Li StarFish S.r.l.
Via Cavour, 35 - 20063 Cernusco S/N (MI), Italy
Tel. +39-02-92150794 - Fax. +39-02-92157285
info@listarfish.it -www.listarfish.it

ANTIGENIC PULMOTEST FOR DETECTION OF BVDV

Sandwich ELISA test

Direct test for tissues lysats

Diagnosis test for bovine viral diarrhoea virus detection

I - INTRODUCTION

BVD--bovine viral diarrhoea--and mucosal disease (MD) are two different clinical entities 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 be detected only by laboratory screening tests.
Diagnosing these infections accurately on clinical grounds is often very difficult. It is thus necessary to perform laboratory tests to establish a diagnosis with certainty. This can be done by revealing the virus in a lung tissue section by means of fluorescein-labelled specific antibody (direct immuofluorescence assay). A diagnosis can also be made by demonstrating clear seroconversion for the virus. To do this, one must take a blood sample during the acute phase of the disease and a second sample 2 to 3 weeks later, that is to say, in the convalescent phase. These paired sera's specific reactions to BVDV antibody will be evaluated by ELISA. Pulmotest BVDV can be used to obtain a diagnosis from a homogenised lung tissue sample taken from a necropsy case. .

II - PRINCIPLE OF THE TEST

The test uses 96-well microtitration plates sensitised by specific antibodies for the BVDV. These antibodies allow specific capture of the corresponding pathogens which are present in the samples (minced lung tissue or culture medium). Rows A, C, E, G have been sensitised with these antibodies and rows B, D, F, H contain non-specific antibodies. These control rows allow the differentiation between specific immunological reactions and non-specific binding so as to eliminate false positives.
Samples are diluted in lysis solution and incubated on the microplate for 1 hour at 21°C +/- 3°C.
After this first incubation step, the plate is washed and incubated for 1 hour with the conjugate, a peroxidase labelled anti-BVDV specific monoclonal antibody. After this second incubation, the plate is washed again and the chromogen (tetramethylbenzidine) is added. This chromogen has the advantages of being more sensitive than the other peroxidase chromogens and not being carcinogenic.
If BVDV is present in the sample, the conjugate remains bound to the corresponding microwells and the enzyme catalyses the transformation of the colourless chromogen into a pigmented compound. The intensity of the resulting blue color is proportionate to the titer of BVDV in the sample. The enzymatic reaction can be stopped by acidification and the resulting optical density at 450 nm recorded using a photometer. The signals recorded for the negative control microwells are subtracted from the corresponding positive microwells. Positive control is provided with the kit so as to validate the test results.

III - COMPOSITION OF THE KIT

- **Microplate**: One 96-well microtitration plate. Rows A, C, E, G are sensitised by anti-BVDV specific antibodies, while rows B, D, F, H are sensitised by the non-specific antibodies (control antibody).
- **Washing solution**: One 100-ml 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. Store the diluted solution between +2°C and +8°C.
- **Lysis buffer**: One 100-ml bottle. The reagent is ready to use. Store the solution between +2°C and +8°C.
- **Conjugate**: One 12 ml vial of coloured conjugate. **This solution is ready to use.**
- **Positive Control:** 1 vial of 2 ml. The reagent is ready to use.
- **Single component TMB** One 12-ml 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 6-ml bottle of the 1 *M* phosphoric acid stop solution.

	BIO K 337/1
Microplate	1
Washing solution	1 X 100 ml (20 X)
Lysis buffer	1 X 100 ml (1 X)
Conjugate	1 X 12 ml (1 X)
Positive control	1 X 2 ml (1 X)
Single component TMB	1 X 12 ml (1 X)
Stopping solution	1 X 6 ml (1 X)

IV - PRECAUTIONS FOR USE

- This test may be used for *in vitro* diagnosis only. It is strictly for veterinary use.
- The reagents must be kept at 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.
- 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.

V – PROCEDURE

A. SAMPLE PREPARATION.

1. **Homogenised tissue.** Collect an approximately 1-gramme sample of lung tissue. Take this sample from areas in the apical lobes that show gross lesions. Place the lung tissue fragment in a Petri dish with 2 ml of lysis solution and snip it into tiny pieces with a pair of scissors. Homogenize, transfer the dish's contents to a test tube, and centrifuge at 500 g for 10 minutes to separate out the insoluble fragments on the bottom of the tube. Collect the supernatant for the ELISA test.
2. **Cell culture.** Pulmotest BVDV may be used to test for viral growth in susceptible host cells (primary cell lines and MDBK cells). In this case you may deposit the culture medium directly on the microplate.

B. ELISA

1- All components must be brought to 21°C +/- 3°C at least 30 minutes before use.
2- Remove the microplate from its wrapper.
3- Distribute the samples into the plate at the rate of 100 µl per well as follows : Sample 1 in wells A1-B1, Sample 2 in wells C1-D1, and so on. Proceed in the same way for the control antigen (ex. : G1-H1).
4- Incubate the plate at 21°C +/- 3°C for 1 hour. Cover the plate with a lid.
5- Rinse the plate with the washing solution prepared as instructed in the 'Composition of the Kit' section. To do this, empty the microplate of its contents by flipping it over suddenly over a sink. Tap the upside-down microplate against a sheet of clean absorbent paper so as to remove all liquid. Fill the used wells with a washing solution either by means of a spray bottle or by plunging the microplate in a suitably-dimensioned container, then empty the plate once again by flipping it over a sink. Repeat the entire operation twice, taking special care to avoid the formation of bubbles in the wells. Upon completing these three washes, go on to the next step.

6- Distribute the conjugate solution at the rate of 100 µl per well. Incubate at 21°C +/- 3°C for 1 hour. Cover the plate with a lid.
7- Wash the plate as described in Step 5.
8- 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. Incubate at 21°C +/- 3°C and away from light for 10 minutes. This time interval is given as a guideline only, for in some circumstances it may be useful to lengthen or shorten the incubation time.
9- Add 50 µl of stop solution to each well.
10- Read the optical densities by means of a microplate spectrophotometer with a 450 nm filter. The results must be read as quickly as possible after the stop solution has been applied, for in the case of a strong signal the chromogen can crystallise and lead to incorrect measurements

VI – INTERPRETING THE RESULTS

Calculate the net optical density of each sample by subtracting from the reading for each sample well the optical density of the corresponding negative control.
Proceed in the same way for the positive control antigen.
The test is validated only if the positive control antigen yields a difference in the optical density at 10 minutes that is greater than the value given on the QC data sheet.

Divide the signal read for each sample well by the corresponding positive control signal and multiply this result by 100 to express it as a percentage. Using the first table in the quality control procedure, determine each sample's status (positive, negative).

$$\text{Val} = \frac{\text{Delta DO spl} * 100}{\text{Delta DO pos}}$$

VII – ORDERING INFORMATION

BIO-X PULMOTEST BVDV:	1 X 48 tests	BIO K 337/1

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