# Evaluation of Elisa rk39 for diagnosis of canine visceral leishmaniasis.

**Código: 1468146**

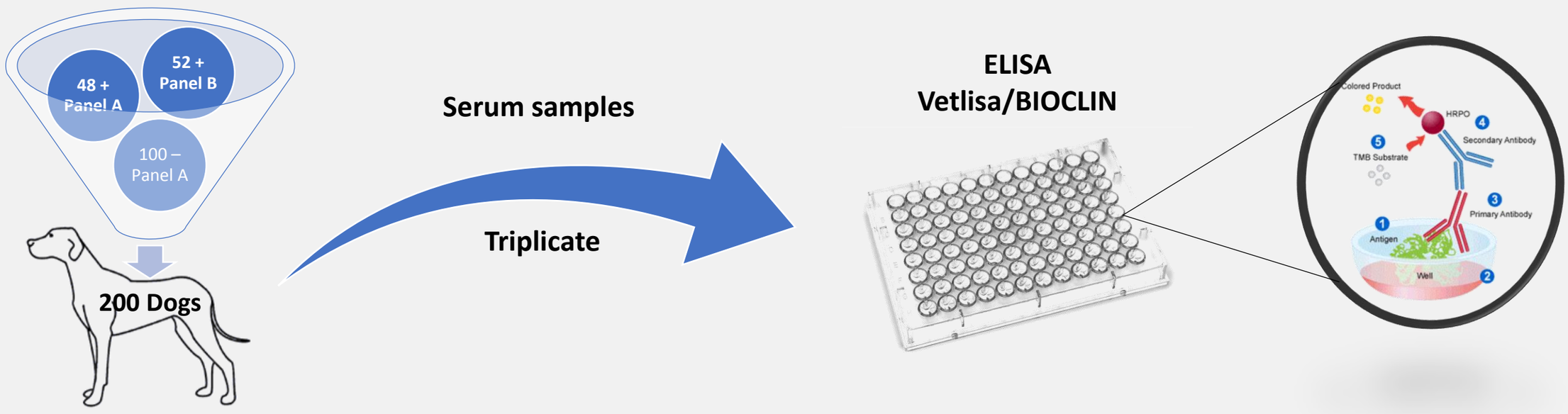
Andreza Pain Marcelino[1]; Fernanda Alvarenga Cardosos Medeiros[2]; Guilherme Loureiro Werneck[3]; Job Alves de Souza Filho[2]; José Ronaldo Barbosa[2]; Gustavo Fontes Paz[4]; Lucas Edel Donato[5]; Fabiano Borges Figueiredo[6]

1. Pesquisadora da Fundação Oswaldo Cruz-Instituto Nacional de Infectologia, Rio de Janeiro-RJ- e-mail: andreza.marcelino@ini.fiocruz.br
2. Fundação Ezequiel Dias-Serviço de Doenças Parasitárias, Belo Horizonte-MG
3. Universidade Estadual do Rio de Janeiro- Instituto de Medicina Social
4. Fundação Oswaldo Cruz, Instituto René Rachou, Belo Horizonte-MG
5. Ministério da Saúde-Coordenação Geral de Doenças Transmissíveis, Brasília-DF
6. Fundação Oswaldo Cruz, Instituto Carlos Chagas, Curitiba-PR

## INTRODUCTION

Visceral leishmaniasis (VL) is caused by protozoan *Leishmania infantum* in dogs and humans and listed as a neglected tropical disease. Brazil is one of the six countries where more than 90% of cases of this disease occur (Organization, 2018). The VL is transmitted in the Americas by the bite of a phlebotomine vector, mainly *Lutzomyia longipalpis* (De Oliveira et al., 2013) and dogs are the main urban reservoirs (Werneck, 2014). Control measures include early diagnosis and treatment of human cases, reactive chemical control of the vector, education activities and control the canine reservoir. Accurate serological diagnosis of canine visceral leishmaniasis (CVL) is of major importance to the epidemi ological surveys in endemic areas.

## MATERIAL AND METHODS




**Panel A**. Fragments of healthy skin and, when present, of skin lesions were collected for parasitological culture, immunohistochemistry, and histopathology according to the protocol by Madeira et al. (2006) and Menezes et al. (2013). The parasites that were isolated in culture wer e characterized by isoenzymes based on protocols previously defined by Cupolillo et al. (1994) and identify of *L. infantum.*
**Panel B**. Fragments of healthy skin, popliteal lymph node and bone marrow aspirates were collected for parasitological culture and direct ex ams. In addition, postmortem immunohistochemistry and PCR examinations allowed for the assessment of infection according to the protoc ol by Tafuri et al. (2004) and Volpini et al. (2004) respectively. The characterization was performed to determine the species of CVL in each c ase and to positively identify cases of *L. infantum* by PCR/RFLP.

