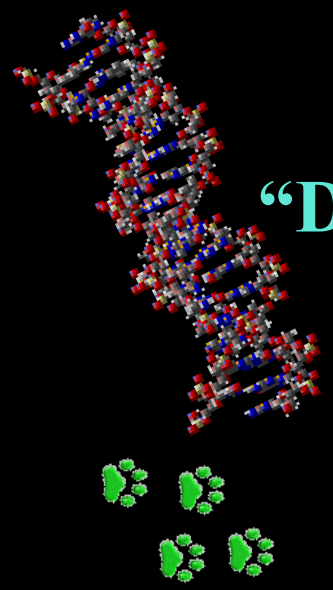
La Leishmaniosi in ottica
One-Health

23 maggio 2023 – Regione Emilia Romagna

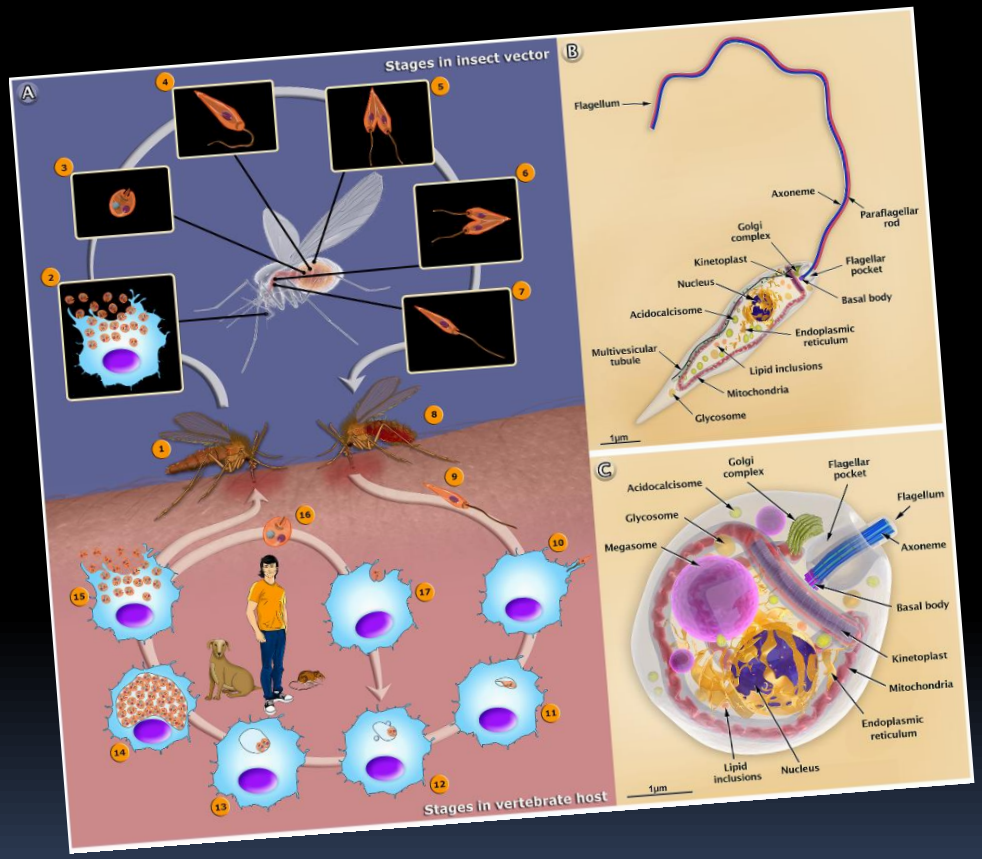


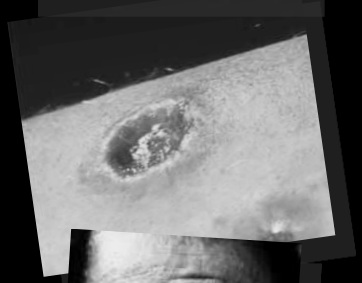
“Diagnostica di laboratorio della leishmaniosi canina”

Germano Castelli

Istituto Zooprofilattico Sperimentale della Sicilia
Centro di Referenza Nazionale per le Leishmaniosi (C.Re.Na.L.)
Laboratorio di Riferimento WOAH

Leishmaniosi





Gli esami di laboratorio sono di fondamentale importanza al fine di emettere correttamente la diagnosi, ma anche a fini prognostici e come monitoraggio durante la terapia.

Nella diagnosi della Leishmaniosi si utilizzano esami ….

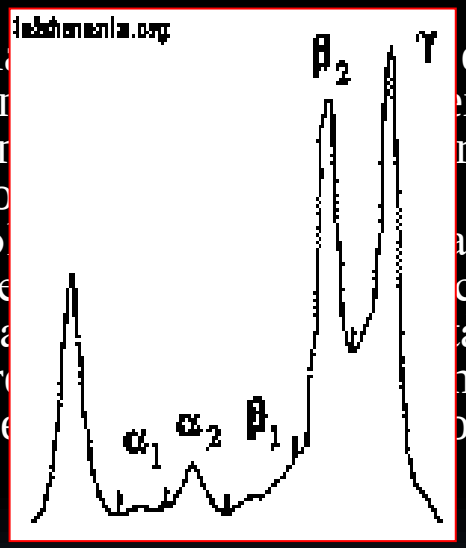
Esami aspecifici

Esami specifici

Aspecifici: pool di test di base che si ritiene indispensabile come approccio diagnostico/prognostico in caso di sospetto clinico di Leishmaniosi; sono esami di routine per indagini ematologiche e di laboratorio non solo in caso di Leishmaniosi. Per es. Protidemia totale (valori normali: 6-8 g/100 ml) e frazionata (protidogramma) sono test indicativi dello stato di salute generale dell'organismo, ma non determinanti per la diagnosi.

Esame aspecifico: PROTIDOGRAMMA

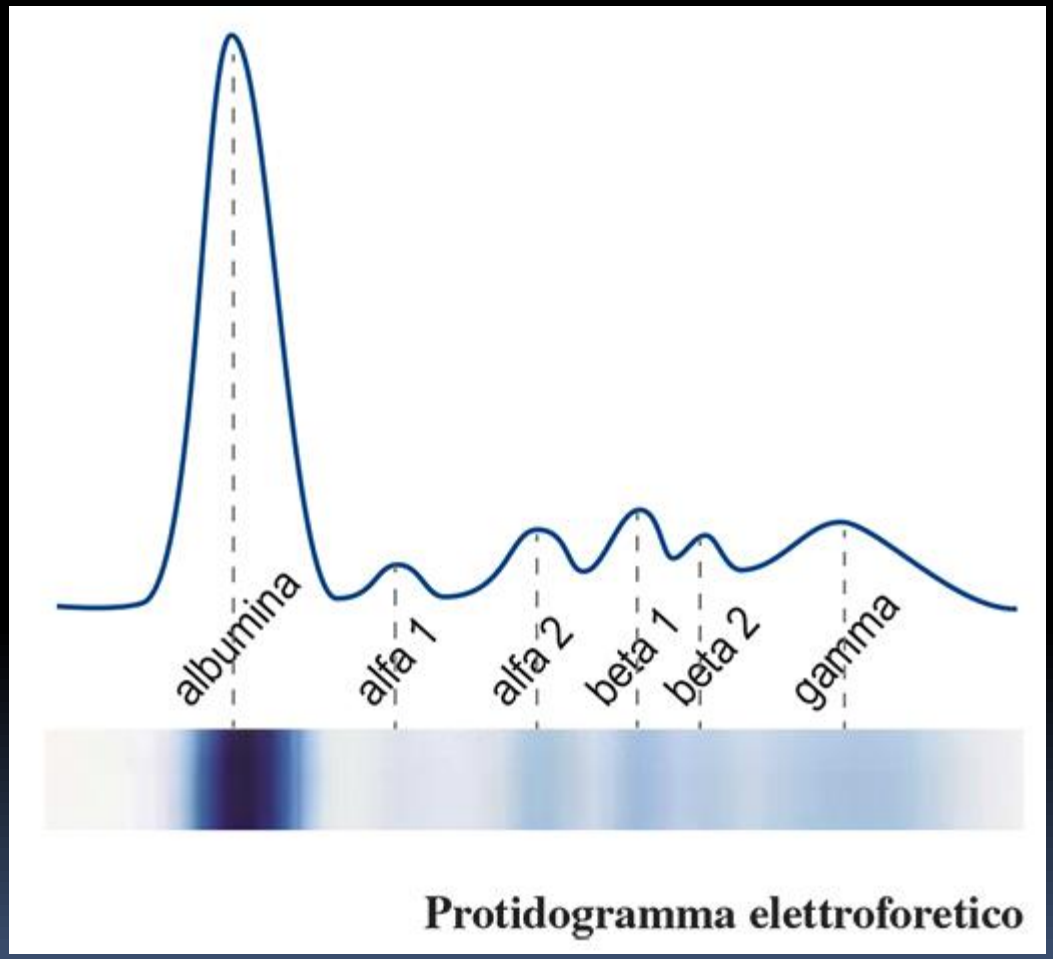
Il tracci essere
contin i cani
leishr rapia,
po di
contro amento
o l'eve cidive,
come a à della
ripr nti o
vice one.



Ipoalbuminemia e picco oligoclonale

aspetto ad *orecchie di gatto*

(infezione di vecchia data).



Esame aspecifico: nei cani…

LABORATORY ABNORMALITIES

CBC*/Hemostasis

- Mild to moderate non-regenerative anemia
- Leukocytosis or leukopenia: lymphopenia, neutrophilia, neutropenia
- Thrombocytopathy
- Thrombocytopenia
- Impaired secondary hemostasis and fibrinolysis

Serum biochemical profile with proteins electrophoresis

- Hyperproteinemia
- Hyperglobulinemia (polyclonal beta and/or gammaglobulinemia)
- Hypoalbuminemia
- Decreased albumin/globulin ratio
- Renal azotemia
- Elevated liver enzyme activities
- Proteinuria

* CBC: complete blood count

Table 1 Clinical manifestations and laboratory abnormalities found in canine leishmaniosis due to *L. infantum*

Clinical manifestations	Laboratory abnormalities
General ○ Generalized lymphadenomegaly ○ Loss of body weight ○ Decreased or increased appetite ○ Lethargy ○ Mucous membranes pallor ○ Splenomegaly ○ Polyuria and polydypsia ○ Fever ○ Vomiting ○ Diarrhea (including chronic colitis)	Serum proteins and electrophoretogram ○ Hyperglobulinemia Polyclonal beta and/or gammaglobulinemia ○ Hypoalbuminemia ○ Decreased albumin/globulin ratio
Cutaneous ○ Non-pruritic exfoliative dermatitis with or without alopecia ○ Erosive-ulcerative dermatitis ○ Nodular dermatitis ○ Papular dermatitis ○ Pustular dermatitis ○ Onychogryphosis	CBC/Hemostasis ○ Mild to moderate non-regenerative anemia ○ Leukocytosis or leukopenia ○ Thrombocytopathy ○ Thrombocytopenia ○ Impaired secondary hemostasis and fibrinolysis
Ocular ○ Blepharitis (exfoliative, ulcerative, or nodular) and conjunctivitis (nodular) ○ Keratoconjunctivitis, either common or sicca ○ Anterior uveitis/Endophtalmitis	Biochemical profile/urinalysis ○ Mild to severe proteinuria ○ Renal azotemia ○ Elevated liver enzyme activities
Other ○ Mucocutaneous and mucosal ulcerative or nodular lesions (oral, genital and nasal) ○ Epistaxis ○ Lameness (erosive or non-erosive polyarthritis, osteomyelitis, polymyositis) ○ Atrophic masticatory myositis ○ Vascular disorders (systemic vasculitis, arterial thromboembolism) ○ Neurological disorders	

Fonte: LeishVet

Esame aspecifico: nei gatti…

Table 4
Frequency of clinicopathological abnormalities in domestic cats (*Felis catus*) with leishmaniosis caused by *Leishmania infantum*

Parameter	Frequency (%)[a]	Reference
Hemogram		
Anaemia	31	Hervás et al. (1999); Pennisi et al. (2004); Marcos et al. (2009); Richter et al. (2014); Pereira Mondolfi et al. (2019); Fernandez-Gallego et al. (2020); Pimenta et al. (2015)
Neutrophilia	19	Poli et al. (2002); Leiva et al. (2005); da Silva et al. (2010); Verneuil (2013); Fernandez-Gallego et al. (2020); Silva et al. (2020)
Thrombocytopaenia	17	Pennisi et al. (2004); Marcos et al. (2009); Richter et al. (2014); Pimenta et al. (2015); Basso et al. (2016); Pereira Mondolfi et al. (2019)
Leukocytosis	10	Ozon et al. (1998); da Silva et al. (2010); Fernandez-Gallego et al. (2020)
Leukopaenia	10	Pennisi et al. (2004); Rüfenacht et al. (2005); Richter et al. (2014)
Eosinophilia	7	Ozon et al. (1998); Hervás et al. (1999); Marcos et al. (2009); Altuzarra et al. (2020)
Neutropaenia	5	Fernandez-Gallego et al. (2020)
Lymphopaenia	2	Rüfenacht et al. (2005)
Monocytosis	2	Leiva et al. (2005)
Blood chemistry		
Hyperproteinaemia	36	Ozon et al. (1998); Hervás et al. (1999); Poli et al. (2002); Pennisi et al. (2004); Pimenta et al. (2015); Attipa et al. (2017a,b); Leal et al. (2018); Brianti et al.

