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User's Manual

Aujeszky's Disease Virus gE Ab ELISA

Enzyme Immunoassay for the detection of Pseudorabies Virus (Aujeszky's Disease Virus) gE antibodies in serum samples

REF DE2493

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Please use only the valid version of the package insert provided with the kit.
Verwenden Sie nur die jeweils gültige, im Testkit enthaltene, Arbeitsanleitung.
Si prega di usare la versione valida dell'inserto del pacco a disposizione con il kit.
Por favor, se usa solo la version valida de la metodico técnico incluido aqui en el kit.

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1 INTRODUCTION

In piglets, a variety of neurological signs are associated with the disease, but respiratory signs are often the most striking clinical feature, The disease is less pronounced in older pigs and, after recovery, i.e. in adult pigs, a lifelong latent infection is established. From such asymptotic pigs, ADV has been isolated from cranial ganglia and lymphoid tissue. The virus can be transmitted by physical contact with infected animals or through maternal infection of foetal or suckling pigs by reactivated virus in lately infected sows.

All herds in endemic regions should be monitored for the presence of infection and uninfected herds protected by control measures. Some countries practice vaccination, while some others try to control the spread by culling sero positive pigs.

Pigs infected with pseudorabies field strains (mostly adult lately infected pigs) or vaccinated with gE+ vaccine, produce antibodies against pseudorabies glycoprotein gE.

This test kit is designed to detect these antibodies against this gE glycoprotein by use of a blocking Enzyme Immuno Assay (ELISA). This test kit meets the requirements of the EC-program.

2 INTENDED USE OF THE TESTKIT

The principle of the test is based on the reaction of 2 monoclonal antibodies with 2 different antigenic determinants on the gE glycoprotein of Aujeszky's disease virus (ADV). Whereas a negative sample does not block the reaction, a sample containing antibodies to gE does block there action. However, pigs immunized with vaccines lacking gE expression do not block the reaction and thus are scored negative.

3 PRINCIPLE OF THE TEST KIT

A first monoclonal anti-gE antibody (the catching antibody) is used for coating the wells of a 96-well microtiter plate. Test sample and antigen, consisting of ADV infected cell cultures, are incubated in the microtiter test plate after 2 hours. A second monoclonal anti gE antibody, conjugated with horseradish peroxidase (HRPO) is added to the wells. This monoclonal antibody recognizes a different antigenic determinant on gE than the catching antibody.

After incubation and washing, the substrate is added. If the test sample is negative, i.e. does not contain antibody to gE, HRPO and substrate will produce a colour reaction.

If the test sample is positive, the binding of the antigen to one or both monoclonal antibodies will be blocked and the colour reaction fails to appear.

A test sample is defined positive if the extinction is below 60% of the mean of that of the negative control which is included in this test kit.

4 CONTENTS

- 1 x 96 well **microtiter strip plate** coated with the monoclonal catching antibody.
- 1 x 13 ml **HRPO-conjugated** anti-ADV-gE monoclonal **antibody**
- 1 x 7 ml inactivated **ADV antigen**
- 1 x 1 ml inactivated **positive control** serum (freeze-dried)
- 1 x 1 ml inactivated **negative control** serum (freeze-dried)
- 1 x 20 ml **wash solution** 200 x concentrated, must be diluted in deionized water before use!
- 1 x 13 ml **Diluent**
- 1 x 7 ml **Substrate Buffer A**
- 1 x 7 ml **Substrate Buffer B**
- 1 x 7 ml **Stop Solution**
- 1 x plastic cover seal

5 HANDLING AND STORAGE OF SPECIMENS

The kit should be stored at +4 °C. An open packet should be used within 10 days.

Samples may be used fresh or may be kept frozen below -20 °C before use.

Positive and negative controls may be stored after reconstitution in aliquots at -20 °C and used until the expiry date. Avoid repeated freezing and thawing as this increases non-specific reactivity

6 WASH PROTOCOL

In ELISAs, un-complexed components must be removed efficiently between each incubation step. This is accomplished by appropriate washing. It should be stressed that each washing step must be carried out with care to guarantee reproducible inter- and intra-assay results. It is essential to follow the washing procedures outlined below. Washing may be done manually or with automatic equipment. Automatic washing equipment usually gives better results.

