PrioCHECK™ L. Hardjo Ab Strip Kit

ELISA for *in vitro* detection of antibodies directed against *Leptospira interrogans* serovar Hardjo in serum and milk of cattle

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support.**

WARNING! POTENTIAL BIOHAZARD. Read the biological hazard safety information at this product's page at **thermofisher.com.** Wear appropriate protective eyewear, clothing, and gloves.

Introduction

Leptospirosis is a contagious disease of animals and humans caused by infection with the spirochete *Leptospira*. The genus *Leptospira* is at present divided into two species *L. interrogans* (parasitic) and *L. biflexa* (saprophytic). Many serovars appear to have a certain animal species as a natural host, but animals and humans can be infected with a wide variety of serovars. Leptospirosis occurs worldwide. In many European countries, *Leptospira interrogans* serovar Hardjo (*L.* Hardjo) is the most common cause of bovine Leptospirosis. The indirect ELISA described by Bercovich et al has been modified into a sensitive, specific and robust ELISA that is suitable for large scale screening. The Applied Biosystems™ PrioCHECK™ L. Hardjo Ab Strip Kit can be used for: *L.* Hardjo eradication programs; Monitoring of the *L.* Hardjo free status of cattle herds; Monitoring of *L.* Hardjo antibody level in cattle after vaccination; Individual and herd diagnosis.

Test principle

The PrioCHECK™ L. Hardjo Ab Strip Kit is an indirect ELISA and detects antibodies (Ab) against *Leptospira interrogans* serovar Hardjo (*L.* Hardjo) in cattle. A microtiter plate is coated with inactivated antigen. Serum- and/or milk samples are dispensed in the coated wells of a Test Plate. Antibodies directed against *L.* Hardjo that are present in the test sample will bind to the antigen during incubation. The bound antibodies are detected using an anti-bovine monoclonal antibody conjugated to the enzyme horseradish-peroxidase. Subsequently, the bound Conjugate is visualized by incubation with the Chromogen (TMB) Substrate. Finally, color development is stopped and measured at a wavelength of 450 nm.

Kit components

5 plate kit for 440 samples. Store kit at 5±3°C until expiry date. See kit label for actual expiry date. The shelf life of diluted, opened or reconstituted components is noted below, when appropriate.

Component	Description
1: Test Plate	Five Test Plates are delivered in vacuum bags which contain a desiccant sachet.
2: Conjugate (30x)	30x concentrated, dilute before use. One vial contains 2.5 mL Conjugate. Diluted conjugate is not stable, prepare just before use.
3: Dilution Buffer (5x)	5x concentrated, dilute before use. One vial contains 60 mL Dilution Buffer. Shelf life of dilution buffer working solution: 4 hours at 22±3°C.
4: Horse Serum	Lyophilized. One vial contains 3.5 mL lyophilized Horse Serum. Shelf life of reconstituted horse serum: until expiry date at –20°C.
5: Demineralized Water	One vial contains 10 mL Demineralized Water.
6: Washing Fluid (200x)	200x concentrated, dilute before use. One vial contains 60 mL Washing Fluid. Shelf life of washing solution: 1 week at 22±3°C.
7: Reference Serum 1	Lyophilized. One vial contains 0.5 mL Reference Serum 1 (positive control). Shelf life of reconstituted serum: until expiry date at –20°C.
8: Reference Serum 2	Lyophilized. One vial contains 0.5 mL Reference Serum 2 (negative control). Shelf life of reconstituted serum: until expiry date at –20°C.
9: Reference Serum 3	Lyophilized. One vial contains 0.5 mL Reference Serum 3 (weak positive control). Shelf life of reconstituted serum: until expiry date at –20°C.
10: Chromogen (TMB) Substrate	Ready-to-use. One vial contains 60 mL Chromogen (TMB) Substrate.
11: Stop Solution	Ready-to-use. One vial contains 60 mL Stop Solution.
Additional Kit Contents	• Package Insert • 10 plate sealers

Additional material required

Use	Description[1]
Dummy plates	Dummy plates to make pre-dilutions of the milk and serum samples. We recommend U-bottom shaped plates (Cat. No. 267245). However, also other non-binding plates or tubes can be used.
General	Laboratory equipment according to national safety regulations.
Analysis of Results	Plate Reader e.g. Multiskan EX or equivalent. The reader has to have an appropriate filter set to read the plates at 450 nm.
Optional	Plate washer.

[1] Unless otherwise indicated, all materials are available through **thermofisher.com**.

Test procedure

Precautions

- National Safety Regulations must be strictly followed.
- The PrioCHECK™ L. Hardjo Ab Strip Kit must be performed in laboratories suited for this purpose.
- Samples should be considered as potentially infectious and all items which contact the samples as potentially contaminated.

