Validation of a new rapid immunochromatographic test for *Leishmania infantum* diagnosis of in dogs

Sponsor: Bigstart Italia – S.r.l.
Study's pattern: Diagnostic Test Validation
Animals: Dogs
Objective: To evaluate the sensitivity and specificity of cLSH-Ab in serum samples of dogs naturally infected by *Leishmania infantum*.

Testing equipment: VET IMMUNOFLUORESCENCE QUANTITATIVE ANALYZER VETIVD™ MIG 300 by **Imhotep (DongGuan) Medical Industry Investment Co, LTD**

Product: cLSH-Ab, by **Imhotep (DongGuan) Medical Industry Investment Co, LTD**

Countries: Italy
Specific protocol for (country): Italy

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TABLE OF CONTENTS

1 STUDY TITLE *3*
2 STUDY SITES *4*
3 OBJECTIVE OF THE STUDY *4*
4 JUSTIFICATION *5*
5 Type of study and study DESIGN *5*
6 ANIMAL SELECTION AND IDENTIFICATION *6*
6.1 Inclusion Criteria: **6**
6.2 Exclusion Criteria: **6**
7 STUDY procedures *7*
7.1 Clinical examination and sample collection **7**
8 Data collection, management, STATISTICAL ANALISYS AND COMPUTING *8*
8.1 Data management **8**
8.2 Handling of records (recording, reporting, storage of study data) **8**
8.3 Statistical analysis **8**
9 RESULTS *9*
9.1 Study Population **9**
12. REFERENCES *15*

1 STUDY TITLE

Validation of a new rapid immunochromatographic tests for the diagnosis of *Leishmania infantum* in dogs

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2 STUDY SITES

The study was conducted in Sicily, southern Italy. Veterinarians, expert in general veterinary medicine and parasitology, act as Investigators and performed clinical examination of dogs and all the diagnostic procedures of the study.

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3 OBJECTIVE OF THE STUDY

The objective of this study was to comparatively evaluate the performance of cLSH-Ab and a commercial rapid test, using the device VET IMMUNOFLUORESCENCE QUANTITATIVE ANALYZER VETIVD™ MIG 300v by **Imhotep (DongGuan) Medical Industry Investment Co, LTD**, by accessing the sensitivity and specificity these two tests, in serum samples of dogs naturally infected by *L. infantum*. The gold standard was a commercial ELISA (ID Screen® Leishmaniasis Indirect, IDVet). The study was conducted in accordance with Veterinary Good Clinical Practice (GCPV) on owned and shelter animals.

4 JUSTIFICATION

Leishmaniosis is a vector borne disease caused, in Mediterranean countries, by protozoan *Leishmania infantum*. Infected dogs remain the main reservoir host of the disease. Clinical diagnosis of canine leishmaniasis (CanL) is still a challenge for the clinician due to great variety of clinical signs and high percentage of asymptomatic dogs. Thus, the use of an accurate diagnostic test for CanL is necessary to achieve a definitive diagnosis.

The gold standard for the diagnosis of CanL is the detection of anti-*Leishmania* antibodies using serological methods (i.e., immunofluorescent assay, IFA, and enzyme linked unosorbent assay, ELISA), since parasitological examination is an invasive and a laborious technique, that usually presents lower sensitivity. Commercial rapid detection assays for anti-*Leishmania* antibodies in dogs, shown lower sensitivity and specificity when compared to conventional serological methods, although these commercial tests are very attractive as screening tests due to their simple and rapid use in clinical practice. Several rapid detections assays have been launched in the market with different performances in terms of sensitivity and specificity.

Recently a new product, the VET IMMUNOFLUORESCENCE QUANTITATIVE ANALYZER VETIVD™ MIG 300 with the rapid quantitative test: cLSH-Ab, by **Imhotep (DongGuan) Medical Industry Investment Co, LTD**, has been proposed in the market. The immunofluorescence chromatography technology of this test is based on the use of fluorescent microspheres wrapped with Europium (Eu) lanthanide as a marker. Europium lanthanide has two special fluorescence properties of time resolution and wavelength resolution, which can minimize the interference of background fluorescence; the fluorescence intensity is much more marked than ordinary fluorescence, thus significantly improving the sensitivity and specificity of the test.

