BIO-X BHV-1 ELISA KIT (BIO K 132)

(Indirect test for blood sera)

ELISA KIT FOR SERODIAGNOSIS OF INFECTIOUS BOVINE RHINOTRACHEITIS IN CATTLE

I - INTRODUCTION

Infectious bovine rhinotracheitis (IBR) is an infectious disease caused by a herpesvirus, BHV-1. The syndrome usually includes fever and eye and nasal discharges. The disease may be accompanied by encephalitis and abortion. The causal virus is identical to the virus that causes infectious pustulo-vaginitis in cattle. It is usually rather easy to make the clinical diagnosis of the disease. Serodiagnosis makes sense only if a sharp increase in the titre in paired serum samples is seen. Prevention may be based on vaccination or elimination of the seropositive animals.

II – PRINCIPLE OF THE TEST

The test uses 96-well microtitration plates sensitised by monoclonal antibodies specific to one of the antigenic determinants of IBR 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 antiviral 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 conjugate, a 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 IBR 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 +++++.
III - COMPOSITION OF THE KIT

- **Microplates**: Two 96-well microtitration plates. The odd columns (1, 3, 5, 7, 9 and 11) are sensitised by the IBR viral antigen and the even columns (2, 4, 6, 8, 10 and 12) by the cells lysate.
- **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.
- **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 months. If a deposit forms at the bottom of the receptacle filter the solution on Whatman filter paper.
- **Conjugate**: One bottle of anti-bovine immunoglobulin-peroxidase conjugate (horseradish peroxidase-labelled anti-bovine IgG1 monoclonal antibody).
- **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.
- **Chromogen solution**: One 2-ml drop-dispenser bottle of the chromogen tetramethylbenzidine. Store at 4°C protected from the light.
- **Substrate solution**: One 30-ml bottle of the hydrogen peroxide substrate solution. Store this reagent at 4°C.
- **Stop 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 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.

V - PROCEDURE

1. Bring all the reagents at room temperature at least half an hour before use.
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 serum in wells A1 and A2, sample 1 in wells B1 and B2, sample 2 in wells C1 and C2 etc...

VI - 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 (+).