Notes

To achieve optimal results with the PrioCHECK™ L. Hardjo Ab Strip Kit, the following aspects must be considered:

- **The Test Procedure protocol must be strictly followed.**
- All reagents of the kit must be equilibrated to room temperature (22±3°C) before use.
- Pipette tips have to be changed for every pipetting step.
- Separate solution reservoirs must be used for each reagent.
- Kit components must not be used after their expiry date or if changes in their appearance are observed.
- Kit components of different kit lot numbers must not be used together.
- Demineralized or water of equal quality must be used for the test.

Solutions to be made in advance

Dilution buffer working solution

The Dilution Buffer (5x) (Component 3) must be diluted 1:5 in demineralized water (1 part Dilution Buffer + 4 parts demineralized water).

Horse serum

Equilibrate the vial to 22±3°C and reconstitute the Horse Serum (Component 4) with 3.5 mL Demineralized Water (Component 5). Can be stored at –20°C until expiry date.

Reconstitution of lyophilized reagents should be performed as follows:

1. Equilibrate the vials to 22±3°C.
2. With the vial in an upright position, tap the vial gently against the worktop to ensure that the content is on the bottom of the vial.
3. Open the vial.
4. Add the required amount of Demineralized Water (see label on vial).
5. Replace the stopper on the vial and gently rotate the vial so that any remaining dry material will be dissolved.
6. Allow the lyophilized material to stand for 15 minutes at 22±3°C.
7. Occasionally gently invert the vial (formation of foam should be avoided).

ELISA buffer

Dilute reconstituted horse serum 1:100 in dilution buffer working solution; e.g. for 1 plate prepare 40 mL (add 400 µL horse serum to 39.6 mL dilution buffer working solution). The ELISA buffer can be stored up to 4 hours at 22±3°C.

Conjugate dilution

Dilute the Conjugate (30x) (Component 2) 1:30 in dilution buffer working solution; e.g. for 1 plate prepare 12 mL (add 400 µL Conjugate to 11.6 mL dilution buffer working solution).

Note: The working dilution must be prepared just before use.

Reference sera

Reconstitute the sera (Component 7– 9) with 0.5 mL Demineralized Water (Component 5). Reconstituted reference sera are preferably aliquoted and can be stored at –20°C until expiry date. Mix sera gently after thawing and do not refreeze.

Washing solution

The Washing Fluid (200x) (Component 6) must be diluted 1:200 in demineralized water and is sufficient for a final volume of 12 liters washing solution. Stability of washing solution: 1 week stored at 22±3°C.

Note: See Appendix B for sample preparation procedure and storage.

Pre-treatment of the test plates

Note: Pre-treatment is only necessary when milk samples are tested and not for serum samples. However, sera may be tested on a pre-treated plate (test results are not affected).

1. Dispense 100 µL ELISA buffer to all wells of the Test Plate (Component 1).
2. Seal or cover the Test Plate and incubate for 60±2 minutes at 37±1°C.
3. Discard the ELISA buffer and wash the Test Plate 6 times with washing solution.

Pre-dilution of reference sera and test sera

- Make a 1:20 dilution of Reference Serum 1, 2, 3 and of the test sera in a dummy plate by mixing 10 µL serum with 190 µL ELISA buffer.

Incubation of test samples

1. In case of testing serum samples, unpack the required number of Test Plates. In case of testing milk samples use the plates as described under "Pre-treatment of the test plates."
2. Dispense 100 µL of ELISA buffer to wells A1 and B1 of the Test Plate (= blanks).
3. Dispense 90 µL of ELISA buffer to wells C1 to H1.
4. Dispense 10 µL of 1:20 diluted reference serum 1 (= positive control) to wells C1 and D1. Final serum dilution 1:200.
5. Dispense 10 µL of 1:20 diluted reference serum 2 (= negative control) to wells E1 and F1. Final serum dilution 1:200.
6. Dispense 10 µL of 1:20 diluted reference serum 3 to (= weak positive control) wells G1 and H1. Final serum dilution 1:200.
7. When testing **serum samples**, dispense 90 µL ELISA buffer into the remaining wells. Dispense 10 µL of 1:20 diluted test sera in each of these wells. Final serum dilution 1:200. Serum samples can be titrated by making two-fold serial dilutions in dilution buffer.
8. When testing **individual milk samples**, dispense 75 µL ELISA buffer into the remaining wells. Dispense 25 µL of milk sample in each of these wells of the plate. Final milk dilution 1:4. Take defatted milk sample from below the creamy layer.
9. When testing **bulk milk** samples, dispense 100 µL of milk sample into the remaining wells. Take defatted milk sample from below the creamy layer.
10. Seal and shake the test plate gently and incubate for 60±5 minutes at 37±1°C.