Accordingly, the aim of this study was to comparatively evaluate the performance of the VET IMMUNOFLUORESCENCE QUANTITATIVE ANALYZER VETIVD™ MIG 300 with the cLSH-Ab and a commercial rapid test (*Leishmania* IgG/IgM Rapid Test Cassette, Citest Diagnostic imminc., Vancuver Canada), by accessing the sensitivity and specificity of these two tests, in serum samples of dogs naturally infected by *L. infantum*.

5 TYPE OF STUDY AND STUDY DESIGN

The study was conducted in Sicily, southern Italy, an hyperendemic area for CanL.

The number of dogs included in the study was at least 50 dogs, of which 25 truly seropositive to *L. infatum* at different ELISA optical density, and 25 truly seronegative. Seropositive and seronegative samples were selected and assessed by a commercial indirect ELISA test for the detection of anti-*Leishmania infantum* antibodies (ID Screen® Leishmaniasis Indirect Test, VET- Innovate ID Diagnostics, France). The test was performed following manufacturer's instructions by an operator blind to the status of the testing sera. The same sera were tested using the commercially available test *Leishmania* IgG/IgM Rapid Test Cassette (Citest Diagnostic imminc., Vancuver Canada) and using VET IMMUNOFLUORESCENCE QUANTITATIVE ANALYZER VETIVD™ MIG 300 with the cLSH-Ab.

6 ANIMAL SELECTION AND IDENTIFICATION

Animals included in the study were privately owned adult dogs, aged twelve months or over, belonging to different breeds, with or whitout clinical symptoms and/or clinical pathological findings referable to CanL. Only the animals that fully satisfied the following criteria were included in the study:

6.1 Inclusion Criteria:

1. Dogs older than 12 months;
2. Dogs positive at ELISA test for *Leishmania infantum*
3. Dogs negative at ELISA for *Leishmania infantum*

6.2 Exclusion Criteria:

1. Dogs vaccinated against *Leishmania infantum;*
2. Dogs elder than 12 months.

7 STUDY PROCEDURES

7.1 Clinical examination and sample collection

Each enrolled dog underwent a complete physical examination using a physician-based scoring system considering sixteen clinical signs. A blood sample was collected from a peripheral vein (jugular or cephalic) using a standard technique. An aliquot of about 3 mL of blood was collected in tube with cloth activator, processed by centrifugation (1.678 *g* x 10 min). Aliquots of serum were used to perform to perform ELISA, *Leishmania* IgG/IgM Rapid Test Cassette and VET IMMUNOFLUORESCENCE QUANTITATIVE ANALYZER VETIVD™ MIG 300 with **cLSH-Ab** test (Fig.1).

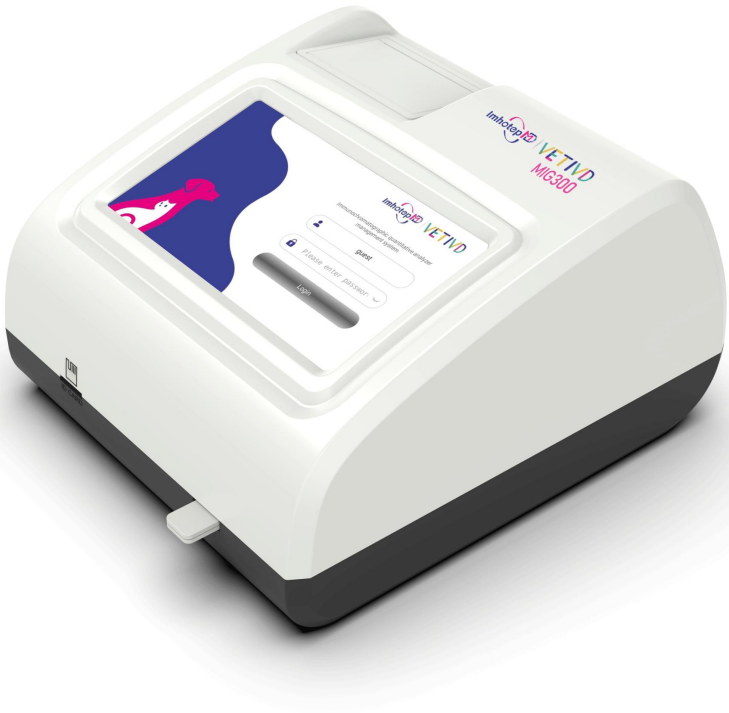

Figure 2. VET IMMUNOFLUORESCENCE QUANTITATIVE ANALYZER VETIVD™ MIG 300 of **Imhotep (DongGuan) Medical Industry Investment Co, LTD.**

