General information

African horse sickness (AHS) is a viral infection of equids that is transmitted by midges (*Culicoides* spp.) and caused by an Orbivirus, with nine different serotypes described. It causes fever, nasal discharge, oedema and death of horses in 80% to 90% of cases, while donkeys show almost no symptoms.

AHS is a notifiable disease listed by the World Organisation for Animal Health (WOAH) due to the high mortality rate and potential for rapid spread.

The AHS virus (AHSV) is endemic to sub-Saharan Africa but completely absent from many areas, including Europe and North America. It is a threat for equine industry regarding all international horse movements. Thus, serological tests are useful to control AHSV spread in the world.

This diagnostic kit is designed to detect antibodies directed against the VP7 protein, which is conserved among all 9 AHSV serotypes.

It can be used with individual serum or plasma samples from horses and donkeys.

Description and principle

Microwells are coated with AHSV VP7 recombinant protein.

Samples to be tested and controls are added to the microwells. Anti-AHSV antibodies, if present, form an antibody-antigen complex.

After washing, an anti-equine IgG horseradish peroxidase (HRP) conjugate is added to the microwells. It fixes to the anti-AHSV antibodies, forming an antigen-antibody-conjugate-HRP complex.

The excess conjugate is then removed by washing, and the Substrate Solution (TMB) is added.

The resulting coloration depends on the quantity of specific antibodies present in the sample to be tested:

- In the presence of antibodies, a blue coloration appears which becomes yellow after addition of the Stop Solution.
- In the absence of antibodies, no coloration appears.

The microplate is read at 450 nm.

Kit components

Reagents*
Microplates coated with AHSV VP7 recombinant protein
Concentrated Conjugate (10X)
Positive Control
Negative Control
Dilution Buffer 14
Dilution Buffer 3
Wash Concentrate (20X)
Substrate Solution
Stop Solution (0.5 M)

** Quantities supplied are indicated on the kit label.*

1. The Conjugate, Controls and Substrate solution must be stored at 5°C (± 3°C).
2. The other reagents can be stored between +2°C and +26°C.
3. For detailed storage conditions of opened and/or diluted components, please refer to www.innovative-diagnostics.com/storage-conditions.
4. Wash and Stop solutions can be used for the entire IDvet product range. Substrate solutions and Dilution buffers with same batch numbers are interchangeable.

Materials required but not provided

1. Mono or multi-channel pipettes capable of delivering volumes of 20 µL, 100 µL, and 500 µL.
2. Disposable tip.
3. 96-well microplate.
4. Distilled or deionized water.
5. Manual or automatic wash system.
6. 96-well microplate reader.

Precautions

1. Do not pipette by mouth.
2. Contains components that can be harmful to the skin and eyes and may cause sensitisation by skin contact. Avoid contact with skin and eyes. Use protective lab coat, one-way gloves and safety glasses. The Stop Solution (0,5 M acid) may be harmful if swallowed.
3. Do not expose the substrate solution to bright light nor to oxidizing agents.
4. All waste should be properly decontaminated prior to disposal. Dispose in accordance with local regulations.

Please refer to the Material Safety Data Sheet, available upon request at info@innovative-diagnostics.com, for more detailed information.

Sample preparation

In order to avoid differences in incubation times between specimens, it is possible to prepare a 96-well microplate containing the test and control specimens, before transferring them into an ELISA microplate using a multichannel pipette.

Wash Solution preparation

If necessary, bring the **Wash Concentrate (20X)** to room temperature and mix thoroughly to ensure that it is completely solubilized.

Prepare the **Wash Solution (1X)** by diluting the **Wash Concentrate (20X)** to 1:20 in distilled/deionized water.

The quality of the wash step may influence results. Ensure that wells are completely empty between washes. If using an automatic washer, it is extremely important to correctly parameter the machine (mode, type of aspiration, aspiration height). For more information, please consult the "IDvet Washing Guide", available upon request.

