VetBlot Leishmania
LineBlot
(LEIVT2310)

Performance Characteristics

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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 Performance Characteristics

4.1 Reproducibility (Precision)

Material

VetBlot Leishmania Lineblot		Lot:	T-20220304
Production date:	2022-03	Expiry date:	2023-03-31
VetBlot Leishmania Lineblot		Lot:	T-20220309
Production date:	2022-03	Expiry date:	2023-03-31
VetBlot Leishmania Lineblot		Lot:	T-20220517
Production date:	2022-05	Expiry date:	2023-05-31

Leishmania positive samples

Test Description

The reproducibility of the VetBlot Leishmania LineBlot was determined by comparing ideally 10-20 replicates of a sample in one assay (within-run) and
by comparing one sample assayed ideally in 10 different runs (between-run). The measurements for the within-run precision and the between-run precision are done with three lots.

Acceptance criterion:

Band intensities of controls and antigen signal have to be constant when analyzed visually.
The qualitative results have to be coincident.

Results

Table 1: Within-Run Precision

Sample	n	result	Lot
1	20	20x pos	T-20220517
2	20	20x pos	T-20220309
3	20	20x pos	T-20220304

Table 2: Between-Run Precision

Sample	n	result	Lot
1	10	10x pos	T-20220517
1	10	10x pos	T-20220309
1	10	10x pos	T-20220304

Conclusion

No relevant differences in band intensities could be detected.
The acceptance criterion is met for both studies.

4.2 Analytical Specificity

4.2.1 Cross-Reactivity

Material

VetBlot Leishmania LineBlot Lot: T-20229309
Production date: 2022-03 Expiry date: 2023-03-31

16 potentially cross-reactive samples

A panel of 16 specimens with confirmed diseases other than Leishmania disease was tested to establish the analytical specificity of the VetBlot Leishmania LineBlot.

The specimens are positive for *Trypanosomas cruzi*, or other diseases which were transmitted by secondary vectors like ticks.

Results

Table 3: Cross-Reactivity (Summary)

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

Additional results:

The test is considered positive if all bands reacted, so these samples were also evaluated as negative. Native signals are probably cross-reactions, or an indication for a vaccination.

3 samples caused a signal at the native antigen (two Chagas positive samples and one Babesia positive sample). But they were considered a negative as well.

Conclusion

None of the tested specimens with confirmed infections other than Leishmania gave a positive result in the VetBlot Leishmania LineBlot.

The VetBlot Leishmania LineBlot is suitable for checking in ELISA unclear specimens to cross-reactions or vaccination.

4.3 Diagnostic Sensitivity and Specificity

Introduction

The purpose of this study was to determine the efficiency of the assay to discriminate between positive and negative clinical samples.

Acceptance Criteria:	Diagnostic Sensitivity:	> 85 %
	Diagnostic Specificity:	> 85 %
	Agreement:	> 90 %

To evaluate the diagnostic performance of the VetBlot Leishmania LineBlot, internal studies were conducted by NovaTec in comparison to an immunoassay already established on the market. In addition, an external study was conducted.

Part I (In-house Study)

The evaluation of the diagnostic performance of the VetBlot Leishmania LineBlot was performed in comparison to well defined samples of different dog sera which were previously tested with the VetLine Leishmania ELISA (LEIVT0310).

This internal study was conducted at NovaTec.

Material

VetBlot Leishmania LineBlot		Lot:	T-20211221-01
Production date:	2021-12	Expiry date:	2022-12-31
VetBlot Leishmania LineBlot		Lot:	T-20220304
Production date:	2022-03	Expiry date:	2023-03-31
VetBlot Leishmania LineBlot		Lot:	T-20220309
Production date:	2022-03	Expiry date:	2023-03-31

50 Leishmania positive and 54 negative samples

Results

Table 4: Diagnostic Sensitivity and Specificity (Part I)

		Demand		
		positive	negative	**Σ**
VetBlot Leishmania LineBlot	positive	50	0	**50**
	negative	0	54	**54**
	Σ	**50**	**54**	**104**

Diagnostic Sensitivity: 100 % (50/50) (95%-width of Confidence Interval: 92.89 – 100 %)
Diagnostic Specificity: 100 % (54/54) (95%-width of Confidence Interval: 93.40 – 100 %)
Agreement: 100 % (104/104)

Part II (External Evaluation)

The evaluation of the diagnostic performance of the VetBlot Leishmania LineBlot was performed in comparison to samples predetermined by a cooperation partner. No further information of the predetermination is available. The samples were collected of healthy and symptomatic dogs in Brazil.

Material

VetBlot Leishmania LineBlot — Lot: T-20220304
Production date: 2022-03 — Expiry date: 2023-03-31

53 defined samples (24 positive, 29 negative samples).

Results

Table 5: Diagnostic Sensitivity and Specificity (Part II)

		Demand		
		positive	negative	**Σ**
VetBlot Leishmania LineBlot	positive	21	0	**21**
	negative	3	29	**32**
	Σ	**24**	**29**	**53**

Diagnostic Sensitivity: 87.50 % (21/24) (95%-width of Confidence Interval: 67.64 – 97.34 %)
Diagnostic Specificity: 100 % (29/29) (95%-width of Confidence Interval: 88.06 – 100 %)
Agreement: 94.34 % (50/53)

Diagnostic Sensitivity and Specificity (Parts I-II)

Table 6: Summary parts I-II

		Demand		
		positive	negative	**Σ**
VetBlot Leishmania LineBlot	positive	71	0	**71**
	negative	3	83	**86**
	Σ	**74**	**83**	**157**

Diagnostic Sensitivity: 95.95 % (95%-width of Confidence Interval: 88.61 – 99.16 %)
Diagnostic Specificity: 100 % (95%-width of Confidence Interval: 95.65 – 100 %)
Agreement: 98.09 %

Conclusion

The evaluation of the diagnostic performance of the VetBlot Leishmania LineBlot was conducted at NovaTec and by an external study in Brazil.

The diagnostic sensitivity was 95.95 % and the diagnostic specificity was 100 % (agreement: 98.10 %)

Therefore, the acceptance criteria are met.