LEPTOSPIRA ELISA IgM

REF M1003 Σ 96

CE For *in vitro* diagnostic use

INTENDED PURPOSE

Indirect immunoenzyme assay to test IgM antibodies against *Leptospira interrogans* in human serum/plasma.
The test is a qualitative and manual or alternatively automated assay, intended to be used as an aid to diagnosis.

INTRODUCTION

Leptospirosis is a zoonotic disease with epidemic potential caused by bacteria of the genus *Leptospira*. Among these, *Leptospira interrogans* is pathogenic to humans and animals, and includes a large number of serovars. Humans usually acquire leptospirosis through direct contact with the urine of infected animals or a urine-contaminated environment, where the bacteria can survive for weeks to months. A wide variety of animal species, primarily mammals (including rodents and domestic animals), may serve as sources of human infection. Leptospirosis occurs worldwide, but is endemic mainly in countries with humid subtropical and tropical climates. It is an occupational hazard for many people who work outdoors or with animals. Leptospirosis may present with a wide variety of clinical manifestations and a wide range of symptoms. Typically, the disease presents in four clinical categories: (i) a mild, influenza-like illness; (ii) Weil's syndrome characterized by jaundice, renal failure, haemorrhage and myocarditis with arrhythmias; (iii) meningitis/meningoencephalitis; (iv) pulmonary haemorrhage with respiratory failure. Due to the varied and non-specific clinical presentation, it can be mistaken with many other infectious diseases.
The disease is usually diagnosed in the laboratory by detecting antibodies, (serodiagnosis), or by direct methods (culture, direct immunofluorescence, PCR). Seroconversion may occur as early as 5–7 days after the onset of disease; IgM class antibodies usually appear somewhat earlier than IgG class antibodies. The microscopic agglutination test (MAT) and enzyme immunoassays (EIA) are two serologic tests used for laboratory diagnosis of leptospirosis. Panels of live leptospires belonging to different serovars must be maintained in the laboratory to be used as antigens in the MAT. EIAs usually only detect antibodies reacting with broadly reactive genus-specific antigens and thus give no indication of the causative serovar or serogroup.

TEST PRINCIPLE

The ELISA method is based upon the reaction of antibodies in the sample tested with the antigen adsorbed on the polystyrene surface. Unbound immunoglobulins are washed off. An enzyme-labelled anti-human globulin binds the antigen-antibody complex in a second step. After a new washing step, bound conjugate is developed with the aid of a substrate solution (TMB) to render a blue coloured soluble product which turns into yellow after adding the acid stopping solution.

KIT FEATURES

All reagents, except for the washing solution, are supplied ready to use.
Serum dilution solution and conjugate are coloured to help in the performance of the technique.
Sample predilution is not necessary.
Break-apart individual wells are supplied, so that the same number of wells is consumed than the number of tests performed.

MATERIALS PROVIDED

[1] VIRCELL LEPTOSPIRA PLATE: 1 96-wells plate coated with antigen of *Leptospira interrogans*. Contains inactivated antigen. Contains material of animal origin.
[2] VIRCELL SERUM DILUENT: 25 ml of serum dilution solution: a blue coloured phosphate buffer containing protein stabilizers. Contains 2-Methyl-2H-isothiazol-3-one and 5-bromo-5-nitro-1,3-dioxane. Contains material of animal origin. Ready to use.
[3] VIRCELL IgM POSITIVE CONTROL: 500 µl of positive control serum. Contains 2-Methyl-2H-isothiazol-3-one and 5-bromo-5-nitro-1,3-dioxane. Contains material of human origin. Contains material of animal origin.
[4] VIRCELL IgM CUT OFF CONTROL: 500 µl of cut off control serum. Contains 2-Methyl-2H-isothiazol-3-one and 5-bromo-5-nitro-1,3-dioxane. Contains material of human origin. Contains material of animal origin.
[5] VIRCELL IgM NEGATIVE CONTROL: 500 µl of negative control serum. Contains 2-Methyl-2H-isothiazol-3-one and 5-bromo-5-nitro-1,3-dioxane. Contains material of human origin. Contains material of animal origin.
[6] VIRCELL IgM CONJUGATE: 2 x 7.5 ml of an orange-coloured anti-human IgM peroxidase conjugate dilution. Contains 2-Methyl-2H-isothiazol-3-one and 5-bromo-5-nitro-1,3-dioxane. Contains material of animal origin. Ready to use.
[7] VIRCELL TMB SUBSTRATE SOLUTION: 15 ml of substrate solution containing tetramethylbenzidine (TMB) and 2-Pyrrolidinone. Ready to use.
[8] VIRCELL STOP REAGENT: 15 ml of stopping solution: 0.5 M sulphuric acid.
[9] VIRCELL WASH BUFFER (20x): 50 ml of 20x washing solution: a phosphate buffer containing Tween®-20 and Reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1).
[10] VIRCELL LB REAGENT: 1.5 ml of *Leptospira biflexa* extract. Contains 2-Methyl-2H-isothiazol-3-one and 5-bromo-5-nitro-1,3-dioxane. Contains inactivated antigen. Contains material of animal origin.