Manual washing

1. Empty each well by turning the microtiter plate upside down, followed by a firm vertical downward movement to remove the buffer.
2. Fill all the wells with 250 µL washing solution.
3. This washing cycle (1 and 2) should be carried out at least 4 times
4. Turn the plate upside down and empty the wells with a firm vertical movement
5. Place the inverted plate on absorbent paper towels and tap the plate firmly to remove any residual washing solution in the wells.
6. Take care that none of the wells dry out before the next reagent is dispensed

Washing with automatic equipment

When automatic plate washing equipment is used, check that all wells are aspirated completely and that the washing solution is correctly dispensed, reaching the rim of each well during each rinsing cycle. The washer should be programmed to execute at least 4 washing cycles.

7 TEST PROTOCOL

1. Bring all reagents to room temperature.
2. Colostrum or serum is required for each sample well.
3. Colostrum samples must be centrifuged for 15 minutes at 2000 g to remove the lipid layer. Take the colostrum sample from under the lipid layer.
4. For confirmation purposes it is recommended to run a duplicate for each test sample.
5. The wash solution must be diluted 200x times in aqua bidest.!
6. Reconstitute the positive control and the negative control in 1 ml deionized water store in aliquots at -20 °C
7. Positive control: add 50 µL of the positive control in 3 wells (A1, B1 and C1) of the coated plate.
   Negative control: add 50 µL of the negative control in 3 wells (D1, E1 and F1) of the coated plate.
   Blank: add 50 µL of diluent (not provided) in 2 wells (G1 and H1) of the coated test plate.
8. Add 50 µL of each test sample into the remaining wells of the microtiter plate.
   If samples are tested individually, 88 samples can be tested on one plate.
9. Dispense 50 µL of antigen in all wells of the plate, except for the two blanks. Add 50 µL diluent to the blanks.
10. Mix the contents of the well gently, seal the plate and incubate for 2 hours at 37 °C.
    (Or overnight 12-18 hours at 4 °C.)
11. Add 100 µL of the HRPO-conjugate to all wells (with serum/antigen mixture) of the coated plate.
    Do not wash the plate!
12. Mix the contents of the wells carefully. Seal the plate and incubate for 1 hour at 37 °C.
13. Wash as pointed out in wash protocol. Flick the plate dry.
14. Mix equal parts of buffer A and buffer B with gentle shaking. Prepare immediately before use!
    Dispense 100 µL substrate solution to each well.
    Incubate 15-25 min. at room temperature (20-25 °C) in the dark.
15. Add 50 µL stop solution into each well.
16. Read the OD of each well immediately (within 10 min.!) at 450 nm. Use 620 nm as a reference.

N.B. Contaminated or lipemic sera can result in very high or low OD.
Pigs of < 6 months might have maternal antibodies.

8 PRECAUTIONS

- Handle all biological material as though capable of transmitting ADV.
- Do not pipette by mouth.
- Do not eat, drink, smoke or prepare foods, or apply cosmetics within the designated working area.
- TMB substrate (buffer A/B) is toxic by inhalation, through contact with skin or when swallowed; observe care when handling the substrate.
- Do not use components past the expiry date and do not mix components from different serial lots.
- Optimal results will be obtained by strict adherence to this protocol. Careful pipetting and washing throughout this procedure are necessary to maintain precision and accuracy.
- Each well is ultimately used as an optical cuvette. Therefore, do not touch the under-surface of the microtiter plate and protect it from damage and dirt.

9 VALIDATION OF THE TEST

In order to confirm appropriate test conditions, the positive control should give an extinction ≤ 0.500 OD.

The negative control should give an OD ≥ 0.900 OD.

10 INTERPRETATION OF TEST RESULTS

The test is only valid when OD negative 0,9 < X <1,8.

$$\text{Calculation of inhibition (I) \% :} \quad \frac{\text{OD negative - OD sample}}{\text{OD negative}} \times 100$$

Low prevalence:

When in a certain area, region or business of a country > 80% of the animals score negative. This is dependent on public rules.

A test sample is positive, i.e. contains antibodies to gE, if I > 40%.

A test sample is negative, i.e. contains antibodies to gE if I < 30%

Repeat test if 30 < I < 40. If the sample scores again between these values, take a new sample and repeat the test.

<u>High prevalence</u>:

When in a certain area, region or business of a country > 20% of the animals score positive. This is dependent on public rules.

A test sample is positive, i.e. contains antibodies to gE, if I > 30%.

A test sample is negative, i.e. contains antibodies to gE if I < 30%

The entire risk as to the performance of these products is assumed by the purchaser. DEMEDITEC shall not be liable for indirect, special or consequential damages of any kind resulting from use of the products.

SYMBOLS USED WITH DEMEDITEC ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità