10- Add 50 µl of stop solution to each well.
11- 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.

The limit of positivity for the antigen is 0.150.
Any sample that yields a difference in optical density that is greater than or equal to 0.150 is considered positive.
Conversely, any sample that yields a difference in the optical density that is less than 0.150 is considered negative.

研究用

Bio-X Diagnostics

BIO-X PULMOTEST ADENOVIRUS 3 (BIO K 199)

(Sandwich ELISA test for Adenovirus 3 virus detection)

KIT FOR ANTIGENIC DIAGNOSIS OF BOVINE ADENOVIRUS 3 BY ELISA

I - INTRODUCTION

Bovine adenoviruses (BAV) are classified into nine serotypes (species) so far. These nine serotypes are divided into two subgroups mainly on the basis of replication in either calf kidney or testicle cells (subgroup I, serotypes 1-3 and 9) or only in testicle cells (subgroup II, serotypes 4-8). The two subgroups also differ in their antigenic properties. The distribution of BAV in cattle populations is worldwide. Serological evidence suggests a high incidence of infection. Adenovirus are generally present in large herds. Viruses are excreted by different forms of shedding and by coughing. Infection occurs most frequently when calves are 3 weeks to 4 months old. In diseased calves respiratory and enteric symptoms, fever and anorexia can be observed. The disease usually starts with respiratory symptoms. Serous excretions from the nose and conjonctivae are accompanied by coughing. Enteric infection becomes manifest by salivation and thin, greyish-yellow feces. The disease can be aggravated by secondary bacterial infections. BIO X PULMOTEST ADENOVIRUS 3 ELISA kit is subgroup-specific and can only detect immune response against subgroup I bovine adenovirus.

II - PRINCIPLE OF THE TEST

The test uses 96-well microtitration plates sensitised by specific antibodies for ADENOVIRUS 3. These antibodies allow specific capture of the corresponding pathogens that are present in the sample (homogenised lung tissue, bronchotracheal fluid, nasal exudate, 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 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 room temperature.
After this first incubation step, the plate is washed and incubated for 1 hour with the conjugate, a peroxidase labelled anti-ADENOVIRUS 3 specific monoclonal antibody. After this second incubation, the plate is washed again and the enzyme substrate (hydrogen peroxide) and the chromogen (tetramethylbenzidine) are added. This chromogen has the advantages of being more sensitive than the other peroxidase chromogens and not being carcinogenic.
If ADENOVIRUS 3 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 colour is proportionate to the titre of ADENOVIRUS 3 in the sample. 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. A control antigen is provided with the kit so as to validate the test results. This control antigen is composed of a lyophilised ADENOVIRUS 3 culture.

III - COMPOSITION OF THE KIT

- **Microplates** : Two 96-well microtitration plates. Rows A, C, E, G are sensitised by anti-ADENOVIRUS 3 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 room temperature 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 50-ml bottle of 5x concentrated buffer. Dilute this concentrated solution 1:5 with distilled or demineralised water. Store the diluted solution at 4°C.
- **Dilution buffer** : One 50-ml bottle of 5x concentrated buffer. Dilute this concentrated dilution buffer 1:5 with distilled or demineralised water. Store the diluted solution at 4°C. If a deposit forms at the bottom of the container filter the solution on Whatman filter paper.
- **Conjugate** : One vial of 50x concentrated anti-ADENOVIRUS 3 -peroxidase conjugate.
- **Control antigen** : 2 vials containing control antigen. Reconstitute this antigen with 0.5 ml of distilled or demineralised water. The reconstituted reagent may be kept at -20°C. Divide the reconstituted antigen into two portions before freezing in order to avoid repeated freezing and thawing. If these precautions are taken the reagent may be kept for several months.
- **Chromogen solution** : One 2-ml drop-dispenser bottle of the chromogen tetramethylbenzidine. Store at 4°C.
- **Substrate solution** : One 30-ml bottle of the hydrogen peroxide substrate solution. Store this reagent at 4°C.
- **Stopping solution** : One 15-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.
- Some of the reagents contain merthiolate or thimerosal. This substance is toxic if it is inhaled or comes in contact with the skin. Take the usual precautions in handling them.
- The stop solution contains 1 M phosphoric acid. Handle it carefully.

V – 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 15-ml test tube, and centrifuge at 2000 rpm for 10 minutes to separate out the insoluble fragments on the bottom of the tube. Collect the supernatant for the ELISA test.
2. **Bronchotracheal sample.** The bronchotracheal sample must be collected by experienced staff (veterinarian) by either a transtracheal puncture or the endonasal route. The liquid used to collect the sample must be isotonic and sterile. After taking the sample, transfer it as quickly as possible to the laboratory, keeping it at a constant 4°C. The sample must be diluted twofold in the lysis solution before undergoing ELISA.
3. **Nasal swab.** (A collection kit is available from Bio-X Diagnostics). Insert a hygienic tampon (OB tampon, for example) into the animal's nostril. Choose a tampon of the right size for the nostril and take care that the animal cannot grab the tampon's string with its tongue. Leave the tampon in place for at least 5 minutes to ensure that it absorbs a sufficient amount of fluid. Remove the tampon, which will be swollen with moisture, from the animal's nostril and place it in a rimmed polypropylene tube with a conical bottom (for example, a Greiner 201250 tube, diam.: 24 mm, height: 90 mm) in which the bottom has been pierced by a white-hot metal spike. Place this tube, without its stopper, in a 50-ml rimless polypropylene tube with a conical bottom (for example, Greiner 227261). Seal this second tube and send the tube-within-a-tube to the laboratory as quickly as possible. As soon as the laboratory receives the tube it must be centrifuged at 1500 rpm for 20 minutes. Remove the inner tube and collect the liquid at the bottom of the outer tube for the test. The liquid must be diluted to half-strength in the dilution buffer before undergoing ELISA.
4. **Cell culture.** Pulmotest ADENOVIRUS 3 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 room temperature 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 room temperature for 1 hour.
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 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- Dilute the conjugate 1:50 in the dilution buffer (for example, for one plate dilute 250 µl of the conjugate stock solution in 12.25 ml of dilution buffer). Distribute the diluted conjugate solution at the rate of 100 µl per well. Incubate at room temperature for 1 hour.
7- Wash the plate as described in Step 5.
8- Prepare the indicator mixture by adding 12 drops (500 µl) of the chromogen to 9.5 ml of the substrate solution (sufficient amount for one plate). Distribute the mixture over the microplate at the rate of 100 µl per well. The solution must be perfectly colourless. If a blue tinge is visible, the solution has been contaminated with peroxidase. If this occurs, the solution must be discarded and a new mixture made up with perfectly clean equipment.
9- Incubate at room temperature 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.