Special materials required but not provided:
-Precision micropipettes.
-ELISA plate washer.
-Thermostatized incubator/water bath.
-ELISA plate spectrophotometer with a 450 nm measuring filter and a 620 nm reference filter.
-Alternatively, an ELISA automated processor.
-Distilled water.
-Human IgG sorbent (ref. Vircell S001).

STORAGE AND HANDLING CONDITIONS

Store at 2-8°C. Do not use the kit reagents beyond the expiration date. This will be valid only if reagents are stored closed and at 2-8°C.

IN-USE STABILITY

VIRCELL WASH BUFFER diluted (1x): 4 months at 2-8°C.
Rest of reagents: Refer to package label for expiration date (at 2-8°C).
Substrate solution is light sensitive. Avoid light exposure and discard if blue colour develops during storage. Substrate solution should not get in contact with oxidizers such as bleach solutions or metals. Make sure that no metal components come in contact with the substrate.
VIRCELL, S.L. does not accept responsibility for the mishandling of the reagents included in the kit.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use only. For professional use only.
2. The product should be limited to personnel who have been trained in the technique.
3. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results.
4. Use only protocols described in this insert. Conditions other than specified may give erroneous results.
5. Wear personal protective equipment when handling samples. Wash hands properly after handling the samples. All procedures must be carried out in accordance with the approved safety standards.
6. Clean pipette tips must be used for every assay step. Use only clean, preferably disposable material.
7. Never pipette by mouth.
8. Do not use in the event of damage to the package.
9. Do not use the kit after expiration date.
10. If the kit or its components are stored in the refrigerator, please bring them at room temperature before use.
11. Do not leave the reagents at temperature different to the recommended longer than absolutely necessary.
12. Keep containers for samples and reagents closed while they are not being handled.
13. Avoid using samples subjected to repeated freeze-thaw cycles.
14. Handle in aseptic conditions to avoid microbial contaminations.
15. Reagents in this kit could include substances of animal and/or human origin and/or inactivated antigen (refer to "Materials provided"). Although materials of human origin have been tested and found negative for Hepatitis B Surface Antigen (HBsAg), Hepatitis C antibodies and Human Immunodeficiency Virus antibodies, all material and patient specimens should be handled and dispose as potentially infectious using safety laboratory procedures. No present method can offer complete assurance that these or other infectious agents are absent. Dispose of unused reagents and waste in accordance with all applicable regulations.
16. This product has been designed for exclusive use in conjunction with VIRCELL human IgG sorbent (Vircell ref. S001).