Table 4 (*continued*)

Parameter	Frequency (%)[a]	Reference
Increased α_2 globulins	13	Basso et al. (2016); Fernandez-Gallego et al. (2020)
Hyperbetaglobulinaemia	3	Hervás et al. (1999)
Urinalysis		
Proteinuria	25	Marcos et al. (2009); Leal et al. (2018); Fernandez-Gallego et al. (2020)
Bilirrubinuria	4	Marcos et al. (2009)
Glycosuria	4	Leiva et al. (2005)

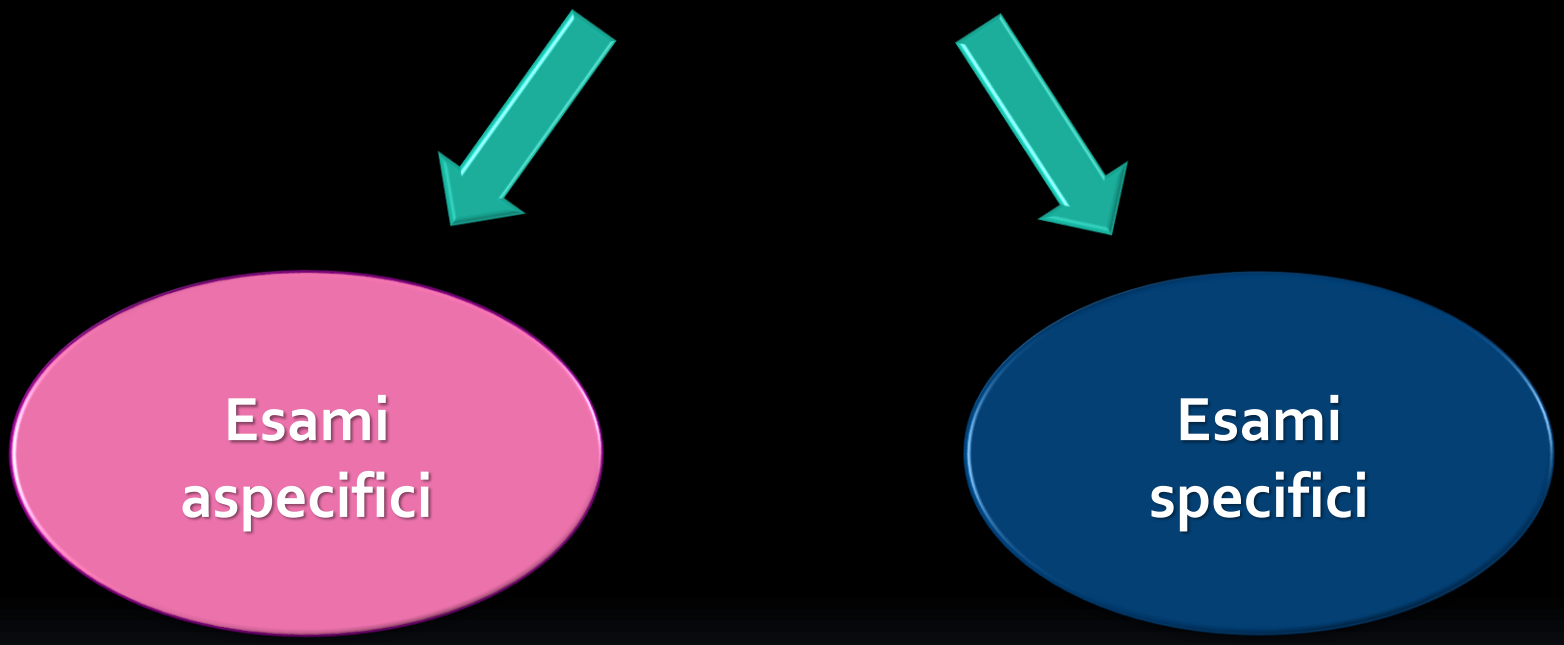
[a] Hemogram, $n = 42$; Blood chemistry, $n = 39$; Serum protein electrophoresis, $n = 32$; Urianalysis, $n = 24$.

Hyperglobulinaemia	31	Pennisi et al. (2004); Leiva et al. (2005); Richter et al. (2014); Pimenta et al. (2015); Brianti et al. (2019); Altuzarra et al. (2020)
Azotemia	21	Pennisi et al. (2004); Leiva et al. (2005); Marcos et al. (2009); da Silva et al. (2010); Leal et al. (2018); Fernandez-Gallego et al. (2020)
Hypoalbuminaemia	10	Hervás et al. (1999; Rüfenacht et al. (2005); Richter et al. (2014); Fernandez-Gallego et al. (2020)
Hyperglycaemia	8	Leiva et al. (2005); Richter et al. (2014); Fernandez-Gallego et al. (2020)
Bilirrubinaemia	5	Fernandez-Gallego et al. (2020)
Hyperphosphataemia	3	Fernandez-Gallego et al. (2020)
Hypophosphataemia	3	Fernandez-Gallego et al. (2020)
Increased alanine aminotransferase	3	Fernandez-Gallego et al. (2020)
Increased aspartate transaminase	3	da Silva et al. (2010)
Increased creatinine kinase	3	Fernandez-Gallego et al. (2020)
Protein electrophoresis		
Hypergammaglobulinaemia	84	Ozon et al. (1998); Hervás et al. (1999); Poli et al. (2002); Pennisi et al. (2004); Leiva et al. (2005); Marcos et al. (2009); Richter et al. (2014); Basso et al. (2016); Leal et al. (2018); Brianti et al. (2019); Pereira Mondolfi et al. (2019); Altuzarra et al. (2020); Fernandez-Gallego et al. (2020)

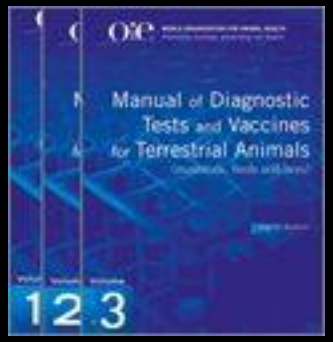
André Pereira, Carla Maia,2021, https://doi.org/10.1016/j.crpvbd.2021.100035.

Anemia non rigenerativa, iperglobulinemia, frazioni di alfa e gammaglobulina elevate e proteinuria sono le anomalie più frequentemente riportate (Pennisi et al., 2015a; Fernandez-Gallego et al., 2020; Urbani et al., 2020; Savioli et al., 2021).

Nella diagnosi della Leishmaniosi si utilizzano esami ….



World Organisation for Animal Health
Founded as OIE



CHAPTER 3.1.11.

LEISHMANIOSIS

SUMMARY

Description and importance of the disease: *Leishmaniosis is not a single entity but comprises a variety of syndromes caused primarily by at least* 20 Leishmania *species affecting humans, transmitted by phlebotomine sand flies belonging to the genera* Phlebotomus *and* Lutzomyia. *Leishmaniosis is associated with and limited by the geographic distribution of its sand fly vectors. Fifteen nosogeographical entities have been classified worldwide, of which 13 have an established or putative zoonotic nature. In recent years, the number of regions becoming* Leishmania*-endemic has grown significantly, accompanied by an increased number of animal and human cases.*

In humans, the clinical spectrum ranges from asymptomatic infections to those with high mortality, with three distinct forms being classically described: visceral (VL), cutaneous (CL) and mucocutaneous (MCL). Dogs are commonly affected by L. infantum *and* L. chagasi *(now regarded as synonyms), which causes a chronic viscero-cutaneous disease in this host (canine leishmaniosis, CanL). Asymptomatic infection in dogs is widespread and contributes to maintaining the long-term presence of the parasite in endemic regions. The clinical appearance and evolution of leishmaniosis is a consequence of complex interactions between the parasite and host immune response. The outcome of infection depends on the ability of host macrophages to effectively destroy the parasite.*

Detection of the agent: *When clinical signs and characteristic lesions are present in affected humans and animals, the demonstration of the parasites in stained smears of splenic, bone marrow and lymph node aspirates, of skin scrapings, and in tissue biopsies, gives a positive diagnosis. If the infection is low grade, detection of parasites is possible only by attempting* in-vitro *isolation or by polymerase chain reaction (PCR). As there are very few morphological differences among various species, the identification of any isolated* Leishmania *organism relies on biochemical and/or molecular methods. Several centres throughout the world are presently using isoenzyme and DNA characterisation to identify the agent.*

Serological tests: *Serology is the preferred method for diagnosis of VL and CanL, even during the early stages of the disease. In subclinical forms, seropositive cases are confirmed by parasitological diagnosis or PCR. Serology is of less value for CL and MCL. Of the several serological techniques available, the indirect fluorescent antibody test and the enzyme-linked immunosorbent assay are the most suitable. Crude antigens for serodiagnostic tests can be prepared in the laboratory from cultured parasites. Tests based on recombinant antigens may also be used and have a high specificity although can be less sensitive.*

The rapid immunochromatographic assay is easy to carry out and can be performed in veterinary clinics, but has lower diagnostic efficiency than the indirect fluorescent antibody test (IFAT) and enzyme-linked immunosorbent assay (ELISA).

Requirements for vaccines: *There is no effective vaccine available worldwide at present for use in dogs or humans. A number of vaccines for use in animals are under evaluation, and four have been authorised for use in dogs (two in Brazil and two in Europe). Beyond other issues to be evaluated, the use of these vaccines is posing present and future challenges to the fields of diagnosis, epidemiology and surveillance of the parasite, especially in countries where the parasite does not occur. Leishmanin skin test antigen is no longer available worldwide and lacks standardisation.*

B. DIAGNOSTIC TECHNIQUES

Leishmaniosis diagnosis must be based on an integrated approach that takes into account signalling, medical history, physical findings, clinical and pathological changes, and results of diagnostic tests. The diagnostic methods currently available are described below.

The list and fitness of each test for different purposes are given in Table 1.

Table 1. *Test methods available for diagnosis of leishmaniosis and their purpose*

Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
Detection of the agent[2]						
Cytological examination	–	–	–	++	–	–
Histological examination	–	–		++	–	
Isolation in culture	–	+		++	–	
Molecular methods	++	+++		++	++	
Detection of immune response						
IFAT	+++	++	–	++	+++	–
ELISA	+++	++		++	+++	
Direct agglutination test	++	++		++	++	
Rapid immuno-chromatographic assay	–	–		++	+	

Key: +++ = recommended for this purpose; ++ recommended but has limitations;
+ = suitable in very limited circumstances; – = not appropriate for this purpose.
PCR = polymerase chain reaction; IFAT = Indirect fluorescent antibody test; ELISA = enzyme-linked immunosorbent assay.

Nella diagnosi della Leishmaniosi si utilizzano esami ….

Esami aspecifici

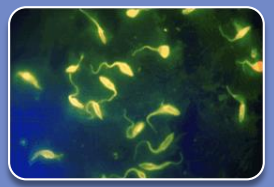
Esami specifici

Esami Diretti (Identificazione agente biologico)

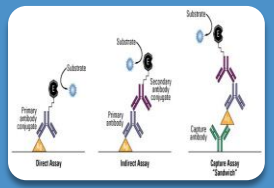
Esami Indiretti (Valutazione della risposta immunitaria)

Specifici: mettono in evidenza la presenza del parassita.

