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User's Manual

Leptospira Hardjo Ab bovine ELISA

Enzyme Immunoassay for the detection of antibodies against an important polysaccharide epitope of Leptospira Hardjo in serum and milk samples

VET

REF DE2498

Σ 96 wells

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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

Leptospira interrogans serovar hardjo and pomona are important pathogens of cattle. Cattle are the primary reservoir hosts for hardjo, which is transmitted by direct contact with contaminated urine and less often through water. Pomona is less host specific and more resistant to environmental conditions. Thus hardjo may be expected to enter a herd through the introduction of infected cattle, whereas pomona may enter either through infected cattle or through contaminated water. (The major recognized site of leptospiral persistence in carrier cattle is the kidney).
The specificity of the monoclonal antibodies used in this test was also determined by modified microscopic agglutination test (MAT). Monoclonal antibodies are standardized reagents which are suitable for use in catching or detecting ELISA systems. Conventional tests for detecting antibodies give many problems of batch-to-batch variation and interpretation.
The ELISA system is intended to use as a rapid screening test for the specific detection of Leptospira hardjo antibodies in serum and milk samples of infected cattle.

2 INTENDED USE OF THE TESTKIT

This diagnostic test for leptospirosis is intended to identify antibodies against sugar antigens of Leptospira in serum and milk samples. In contrast to test systems which make use of unpurified non-specific Leptospira hardjo antigens, this test uses a monoclonal antibody which catches a specific Leptospira hardjo sugar antigen. This monoclonal based ELISA has very high specificity and sensitivity.

3 PRINCIPLE OF THE TEST KIT

An antigen solution antibody mixture is coated to the wells of the micro titer plate after stabilization and drying Plates are vacuum sealed. Diluted milk or serum samples are added to the coated wells. After incubation and appropriate washing a monoclonal anti-bovine conjugate is added and the plates are again incubated. After appropriate washing, substrate is added. Within several minutes the color reaction is stopped and the plates are immediately read at 450 nm.

4 CONTENTS

1 x 96 well **microtiter plate coated** with monoclonal antibodies
1 x 13 mL **conjugate** anti-bovine-HRPO (monoclonal antibody)
1 x 0.5 mL inactivated **positive control** (freeze dried)
1 x 1 mL inactivated **negative control** (freeze dried)
1 x 20 mL **wash-solution 200 x concentrated**, must be diluted in de-ionized water before use!
1 x 20 mL **ELISA buffer**
1 x 7 mL **substrate buffer A**
1 x 7 mL **substrate buffer B**
1 x 8 mL **stop solution**
1 x plastic cover seal.

5 HANDLING AND STORAGE OF SPECIMENS.

The kit should be stored at 2 °C - 8 °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. Wash the micro titer plate with washing solution according to the washing protocol.
   The washing solution provided has to be diluted 200x
2. Reconstitute the positive in 0,5 mL demi-water and negative control in 1 ml demi-water, reconstituted controls should be divided into aliquots (not less than 100 uL)and stored at -20 °C until expiry date
3. Pre dilute the sera to be tested 1:10 (10 µL serum sample + 90 µL buffer) in a round bottomed microtiter plate (not supplied in the test kit),
   also pre dilute the positive and negative control 1:10 (10 µL positive/negative control + 90 µL buffer)
4. Dispense 100 µL ELISA buffer to wells A1 and B1 (blanks) of the Leptospira hardjo coated test plate
5. **Serum samples**:
   Dispense 90 µL ELISA buffer to all remaining wells
   Individual milk samples:
   Dispense only 75 µL ELISA buffer to all remaining wells.
6. **Serum:**
   Transfer 10 µL of pre-diluted samples to the wells of the coated microtiter plate already filled with 90 µL of ELISA-buffer
   Milk:
   Dispense 25 µL from individual milk samples to the wells of the coated microtiter plate already filled with 75 µL of ELISA-buffer.
   If pooled milk samples are used dispense 100 µL skimmed milk sample to the coated well, don't add any buffer
7. Seal and incubate 60 min. at 37 °C.
8. Wash as pointed out in wash protocol.
9. Dispense 100 µL HRPO-conjugated monoclonal antibody to all wells.
10. Seal and incubate 1 hour at 37 °C.
11. Wash as pointed out in wash protocol.
12. With gentle shaking mix equal parts of buffer A and B together. Prepare immediately before use!
    Dispense 100 µL substrate solution to each well.
13. Incubate 10-15 min. at room temperature (21 °C.).
14. Add 50 µL stop solution to each well shake the stop solution firmly before use !!
15. Read the absorbency values (within 10 min.!) at 450 nm.
16. Use as a reference wave length 620 nm.

8 PRECAUTIONS

- Handle all biological material as though capable of transmitting Leptospira Hardjo (human pathogen).
- 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 mean absorption value of the negative control should be < 0.200 OD units (450 nm).
The mean absorption value of the positive control provided should be > 0.800 OD units (450 nm).

10 INTERPRETATION OF TEST RESULTS

In general high prevalence is more than 15% positive animals.

This prevalence can be used for a certain area (f.i. farm, state or country) depending on elimination campaign or other (government) restrictions.

Serum

- **High prevalence**:
  A sample is scored Leptospira hardjo negative if the OD value is below or equal to the average OD value of the mean negative control plus 0.150 OD units.
  Negative: OD samples < OD mean negative control plus 0.150.
  Positive: OD samples > OD mean negative control plus 0.150.

- **Low prevalence**:
  A sample is scored Leptospira hardjo negative if the OD value is below or equal to the average OD value of the mean negative control plus 0.100 OD units.
  Negative: OD samples < OD mean negative control plus 0.100.
  Positive: OD samples > OD mean negative control plus 0.200.
  Doubtful: between mean negative control and 0.200.

Milk

- **High prevalence**:
  A sample is scored Leptospira hardjo negative if the OD value is below or equal to the average OD value of the mean negative control plus 0.050 OD units.
  Negative: OD samples < OD mean negative control plus 0.075.
  Positive: OD samples > OD mean negative control plus 0.075.

- **Low prevalence**:
  A sample is scored Leptospira hardjo negative if the OD value is below or equal to the average OD value of the mean negative control plus 0.050 OD units.

  Negative: OD samples < OD mean negative control plus 0.050.
  Positive: OD samples > OD mean negative control plus 0.100.
  Doubtful: between mean negative control and 0.100.

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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
	European Conformity	CE-Konformitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
	For veterinary use only				
	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
	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