8 DATA COLLECTION, MANAGEMENT, STATISTICAL ANALISYS AND COMPUTING

8.1 Data management

All study data were collected in the Data Capture Forms. Raw data were entered into an Excel® (Microsoft Office 365, Microsoft inc.) database.

8.2 Handling of records (recording, reporting, storage of study data)

All the study observation records were collected and stored into the investigators file.

8.3 Statistical analysis

The percentage of Agreement between test (ELISA Vs *Leishmania* IgG/IgM Rapid Test Cassette and ELISA Vs **cLSH-Ab** test) was calculated for ELISA positive, dubt and negative sera samples; mover the Cohen's kappa coefficient, a statistic that is used to measure inter-rater reliability (and also intra-rater reliability) for qualitative (categorical) items among the tests was calculated.

All the statistical analysis were performed using statistical analysis package (GraphPad Software, San Diego California USA, www.graphpad.com).

9 . RESULTS

9.1 Study Population

A total of 100 dogs underwent to the physical examination and 61 dogs were enrolled in the study. A total of 34 dogs were ELISA positive to *L. infantum*, 5 were ELISA dubt and 22 resulted negative to ELISA test.

Table 1 reports the results of the rapid test (*Leishmania* IgG/IgM Rapid Test Cassette) and the cLSH-Ab tests conducted on ELISA positive samples, the cLSH-Ab tests value expressed as Yu/ml the concordance between the ELISA and the two test and additional comments.

As highlighted in table 1, the concordance is identical in both tests under investigation and is 97.06%.

ID		Name	ELISA	RAPID TEST	**cLSH-Ab** tests	**cLSH-Ab** tests Value Yu/ml	Concordance	Comments
1	P1	Ade	POS	1	1	399.15	1	
2	P2	Ciccio	POS	1	1	>640	1	
3	P3	55bol	POS	1	1	346.81	1	
4	P4	59bol	POS	1	1	>640	1	haemolitic
5	P5	60b	POS	1	1	>640	1	haemolitic
6	P6	62b	POS	1	1	>640	1	
7	P7	67b	POS	1	1	>640	1	
8	P8	70b	POS	1	1	9.43	1	
9	P9	10H	POS	1	1	159.03	1	
10	P10	B12H	POS	1	1	242.66	1	
11	P11	B27H	POS	1	1	49.97	1	
12	P12	B28H	POS	1	1	120.39	1	
13	P13	B32H	POS	1	1	521.13	1	
14	P14	B34H	POS	1	1	256.6	1	
15	P15	B36H	POS	1	1	>640	1	
16	P16	B28HD	POS	1	1	>640	1	
17	P17	B50	POS	1	1	>640	1	
18	P18	B51	POS	1	1	11.58	1	
19	P19	ZEUS	POS	1	1	>640	1	
20	P20	TYSON	POS	1	1	>640	1	
21	P21	SENIA 1	POS	1	1	43.06	1	
22	P22	SENIA 2	POS	1	1	15.01	1	
23	P23	PP6	POS	1	1	>640	1	

24	P24	PP09	POS	1	1	>640	1	
25	P25	LI27	POS	1	1	>640	1	
26	P26	LI18	POS	1	1	>640	1	
27	P27	JJ	POS	1	1	>640	1	
28	P28	BOH	POS	1	1	>640	1	
29	P29	LI51	POS	1	1	12.58	1	
30	P30	CHUCK	POS	1	1	>640	1	
31	P31	RAMBO	POS	1	1	>640	1	
32	P32	RINGO	POS	1	1	>640	1	
33	P33	BIONDO	POS	1	1	538.45	1	
34	P34	LILLI	POS	0	0	<1.5	0	lipemic
				97.06%	97.06%			

Table 1.

