研究用

コスモ・バイオ株式会社

www.biox.com

PULMOTESTS
(Respiratory antigenic ELISA test kits)

BIO K 341/2 - 336/1 - 335/1 - 337/1 - 340/2 - 340/5

Mycoplasma bovis - BRSV - BoHV-1 - BVDV - TETRA

Respiratory disorders are of major concern for bovidae, given the frequency of such infections and the high number of animals affected. These infections occur in all countries that practice intensive livestock farming in which large numbers of animals are confined to small areas. Treatment and diagnosis are both complicated due to the multifactorial character of this diseases etiology. Viruses and bacteria combined with stress due either to transport in overcrowded vans or dirty or poorly-ventilated stabling, for instance, play a key role in triggering acute respiratory infections. These infections are particularly common among young animals, although they also affect adult animals. In most cases the animals that show signs of respiratory ailments harbour several pathogens, some of which may act synergistically. So, it is generally recognised that viruses are the first pathogens to intervene, whereas bacteria act as second invaders to worsen the animal's condition. Shipping fever is a good example of the synergism that can exist between a virus (PI3) and a bacterium, such as *Mannheimia haemolytica*, in the respiratory tract. Respiratory Pulmotest can be used to obtain a diagnosis from a minced lung tissue sample taken from a corps. It is also possible to use tracheobronchial lavage liquid or, lacking that, nasal fluid to carry out this test. However, the diagnosis is less reliable than when lung tissue is used.

Reliable Results

The use of monoclonal antibodies as conjugate ensures excellent specificity and very reliable results.

Ease-of-Use

Minimal hands-on-time
Room temperature incubation
Results available in 140 minutes except for *Mycoplasma bovis* (72 hours).

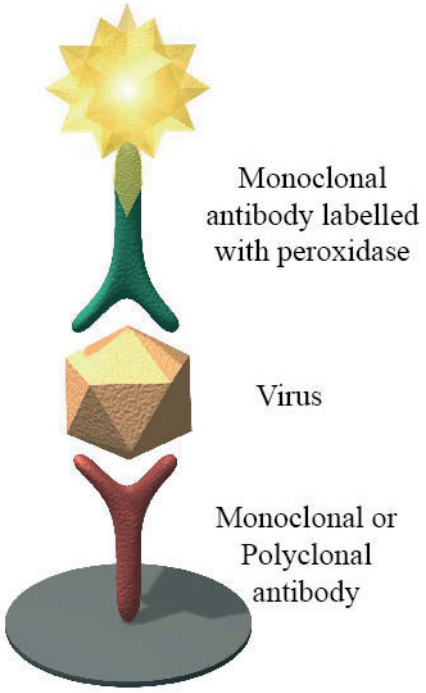
Flexibility

Results can be read visually or spectrophotometrically.

EIA Procedure

(BoHV1, BVDV, BRSV, Tetra)
BIO K 335/1 - BIO K 337/1 -
BIO K 336/1 - BIO K 340/2 /5

1- Microplate coated with monoclonal or polyclonal antibodies
2- Add samples and positive controls.
Incubate 1 hour at 21°C +/- 3°C
Wash
3- Add conjugate(s).
Incubate 1 hour at 21°C +/- 3°C .
Wash
4- Add chromogen (TMB)
Wait 10 minutes.
Add stop solution.
Read at 450 nm



Bio-X Diagnostics
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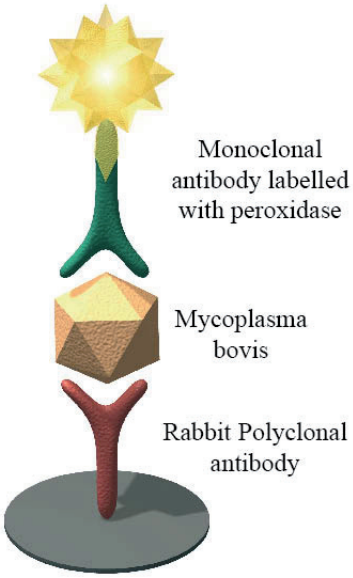
EIA Procedure

BIO K 341/2 - *Mycoplasma bovis*

1- Microplate coated with polyclonal antibody.
2- Add samples and positive controls.
Incubate 72 hours at 37°C +/- 0.5 °C
Wash
3- Add conjugate.
Incubate 1 hour at 21°C +/- 3°C .
Wash
4- Add chromogen (TMB)
Wait 10 minutes.
5- Add stop solution.
Read at 450 nm



Lung tissue specimens were taken in the necropsy room from animals that had died from respiratory ailments. These specimens were cut up into small fragments in the kit's lysis solution with a pair of scissors. They were then tested by the Pulmotest kits, following the kits' protocols. The specimens were also tested by PCR for BoHV1, nested RT PCR for BRSV, and RT PCR for BVDV following internal protocols developed by Bio-X Diagnostics.

Example of results for BoHV1

ELISA BIO K 335/1	PCR +	PCR -	
+	2	2	4
-	0	97	97
	2	99	101

Specificity: 98.0 %
Sensitivity: 100 %

Example of results for BVDV

ELISA BIO K 337/1	PCR +	PCR -	
+	3	2	5
-	0	97	97
	3	99	102

Specificity: 98 %
Sensitivity: 100 %

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Example of results for BRSV

ELISA BIO K 336/1 / PCR

	+	-	
+	23	1	24
-	2	76	78
	25	77	102

Specificity: 98.7 %
Sensitivity: 92.0 %

Parainfluenza 3:

2 positive samples
102 negative samples

Mycoplasma bovis:

6 positive samples
69 negative samples

The BIO K 341/2 kit was used to test for *Mycoplasma bovis*. The lung tissue samples were cut up in the lysis solution. Four serial ninefold dilutions of the samples were then made in the kit's Hayflick's medium with the addition of a mixture of antibiotics and antimycotic. following the kit's instructions, then the diluted samples were incubated on the microplate at 37°C for 72 hours.

