

DRG® Leptospira hardjo (EIA-2498)

Revised 29 May 2009 (Vers. 4.0)

For Veterinary Use Only

*Please use only the valid version of the package insert provided with the kit***INTRODUCTION****For Veterinary use only!**

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. This 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.

INTENDED USE OF THE TEST KIT

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.

PRINCIPLE OF THE TEST KIT

An antigen solution antibody mixture is coated to the wells of the microtiter plate. After stabilization and drying plates are vacuum sealed.

Diluted milk or serum samples are added to the 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.

CONTENTS

- 1 x 96 well microtiter plate coated with monoclonal antibodies
- 1 vial 100X concentrated HRPO conjugated (anti-bovine) monoclonal antibody
- 1 x 13 ml Conjugate Diluent
- 1 x 1 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 deionized 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

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HANDLING AND STORAGE OF SPECIMEN

The kit should be stored at 4°C. After reconstitution, the lyophilized reagents should be used immediately. The test kit is stable until the date given on the label. Positive and negative control sera can be stored in aliquots at -20°C and used until the expire date. Repeated freezing and thawing must be avoided.

Fresh samples can be used without restrictions. Addition of 0,1% sodium azide to the samples has no influence on the test results. For prolonged storage, samples should be frozen as soon as possible and stored at -25°C until use. Avoid repeated freezing and thawing as this increases non-specific reactivity and decreases end-point titers.

WASH PROTOCOL

In ELISA's un-complexed components must be removed efficiently between each immunological 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 advised to follow the washing procedures outlined below carefully.

Both manual washing and washing with automatic equipment can be performed. (Automatic washing equipment usually gives better results).

Manual washing

1. Empty each well by turning the microtitre plate upside down, followed by a firm vertical movement.
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 by a firm short vertical movement.
5. Place the inverted plate on absorbent paper towels and tap the plate firmly to remove residual washing solution in the wells.
6. Take care that none of the wells dries out before the next reagent is dispensed.

Washing with automatic equipment

When using automatic plate washing equipment, check that all wells can be 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.

PREPARATION OF SPECIMENS***Milk samples - undiluted***

For optimal sensitivity pooled milk samples can be tested undiluted.

To avoid false positive reactions defatted samples must be used. Centrifuge the milk samples for 15 minutes at 2500 x g and take a sample from below the fat layer.

Milk samples - diluted

For optimal specificity individual milk samples should be tested at a 1:4 dilution.

Pooled milk samples, collected from up to 25 individual animals, can also be tested at a 1:2 dilution.

The use of diluted milk samples guarantees minimum false positivity.

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Serum samples

Individual serum samples should be diluted 1:100 in ELISA buffer. (See Test Protocol)

TEST PROTOCOL

1. Wash the microtiter plate with washing solution according to the washing protocol.
The washing solution provided has to be diluted 200x.
Reconstitute positive and negative control with 1 ml bidistilled water, divide in aliquots and store the not immediately used controls at -20°C.
2. Predilute the test sera to be tested 1:10 (10 µl sample + 90 µl Buffer) in a round bottomed microtiter plate (not supplied in the kit), also predilute the positive and negative control 1:10 (10 µl control + 90 µl Buffer).
3. Dispense 100 µL ELISA Buffer to wells A1 and B1 (**blanks**) of the Leptospira hardjo coated plate.

Serum samples: Dispense 90 µl ELISA Buffer to all remaining wells.

Individual milk samples: Dispense only 75 µl ELISA Buffer to all remaining wells.

4. **Serum:** Transfer 10 µl of the prediluted samples to the wells of the coated microtiter plate already filled with 90 µl ELISA Buffer.

Milk: Dispense 25 µl from individual milk samples to the wells of the coated microtiter plate already filled with 75 µl ELISA Buffer.
If pooled milk samples are used, dispense 100 µl skimmed milk sample to the coated well don't add any buffer.
5. Seal and incubate 60 min. at 37°C.
6. Wash as in 1.
7. **Dilute the 100X concentrated HRPO conjugated monoclonal antibody 1:100 in conjugate diluent.**
Dispense 100 µl diluted conjugated antibody to all wells.
8. Seal and incubate 1 hour at 37°C.
9. Wash as in 1.
10. With gentle shaking mix equal parts of buffer A and B together. **Prepare immediately before use!**
Dispense 100 µl substrate solution to each well.
Incubate 10-15 min. at room temperature (21°C.).
11. Add 50 µl stop solution to each well.
12. Read the absorbency values immediately (within 10 min.!) at 450 nm.
Use as a reference wave length 620 nm.

PRECAUTIONS

Handle all biological materials 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 work area.

TMB is toxic by inhalation, through contact with skin or when swallowed; observe care when handling the substrate.

Do not use components past expire date and do not intermix components from different serial lots.

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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 prevent it from damage and dirt.

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).

INTERPRETATION

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: $OD_{\text{samples}} \leq \text{Mean } OD_{\text{negative control}} + 0.150.$

Positive: $OD_{\text{samples}} \geq \text{Mean } OD_{\text{negative control}} + 0.150.$

- 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: $OD_{\text{samples}} \leq \text{Mean } OD_{\text{negative control}} + 0.100.$

Positive: $OD_{\text{samples}} \geq \text{Mean } OD_{\text{negative control}} + 0.200.$

Doubtful: between mean $OD_{\text{negative control}}$ 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: $OD_{\text{samples}} \leq \text{Mean } OD_{\text{negative control}} + 0.075.$

Positive: $OD_{\text{samples}} \geq \text{Mean } OD_{\text{negative control}} + 0.075.$

- 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: $OD_{\text{samples}} \leq \text{Mean } OD_{\text{negative control}} + 0.050.$

Positive: $OD_{\text{samples}} \geq \text{Mean } OD_{\text{negative control}} + 0.100.$

Doubtful: between mean $OD_{\text{negative control}}$ and 0.100.



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



















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

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Symbols used with DRG 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-Konformitätskennzeichnung	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 research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
	For Veterinary Use only				
	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
Symbol	Portugues	Dansk	Svenska	Ελληνικά	
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη	
	Conformidade com as normas europeias	Europeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση	
	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό	
					
					
	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου	
	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος	
		Indeholder tilstrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις	
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstemperatur	Θερμοκρασία αποθήκευσης	
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης	
	Fabricante	Producent	Tillverkare	Κατασκευαστής	
Distributed by					
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο	
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..	