NovaTec Immundiagnostica GmbH

VetBlot Leishmania

LineBlot

Product Number: LEIVT2310 (16 Determinations)

ENGLISH

1. INTRODUCTION

Leishmaniasis is an infectious disease transmitted by sand flies and caused by protozoan parasites, which causes various species of Leishmania. The parasites can infect both humans and canines, and the resulting condition is known as visceral leishmaniasis.
The disease is particularly common in Mediterranean basin (e.g., Italy, Spain and Portugal), the Balkans, central and southwest Asia, north and northwest China, north and sub-Saharan Africa, and parts of Central and South America. The domestic dog seems to be the main reservoir for human visceral leishmaniasis, rendering disease control that much more vital.
In dogs clinical manifestations include chronic wasting, epistaxis, diarrhea, conjunctivitis, ocular signs (anterior uveitis, retinitis), severe muscle atrophy, swollen limbs and joints, lameness, lymphadenopathy, polyarthritis, and protein-losing nephropathy, which may lead to renal failure. Assessment of renal function in all infected dogs is critically important.
Infection may be identified by:

- Microscopy
- Serology: IFA, ELISA, LineBlot

2. INTENDED USE

The VetBlot Leishmania LineBlot is intended for the qualitative determination of IgG antibodies against Leishmania in canine serum.

3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of specific antibodies against Leishmania is based on the immunoblot technique in a LineBlot format. 1 native and 3 recombinant antigens of Leishmania are printed onto a Nitrocellulose membrane together with a control for sample loading and for conjugate function.
The stripes of these membranes are incubated with diluted veterinary samples. Leishmania specific antibodies, if present, will bind to their target antigens. After washing the strips to remove all unbound sample material, a horseradish peroxidase (HRP) labelled Protein A/G conjugate is added. This binds to the Antigen-Antibody-complexes on the membrane. At the end of this second incubation, unbound conjugate is removed by washing and aspiration. The bound conjugate is visualized by the addition of a chromogenic substrate (Tetramethylbenzidine; TMB). Strips are dried and analysed. Using the template supplied with the kit, the position of the stained bands can be correlated with the Leishmania antigen bands and the controls.

4. MATERIALS

4.1. Reagents supplied

- **Test Strips:** 1 envelope containing 16 consecutively numbered LineBlot strips coated with 1 native and 3 recombinant antigens of Leishmania and a Sample Load Control (SLC) and a Conjugate Control (CC).
- **LB Sample Dilution Buffer:** 1 bottle containing 20 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; white vial with white cap, ≤ 0.0015 % (v/v) CMIT/MIT (3:1).
- **Washing Buffer (20x conc.):** 1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2 for washing the Test Strips; white cap.
- **Conjugate:** 1 bottle containing 20 mL of peroxidase Protein A/G, coloured yellow, ready to use; black vial with white cap ≤ 0.02 % (v/v) MIT.
- **TMB Substrate Solution Membrane:** 1 bottle containing 20 mL 3,3',5,5'-tetramethylbenzidine (TMB) (1.2 mM) ready to use; blue cap. NMP (< 0,3 %), H_2O_2 (< 3 mM).

4.2. Materials supplied

- 1 test protocol
- 1 result sheet with a template strip
- 2 incubation trays **- <u>for single use only!</u>**

4.3. Materials and Equipment needed

- Scissors
- Deionised or (freshly) distilled water
- Shaking platform (horizontal fixed-angle rocking shaker)
- Micropipettes and one-way tips (10 and 1000 µL)
- Plastic tweezers for handling the Test Strips
- Vacuum apparatus
- Filter paper

5. STABILITY AND STORAGE

Store the kit at 2...8 °C. The opened reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

6. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20…25 °C) and mix them before starting the test run!