Incubation with conjugate and chromogen (TMB) substrate

1. Wash the Test Plate 6 times with washing solution.
2. Dispense 100 µL of diluted conjugate to all wells.
3. Cover the Test Plate and incubate for 60±5 minutes at 37±1°C.
4. Wash the Test Plate 6 times with washing solution.
5. Dispense 100 µL of the Chromogen (TMB) Substrate (Component 10) to all wells.
6. Incubate the Test Plate for 15 minutes at 22±3°C.
7. Add 100 µL Stop Solution (Component 11).
8. Agitate the Test Plate to mix the content of the wells prior to measuring.

Note: Start the addition of Stop Solution 15 minutes after the first well was filled with Chromogen (TMB) Substrate. Add the Stop Solution in the same order and at the same pace as the Chromogen (TMB) Substrate was dispensed.

Reading of the test and calculating the results

1. Measure the optical density (**OD**) of the wells at 450 nm within 15 minutes of stopping the color development.
2. Calculate the mean OD_{450} value of the blanks (wells A1 and B1).
3. Calculate the **corrected** OD_{450} value of all **samples** by subtracting the mean OD_{450} of the blanks.
4. Calculate the percentage positivity (**PP**) of the reference samples 2, 3 and the **test samples** according to the formula given below.

The **corrected** OD_{450} values of all **samples** are expressed as percentage positivity (**PP**) relative to the **corrected** mean OD_{450} value of **Reference Serum 1** in wells C1 and D1.

$$PP = (\text{corrected } OD_{450\ \text{test sample}} / \text{corrected } OD_{450\ \text{Reference Serum 1}}) \times 100$$

Result interpretation

Validation criteria

1. The mean OD_{450} of the blanks (wells A1 and B1) must be <0.150.
2. The **corrected** OD_{450} of **Reference Serum 1** (wells C1 and D1) must be ≥1.000.
3. The mean **PP** of Reference Serum 2 must be <20.
4. The mean **PP** of Reference Serum 3 must be between 20 and 60.

The above mentioned criteria have to be met in order to validate the results of test samples.

Note: If the **corrected** mean OD_{450} of **Reference Serum 1** is below 1.000 possibly the Chromogen (TMB) Substrate solution is too cold. In that case pre-warm the solution to 22±3°C or incubate up to 30 minutes.

Interpretation of the percentage positivity

Serum samples

PP = <20%	Negative for *L.* Hardjo specific antibodies.
PP = 20%–45%	Inconclusive (antibodies may be present)
PP = >45%	Positive for *L.* Hardjo specific antibodies.

Milk (individual and bulk) samples

PP = 40%	Negative for *L.* Hardjo specific antibodies
PP = 40%–60%	Inconclusive[1] (antibodies may be present)
PP = >60%	Positive for *L.* Hardjo antibodies

[1] Bulk milk samples with a doubtful test result can be retested in the PrioCHECK™ L. Hardjo Ab Strip Kit. When an inconclusive result is confirmed it may be followed by collecting blood samples of the (possibly) infected herd.

Note: Cut off values may need to be refined for the local situation in order to obtain acceptable percentages of false-positive and false-negative results. Testing of individual serum samples (1:200 diluted) has preference over testing of individual milk samples (1:4 diluted), because of non-specific reactions that incidentally may occur when testing individual milk samples.

Appendix A – References

1. International Leptospirosis Society, First meeting, Nantes, France 9–12 September 1996.
2. OIE Manual of Standards for Diagnostic Tests and Vaccines, Third Edition, 1996.
3. Bercovich, Z, Taaijke, R and Bokhout, BA. *Vet. Microbiol.* 21, 255–262, 1990.

Appendix B – Sample preparation procedure and storage

Serum	Milk
• Test sera can be stored at –20°C prior to testing. • Test sera have to be prediluted in ELISA buffer.	• Milk samples can be stored at 5±3°C prior to testing. If milk samples are not tested within 3 days of collection add 0.02% sodium azide as a preservative. • The fluid of the milk sample to be tested should be collected from underneath the creamy layer. • Milk samples (bulk) undiluted. • Individual milk samples 1:4 diluted in ELISA buffer.

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- Worldwide contact telephone numbers
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- Safety Data Sheets (SDSs; also known as MSDSs)

  NOTE: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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Rev.	Date	Description
A.0	15 January 2019	New document. Converted the legacy document (PrioCHECK L. hardjo Ab 7442080 5 plates v1.0_e.doc) to the current document template, with associated updates to the publication number, limited license information, warranty, trademarks, and logos.