In Table 2 are reported the results of the rapid test (*Leishmania* IgG/IgM Rapid Test Cassette) and the cLSH-Ab tests conducted on ELISA doubt samples, the cLSH-Ab test value expressed as Yu/ml the concordance between the ELISA and the two test and additional comments.

As highlighted in Table 2 both the tests under investigation the conceordance is identical and is 40%. However, it is important to remark that the *Leishmania* IgG/IgM Rapid Test Cassette does not consioder doubut results.

	Name	ELISA	RAPID TEST	**cLSH-Ab** tests	**cLSH-Ab** tests Value Yu/ml	Concordance	Comments
35	D1	LI11	DUB	0	0 4.56	0	
36	D2	LI30	DUB	0	0 2.03	0	
37	D3	LI32	DUB	0	0 4.35	0	
38	D4	GALA	DUB	1	1 90.35	0	lipemic
39	D5	B24H	DUB	1	1 51.89	0	

Table 2.

In Table 3 is reported the results of the rapid test (Leishmania IgG/IgM Rapid Test Cassette) and the cLSH-Ab tests conducted on ELISA doubt samples, the cLSH-Ab test value expressed as Yu/ml the concordance between the ELISA and the two test and additional comments.

As highlighted in table 3 both the tests under investigation showed a good concordance with the ELISA but those of the cLSH-Ab test is higher being 81.81% and 90.90% respecivelly.

	Name	ELISA	RAPID	cLSH-	cLSH-Ab	Concordance	Comments

				TEST	Ab tests	tests Value Yu/ml		
40	N1	Darwin	NEG	0	0	<6	1	
41	N2	57bol	NEG	0	0	<6	1	
42	N3	13b	NEG	0	0	<6	1	haemolitic
43	N4	Costa	NEG	0	0	<6	1	
44	N5	Costa	NEG	0	0	<6	1	
45	N6	Marley	NEG	0	0	<6	1	
46	N7	B39	NEG	0	0	<6	1	
47	N8	B42	NEG	0	0	<6	1	
48	N9	B75	NEG	0	0	<6	1	
49	N10	B76	NEG	0	0	<6	1	
50	N11	B78	NEG	0	0	<6	1	
51	N12	B86	NEG	1	0	<6	0	
52	N13	B88	NEG	1	0	<6	0	
53	N14	105B	NEG	1	0	<6	0	
54	N15	TAVOR	NEG	0	0	<6	1	
55	N16	LI28	NEG	0	0	4.0	1	
56	N17	LI50	NEG	0	0	5.15	1	
57	N18	LISA	NEG	0	0	3.34	1	
58	N19	SWAN	NEG	0	0	8.77	0	haemolitic
59	N20	MENA	NEG	0	0	<1.5	1	
60	N21	DAI	NEG	0	0	<1.5	1	
61	N22	PP13	NEG	1	1	16.19	0	haemolitic
				81.81%	90.90%			

Table 3.

In table 4 are reported all the results regardless the ELISA status, the rapid test, the cLSH-Ab tests, the value of the cLSH-Ab test expressed as Yu/ml the concordance between the ELISA and the two test and additional comments; moreover are reported the percentage of agreement and the Cohen's kappa results.

As highlithed in table 4 the *Leishmania* IgG/IgM Rapid Test Cassette showed a substancial agreement (0.743) with the ELISA test while the cLSH-Ab tests with the VET IMMUNOFLUORESCENCE QUANTITATIVE ANALYZER MIG 300 showed a almost perfect agreement with the ELISA test. The value range usefull for the intretation of Cohen's kappa results is reported in figure 2.