6.1. Test Strips

The ready to use LineBlot strips are coated with 1 native and 3 recombinant antigens of Leishmania and the two controls, a Sample Load Control (SLC) and a Conjugate Control (CC). Immediately after removal of needed strips, the remaining strips should be stored in the resalable envelope together with a desiccant at 2...8 °C.

6.2. Washing Buffer (20x conc.)

Dilute Washing Buffer 1 + 19; e. g. 10 mL Washing Buffer + 190 mL distilled water. The diluted buffer is stable for 5 days at room temperature (20…25 °C). In case crystals appear in the concentrate, warm up the solution to 37 °C e.g. in a water bath. Mix well before dilution.

6.3. TMB Substrate Solution Membrane

The reagent is ready to use and has to be stored at 2...8 °C, away from the light. The solution should be colourless to pale yellow. If the substrate turns into blue, it may have become contaminated and should be thrown away.

7. SAMPLE COLLECTION AND PREPARATION

Use canine serum samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise, they should be aliquoted and stored deep-frozen (-70…-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
Heat inactivation of samples is not recommended.

8. ASSAY PROCEDURE

Please read the test protocol carefully **before** performing the assay. Result reliability depends on strict adherence to the test protocol as described. The following test procedure is only validated for manual procedure. Prior to commencing the assay, the assignment for all specimens to antigen strips should be carefully established on the result sheet supplied with the kit.
Perform all assay steps in the correct order and without any appreciable delays between the steps.
A clean, disposable tip should be used for dispensing each sample.
Protect strips from light during incubation by covering the incubation tray.

1. Using tweezers and scissors, carefully cut off the number of required Test Strips in between the 2 labels from the map and place them in the channels of the incubation tray. One Strip per channel and sample. The **numbered side of the strip must face up**; antigens are bound to this side of the membrane.
2. Pipet **1 mL** of **LB Sample Dilution Buffer** to each strip. Check that all Test Strips are **completely immersed** and, if needed, gently shake the tray or push delicately the strips into the solution with a clean pipette tip.
3. Add **10 µL** of **sample** to the LB Sample Dilution Buffer and strip in the channel. Avoid direct dispensation of sample on top of the strip.
4. Incubate the strips at room temperature for **60 min** on a shaking platform (approx. 10 cpm).
5. Wash procedure
   - Carefully aspirate the contents of each channel, add **1 mL** of **Washing Buffer**.
   - Place the incubation tray on the shaking platform for **5 min** at room temperature.
   - Aspirate the contents of each channel and repeat the wash procedure two more times.

   Note: Washing is critical! Insufficient washing results in poor precision and falsely emerging results.
6. Add **1 mL** of **Protein A/G Conjugate** to the appropriate channels and incubate at room temperature for **30 min** on the shaking platform (approx. 10 cpm).
7. Repeat step 5.
8. Add **1 mL** of chromogenic **TMB Substrate Solution Membrane** to the appropriate channels and incubate for **15 min** at room temperature on the shaking platform (approx. 5 - 10 rpm). Do not stop the reaction earlier, even when the strips become blue. This background colouring will fade during the drying process.
9. Stop the reaction by aspirating the contents of each channel and wash 3 times with deionised water by completely filling the channels and decanting.
10. Remove the strips from the incubation tray and place them with the number face up on filter paper to dry (at least 30 min at room temperature). Do not evaluate strips when not completely dried!

IMPORTANT: to prevent fading, the strips should be protected from exposure to light.

9. RESULTS

9.1. Run Validation Criteria

In order for an assay to be considered valid, the following criterium must be met:

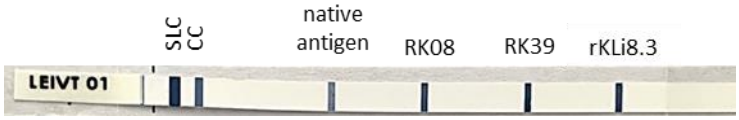
- **Sample Load Control:** intensively coloured band
- **Conjugate Control:** intensively coloured band

If these criteria are not met, the test is not valid and must be repeated.