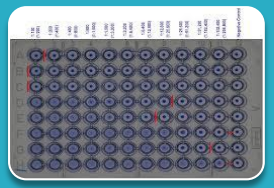
TECNICHE INDIRETTE
(Valutazione della risposta immunitaria)



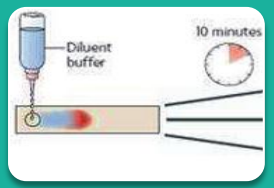
Immunofluorescenza indiretta (IFAT)



TEST ELISA

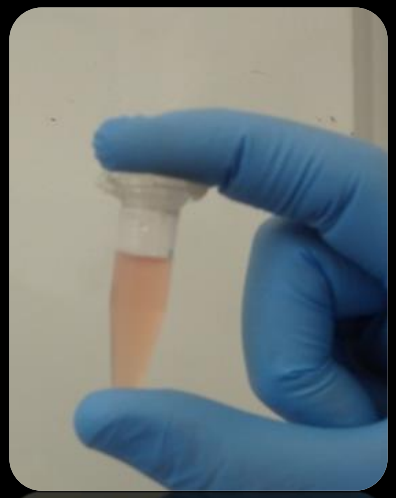


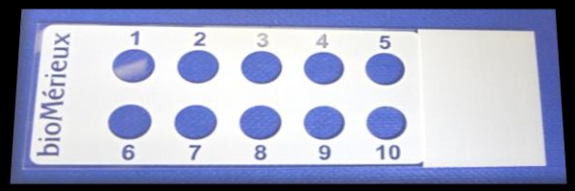
Test di agglutinazione diretta (DAT)

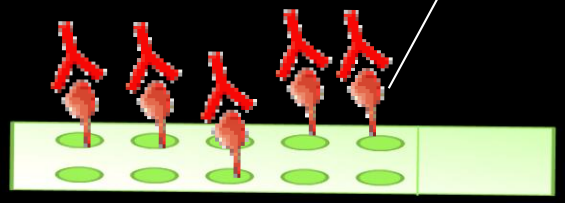


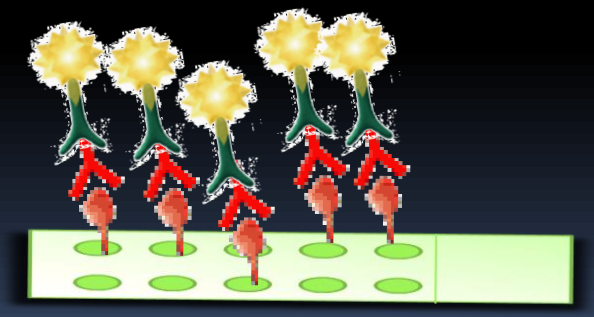
Saggio immuno-cromatografico rapido

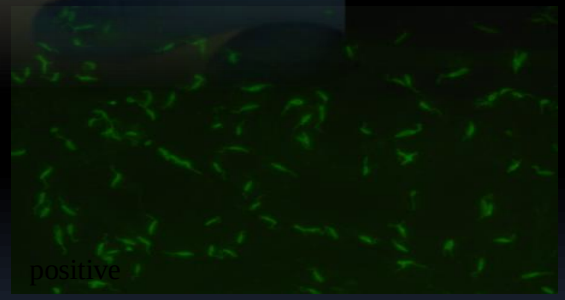
Immunofluorescenza indiretta (IFAT)

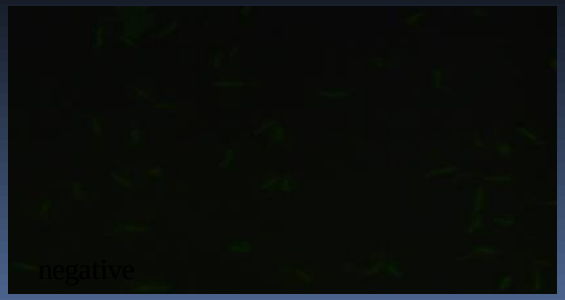




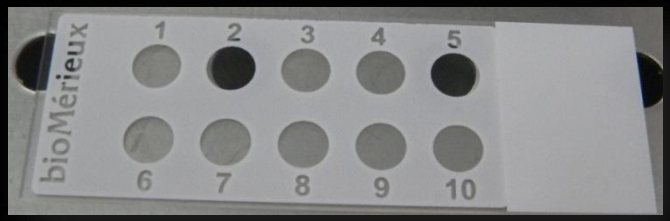




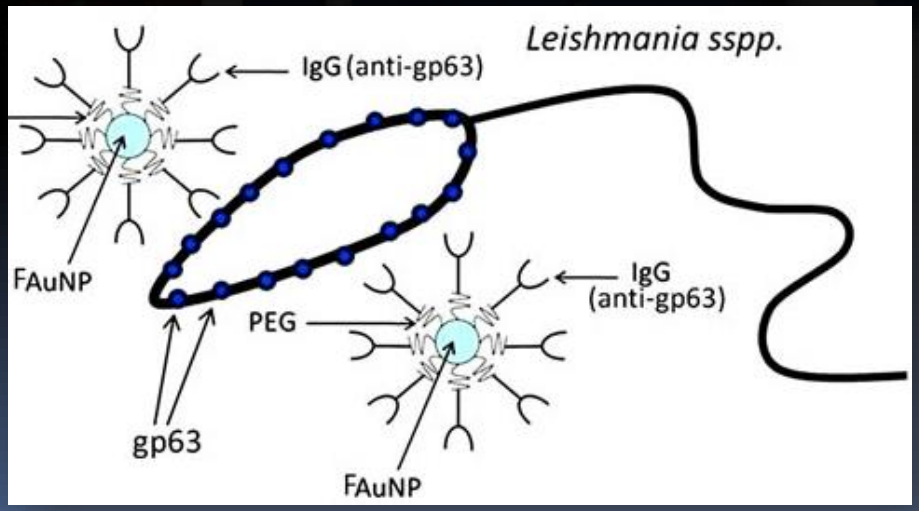




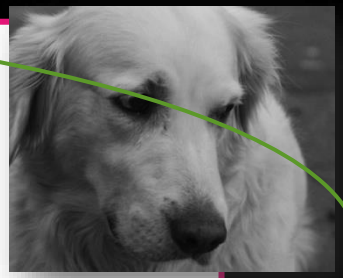
Immunofluorescenza indiretta (IFAT)



Vetrini multipozzetto sensibilizzati con il ceppo di riferimento nazionale MHOM/TN/80/IPT1 di Leishmania infantum. Sieri di controllo + e Ø Anti IgG di specie coniugate FITC



La sierologia consente di determinare anticorpi specifici nei confronti del parassita, in particolare nei confronti della **glicoproteina gp63**

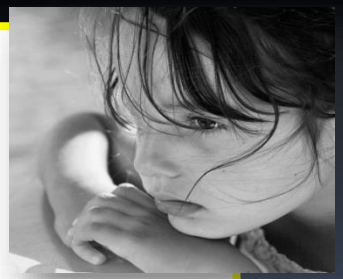


- Titolo < 1:40 — Negativo
- Titolo da 1:40 a 1:80 — Sospetto *
- Titolo ≥ 1:160 — Positivo

Il titolo Sospetto indica una reattività anticorpale dell'animale, è opportuno riprelevare il siero a distanza di 21-30 giorni per stabilire se il campione esaminato presenta un movimento anticorpale e quindi se il cane sia positivo o negativo.

- Titolo < 1:80 — Negativo
- Titolo 1:80 — Sospetto *
- Titolo ≥ 1:160 — Positivo

Il titolo Sospetto indica una reattività anticorpale dell'animale, è opportuno riprelevare il siero a distanza di 21-30 giorni per stabilire se il campione esaminato presenta un movimento anticorpale



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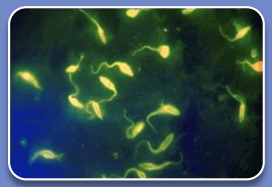
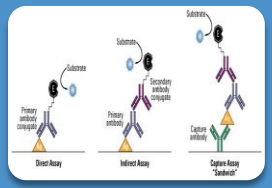
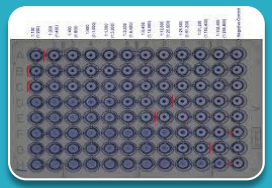
TECNICHE INDIRETTE
(Valutazione della risposta immunitaria)



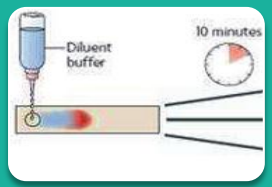
Immunofluorescenza indiretta (IFAT)



TEST ELISA

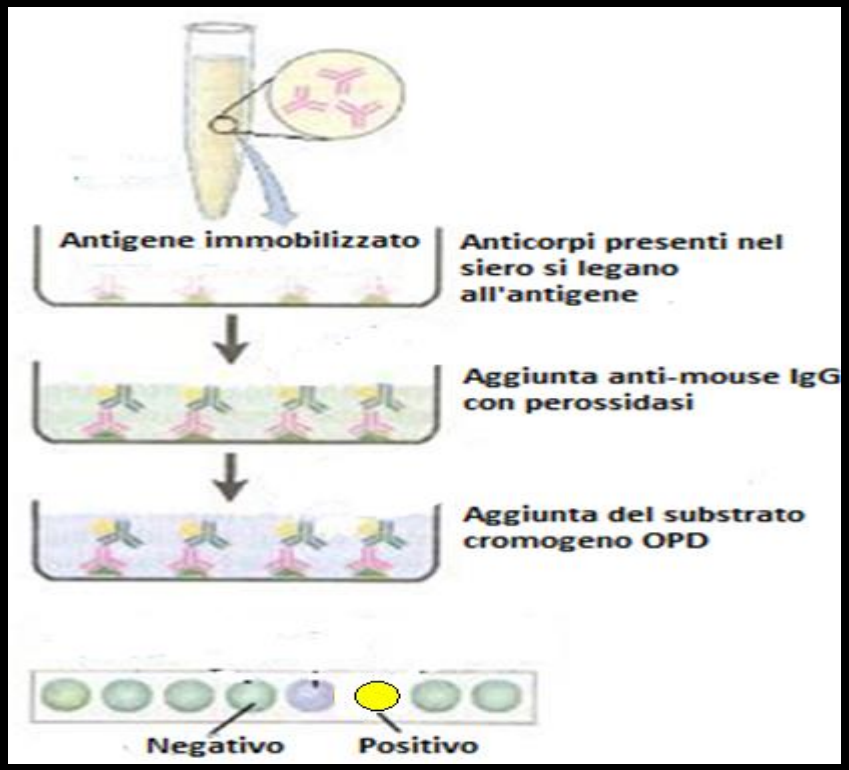


Test di agglutinazione diretta (DAT)

