MONOSCREEN Ag ELISA

BRSV

ELISA kit for antigenic diagnosis of
Bovine respiratory Syncytial Virus (BRSV)
Sandwich test for lung lysates
Diagnostic test for cattle
Double wells

I - INTRODUCTION

In Europe, BRSV is the most important etiological agent responsible for respiratory affections in young cattle. In cattle as in children, respiratory syncytial viruses can cause a very deep attack of the respiratory tree. The affection is often provoking very severe injuries, which are responsible for important economic losses. As a matter of fact, in Europe, 7 million calves suffer from infections diseases yearly, 60% of which are caused by respiratory pathogens. One million calves dye of respiratory diseases in the European Community each year. The cost of these diseases, including medical treatments, growth delays and mortality, is about 450 million EURO per year for calves under one year. For dairy cattle, the cost of BRSV has been evaluated at around 25 EURO per animal.
BRSV principally affects young cattle. Beef cattle are especially vulnerable because of the high proportion of muscle mass compared with the pulmonary volume in such animals. The clinical manifestations can be dramatic. Often signs of severe pneumonia such as polypnoea, abdominal breathling and hyperthermia are present. Reinfections may be observed but most often they remain subclinical. Clinical diagnosis is very difficult and laboratory assistance is required for a precise diagnosis. The virus can be detected in lung tissue by fluorescein labelled specific antibodies.
Diagnosis can also be achieved by measuring a virus specific seroconversion. To do so, a first sample will be collected during the acute phase of the disease and a second sample will be collected 2 or 3 weeks later.
These two samples will be evaluated for their content in specific antibodies against BRSV by ELISA.
The BRSV kit can be used to obtain a diagnosis from a minced lung tissue sample taken from a corpse.

II - PRINCIPLE OF THE TEST

The test uses 96-well microtitration plates sensitised by specific antibodies for the BRSV. 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-BRSV 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 BRSV 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 BRSV 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-BRSV 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 336/1
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 - 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, scissors, Petri dishes, microplate reader, and microplate washer and shaker (optional), scale.

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 may be stored at room temperature. Once diluted, this solution 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

A. SAMPLE PREPARATION.

1. **Homogenised tissue.** Collect an approximately 1 gram 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.** The BRSV kit may be used to test for viral growth in susceptible host cells (primary cell lines, HEP2 and VERO 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 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- Cover with a lid and incubate the plate at 21°± 3°C for one hour.
5- 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 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. Cover with a lid and incubate the plate at 21°± 3°C for one hour.
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. Do not cover. 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. The blue colour will change into a yellow colour.
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.

VII – 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).

$$\text{Val(ue)} = \frac{\text{Delta OD Sample} * 100}{\text{Delta OD positive}}$$

VIII – ORDERING INFORMATION

Monoscreen AgELISA BRSV: 1 X 48 tests BIO K 336/1