Frequency (from ELISA results)	
BRSV	23,5 %
Mycoplasma bovis	8 %
BVDV	4,9 %
BHV-1	4 %
PI3	1,9 %

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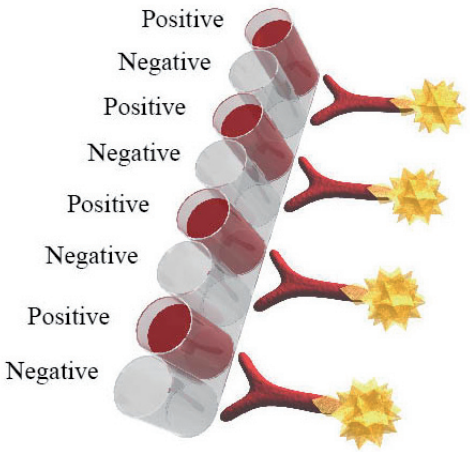
Conclusions

The various Pulmotest kits (individual and combined tests) offer interesting opportunities for laboratories that do not have the necessary equipment for carrying out immunofluorescence assays on organ slices (cryostat, microscope equipped with epifluorescence optics, etc.). The Pulmotests can also enable one to dispense with expensive and fastidious methods such as PCR and cell culturing.

Comparisons between the Pulmotest ELISAs and PCR have shown that the Pulmotests are sufficiently specific. The BRSV Pulmotest (BIO K 336/1) can be considered to be sufficiently sensitive, to the extent that PCR's capacity for amplification is excessive, as a result of which there is a risk of detecting amounts of viral particles that are actually too small to explain the clinical cases studied. The tests conducted using the Pulmotest kits have made it possible to confirm that BRSV is the pathogen most often associated with serious enzootic bronchopneumonia. *Mycoplasma bovis* is also an important pathogen, the detection of which is all the more important in that it can be controlled by the appropriate antibiotics. BVDV is not considered to be a primary cause of enzootic bronchopneumonia in cattle, but its immunosuppressive effect on the immune system boosts the development of other pathogens. The low frequency of BoHV-1 detection is doubtless due to the active control policy that Belgium is currently waging against this virus.

Composition of the kits

BIO K335/1 - BIO K 336/1 - BIO K 337/1	
Microplate	1 - 12 strips (48 tests)
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)



Stability : One year between +2°C and +8°C

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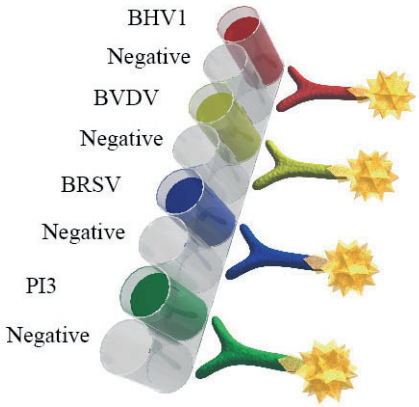
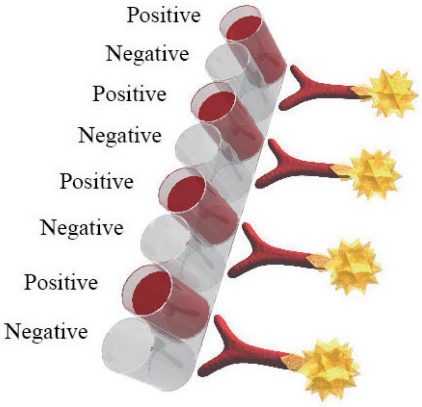
PULMOTEST TETRA	BIO K 340/2	BIO K 340/5
Microplate	2 (24 tests)	5 (60 tests)
Washing solution	1 X 100 ml (20 X)	1 X 250 ml (20 X)
Lysis buffer	1 X 50 ml (5 X)	1 X 125 ml (5 X)
Conjugate	4 X 6 ml (1 X)	4 X 15 ml (1 X)
Positive control	1 X 4 ml (1 X)	1 X 10 ml (1 X)
Single component TMB	1 X 25 ml (1 X)	1 X 55 ml (1 X)
Stopping solution	1 X 15 ml (1 X)	1 X 30 ml (1 X)



BIO K 341/2 - PULMOTEST *Mycoplasma bovis*	
Microplate	2 - 12 strips (24 tests)
Washing solution	1 X 100 ml (20 X)
Conjugate	1 X 25 ml (1 X)
Control antigen	2 X 0.5 ml (1 X)
Single component TMB	1 X 25 ml (1 X)
Stopping solution	1 X 15 ml (1 X)
Hayflick medium	1 X 30 ml
Antibiotic - antimycotic mixture	2



Stability : One year between +2°C and +8°C

Certified ISO 9001 by BVQI n°12345

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Bio-X Diagnostics

ANTIGENIC PULMOTEST FOR DETECTION OF BRSV

Sandwich ELISA test

Direct test for tissues lysats

Diagnosis test for bovine respiratory syncytial virus detection

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.

Pulmotest BRSV can be used to obtain a diagnosis from a minced lung tissue sample taken from a corps.

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 - 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 BRSV 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 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. 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.
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).

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

VII – ORDERING INFORMATION

BIO-X PULMOTEST BRSV :	1 X 48 tests	BIO K 336/1