DRG® PI3 Bovine ELISA (Parainfluenza 3) (EIA-4429)

REVISED 17 AUG. 2007 **FOR VETERINARY USE ONLY**

NAME AND INTRODUCTION

Parainfluenza 3 was first isolated in the USA from the nasal mucus of cattle showing clinical signs of shipping fever. Its distribution in the 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 during the months 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 submit animals from the outbreak for necropsy to facilitate immunocytochemical examinations of the lower respiratory tract. PI3 virus infection in an outbreak of respiratory disease can be detected by the demonstration of a rise in serum antibody titer to the virus between acute and convalescent phase serum samples (seroconversion).

PRINCIPLE OF THE TEST

The test uses 96-well microtitration plates sensitised by monoclonal antibodies specific to one of the antigenic determinants of PI3 virus. This antibody is used to trap the virus as well as to purify it from lysate of the cells in which the virus was grown. The plate's odd columns (1, 3, 5, 7, 9 and 11) contain the virus, whereas the even columns (2, 4, 6, 8, 10 and 12) contain a lysate of bovine kidney cell line that was used as a substrate to propagate the virus. We thus have a genuine negative control to differentiate the specific anti-viral antibody from the antibodies directed against the antigenic determinants of the bovine kidney cells used for its replication. Using such a control reduces the number of false positives considerably.

The test blood sera are diluted in the buffer for dilution. The plate is incubated and washed, then the conjugated peroxidase-labelled anti-bovine IgG1 monoclonal antibody-is added to the wells. The plate is then incubated a second time at room temperature and washed again and the enzyme's substrate (hydrogen peroxide) and the chromogen tetramethylbenzidine (TMB) are added. This chromogen has the advantages of being more sensitive than the other peroxidase chromogens and not being carcinogenic. If specific PI3 immunoglobulins are present in the test sera the conjugate remains bound to the microwell that contains the viral antigen and the enzyme catalyses the transformation of the colorless chromogen into a pigmented compound. The intensity of the resulting blue colour is proportionate to the titre of specific antibody in the sample. The signal read off the negative control microwell is subtracted from that of the positive microwell sensitised by the viral antigen. It is possible to quantify the reactivity of an unknown serum on a scale ranging from 0 to +++++.

COMPOSITION OF THE KIT

1. **Microplates** : Two 96-well microtitration plates. The odd columns (1, 3, 5, 7, 9 and 11) are sensitised by the PI3 viral antigen and the even columns (2, 4, 6, 8, 10 and 12) by the culture medium.
2. **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 so that all the crystals disappear. 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.
3. **Dilution buffer** : One 50-ml bottle of 5x concentrated buffer for diluting the blood sera and conjugate. The bottle's contents is to be diluted with distilled or demineralised water. This solution will keep at 4°C for at least 3 month. If a deposit forms at the bottom of the receptacle filter the solution on Whatman filter paper.

4. **Conjugate (50x conc.)** : 1 x 0.5 ml bottle of anti-bovine immunoglobulin-peroxidase conjugate (horseradish peroxidase labelled anti-bovine IgG1 monoclonal antibody). Store at 4°C.
5. **Positive reference** : One bottle of positive serum. Reconstitute this serum with 0.5 ml of distilled or demineralised water. The reconstituted serum may be kept at -20°C. Divide the reconstituted serum into several portions before freezing in order to avoid repeated freezing and thawing. If these precautions are taken the reagent may be kept for several months.
6. **Chromogen solution** : One 2-ml drop-dispenser bottle of the chromogen tetramethylbenzidine. Store at 4°C protected from the light.
7. **Substrate solution** : One 30-ml bottle of the hydrogen peroxide substrate solution. Store this reagent at 4°C.
8. **Stop solution** : One 15-ml bottle of the 1 M phosphoric acid stop solution.

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 positive serum must be kept at -20°C once it is reconstituted. The reagents cannot be guaranteed if the shelf-life dates have expired or 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 make up 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 carefully.