9.2. Interpretation of Results

Once dried, attach the strips to the result sheet using clear tape.

- The LineBlot utilizes a template displaying the positions of all bands on the Test Strip. Identification of the reactive bands is based upon comparison with the bands on the template. The Sample Load Control (SLC) and the Conjugate Control (CC) at the top of the strip must be aligned with the reference line near the top of the template strip. Native signals could be cross-reactions or an indication for a vaccination, so the test is just considered positive, if all bands reacted. If a sample display only a native antigen signal further diagnostics or anamnesis must be carried out.



10. SPECIFIC PERFORMANCE CHARACTERISTICS

The performance data have been established with canine samples.

For further information about the specific performance characteristics please contact NovaTec Immundiagnostica GmbH.

10.1. Precision

Within-Run Precision

Sample	n	Result	Lot
#1	20	20x pos	#A
#2	20	20x pos	#B
#3	20	20x pos	#C

Between-Run Precision

Sample	n	Result	Lot
#1	10	10x pos	#A
#1	10	10x pos	#B
#1	10	10x pos	#C

10.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte.

Diagnostic Specificity canine: 100 % (95% confidence interval: 95.65 – 100 %)

10.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte.

Diagnostic Sensitivity canine: 95.95 % (95% confidence interval: 88.61 – 99.16 %)

10.4. Cross Reactivity

16 potentially cross-reactive samples were tested to evaluate the cross reactivity of the assay.

Cross-Reactivity

Disease Type	Total Specimens	Positive Result
Chagas (*T.cruzi*)	4	0/4
Malaria	1	0/1
Babesia	4	0/4
Ehrlichia and Babesia	1	0/1
Ehrlichia	1	0/1
Anaplasma	5	0/5
Total	16	0/16

Cross reactions cannot be excluded. A positive signal of the native antigen in combination with the absence of signals from the recombinant antigens is an indication of a cross reaction or a vaccination of the dog.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the reaction intensities.

Some samples may cause a colouring (continuous or patterned) over the complete membran strip. This is due to varying factors of the sample which should be analysed with a different serological method. Interpretation of this strips is restricted. White bands on dark background can be rated negative.

12. PRECAUTIONS AND WARNINGS

- All materials should be regarded and handled as potentially infectious.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- The use of a horizontal fixed-angle rocking platform is strongly recommended. Use of an orbital shaker may reduce efficiency of binding as well as the washing performance.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate vial for microbial contamination prior to further use.
- Always use tweezers to handle Test Strips. Grab strips only at the label. Avoid contact to reaction area.
- During incubations and washing, the Test Strips must remain completely covered with fluid and the numbered side of the strips must face up.
- To avoid cross-contamination and falsely elevated results pipette samples and dispense conjugate without splashing accurately to the bottom of channels.
- Do not reuse incubation trays.
- The LineBlot is only designed for qualified personnel who are familiar with good laboratory practice.

12.1. Safety note for reagents containing hazardous substances

Reagents may contain CMIT/MIT (3:1) or MIT (refer to 4.1)
Therefore, the following hazard and precautionary statements apply.

Warning

H317	May cause an allergic skin reaction.
H315	Causes skin irritation
H319	Causes serious eye irritation
P261	Avoid breathing spray
P280	Wear protective gloves/protective clothing/eye and face protection.
P302+P352	IF ON SKIN: Wash with plenty of soap and water.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
P337+P313	If eye irritation persists: Get medical advice/attention
P362+P364	Take off contaminated and wash it before reuse.

Further information can be found in the safety data sheet.

