of the conjugate stock solution in 12.250 ml of diluent).
Add to each well used 100 µl of the conjugate. Cover with a lid and incubate the plate at 37°C for 1/2 hour.

5- Rinse the plate with the washing solution as instructed in step 3.

6- Add 100 µl of the chromogen solution to each well on the plate. The chromogen solution must be absolutely colourless when it is pipetted into the wells. If a blue colour is visible, this means that the solution in the pipette has been contaminated.

7- Incubate for 10 minutes at 21°C +/- 3°C protected from the light and uncovered. This time is given as a guideline only, for in some circumstances it may be useful to lengthen or shorten the incubation time.

8- Add 50 µl of stop solution per microwell. The blue colour will change into a yellow colour.

9- Read the optical densities in the microwells using a plate reader and a 450 nm filter. Results must be read fairly soon after the stopping solution has been added since the chromogen may cristallise in wells with strong signals and distort the results accordingly.

VII – CALCULATING THE RESULTS

Measure the optical densities of the positive and negative sera (OD pos and OD neg) and those of all the samples (OD samples).
Calculate the percent inhibition (% inhib) for each tested sample and the positive serum by means of the following formulas:

% inhib sample = [(OD neg - OD sample)/OD neg]*100
% inhib positive = [(OD neg - OD pos)/OD neg]*100

VIII – VALIDATING THE TEST

The test may be validated only if the following two conditions are met:

- OD neg - OD pos > 0.7
- % inhib positive > 50%

IX – INTERPRETING THE RESULTS

Determine each sample's positivity using the following cut-off:

% inhibition > or = 50 % : positive
% inhibition < à 50 % : negative

X – ORDERING INFORMATION

Monoscreen AbELISA BVDV (NS3)	2x96 tests	BIO K 230/2
	5x96 tests	BIO K 230/5