Equine Herpes Virus type 1 and 4 Discriminating Test (EHV1/EHV4-Ab)  
SVANOVIR®  
ELISA test for the detection of EHV1 and/or EHV4 antibodies in serum

General information

Equine Herpesvirus type 1 and 4 infect horses throughout the world. The nature of the infection differs between the two virus types. EHV4 is the major cause of respiratory disease in young horses while EHV1 is a major cause of abortion. Because polyclonal antibodies to EHV1 and EHV4 are highly cross-reactive, serological determination of the infection caused by either of the two virus types so far has not been possible. This ELISA test discriminates between antibodies to EHV1 and EHV4.

Principle

The SVANOVIR® EHV1, EHV4 Ab-discriminating ELISA Kit is designed to detect and differentiate antibodies specific to EHV1 and EHV4 in sera of infected horses. The kit procedure is based on the indirect Enzyme Linked Immunosorbent Assay (Indirect-ELISA). In this procedure, samples are added to wells coated with noninfectious EHV1 and EHV4 antigen, and also to a well coated with control antigen (altogether three wells per sample) in microtitre strips. If antibodies directed to EHV1 or EHV4 are present in the test sample, they will bind virus antigens in the wells. Addition of horseradish peroxidase (HRP) conjugated rabbit anti horse Ig binds to all horse antibodies. Unbound material is removed by rinsing before the addition of a substrate solution. Subsequently a blue colour develops which is due to the conversion of the substrate by the conjugate. A positive result is indicated by development of the blue colour. The reaction is stopped by addition of the stop solution; the colour changes to yellow. The result can be read visually or by a microplate photometer, where the optical density (OD) is measured at 450 nm. OD values from the samples are compared to those of the positive and negative control solution in parallel.

Contents

- Microtiter plates, coated with EHV1 antigen (row 1, 4, 7, 10), EHV4 antigen (row 2, 5, 8, 11) and control antigen (row 3, 6, 9, 12)
- HRP Conjugate Concentrate (horseradish peroxidase conjugated rabbit anti horse Ig antibodies).
- Sample/Conjugate Dilution Buffer containing 0.05% merthiolate
- PBS-Tween Solution 20 x concentrate
- Substrate Solution – (tetramethylbenzidine in substrate buffer containing H₂O₂) – STORE IN THE DARK?
- Stop Solution – Contains sulphuric acid – CORROSIVE!

Interpretation

Criteria for test validity Serum
To ensure assay validity, the Positive Control Solution should have corrected OD value greater than 0.6. The Negative Control Solution should have a corrected OD value less than 0.1. For invalid tests, technique may be suspect and the assay should be repeated.

<table>
<thead>
<tr>
<th>Interpretation</th>
<th>OD</th>
</tr>
</thead>
<tbody>
<tr>
<td>OD &gt; 0.2</td>
<td>positive</td>
</tr>
<tr>
<td>OD 0.1–0.2</td>
<td>doubtful</td>
</tr>
<tr>
<td>OD &lt; 0.1</td>
<td>negative</td>
</tr>
</tbody>
</table>

Samples with a corrected OD value between 0.1 and 0.2 are considered doubtful and should be retested. If the results are still doubtful, it is recommended to test a second sample from the animal, obtained after a period of 10–14 days.

References


Manufacturer

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This manual covers the following EHV1/EHV4-Ab ELISA kits:  
Article number 10-3100-02
A. EHV1/EHV4 Positive Control Solution diluted in Sample Dilution Buffer containing 0.05% merthiolate.
B. EHV1/EHV4 Negative Control Solution diluted in Sample Dilution Buffer containing 0.05% merthiolate
C. EHV4 Positive Control Solution diluted in Sample Dilution Buffer containing 0.05% merthiolate

Material needed but not provided
1. Precision pipets (ranging from 5 to 200 µl)
2. Disposable pipet tips
3. Distilled water
4. Wash bottle
5. Container: 1 to 2 litres for Washing buffer
6. Microplate photometer, 450 nm
7. Microplate shaker

Specimen information
Undiluted serum or plasma is required for the test. Fresh, refrigerated, previously frozen but not inactivated serum or plasma can be used.

Preparation of reagents
PBS-Tween Buffer: Dilute the PBS-Tween Solution 20 x concentrate 1/20 in distilled water. Prepare 500 ml per plate by adding 25 ml PBST solution to 475 ml distilled water and mix thoroughly. N.B. Please check that there is no crystal precipitation in the bottle. If crystals are seen, please warm and shake well.

Conjugate: Conjugate should be diluted in conjugate dilution buffer according to the given dilution factor on the label and on the inner side of the box. It is recommended to prepare a pre-dilution of the conjugate, (for instance 1/100) for further preparation of the final working dilution.