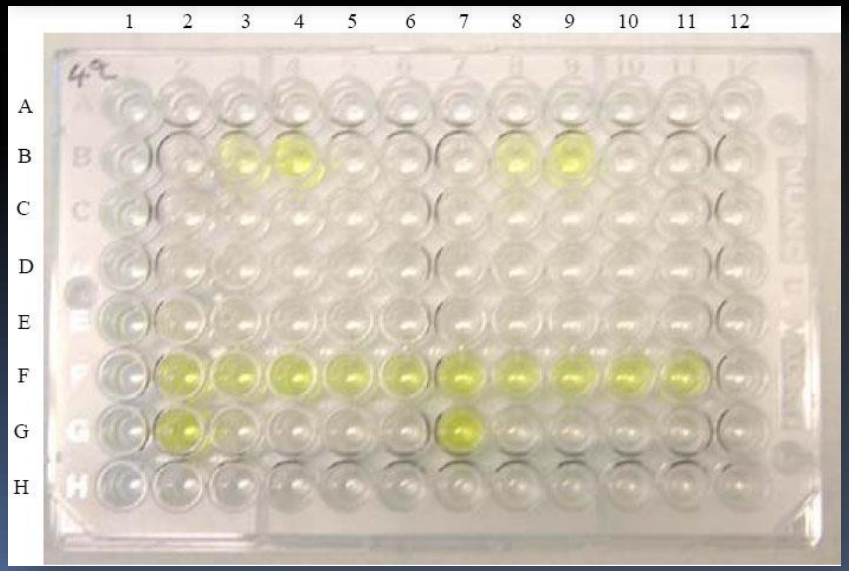


Saggio immuno-cromatografico rapido

ELISA



- Specificità inferiore
- Sensibile inferiore
- Possibili reazioni crociate



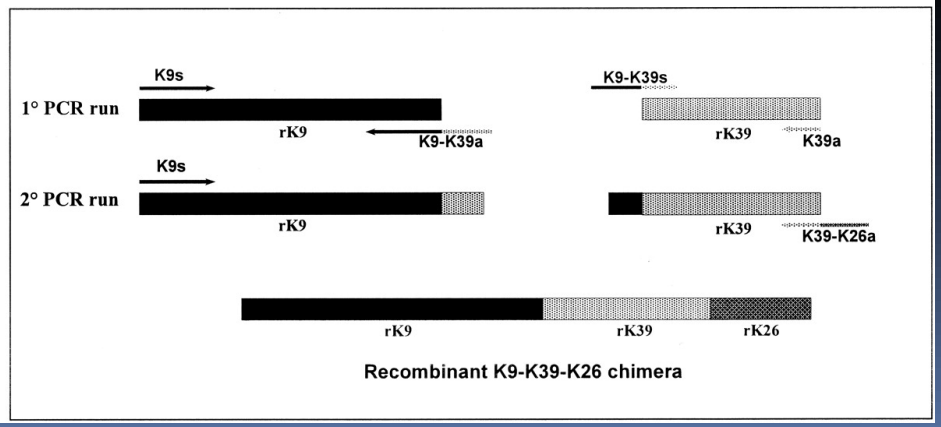
ELISA

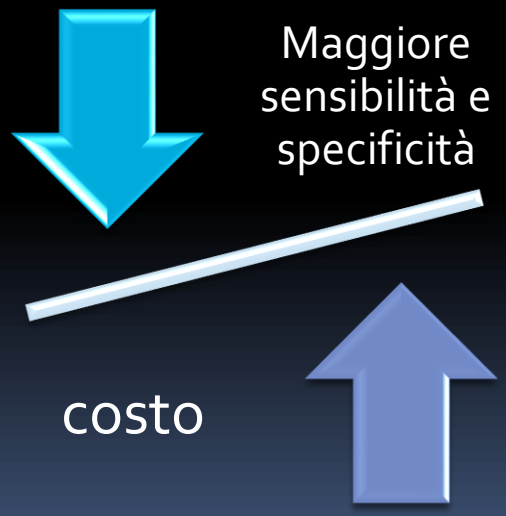
The ELISA methods described above are based on crude antigenic preparations. A recombinant antigen from a cloned protein of *L. infantum*, called rK39, has been reported to be highly reactive to sera from human and canine visceral leishmaniosis cases when run in an ELISA format. Using 25–50 ng of the antigen, 99% specificity and sensitivity was consistently found for dogs with parasitologically proven disease (Scalone *et al.*, 2002). In HIV-positive patients, K39-ELISA showed higher sensitivity (82%) than the IFA test (54%) (Houghton *et al.*, 1998). The K39 antigen, which shows remarkable stability and reproducibility, is commercially available. More recently, a K9-K39-K26 recombinant chimeric antigen has been evaluated as a single ELISA protocol for serological diagnosis of both human and canine *Leishmania* infections (Daprà *et al.*, 2008). In dogs, test specificity and sensitivity were reported to be 99.5% and 98.5%, respectively, with high concordance (K value: 0.98) with standard IFA test.

A

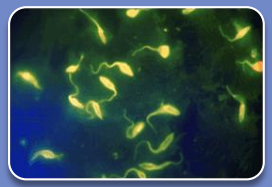
Primer	Sequence (5′-3′)
K9s:	5′-TTGGATCCATGGGAGCCTACTGCACGAAGGAC-3′
K9-K39a:	5′-GCGAAGCTGCTGCTCTAGGTTGCCGGCAACCTGCTC-3′
K9-K39s:	5′-GAGCAGGTTGCCGGCAACCTAGAGCAGCAGCTTCGC-3′
K39a:	5′-TTGAATTCCGTGGCCCTCGTGCTC-3′
K39-K26a:	5′-TTGAATTCGCCATCGCCGTCATTTTTCTGTGTACGGCCGTCCT CCTTAGGGCCATCGCCGTCATTCGTGGCCCTCGTGCTCTCGCG-3′

B

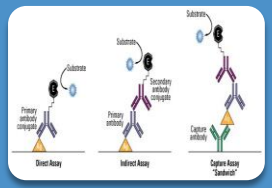




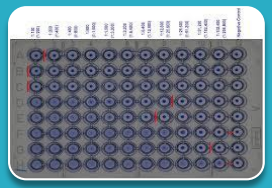
TECNICHE INDIRETTE
(Valutazione della risposta immunitaria)



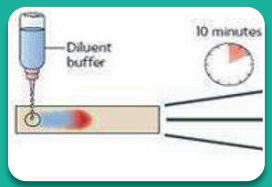
Immunofluorescenza indiretta (IFAT)



TEST ELISA

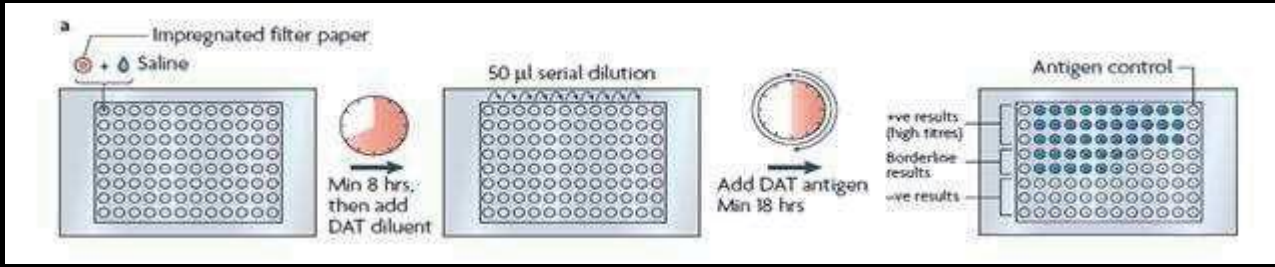


Test di agglutinazione diretta (DAT)

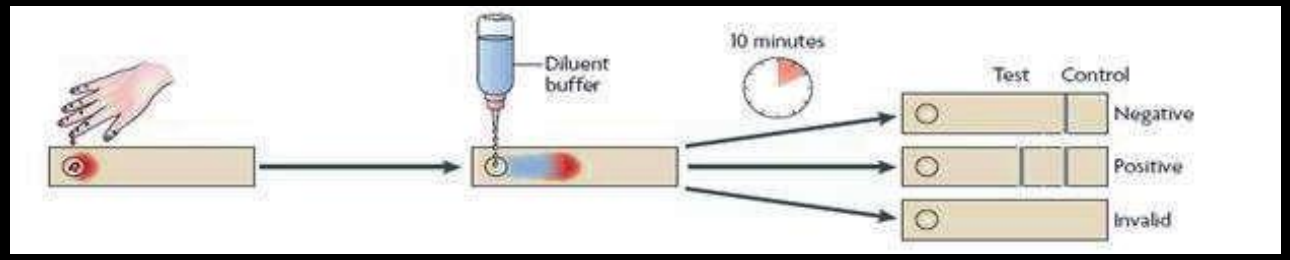


Saggio immuno-cromatografico rapido

Test di agglutinazione diretta (DAT)



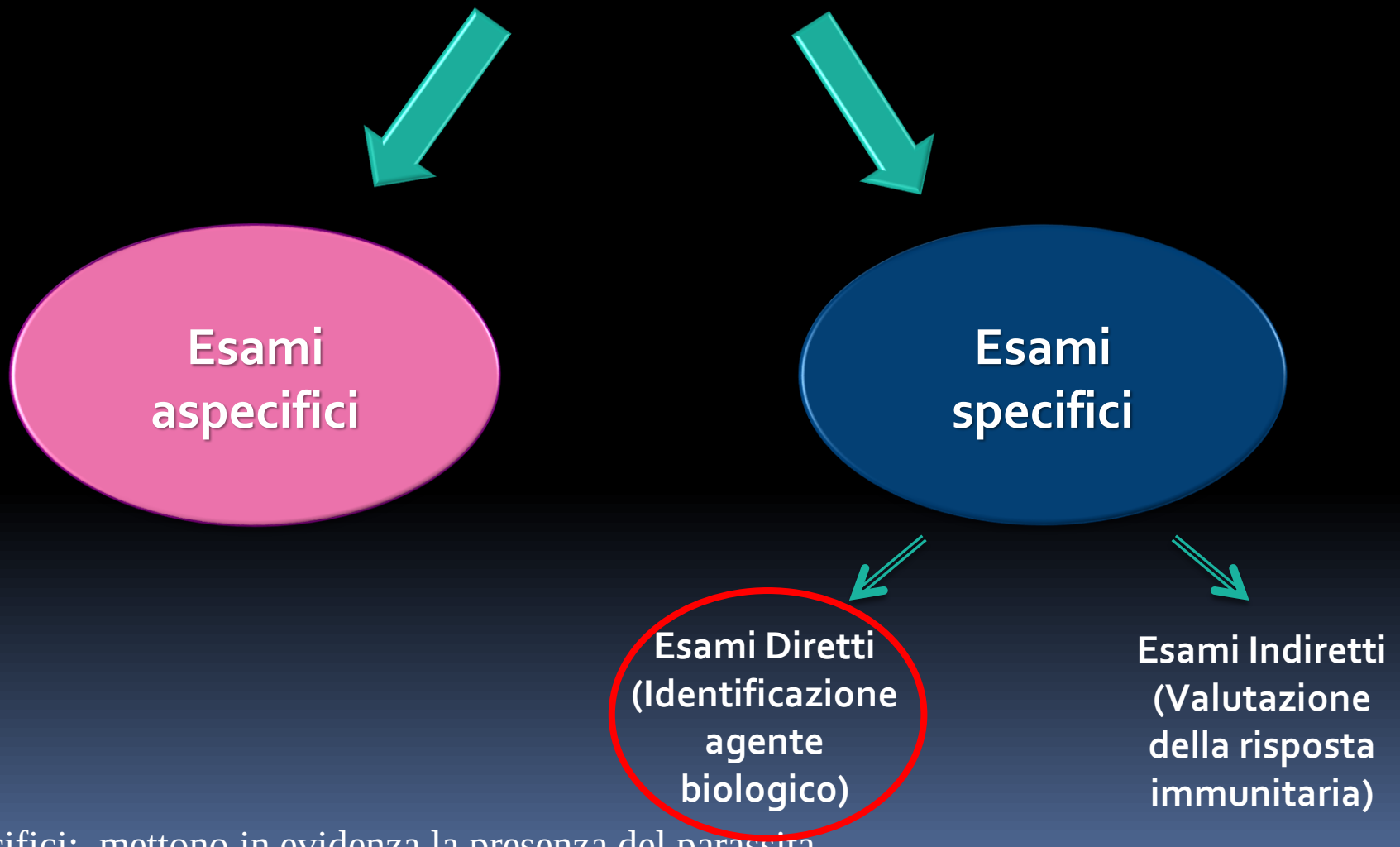
Saggi immuno-cromatografici rapidi



- *Vantaggi: Praticità/velocità*
- *Svantaggi: sensibilità bassa (30-50%)*

I test rapidi in commercio per la rilevazione qualitativa degli anticorpi canini anti-L. *infantum* non sono validati per l'uso con il siero di gatto. Quando uno di questi test rapidi commerciali è stato confrontato con un test IFA validato, sono stati osservati bassi livelli di sensibilità, valori predittivi negativi, specificità e valori predittivi positivi.

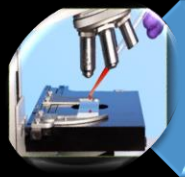
Nella diagnosi della Leishmaniosi si utilizzano esami ….



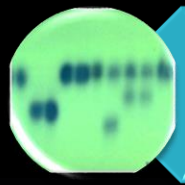
Specifici: mettono in evidenza la presenza del parassita.