PROCEDURE

1. Bring all reagents at room temperature at least 30 minutes before starting the test.
2. Remove the microplate from its wrapper.
3. Place 1-ml aliquots of the dilution solution, prepared as instructed in the "Composition of the Kit" section, in 5- or 10-ml hemolysis tubes.
   Add 10 µl of the serum samples to each of these tubes and shake briefly on a mechanical agitator. Proceed in the same manner for the positive serum.
4. Add 100-µl aliquots of the 1:100 dilute samples to the wells as follows:
   positive reference serum: wells A1 and A2, sample 1 in wells B1 and B2, sample 2 in wells C1 and C2, etc...
5. If a diagnosis based on seroconversion monitoring is required, incubate the plate at room temperature for one hour. If, in contrast, the test's sensitivity is more important (positive-negative test), it is preferable to incubate the microplate at 37°C for one hour or on a plate agitator at room temperature for one hour.
   Shaking or 'stirring' during incubation will improve the test's sensitivity. This will, however, yield signals for certain sera that are greater than 2 optical density units. Not all microplate readers can interpret such values.

6. Rinse the plate with the washing solution, prepared as instructed in the "Composition of the Kit" section, as follows: Empty the microplate of its contents by flipping it over sharply above a sink. Tap the microplate upside down against a piece of clean absorbent paper to remove all the liquid. Fill the used wells with the washing solution using a squeeze bottle or by plunging the plate in a vessel of the right dimensions, then empty the wells once more by turning the plate over above a sink. Repeat the entire operation two more times, taking care to avoid the formation of bubbles in the microwells. After the plate has been washed three times go on to the next step.
   An automatic plate washer may also be used, but in this case particular care must be taken to avoid any contact between the needles and the bottom of the wells to prevent any damage of the reagent layer.
7. Dilute the conjugate 1:50 in the dilution buffer (for example, for one plate dilute 250 µl of the conjugate stock solution in 12.5 ml of diluent).
   Add 100 µl of the dilute conjugate solution to each well.
   Incubate for 1 hour at room temperature, at 37°C or on an agitator, depending on the choice made in Step 5.
8. Wash the plate as described in step 6 above.
9. Prepare 10 ml of indicator solution extemporaneously as follows:
   Add 500 µl (12 drops) of chromogen to 9.5 ml of the substrate solution. Mix thoroughly, then apply to the plate immediately in volumes of 100 µl per microwell.
   At the time of distribution of the chromogen-substrate mixture on the plates the solution must be completely colourless. If a blue colour appears at this stage, the solution is contaminated with peroxidase. If this occurs, the chromogen-substrate solution must be discarded and a new solution made up using absolutely clean glassware and equipment.
10. Incubate for 10 minutes at room temperature. This time is given as a guideline only, for in some circumstances it may be useful to lengthen or shorten the incubation time.
11. Add 50 µl of stop solution per microwell.
12. Read the optical densities in the microwells using a plate reader and a 450 nm filter. Results must be read fairly soon after the stopping solution has been added since the chromogen may crystallize in wells with strong signals and thereby distort the data.

INTERPRETING THE RESULTS

Subtract from each value recorded for the odd columns the signal of the corresponding negative control well and write down the result. In performing this calculation, allow for any negative values that may exist.
Carry out the same operations for the column corresponding to the positive control.
The test can be validated only if the positive serum yields a difference in optical density at 10 minutes that is greater than the value given in the QC data sheet:

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

A reliable diagnosis can be made only if frank seroconversion can be documented using two coupled serum samples taken at 2- to 3-week intervals. The first sample must be taken during the acute phase of the infection.

A frank seroconversion is considered to have occurred if the signal increases by two orders of magnitude (two plusses; for example, ++ -> ++++ or + -> +++). A sample must be considered positive if it yields a result that is greater than or equal to one plus sign (+).