17. Use kit components only. Do not mix components from different kits or manufacturers. Only VIRCELL WASH BUFFER, VIRCELL TMB SUBSTRATE SOLUTION, VIRCELL STOP REAGENT and VIRCELL SERUM DILUENT are compatible with the equivalents in other VIRCELL ELISA references and lots.
18. Use only the amount of product required for the test. Do not return the excess solution into the vial.
19. During incubation times, an adequate sealing of the plates with the adhesive film included in the kit avoids the desiccation of the samples, and guarantees the repeatability of the results.
20. Before incorporating this product onto an automatic processing system, we strongly recommend the performance of a pre-evaluation assay.
21. Any serious incident that occurs in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Safety precautions.
Observe the following safety information. For further information a Material Safety Data Sheet is available.

Materials provided	Hazardous ingredients:	Hazard statements (CLP):
[2] VIRCELL SERUM DILUENT	2-Methyl-2H-isothiazol-3-one CAS-No.: 2682-20-4 EC-No.: 220-239-6	H317 – May cause an allergic skin reaction.
[3] VIRCELL IgM POSITIVE CONTROL	2-Methyl-2H-isothiazol-3-one CAS-No.: 2682-20-4 EC-No.: 220-239-6	H317 – May cause an allergic skin reaction.
[4] VIRCELL IgM CUT OFF CONTROL	2-Methyl-2H-isothiazol-3-one CAS-No.: 2682-20-4 EC-No.: 220-239-6	H317 – May cause an allergic skin reaction.
[5] VIRCELL IgM NEGATIVE CONTROL	2-Methyl-2H-isothiazol-3-one CAS-No.: 2682-20-4 EC-No.: 220-239-6	H317 – May cause an allergic skin reaction.
[6] VIRCELL IgM CONJUGATE	2-Methyl-2H-isothiazol-3-one CAS-No.: 2682-20-4 EC-No.: 220-239-6	H317 – May cause an allergic skin reaction.
[7] VIRCELL TMB SUBSTRATE SOLUTION	2-Pyrrolidinone CAS-No.: 616-45-5 EC-No.: 210-483-1	H360 – May damage fertility or the unborn child.
[8] VIRCELL STOP REAGENT	Sulphuric acid CAS-No.: 7664-93-9 EC-No.: 231-639-5	H314 – Causes severe skin burns and eye damage.
[9] VIRCELL WASH BUFFER (20x)	Reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1) CAS-No.: 55965-84-9	H317 – May cause an allergic skin reaction.
[10] VIRCELL LB REAGENT	2-Methyl-2H-isothiazol-3-one CAS-No.: 2682-20-4 EC-No.: 220-239-6	H317 – May cause an allergic skin reaction.

Hazard statements (CLP):	H314 – Causes severe skin burns and eye damage.
Hazard pictograms (CLP):	 GHS05 Corrosive
Signal word (CLP):	Danger
Precautionary statements (CLP):	P280 – Wear protective gloves/protective clothing/eye protection/face protection. P305+P351+P338 – If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P303+P361+P353 – If on skin or hair: Take off immediately all contaminated clothing. Rinse skin with water or shower. P310 – Immediately call a doctor or a poison centre. P363 – Wash contaminated clothing before reuse.
Hazard statements (CLP):	H317 – May cause an allergic skin reaction.
Hazard pictograms (CLP):	 GHS07 Health hazard/Hazardous to the ozone layer
Signal word (CLP):	Warning
Precautionary statements (CLP):	P261 – Avoid breathing dust/fume/gas/mist/vapours/spray. P272 – Contaminated work clothing should not be allowed out of the workplace. P280 – Wear protective gloves/protective clothing/eye protection/face protection. P302+P352 – If on skin: Wash with plenty of water. P321 – Specific treatment (see supplemental first aid instruction on this label). P333+P313 – If skin irritation or rash occurs: Get medical advice/attention.
Hazard statements (CLP):	H360 – May damage fertility or the unborn child.
Hazard pictograms (CLP):	 GHS08 Serious health hazard
Signal word (CLP):	Danger
Precautionary statements (CLP):	P202 – Do not handle until all safety precautions have been read and understood. P280 – Wear protective gloves/protective clothing/eye protection/face protection. P308+P313 – If exposed or concerned: Get medical advice/attention. P501 – Dispose of contents/container to an approved hazardous/special waste disposal facility in accordance with local and national regulations.