TECNICHE DIRETTE
(Identificazione agente biologico)

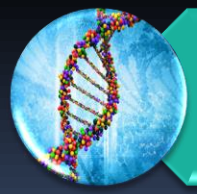
Colorazione May-Grümwald Giemsa



Isolamento colturale



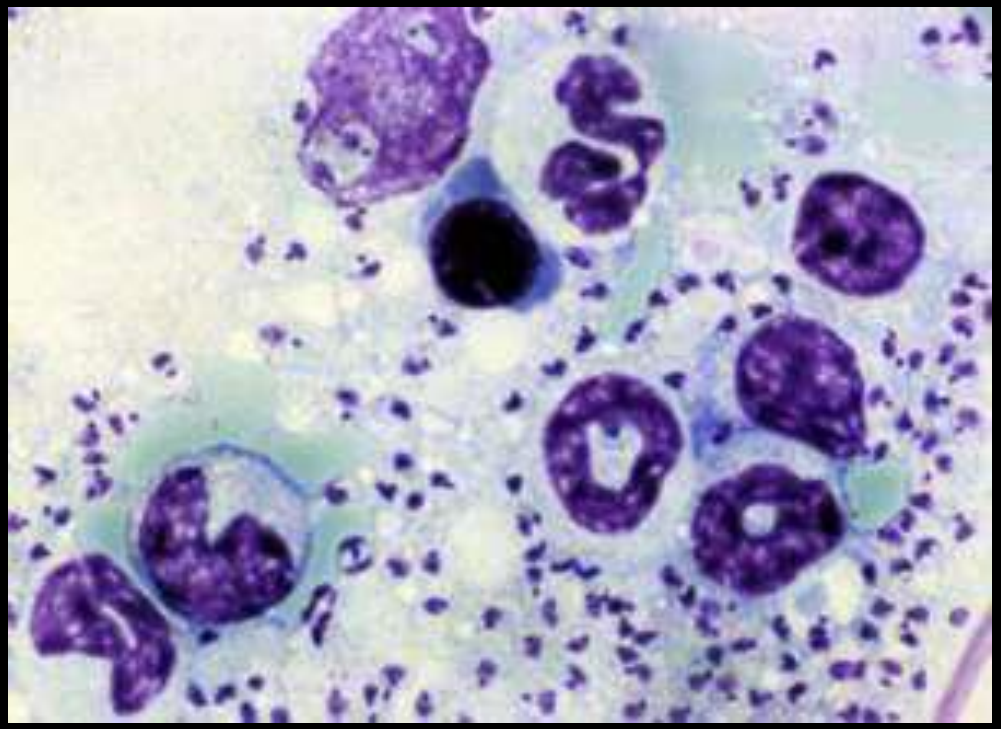
Tipizzazione isoenzimatica



Test molecolari

Colorazione May-Grümwald Giemsa

1. *May-Grümwald per 3'*
2. *Risciacquo*
3. *Giemsa 1:10 per 20'*
4. *Risciacquo*

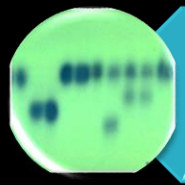


- **Vantaggi**: economica, pratica, veloce, specificità molto elevata in particolare all'inizio della evoluzione della patologia quando la carica parassitaria è notevole
- **Svantaggi**:bassa sensibilità.
- Nelle frequenti paucinfezioni infatti il numero di protozoi risulta scarso

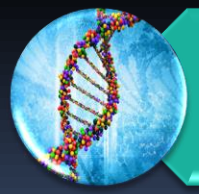
TECNICHE DIRETTE
(Identificazione agente biologico)

Colorazione May-Grümwald Giemsa

Isolamento colturale

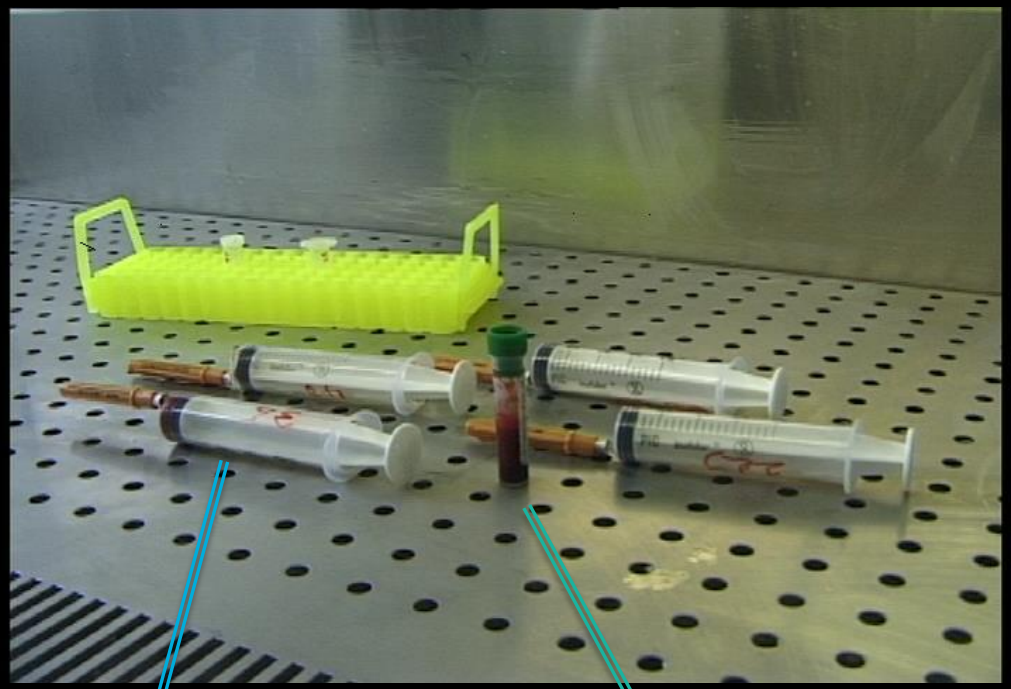


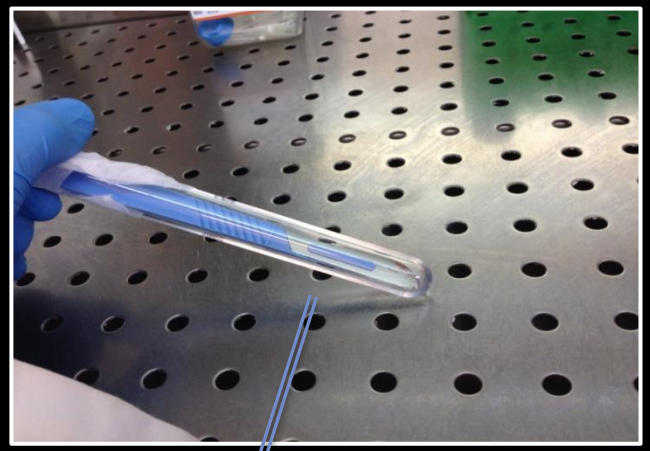
Tipizzazione isoenzimatica



Test molecolari

Isolamento delle Leishmanie: promastigoti





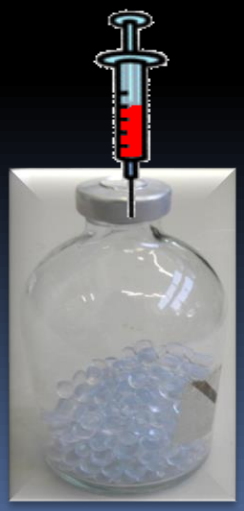
Agoaspirato linfonodale

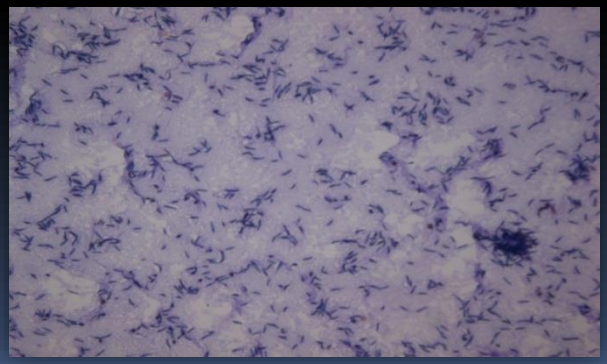
Agoaspirato midollare

Raschiato Cutaneo

Terreni di coltura

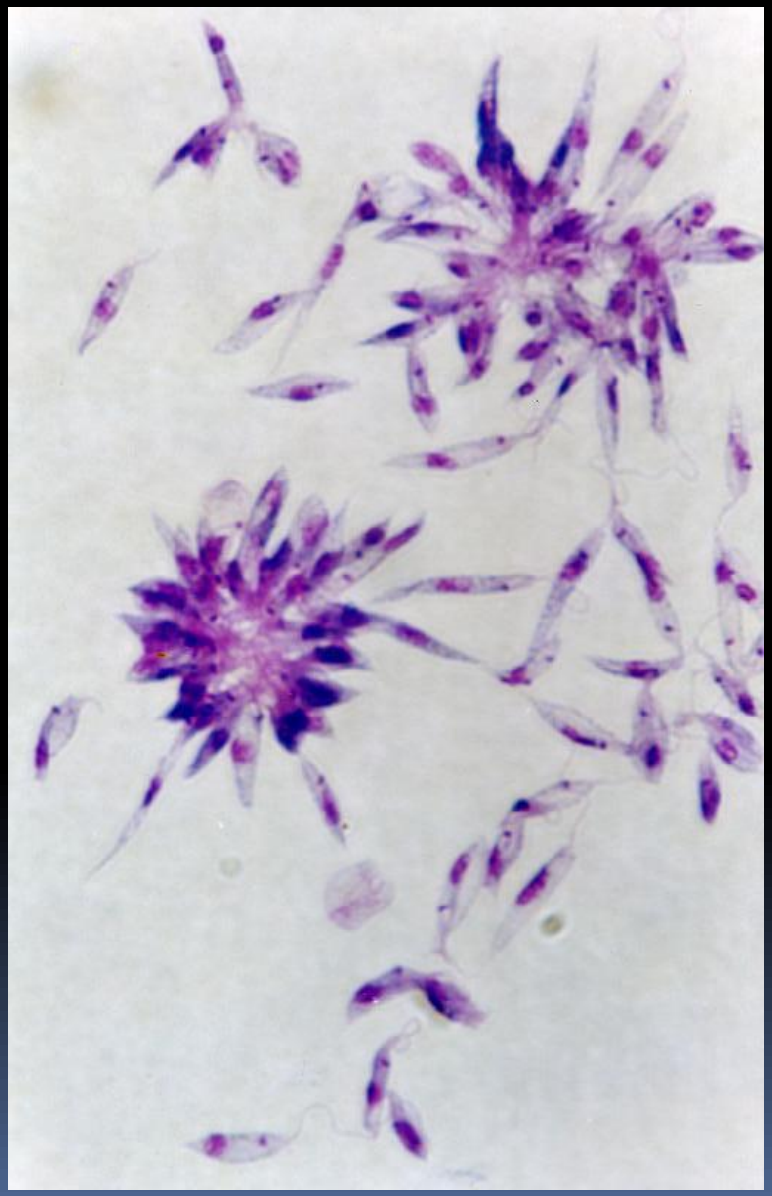
- Bifasici
  - EMTM
  - NNN
- Liquidi
  - RPMI
  - P-Y
  - RPMI-PY





ISOLAMENTO

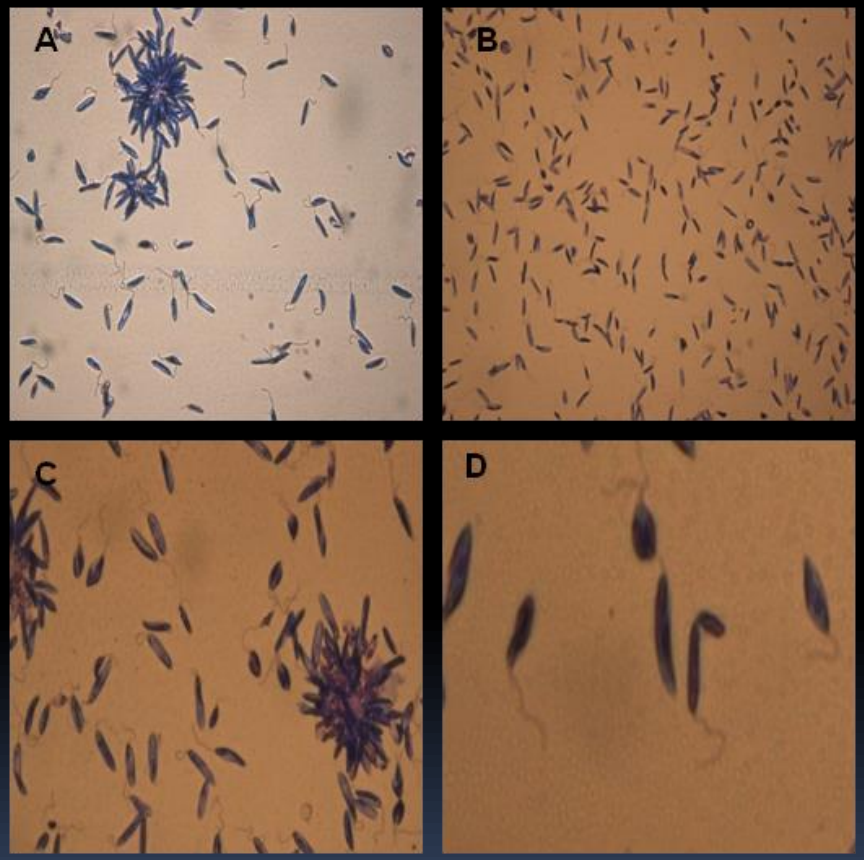
- Se il campione si mantiene negativo eseguire 3 passaggi nell'arco di 2 mesi.
- Quando il campione si positivizza, proseguire vari passaggi finalizzati all'amplificazione del ceppo isolato.
- Finalità dell'isolamento (diagnostica,conservazione del ceppo in azoto liquido, DNA, tipizzazione isoenzimatica e/o molecolare)



