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).

Use of the kit
The kit is designed to follow seroconversion on paired sera.

Reliable Results
The use of monoclonal antibody as conjugate ensures excellent specificity and very reliable results. The use of monoclonal antibodies to purify the virus on the plate also makes it possible to obtain an excellent specificity.

Ease-of-Use
Minimal hands-on-time
Room temperature incubation
Results available in 140 minutes for single or batch testing.

EIA Procedure

1- Microplate coated with monoclonal antibody and inactivated virus.
2- Add samples and positive control. Incubate 1 hour at 21°C+-3°C. Wash.
3- Add conjugate. Incubate 1 hour at 21°C+-3°C. Wash.
4- Add chromogen + Substrate. Wait 10 minutes. Add stop solution. Read at 450 nm.
A batch of 12 calves of approximately 6 months was divided into two groups. The first group was vaccinated with an inactivated commercial vaccine. The second group was not vaccinated. Before vaccination, the 12 calves were blood sampled. After the second vaccination, the 12 calves underwent a blood sampling. The paired sera were tested with the Bio K 062 kit of Bio-X Diagnostics.

**Example of results**

**Vaccinated group**

<table>
<thead>
<tr>
<th>Calves numbers</th>
<th>Optical density (Before)</th>
<th>Optical density (After)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calf 7</td>
<td>0</td>
<td>3000</td>
</tr>
<tr>
<td>Calf 8</td>
<td>500</td>
<td>2500</td>
</tr>
<tr>
<td>Calf 9</td>
<td>1000</td>
<td>2000</td>
</tr>
<tr>
<td>Calf 10</td>
<td>1500</td>
<td>1500</td>
</tr>
<tr>
<td>Calf 11</td>
<td>2000</td>
<td>1000</td>
</tr>
<tr>
<td>Calf 12</td>
<td>2500</td>
<td>500</td>
</tr>
</tbody>
</table>

**Not vaccinated group**

<table>
<thead>
<tr>
<th>Calves numbers</th>
<th>Optical density (Before)</th>
<th>Optical density (After)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calf 1</td>
<td>0</td>
<td>500</td>
</tr>
<tr>
<td>Calf 2</td>
<td>500</td>
<td>1000</td>
</tr>
<tr>
<td>Calf 3</td>
<td>1000</td>
<td>1500</td>
</tr>
<tr>
<td>Calf 4</td>
<td>1500</td>
<td>2000</td>
</tr>
<tr>
<td>Calf 5</td>
<td>2000</td>
<td>2500</td>
</tr>
<tr>
<td>Calf 6</td>
<td>2500</td>
<td>3000</td>
</tr>
</tbody>
</table>

**BIO K 062 PARAINFLUENZA 3 ELISA**

- 2 plates of 6 strips for a total of 96 tests
- 2 vials of positive reference serum
- 1 vial of conjugate
- 1 bottle of washing solution
- 1 bottle of dilution buffer
- 1 drop-dispenser bottle of chromogen
- 1 bottle of substrate solution
- 1 bottle of stopping solution

1 year of stability at 4°C
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).

II - 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.

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 pluses; for example, ++ -> ++++ or + -> +++). A sample must be considered positive if it yields a result that is greater than or equal to one plus sign (+).
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. If, in contrast, the test's sensitivity is more important (positive-negative test), it is preferable to incubate the plate at room temperature for one hour. If the results are contaminated with bacteria or fungi, the plate may be kept for several months. 

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.

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 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 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 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 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 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 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 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 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 plate at room temperature for one hour.

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 filling 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 take care that the needles do not get too close to the bottoms of the wells to prevent damaging the reagent layer.

7- Dilute the conjugate 1:50 in the buffer for dilution (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 12 drops (500 µl) 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 protected from the light. 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 to each 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.

V - PROCEDURE

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,