CONDITIONS FOR COLLECTION, HANDLING AND PREPARATION OF THE SPECIMEN

Blood should be collected aseptically using venipuncture techniques by qualified personnel. Use of sterile or aseptic techniques will preserve the integrity of the specimen. Serum/plasma samples are to be refrigerated (2-8°C) upon collection or frozen (-25- -15°C) if the test cannot be performed within 7 days. Samples should not be repeatedly frozen and thawed. Do not use hyperlipemic, hemolysed or contaminated samples. Samples containing particles should be clarified by centrifugation. The kit is suitable for use with serum or plasma.

PREPARATORY TREATMENT OF THE DEVICE

Only the VIRCELL WASH BUFFER must be prepared in advance. Fill 50 ml of VIRCELL WASH BUFFER (20x) up to 1 litre with distilled water. Should salt crystals form in the washing concentrate during storage, warm the solution to 37°C before diluting.

ASSAY PROCEDURE

1. Set incubator/water bath to 37±1°C.
2. Bring all reagents to room temperature before use (approximately 1 hour), without removing the plate from the bag.
3. Shake all components.
4. Remove the plate [1] from the package. Determine the numbers of wells to be employed counting in 4 wells for the controls: two for the cut off control and one each for the negative and positive control. Wells not required for the test should be returned to the pouch, which should then be sealed.
5. Add 25 µl of VIRCELL IgG sorbent (ref. S001) and 10 µl of VIRCELL LB REAGENT [10] to each of the required wells, except for the wells where controls will be dispensed. Add 5 µl of sample and then 65 µl of the serum diluent [2] to each well.

6. Prepare the control wells by adding first 100 µl of the serum diluent [2] to each well and then 5 µl of positive control [3], 5 µl of cut off control [4] (in duplicate) and 5 µl of negative control [5] to the corresponding wells.
7. If the assay is performed manually, shake the plate in a plate shaker (2 minutes) in order to achieve a homogenous mixture of the reagents. If for some reason correct shaking cannot be guaranteed, a pre-dilution of the sample in a separate tube or plate should be made, using double volume of reagents and sample. Mix homogenously with the pipette and dispense 105 µl of each diluted sample to the wells [1].
8. Cover with a sealing sheet and incubate at 37±1°C for 45 minutes.
9. Remove the seal, aspirate liquid from all wells and wash five times with 0.3 ml of washing solution [9] per well. Drain off any remaining liquid.
10. Immediately add 100 µl of conjugate solution [6] into each well.
11. Cover with a sealing sheet and incubate at 37±1°C for 30 minutes.
12. Remove the seal, aspirate liquid from all wells and wash five times with 0.3 ml of washing solution [9] per well. Drain off any remaining liquid.
13. Immediately add 100 µl of substrate solution [7] into each well.
14. Incubate at room temperature for 20 minutes protected from light.
15. Add immediately 50 µl of stopping solution [8] into all wells.
16. Read with a spectrophotometer at 450/620 nm within 1 hour of stopping.

INTERNAL QUALITY CONTROL

Each batch is subjected to internal quality control (Q.C.) testing before batch release complying with specifications stricter than validation protocol for users. Final Q.C. results for each particular lot are available.
The control material is traceable to reference sera panels internally validated.

VALIDATION PROTOCOL FOR USERS

Positive, negative and cut off controls must be run with each test run. It allows the validation of the assay and kit.
Optical densities (OD) must fall in the following ranges. Otherwise, the test is invalid and must be repeated.

Control	OD
Positive control	OD > 0.90
Cut off control	0.55 < OD < 1.50
Negative control	OD < 0.50

CALCULATIONS AND INTERPRETATION OF RESULTS

Calculate the mean OD for cut off serum.

Antibody index=(sample OD/cut off serum mean OD) x 10

Index	Interpretation
<9	Negative
9-11	Equivocal
>11	Positive

Samples with equivocal results must be retested and/or a new sample obtained for confirmation.
Samples with indexes below 9 are considered as not having antibodies of the specificity and class measured by this kit.
Samples with indexes above 11 are considered as having antibodies of the specificity and class measured by this kit.