		Name	ELISA	RAPID TEST	cLSH-Ab tests	cLSH-Ab tests Value Yu/ml	Concordance	Comments
1	P1	Ade	POS	1	1	399.15	1	
2	P2	Ciccio	POS	1	1	>640	1	
3	P3	55bol	POS	1	1	346.81	1	
4	P4	59bol	POS	1	1	>640	1	haemolitic
5	P5	60b	POS	1	1	>640	1	haemolitic
6	P6	62b	POS	1	1	>640	1	
7	P7	67b	POS	1	1	>640	1	
8	P8	70b	POS	1	1	9.43	1	
9	P9	10H	POS	1	1	159.03	1	
10	P10	B12H	POS	1	1	242.66	1	
11	P11	B27H	POS	1	1	49.97	1	
12	P12	B28H	POS	1	1	120.39	1	
13	P13	B32H	POS	1	1	521.13	1	
14	P14	B34H	POS	1	1	256.6	1	
15	P15	B36H	POS	1	1	>640	1	
16	P16	B28HD	POS	1	1	>640	1	
17	P17	B50	POS	1	1	>640	1	
18	P18	B51	POS	1	1	11.58	1	
19	P19	ZEUS	POS	1	1	>640	1	
20	P20	TYSON	POS	1	1	>640	1	
21	P21	SENIA 1	POS	1	1	43.06	1	
22	P22	SENIA 2	POS	1	1	15.01	1	
23	P23	PP6	POS	1	1	>640	1	
24	P24	PP09	POS	1	1	>640	1	
25	P25	LI27	POS	1	1	>640	1	
26	P26	LI18	POS	1	1	>640	1	
27	P27	JJ	POS	1	1	>640	1	
28	P28	BOH	POS	1	1	>640	1	
29	P29	LI51	POS	1	1	12.58	1	
30	P30	CHUCK	POS	1	1	>640	1	
31	P31	RAMBO	POS	1	1	>640	1	
32	P32	RINGO	POS	1	1	>640	1	
33	P33	BIONDO	POS	1	1	538.45	1	

34	P34	LILLI	POS	0	0	<1.5	0	LIPEMIC
35	D1	LI11	DUB	0	0	4.56	0	
36	D2	LI30	DUB	0	0	2.03	0	
37	D3	LI32	DUB	0	0	4.35	0	
38	D4	GALA	DUB	1	1	90.35	0	LIPEMIC
39	D5	B24H	DUB	1	1	51.89	0	
40	N1	Darwin	NEG	0	0	<6	1	
41	N2	57bol	NEG	0	0	<6	1	
42	N3	13b	NEG	0	0	<6	1	haemolitic
43	N4	Costa	NEG	0	0	<6	1	
44	N5	Costa	NEG	0	0	<6	1	
45	N6	Marley	NEG	0	0	<6	1	
46	N7	B39	NEG	0	0	<6	1	
47	N8	B42	NEG	0	0	<6	1	
48	N9	B75	NEG	0	0	<6	1	
49	N10	B76	NEG	0	0	<6	1	
50	N11	B78	NEG	0	0	<6	1	
51	N12	B86	NEG	1	0	<6	0	
52	N13	B88	NEG	1	0	<6	0	
53	N14	105B	NEG	1	0	<6	0	
54	N15	TAVOR	NEG	0	0	<6	1	
55	N16	LI28	NEG	0	0	4.0	1	
56	N17	LI50	NEG	0	0	5.15	1	
57	N18	LISA	NEG	0	0	3.34	1	
58	N19	SWAN	NEG	0	1	8.77	0	haemolitic
59	N20	MENA	NEG	0	0	<1.5	1	
60	N21	DAI	NEG	0	0	<1.5	1	
61	N22	PP13	NEG	1	1	16.19	0	haemolitic
% of agreement:				87.93%	91.07%			
Cohen's *k*:				0.743	0.81			

Table 4.

Guidelines to Interpretate of Cohen's kappa

- 0.01-0.20 slight agreement
- 0.21-0.40 fair agreement
- 0.41-0.60 moderate agreement
- 0.61-0.80 substantial agreement
- 0.81-1.00 almost perfect or perfect agreement

Figure 2. Interpretation of Cohen's kappa results.

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The present report represents only a summary of the study results and is intended for the internal use of the sponsor and the investigators. The divulgation of the this report in the present form is not allowed whitout a previous signed agreement betwent the sponsor and the investigators. This report should not be published or used for publicity or promotional purposes.

Realising day of the final report 26 January 2024.