Isolamento

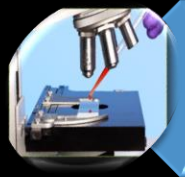
Fattori influenzanti l'esito dell'isolamento:

- Carica parassitaria del campione
- Virulenza del ceppo
- Idonee condizioni di coltura (terreno e temperatura)

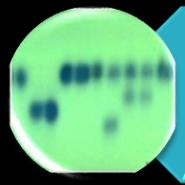


TECNICHE DIRETTE
(Identificazione agente biologico)

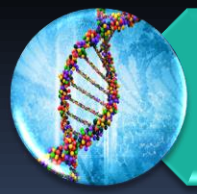
Colorazione May-Grümwald Giemsa



Isolamento colturale

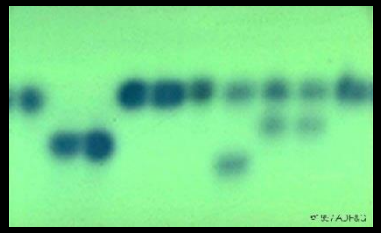


Tipizzazione isoenzimatica



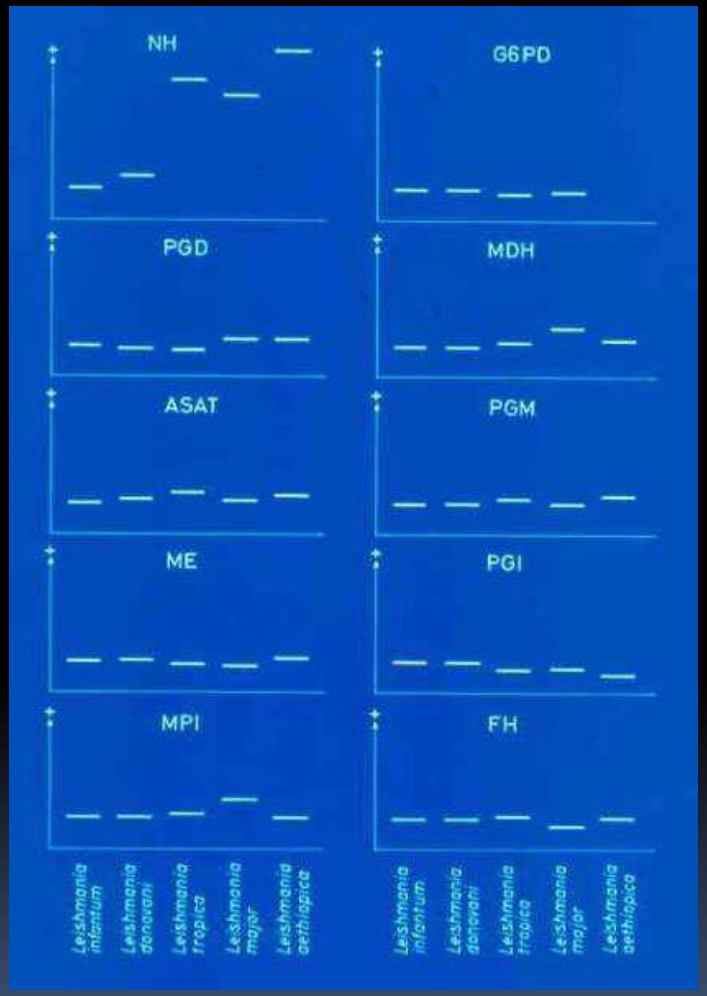
Test molecolari

TIPIZZAZIONE ISOENZIMATICA



Utilizzati per caratterizzare singoli individui facenti parte di un gruppo tassonomico

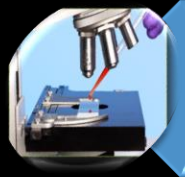
- Gli isoenzimi di uno stesso enzima catalizzano la stessa reazione, ma hanno una struttura chimica differente e diverse proprietà chimico-fisiche (punto isoelettrico, mobilità elettroforetica)
- Gli isoenzimi possono derivare da:
  - Geni differenti
  - Modificazioni post-traduzionali
  - Assemblaggi diversi



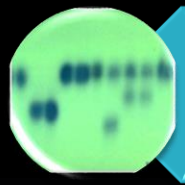
L'analisi viene condotta per confronto di bande elettroforetiche e riferite a ceppi standard di riferimento WHO, definiti biochimicamente e molecolarmente

TECNICHE DIRETTE
(Identificazione agente biologico)

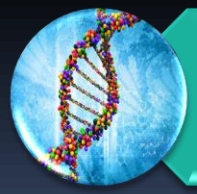
Colorazione May-Grümwald Giemsa



Isolamento colturale



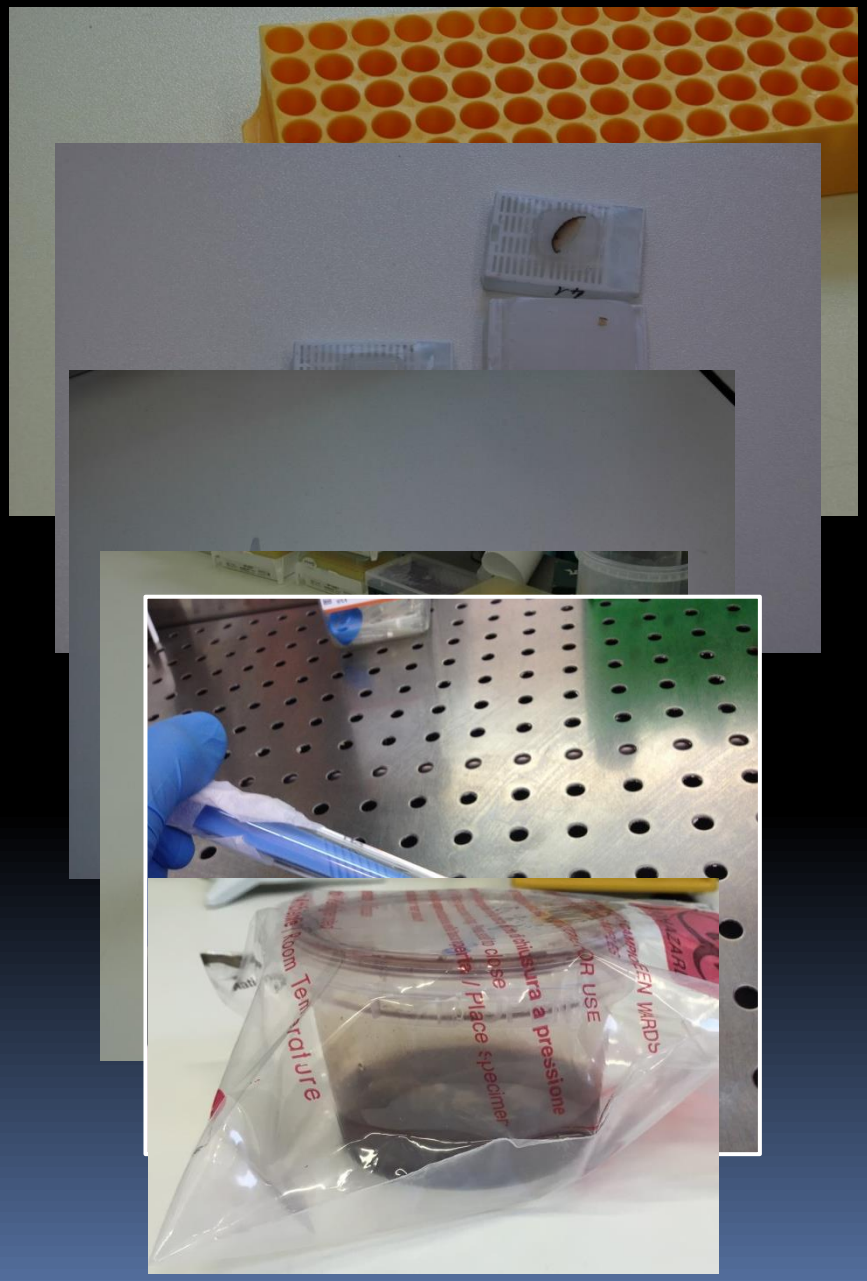
Tipizzazione isoenzimatica



Test Molecolari

Campioni di partenza:

- Sangue intero preferibilmente in EDTA
- Preparati istologici (campioni in paraffina, o in formalina)
- Ago aspirati linfonodali o midollari
- Tamponi oculo-congiuntivali, urine
- Raschiati cutanei
- Organi refrigerati o congelati

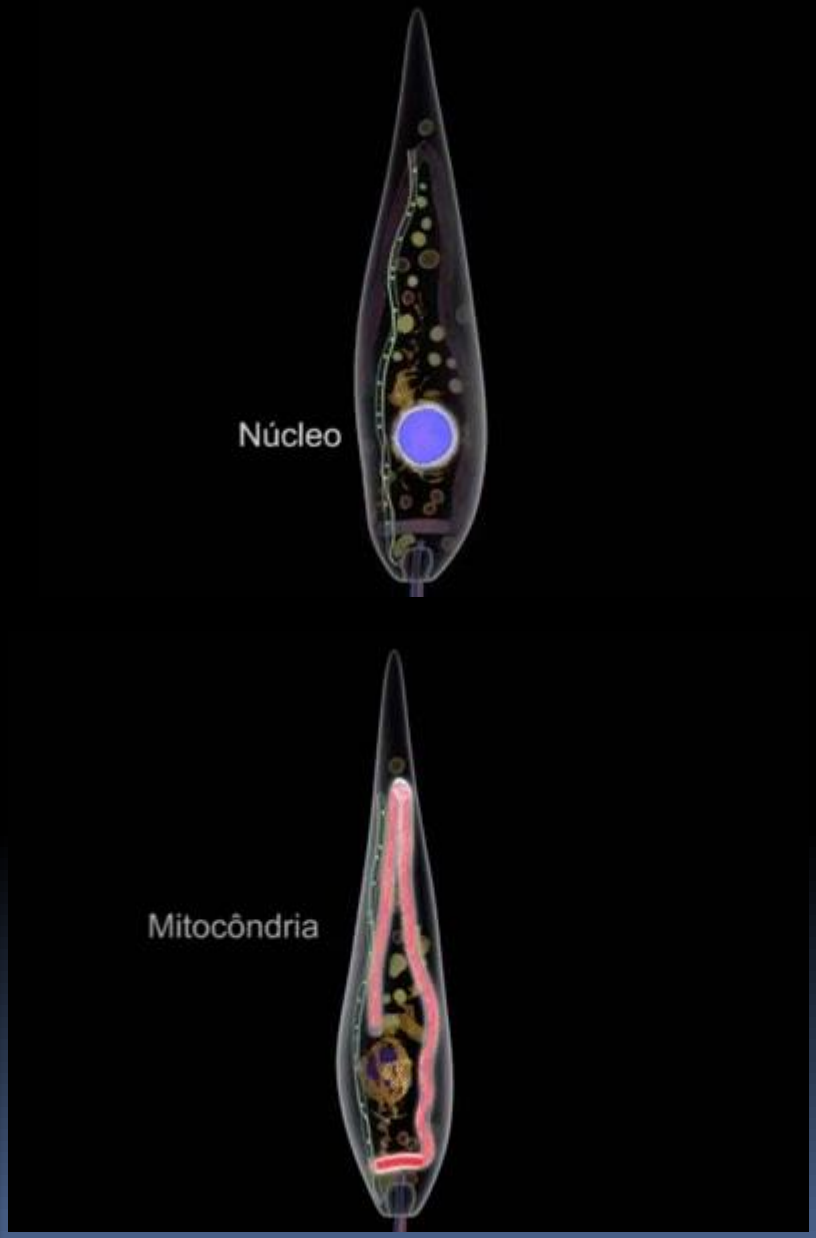


Scelta della sequenza PCR target

- Leishmania e altri trypanosomatidi hanno un unico mitocondrio che contiene una grande massa di DNA mitocondriale conosciuto come DNA del cinetoplasto (kDNA).