LIMITATIONS OF USE

1. This kit is intended to be used with human serum/plasma.
2. The results of samples should be used in conjunction with clinical evaluation and other diagnostic procedures. A definitive diagnosis should be made by direct diagnostic techniques.
3. This test will not indicate the site of infection. It is not intended to replace isolation.
4. Samples collected at the beginning of infection may not have detectable levels of antibodies. In these cases it is recommended to obtain a second sample between 14 and 21 days to be tested in parallel with the original sample, in order to determine a seroconversion.
5. Results in IgG detection in neonates must be interpreted with caution, since maternal IgG is transferred passively from the mother to the foetus before birth. IgM assays are generally more useful indicators of infection in children below 6 months of age.
6. A negative result in immunosuppressed patients does not always exclude the possibility of infection.
7. Lack of a detectable antibody level does not exclude the possibility of infection.
8. Reliable results are dependent on adequate specimen collection, transport, storage and processing procedures.
9. The performance of this test has not been evaluated for use in patients without clinical signs and symptoms of infection.
10. Low levels of IgM antibodies may occasionally persist for more than 12 months post-infection.
11. For IgM testing, human IgG sorbent must be used. Otherwise, false positive results may be obtained due to presence of rheumatoid factor or false negative results may be obtained due to an excess of IgG antibodies.
12. Positive and negative predictive values are highly dependent on prevalence. False negative test results are more likely when prevalence of disease is high. False positive test results are more likely in low prevalence scenarios.
13. The performance results showed correspond to comparative studies with commercial predicate devices in a defined population sample. Small differences can be found with different populations or different predicate devices.

PERFORMANCE CHARACTERISTICS

SENSITIVITY AND SPECIFICITY

Serum/plasma samples were assayed against commercial ELISA and immunofluorescence kits.
The results were as follows:

Samples No.		87
Sensitivity (%)		82
	95% CI	64-92
Specificity (%)		94
	95% CI	85-98
PPV (%)		88
NPV (%)		91
LR+/LR-		-0.88/-0.86

CI: Confidence intervals
PPV: Positive predictive value
NPV: Negative predictive value
LR+: Positive likelihood ratio
LR-: Negative likelihood ratio

WITHIN-RUN PRECISION

3 samples were individually pipetted 10 times each one in a single assay performed by the same operator in essentially unchanged conditions.
The results were as follows:

Sample	% CV
Positive control	10.7
Cut off control	5.3
Negative control	16.9

CV: Coefficient of variation

BETWEEN-RUN PRECISION

3 samples were individually pipetted on 5 consecutive days by 2 different operators.
The results were as follows:

Sample	% CV
Positive control	9.0
Cut off control	9.3
Negative control	22.7

CV: Coefficient of variation

INTERFERENCES

Interferences – ANA/RF

19 samples known to be positive for antinuclear antibodies and rheumatoid factor were assayed. Interferences with antinuclear antibodies (2 out of 8 samples tested) were found. No interferences with rheumatoid factor (11 samples tested) were found.

Interferences – Endogenous substances

3 samples were tested with each interferent. Specifications were fulfilled in all cases. No interferences were found with haemolytic (8.5 g/L hemoglobin), icteric (6 g/L bilirubin) and hyperlipemic (4 g/L cholesterol and 2 g/L tributyrin) samples.

CROSS REACTIVITY

28 samples known to be positive for other microorganisms (Epstein-Barr virus, *Treponema pallidum* and *Borrelia burgdorferi*) were assayed.
No cross-reactivity with Epstein-Barr VCA virus (10 samples tested), *Treponema pallidum* (9 samples tested) and *Borrelia burgdorferi* (9 samples tested) was found.

SYMBOLS USED IN LABELS

IVD — *In vitro* diagnostic medical device


Use-by (expiry date)

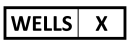
Store at x-y°C

Contains sufficient for <n> test

Batch code

Catalogue number


Consult instructions for use



<X> wells


Manufacturer

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