12.2. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

13. ORDERING INFORMATION

Prod. No.:	LEIVT2310	VetBlot Leishmania LineBlot (16 Determinations)

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ABBREVIATIONS / ABKÜRZUNGEN / ABRÉVIATIONS / ABBREVIAZIONI / ABREVIACIÓNES / ABREVIATURAS

CMIT	5-Chloro-2-methyl-2H-isothiazol-3-one
MIT	2-Methyl-2H-isothiazol-3-one
NMP	1-Methyl-2-pyrrolidone
H_2O_2	Hydrogen peroxide

SYMBOLS KEY / SYMBOLSCHLÜSSEL / EXPLICATION DES SYMBOLES / LEGENDA / SÍMBOLOS / TABELA DE SÍMBOLOS

	Manufactured by / Hergestellt von / Fabriqué par / Prodotto da / Fabricado por / Fabricado por
LOT	Lot Number / Chargenbezeichnung / Numéro de lot / Lotto / Número de lote / Número de lote
	Expiration Date / Verfallsdatum / Date de péremption / Scadenza / Fecha de caducidad / Data de Validade
	Storage Temperature / Lagertemperatur / Température de conservation / Temperatura di conservazione / Temperatura de almacenamiento / Temperatura de Armazenamento
REF	Catalogue Number / Katalog Nummer / Référence du catalogue / Numero di codice / Número de Catálogo / Número de Catálogo
	Consult Instructions for Use / Arbeitsanleitung beachten / Consulter la notice d'utilisation / Consultare le istruzioni per l'uso / Consulte las Instrucciones de Uso / Consultar as Instruções de Utilização
CONJL	Conjugate / Konjugat / Conjugué / Coniugato / Conjugado / Conjugado
STRIP	Test Strip / Teststreifen / Bandelettes réactives / strisce reattive / tiras de ensayo / tiras de teste
DIL BG	IgG-LB Sample Dilution Buffer / IgG-LB Probenverdünnungspuffer / LB Tampon diluant pour échantillon IgG / LB Tampone diluente per i campioni IgG / LB Tampón diluyente para muestras IgG / LB Tampão diluente para amostras IgG
SUB TMBM	TMB Substrate solution membrane / TMB-Substratlösung Membran / Solution de Substrat TMB membrane / soluzione substrato TMB membrana / solción substrato TMB membrana / Solução substrato TMB membrana
WASH BUF 20x	Washing Buffer 20x concentrated / Waschpuffer 20x konzentriert / Tampon de lavage concentré 20 x / Tampone di lavaggio concentrazione x20 / Tampón de lavado concentrado x20 / Tampão de lavagem concentrada 20x
	Contains sufficient for "n" tests / Ausreichend für "n" Tests / Contenu suffisant pour "n" tests / Contenuto sufficiente per "n" saggi / Contenido suficiente para "n" tests / Conteúdo suficiente para "n" testes
	Single use only / nicht zur Wiederverwendung/ uso unitario

SUMMARY OF TEST PROCEDURE / KURZANLEITUNG TESTDURCHFÜHRUNG / RÉSUMÉ DE LA PROCEDURE DE TEST / SCHEMA DELLA PROCEDURA / RESUMEN DE LA TÉCNICA / RESUMO DO PROCEDIMENTO DE TESTE

SCHEME OF THE ASSAY

VetBlot Leishmania LineBlot

Test Preparation

Prepare reagents and samples as described. Establish the identification plan for all specimens on the result sheet supplied in the kit. Select the required number of test strips and separately place them into the incubation chambers.

Assay Procedure

pipette **1 mL LB Sample Dilution Buffer** to each strip
⇓
add **10 µL** of **sample** per test and strip
⇓
incubate for **60 min** at room temperature while shaking gently
⇓
wash **3 times** for **5 min** with **1 mL Washing Buffer** while shaking
⇓
add **1 mL Conjugate**
⇓
incubate for **30 min** at room temperature while shaking gently
⇓
wash **3 times** for **5 min** with **1 mL Washing Buffer** while shaking
⇓
add **1 mL TMB Substrate Solution**
⇓
incubate for **15 min** at room temperature while shaking gently
⇓
wash 3 times with deionised water
dry and read

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