- Il kDNA è costituito da una gigantesca rete di minicircoli e maxicircoli concatenate. Ci sono circa 10.000 minicircoli e 50 maxicircoli per rete.

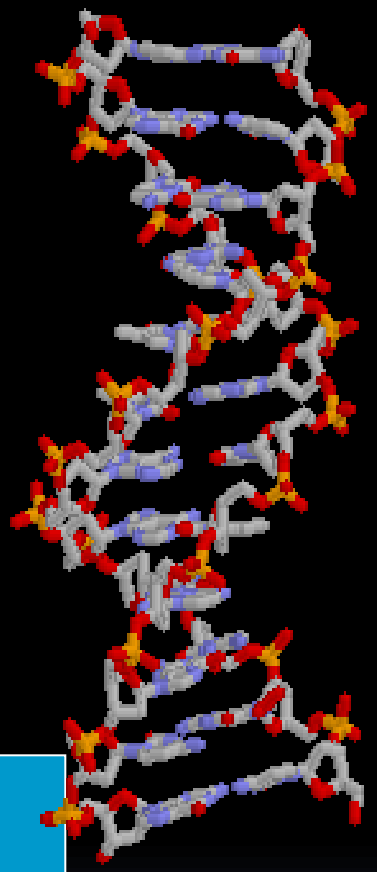
- Il kDNA mostra caratteristiche che lo rendono quasi ideale per la costruzione di sonde molecolari, dal momento che sono presenti in un alto numero di copie e contengono sequenze conservate.



PCR classica

La PCR è una tecnica diretta indipendente dallo status immunitario del cane

Oltre ad essere specifica è anche nettamente più sensibile rispetto alle altre tecniche diagnostiche sia nelle fasi precoci della malattia che nei casi asintomatici



Amplificazione selettiva in vitro di una sequenza di DNA target

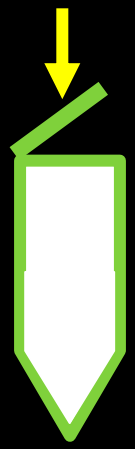
COMPONENTI DELLA REAZIONE DI PCR:

1) Sequenza di DNA target
2) DNA polimerasi termostabile(Termophilus aquaticus, Taq polimerasi)
3) Due oligonucleotidi (15-25 bp) specifici per la sequenza target (Primers)
4) dNTPs

Dalla teoria…

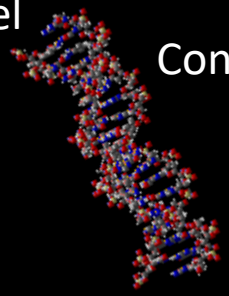
…alla pratica

PCR convenzionale



Estrazione del DNA

30’



Controllo spettrofotometrico

10’

PCR

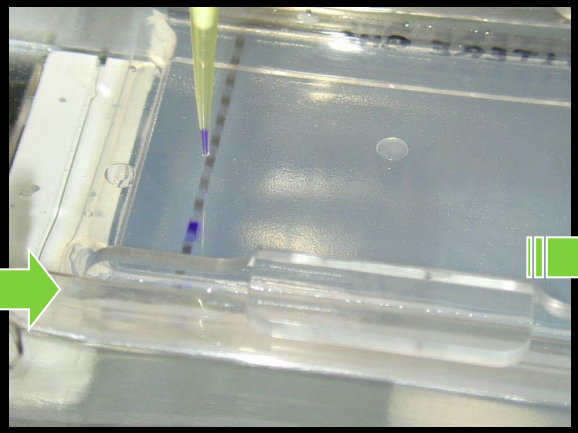
3-5 h

Termociclatore

Prodotti amplificati

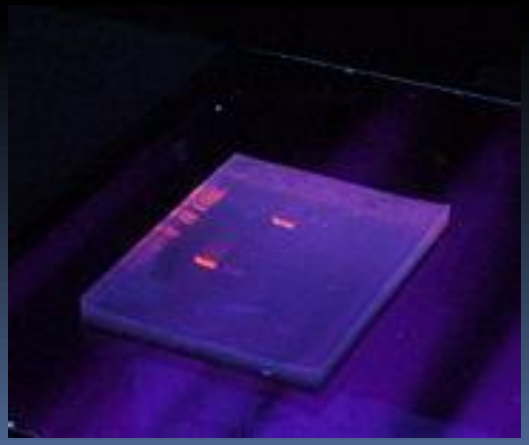
PCR convenzionale

Preparazione Gel di agarosio

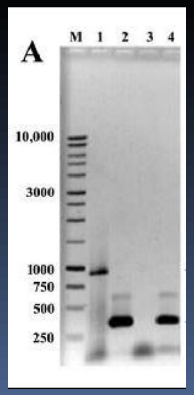


Caricamento

Elettroforesi del DNA



Lettura



Analisi dei risultati

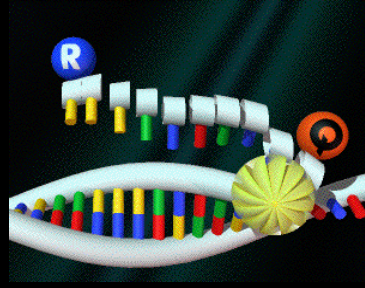
LIMITI DELLA PCR standard

- Numerosi step che possono aumentare la possibilità di errore
- Utilizzo di sostanze tossiche (etidio bromuro….)
- Possibile *cross-contamination* del DNA amplificato
- Non consente una quantificazione precisa del DNA target

Sensibilità → vantaggio ?

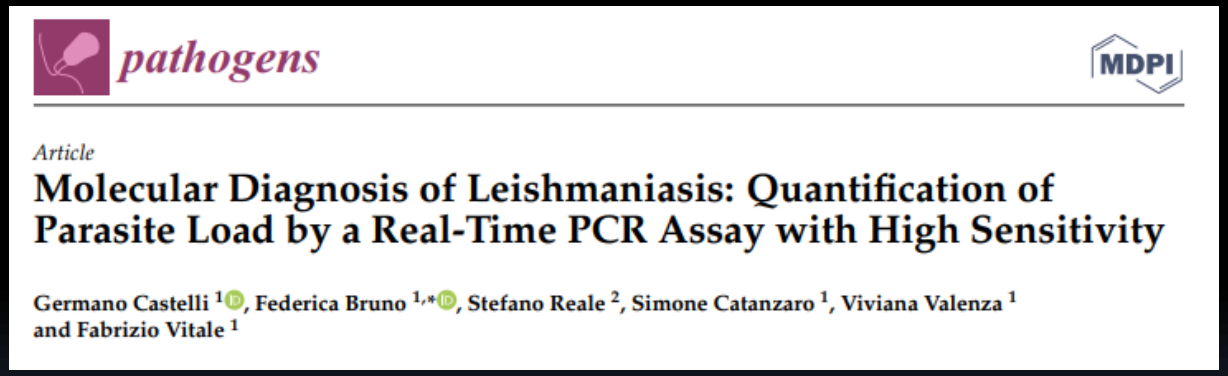
Sensibilità → svantaggio ?

Real time PCR



Misura l'amplificazione in tempo reale durante la fase esponenziale della PCR, quando cioè l'efficienza di amplificazione è influenzata minimamente dalle variabili di reazione, permettendo di ottenere risultati molto più accurati rispetto alla PCR tradizionale "end point"

- Rilevamento della fluorescenza associata all'amplificazione
- Il prodotto di PCR non viene analizzato su gel di agarosio
- Analisi del prodotto di fluorescenza tramite computer



pathogens MDPI

Article

Molecular Diagnosis of Leishmaniasis: Quantification of Parasite Load by a Real-Time PCR Assay with High Sensitivity

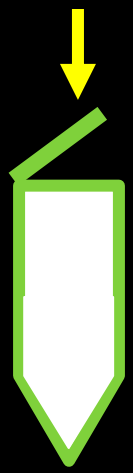
Germano Castelli [1], Federica Bruno [1,*], Stefano Reale [2], Simone Catanzaro [1], Viviana Valenza [1] and Fabrizio Vitale [1]

COMPONENTI DELLA REAZIONE RT-PCR:

1) Sequenza di DNA target
2) Master mix (DNA polimerasi, dNTPs)
3) Due oligonucleotidi (15-25 bp) specifici per la sequenza target (Primers)
4) Sonda (oligonucleotide di 12 bp).

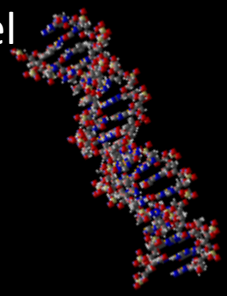
Riassumendo

Sample



Estrazione del DNA

30’



Controllo spettrofotometrico

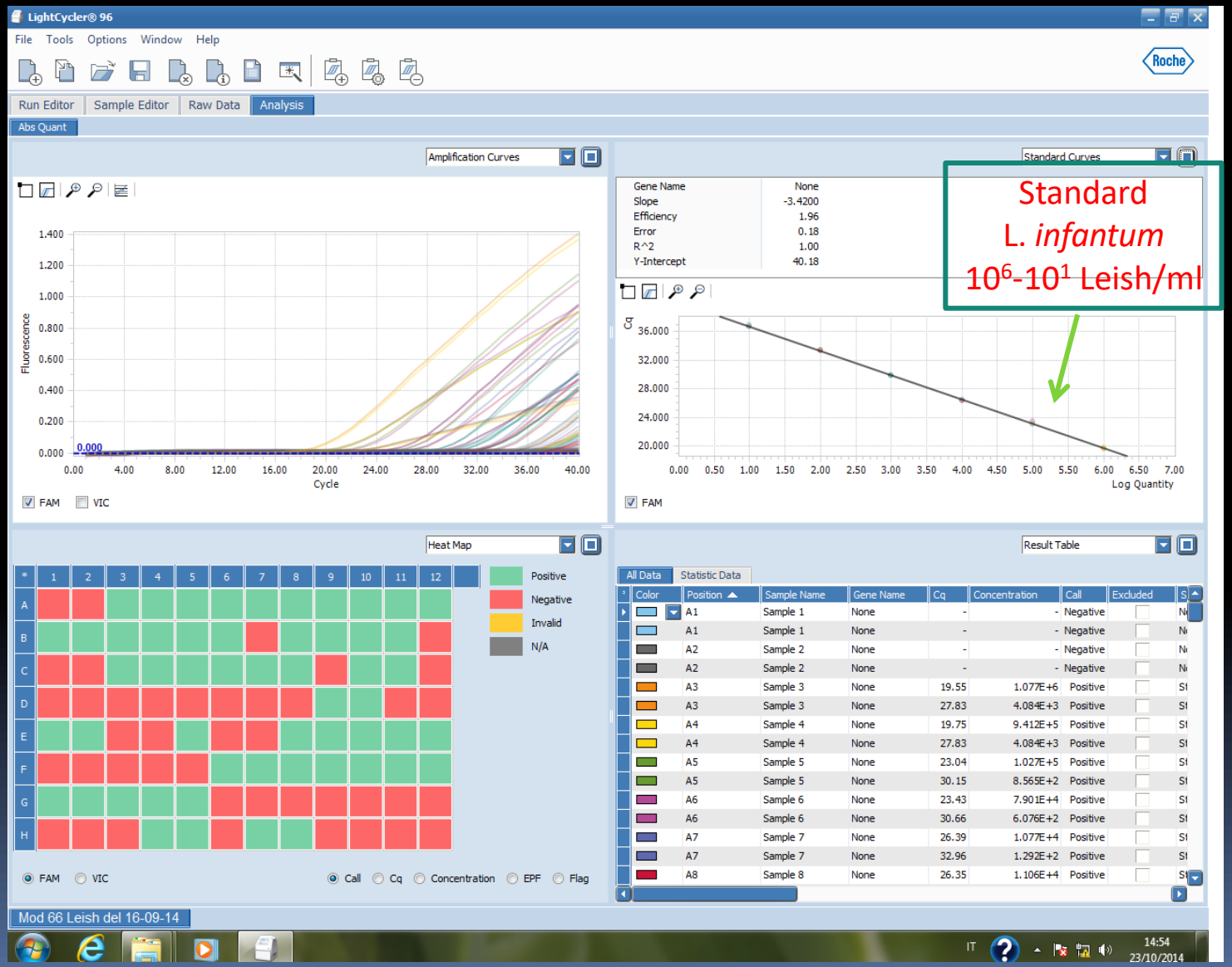
10’

Real time PCR

1h 28’

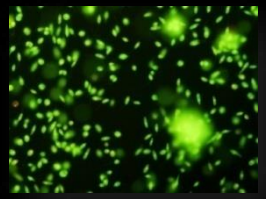
Lettura ed interpretazione dei Dati

Lettura ed interpretazione



DIAGNOSI?? VANTAGGI METODI DIAGNOSTICI SIEROLOGICI:

IFAT



Specificità del 100% per titoli anticorpali superiori a 160

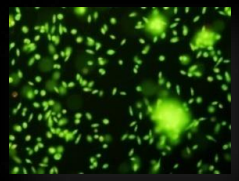
l'IFAT rileva con maggiore precisione gli anticorpi negli stadi avanzati dell'infezione, sia negli animali sintomatici sia in quelli asintomatici

Titoli fra 40 e 80, in assenza di sintomi, devono essere considerati dubbi (controlli mensili)

ELISA

Migliore standardizzazione / interpretazione

DIAGNOSI?? SVANTAGGI METODI DIAGNOSTICI SIEROLOGICI:



Il 5-10% dei cani sviluppano una quantità di anticorpi impossibili da rilevare attraverso i metodi tradizionali (20-50% nell'uomo).

