Bio-X Diagnostics

BIO-X PULMOTEST PARAINFLUENZA 3 (BIO K 338)

(Sandwich ELISA test for PI3 virus detection)

KIT FOR ANTIGENIC DIAGNOSIS OF BOVINE PARAINFLUENZA 3 VIRUS BY ELISA

I - INTRODUCTION

Parainfluenza 3 was first isolated in the USA from the nasal mucus of cattle showing clinical signs of shipping fever. Its distribution in cattle has been found to be worldwide. Most reports of bovine PI3 virus activity have been in groups of young cattle with respiratory diseases such as enzootic calf pneumonia and shipping fever. Bovine PI3 virus infections are not invariably associated with disease, and subclinical infections often occur. In European countries PI3 infection mostly occurs from October to March. PI3 virus infection may be accompanied by concurrent infection of the respiratory tract by other viruses such as respiratory syncytial virus, adenovirus or BVDV. In outbreaks of bovine respiratory disease it is not possible to diagnose PI3 virus infection on clinical grounds alone. To establish a diagnosis, it is necessary to take paired sera from infected animals or to necropsy animals from the outbreak to facilitate immunocytochemical examination of the lower respiratory tract. PI3 virus infection in an outbreak of respiratory disease can be detected by demonstrating a rise in the serum antibody titre to the virus between acute and convalescent phase serum samples (seroconversion).
Pulmotest PI3 can be used to diagnosis the infection in homogenised lung tissue taken from a necropsy case.

II - PRINCIPLE OF THE TEST

The test uses 96-well microtitration plates sensitised by specific antibodies for the PI3. 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-PI3 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 PI3 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 PI3 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

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recorded for the negative control microwells are subtracted from the corresponding positive microwells. A control antigen is provided with the kit so as to validate the test results. This control antigen is composed of a lyophilised and inactivated PI3 culture.

III - COMPOSITION OF THE KIT

- **Microplate** : One 96-well microtitration plate. Rows A, C, E, G are sensitised by anti-Bovine Parainfluenza 3 virus 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 at 4°C.
- **Lysis buffer** : One 100-ml bottle. The reagent is ready to use. Store the solution at 4°C.
- **Conjugate** : One 12 ml vial of coloured conjugate. **This solution is ready to use.**
- **Control antigen** : 1 vial containing control antigen. Reconstitute this antigen with 1 ml of distilled or demineralised water. The reconstituted reagent may be kept at -20°C. Divide the reconstituted antigen into four portions before freezing in order to avoid repeated freezing and thawing. If these precautions are taken the reagent may be kept for several months.
- **Single component TMB** One 12-ml bottle of the chromogen tetramethylbenzidine (TMB). Store at 4°C protected from light. **This solution is ready to use.**
- **Stopping solution** : One 6-ml bottle of the 1 *M* phosphoric acid stop solution.

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 4 and 8°C. The control antigen must be kept at -20°C once reconstituted. 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 PI3 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).

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

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