Pre-dilution of samples: For testing, the samples should be pre-diluted 1/100 in sample dilution buffer, (for example 5 µl sample into 495 µl of sample dilution buffer)

Precautions
1. Carefully read and follow all instructions.
2. Store the kit and all reagents at +2 to +8°C (35 to 45°F)
3. All reagents should equilibrate to room temperature 18 to 25°C (64 to 77°F) before use.
4. Handle all materials according to the Good Laboratory Practice.
5. Do not mix components or instruction booklets from different test kit batches.
6. Care should be taken to prevent contamination of kit components.
7. Do not use test kit beyond date of expire.
8. Do not eat, drink, or smoke where specimens or kit reagents are handled.
9. Use a separate pipet tip for each sample.
10. Do not pipet by mouth.
11. Include positive and negative control solutions on each plate or test strip series.
12. Use only distilled water for preparation of reagents.
13. The Stop Solution contains sulphuric acid, which is corrosive.
14. All unused biological materials should be disposed according to the local, regional and national regulations.

Recommendation!
The volume of the reagents is sufficient for at least 4 separate test occasions.
Strips with broken seal can be stored at +2 to +8°C for up to 4 weeks.

Procedure
1. All reagents should equilibrate to room temperature 18 to 25°C (64 to 77°F) before use. Label each strip with a number.
2. Pre-dilute serum samples 1/100 with sample dilution buffer.
3. Add 100 µl of Positive Control Solution and 100 µl of Negative Control Solution to selected wells. For confirmation purposes it is recommended to run the control solutions in duplicates, (proposed layout, please see figure #1).
4. Add 100 µl of pre-diluted serum sample to each well, (proposed layout, please see figure #1).
5. Seal strips and incubate for 2 hours at room temperature 18 to 25°C (64 to 77°F) under shaking.
6. Rinse the plates/strips 4 times with PBS-Tween Buffer: at each rinse cycle fill up the wells, empty the plate and tap hard to remove all remains of fluid.
7. Add 100 µl of diluted HRP conjugate to each well
8. Incubate the plate for 1 hour at room temperature 18 to 25°C (64 to 77°F) under shaking.
10. Add 100 µl of Substrate Solution to each well. Incubate for 10 minutes at room temperature 18 to 25°C (64 to 77°F). Begin timing when the first well is filled.
11. Stop the reaction by adding 50 µl Stop Solution to each well. Add the Stop Solution in the same order as the Substrate Solution in step #10.
12. Measure the optical density (OD) of the controls and samples at 450 nm in a microplate photometer (use air as blank). Measure the OD within 15 minutes after the addition of Stop Solution to prevent fluctuation in OD values.

Calculation of results
The optical density (OD) values in wells coated with EHV1 and EHV4 are corrected by subtracting the OD values of the corresponding wells containing the control antigen, (OD corr).
All controls and samples OD values should be corrected before results are interpreted.

\[
\text{OD corr} = \text{OD Antigen} - \text{OD Control antigen}
\]

Figure 1; Proposed testlayout

<table>
<thead>
<tr>
<th></th>
<th>A1</th>
<th>A2</th>
<th>A3</th>
<th>A4</th>
<th>A5</th>
<th>A6</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>Control A</td>
<td>Control A</td>
<td>Control A</td>
<td>Sample a3</td>
<td>Sample a3</td>
<td>Sample a3</td>
</tr>
<tr>
<td>B2</td>
<td>Control B</td>
<td>Control B</td>
<td>Control B</td>
<td>Sample a4</td>
<td>Sample a4</td>
<td>Sample a4</td>
</tr>
<tr>
<td>C1</td>
<td>Control C</td>
<td>Control C</td>
<td>Control C</td>
<td>Sample a5</td>
<td>Sample a5</td>
<td>Sample a5</td>
</tr>
<tr>
<td>C2</td>
<td>Control C</td>
<td>Control C</td>
<td>Control C</td>
<td>Sample a6</td>
<td>Sample a6</td>
<td>Sample a6</td>
</tr>
<tr>
<td>D1</td>
<td>Sample a7</td>
<td>Sample a7</td>
<td>Sample a7</td>
<td>Sample a8</td>
<td>Sample a8</td>
<td>Sample a8</td>
</tr>
<tr>
<td>D2</td>
<td>Sample a9</td>
<td>Sample a9</td>
<td>Sample a9</td>
<td>Sample a10</td>
<td>Sample a10</td>
<td>Sample a10</td>
</tr>
</tbody>
</table>

The optical density (OD) values in wells coated with EHV1 and EHV4 are corrected by subtracting the OD values of the corresponding wells containing the control antigen, (OD corr). All controls and samples OD values should be corrected before results are interpreted.