## RESULTS

In this study, the prototype kit showed a 99% sensitivity (95% CI: 94-100%) and a 100% specificity (95% CI: 96-100%). The sensitivity of the prototype kit did not vary significantly with the clinical status of the dogs. The area under the ROC curve was 0.99. Agreement between re peated tests was perfect (kappa = 1.00).

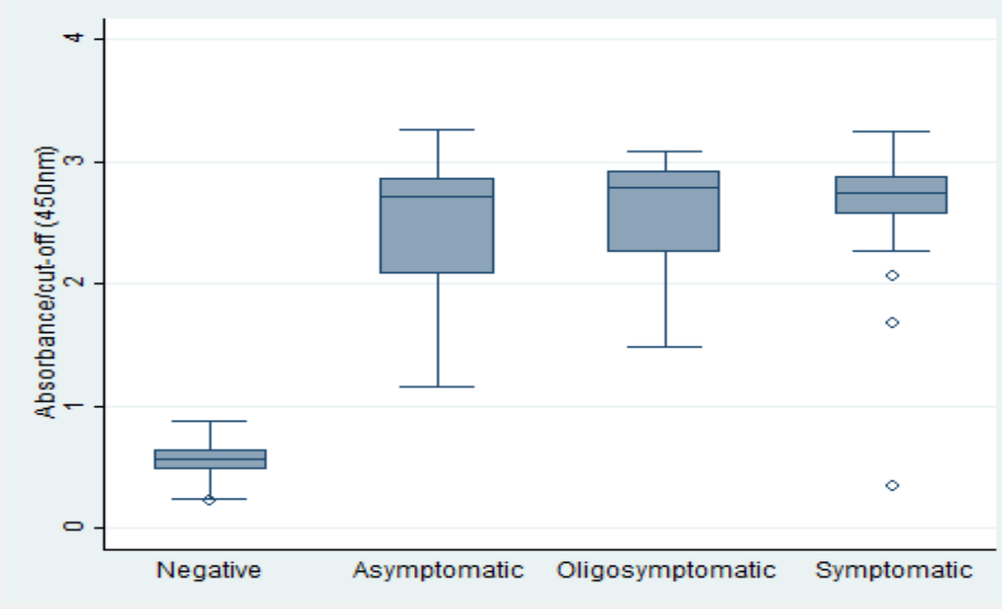



**Figure 1**.ELISA results (Absorbance/Cut-off 450nm) in canine sera with CVL with diferente clinical status.

## CONCLUSION

The results presented here indicate that prototype Vetlisa/BIOCLIN may be an option of ELISA test to be used in association with other methods for the diagnosis of CVL. The increased the options of diagnostic test are important, principal for private laboratories and veterinary services in Brazil, given the increasing demand and public health impact.

## REFERENCES

Organization, W.H., 2018. Leishmaniasis, Epidemiological situation.

De Oliveira, E.F., Silva, E.A., Casaril, A.E., Fernandes, C.E.S., Paranhos Filho, A.C., Gamarra, R.M., Ribeiro, A.A., Brazil, R.P., Oliveira, A.G., 2013. Behavioral Aspects of *Lutzomyia longipalpis* (Diptera: Psychodidae) in Urban Area Endemic for Visceral Leishmaniasis. J. Med. Entomol. 50, 277–284.

Werneck, G.L., 2014. Visceral leishmaniasis in Brazil: Rationale and concerns related to reservoir control. Rev. Saude Publica 48, 851–856.

De Fátima Madeira, M., de O. Schubach, A., Schubach, T.M.P., Pereira, S.A., Figueiredo, F.B., Baptista, C., Leal, C.A., Melo, C.X., Confort, E.M., Marzochi, M.C.A., 2006. Post mortem parasitological evaluation of dogs seroreactive for *Leishmania* from Rio de Janeiro, Brazil. Vet. Parasitol. 138, 366–370.

Tafuri, W.L., Santos, R. de L., Arantes, R.M.E., Gonçalves, R., de Melo, M.N., Michalick, M.S.M., Tafuri, W.L., 2004. An alternative immunohistochemical method for detecting *Leishmania* amastigotes in paraffin-embedded canine tissues. J. Immunol. Methods 292, 17–23