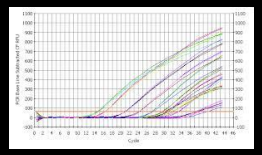
Possibile fase precoce di infezione (primi mesi) o infezione

E' possibile ottenere falsi positivi a causa di reazioni incrociate con altre infezioni

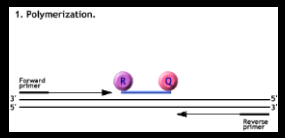
Infezione di transizione, successivamente debellata dal cane

Il territorio influenza l'interpretazione di questi dati

DIAGNOSI?? VANTAGGI REAL TIME PCR:



Alta sensibilità (teoricamente <1 parassita)



Specificità: quasi assoluta (Assenza di interferenze da parte di altri microrganismi)

Automazione e riproducibilità

Tempi di risposta più rapidi e quantizzazione del parassita.

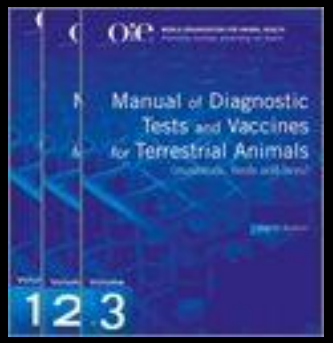
SVANTAGGI:

Scelta della matrice biologica idonea

MATRICI IDONEE:
linfonodo e midollo osseo e raschiati cutanei

Ogni paziente è un "protocollo diagnostico”

World Organisation for Animal Health
Founded as OIE



B. DIAGNOSTIC TECHNIQUES

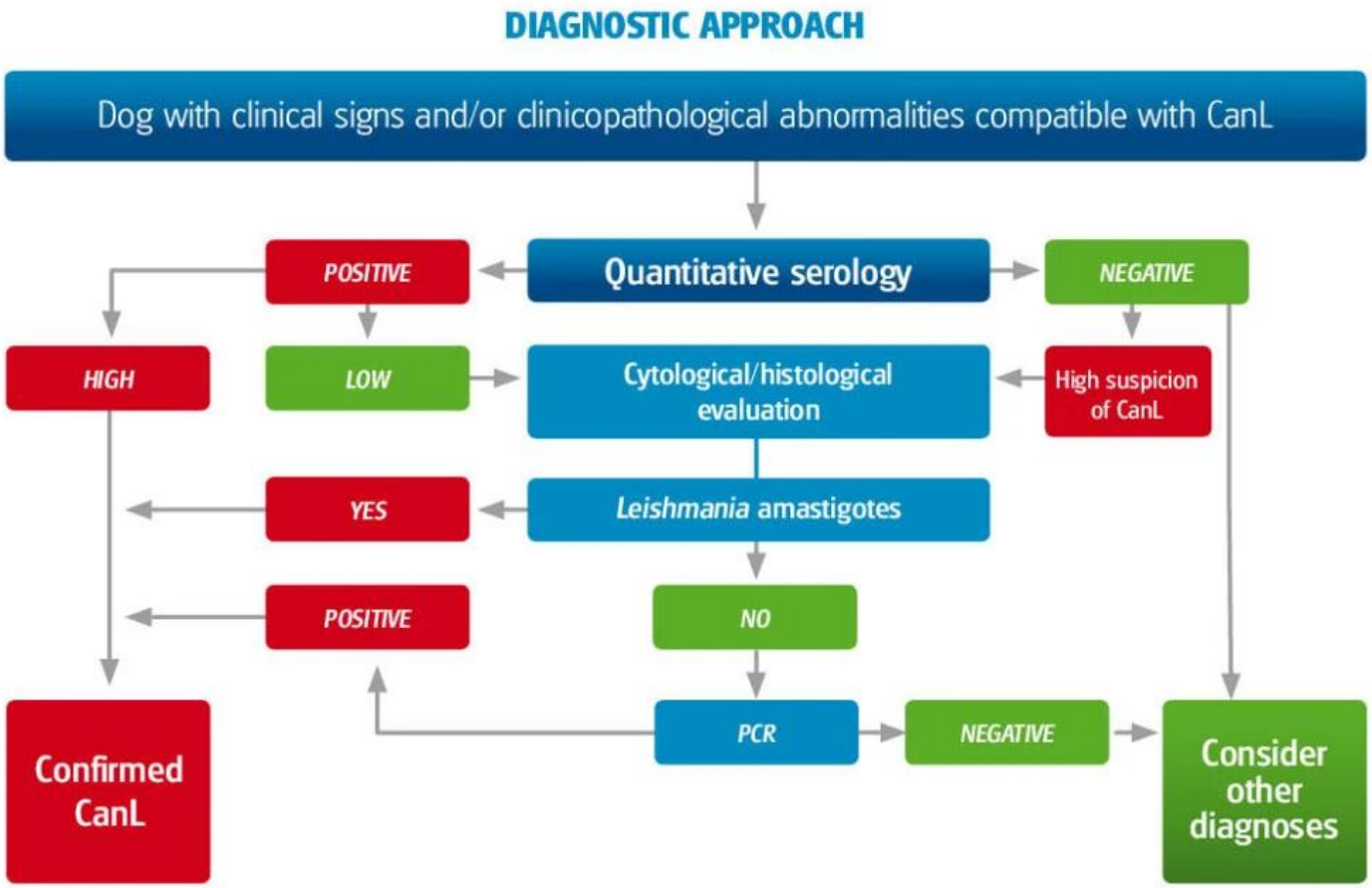
Leishmaniosis diagnosis must be based on an integrated approach that takes into account signalling, medical history, physical findings, clinical and pathological changes, and results of diagnostic tests. The diagnostic methods currently available are described below.

The list and fitness of each test for different purposes are given in Table 1.

Table 1. *Test methods available for diagnosis of leishmaniosis and their purpose*

Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
Detection of the agent[2]						
Cytological examination	–	–	–	++	–	–
Histological examination	–	–		++	–	
Isolation in culture	–	+		++	–	
Molecular methods	++	+++		++	++	
Detection of immune response						
IFAT	+++	++	–	++	+++	–
ELISA	+++	++		++	+++	
Direct agglutination test	++	++		++	++	
Rapid immuno-chromatographic assay	–	–		++	+	

Key: +++ = recommended for this purpose; ++ recommended but has limitations;
+ = suitable in very limited circumstances; – = not appropriate for this purpose.
PCR = polymerase chain reaction; IFAT = Indirect fluorescent antibody test; ELISA = enzyme-linked immunosorbent assay.



Fonte: LeishVet

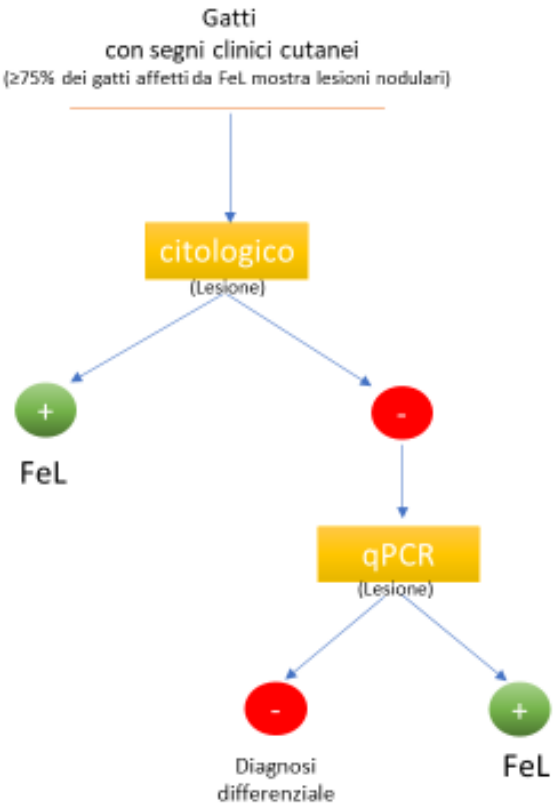
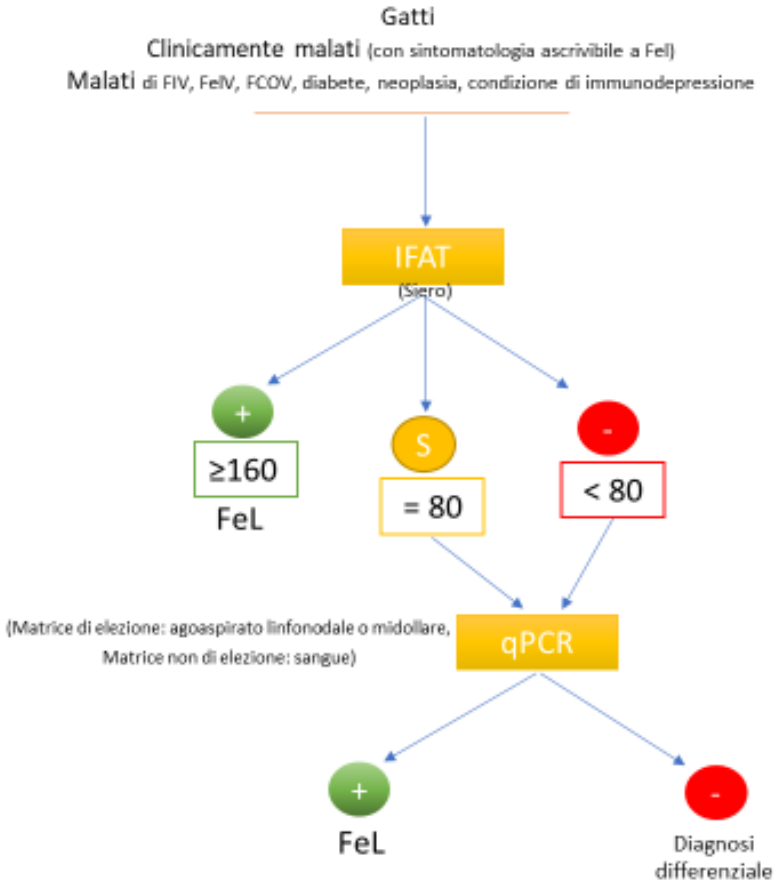
KEY POINTS… Leishmaniosi Felina

Table 4 Frequency (%) of clinical manifestations described in a total of 46 case reports and 15 histopathological case descriptions of feline leishmaniosis from European countries (1989–2014)

Frequency of clinical manifestations (%)		
~50 %	20–30 %	<10 %
Lymph node enlargement	Ocular lesions (mainly uveitis)	Pale mucous membranes
Skin and/or mucocutaneous lesions (mainly ulcerative or nodular)	Oral lesions	Hepatomegaly
	Weight loss	Icterus
	Anorexia	Cachexia
	Lethargy	Fever
	Dehydration	Vomiting
		Diarrhea
		Chronic nasal discharge
		Splenomegaly
		Polyuria/polydipsia
		Itching
		Dyspnea
		Wheezing
		Abortion
		Hypothermia

Pennisi et al. Parasites & Vectors (2015) 8:302 DOI 10.1186/s13071-015-0909-z

La citologia, l'istologia, l'isolamento o la PCR su campioni cutanei, linfonodi, sangue o qualsiasi altro tessuto colpito sono utili per la diagnosi diretta.

La sierologia è utile per valutare le frequenze di infezione.

Algoritmo diagnostico
Leishmaniosi felina (FeL)