Testing procedure

Allow the reagents to come to room temperature (21°C ± 5°C) before use. Homogenize all reagents by inversion or by vortex.

1. In the ELISA microplate, add:
   - 90 µL of **Dilution Buffer 14** to each microwell.
   - 10 µL of the **Negative Control** to wells A1 and B1.
   - 10 µL of the **Positive Control** to wells C1 and D1.
   - 10 µL of **each sample to be tested** to the remaining wells.
2. Cover the plate and incubate **45 min ± 5 min** at 21°C (± 5°C).
3. Prepare the **Conjugate 1X** by diluting the **Concentrated Conjugate 10X** to 1:10 in **Dilution Buffer 3.**
4. Empty the wells. Wash each well 3 times with at least 300 µL of the **Wash Solution**. Avoid drying of the wells between washes.
5. Add 100 µL of the **Conjugate 1X** to each well.
6. Cover the plate and incubate **30 min ± 3 min** at 21°C (± 5°C).
7. Empty the wells. Wash each well 3 times with at least 300 µL of the **Wash Solution**. Avoid drying of the wells between washes.
8. Add 100 µL of the **Substrate Solution** to each well.
9. Cover the plate and incubate **15 min ± 2 min** at 21°C (± 5°C) in the dark.
10. Add 100 µL of the **Stop Solution** to each well, in the same order as in step No. 8, to stop the reaction.
11. Read and record the O.D. at 450 nm.

Validation

The test is validated if:

✓ the mean O.D. value of the Positive Control (OD_{PC}) is greater than 0.350.

$$\mathbf{OD_{PC} > 0.350}$$

✓ the ratio of the mean OD values of the Positive Control (OD_{PC}) and Negative Control (OD_{NC}) is greater than 3.

$$\mathbf{OD_{PC}/OD_{NC} > 3}$$

Interpretation

For each sample, calculate the S/P percentage (S/P%):

$$\mathbf{S/P\,\% = \frac{OD_{sample} - OD_{NC}}{OD_{PC} - OD_{NC}} \times 100}$$

Samples presenting a S/P%:

- less than or equal to 60% are considered negative;
- greater than 60% and inferior or equal to 70% are considered doubtful;
- greater than 70% are considered positive.

Result	Status
S/P % ≤ 60 %	NEGATIVE
60% < S/P % ≤ 70%	DOUBTFUL
S/P % > 70 %	POSITIVE

Note 1: The S/P% positivity cut-off of this test has been established at 60-70%. However, the uncertainty of the measurement applicable to a test depends on the kit itself and on the laboratory implementing it (*). The laboratory is therefore invited to estimate its measurement uncertainty around the cut-off. Samples with S/P% values found within this uncertainty zone must be considered separately and distinguished from samples found to be positive or negative with certainty. It is recommended to implement this ELISA technique under a quality assurance system and, if possible, under ISO 17025 accreditation.

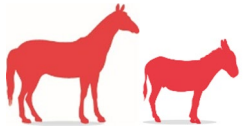
(*) WOAH Terrestrial Manual, Chapter 2.2.4. Measurement uncertainty, version adopted in May 2014 [consulted online on January 11, 2024])"

Note 2: The IDSoft™ data analysis program is available free-of-charge. Please contact, for more information, support.software@innovative-diagnostics.com

This software program can calculate many parameters (validation criteria, S/P or S/N values, titers, vaccination age, groups) and offers a graphic representation of the serological profiles of the animals tested.

Certified management system

ID Screen®
African Horse Sickness Indirect



Indirect ELISA for the detection of antibodies directed against the VP7 protein of the African Horse Sickness virus in serum or plasma from horses and donkeys

For *in vitro* use

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Innovative Diagnostics, 310, rue Louis Pasteur – Grabels - FRANCE
Tel:+ 33 (0)4 67 41 49 33 - Fax: + 33 (0)4 67 45 36 95
www.innovative-diagnostics.com - E-mail: info@innovative-diagnostics.com