



"Specific antibody" as an indicator for reliable detection of cutaneous forms of Leishmania infection in different endemic areas

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Background

More than 90% of the world's cases of CL are in Afghanistan, Algeria, Brazil, Iran, Iraq, Peru, Saudi Arabia and Syria. Nearly 1.5 million new CL cases occur every year and several Leishmania species are incriminated [Box -1]. CL is debilitating, disfiguring and economically important. It has a DALY burden of nearly one million. Recently CL infection has flared-up in Afghanistan due to war, civil unrest and refugee movements and an estimated 200,000 cases have occurred in Kabul. Unexpected and very high incidence of CL occurred in military personnel during recent Iraq war. Detection of infection before the onset of clinical symptoms is required

Objectives:

1. To develop a reliable and specific serology test (Antibody detection ELISA) uniformly applicable to all CL-endemic countries.
2. To provide a reliable tool for epidemiological studies.
3. To identify individuals exposed to infection.
4. To provide a reliable preclinical tool for detection of CL infection.

Antibody detection ELISA

- Reliability of antibody detection depends upon the techniques and specific reagents employed in the assay (Fig 1).
- We constructed an antibody detection ELISA based on the exo-antigens (secreted, excreted) derived from Leishmania promastigotes.
- A special medium free from serum and proteins is used for harvesting exoantigens.
- Assay is based on indirect detection of specific IgG in serum and blood samples.
- Anti-human monoclonal IgG – HRP conjugate is used to overcome non-specific reactivity.
- TMB was employed as a substrate to increase the sensitivity of the assay.

Observations:

Step 1: Initially we used exo-antigens from one species (*L. mexicana*) for detecting antibodies in serum samples. Exo-antigens of *L. mexicana* were found to be sensitive for samples from Brazil, Mexico but not found to be sensitive enough for other endemic areas such as Colombia and Panama.

Step 2: Then we used a cocktail of antigens from three species (*L. mexicana*, *L. tropica*, and *L. panamensis*) and tested the samples. Cocktail antigens were found to be satisfactory.

Further we examined the incorporation of exoantigens from *L. major* and *L. braziliensis*. No further improvement was seen.

Step 3: A three species cocktail antigen was found to be satisfactory for testing the samples from different endemic areas (Fig 2).

The CL-IgG ELISA was further tested with defined clinically proven samples from patients showing ulcerous, plaque, papular, verruciform and nodular conditions. Our ELISA assay has detected antibody levels in different forms of cutaneous lesions (Fig 3).

Future work:

- Present assay is found to be useful for testing CL samples in different endemic areas.
- Limited work has been done on possible cross reactivity with the co-endemic infections (Fig 2).
- Additional studies are planned to investigate the degree of cross reactivity with the Chagas infection.

Conclusions

- A uniform serology testing method is described for detecting different forms of cutaneous leishmaniasis.
- The ELISA test is based on the cocktail of antigens obtained from three major cutaneous leishmania species.
- Appropriate reagents are employed to construct highly reliable, sensitive and specific antibody detection tool.
- Serum samples from different endemic areas (Brazil, Panama, Peru, Colombia, and Mexico) were tested and evaluated. A very high level of sensitivity was observed. This test is useful for detecting all clinical forms of cutaneous Leishmaniasis.
- This test designated CL-IgG CELISA is in commercial format and can be supplied in the form of ready-to-use-kits.
- The kit can be used for epidemiological investigations.
- CL-IgG CELISA is clinically useful for determining the exposure to infection and for patient management.



Fig 1

Box 1: Cutaneous Leishmania parasites

- Old World Species
L. tropica, *L. major*, *L. aethiopica*
- New world species
mexicana complex:
L. mexicana, *L. amazonensis*
braziliensis complex:
L. braziliensis, *L. peruviana*,
L. venezuelensis, *L. panamensis*
L. guyanensis,

Cutaneous Leish specific IgG detection ELISA

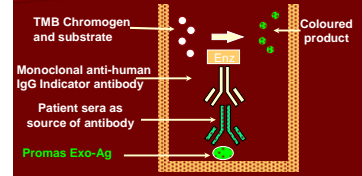
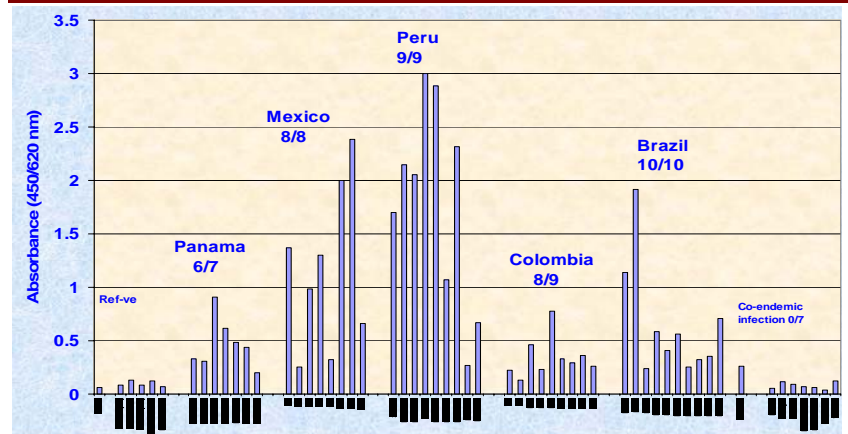


Fig 2

CL-Testing sera from different endemic areas



Detection of specific IgG Antibody in CL patients showing different clinical symptoms (Colombia : Diana Isaza)

Fig 3

