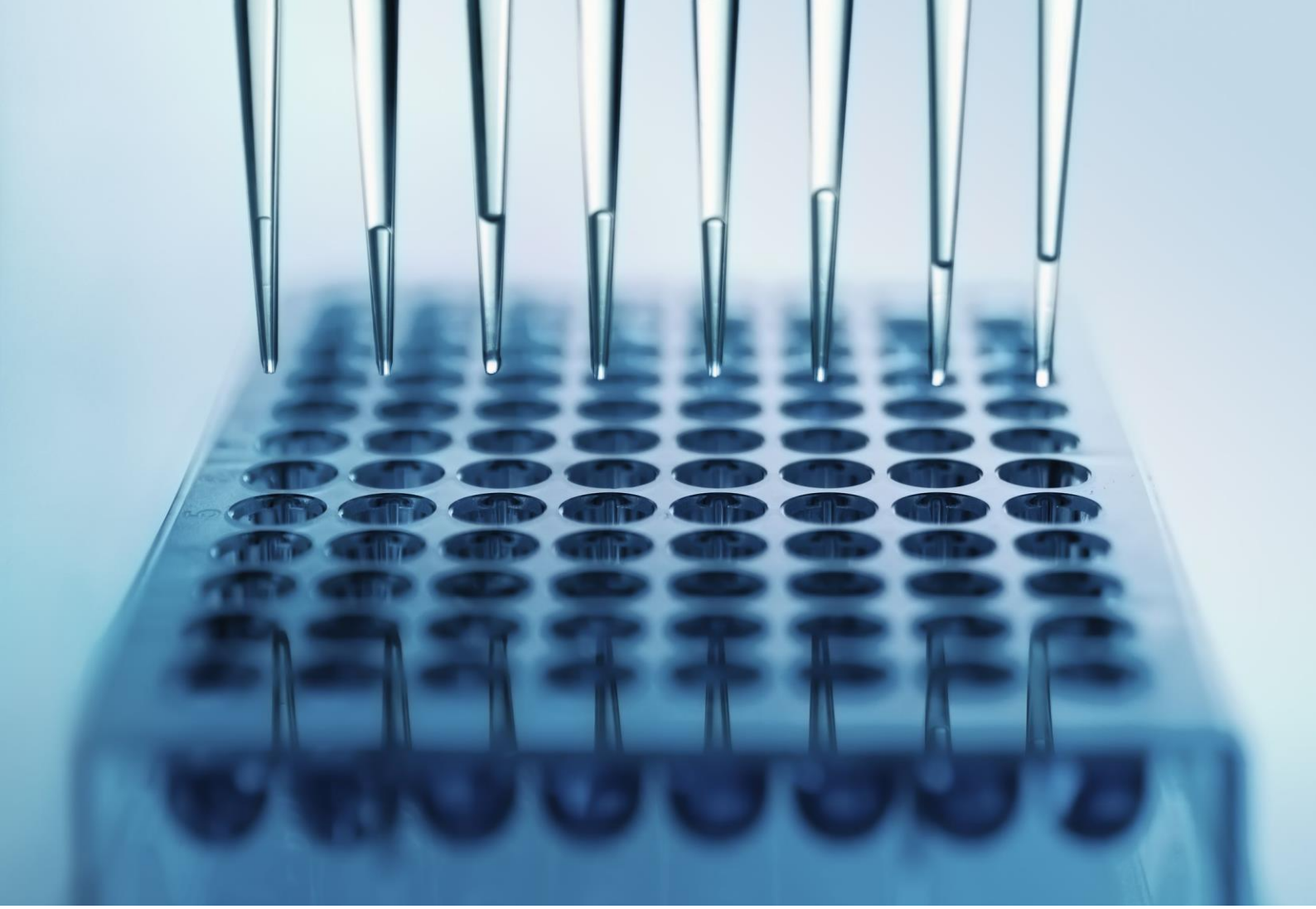

Technical Reports

LEISHMANIA RECOMBINANT ANTIGENS KMP11 AND K39

rekom biotech

The aim of the research presented in this technical report is to show the evaluation of the *Leishmania* recombinant antigens KMP-11 and K39 produced in Rekom Biotech in a diagnostic assay, their purity, integrity and specificity/sensitivity in an "in house" ELISA test.

Leishmaniasis is a disease caused by protozoan parasites transmitted through the bites of infected sandflies like *Phlebotomus* and *Lutzomya*. This disease has a wide range of clinical symptoms. The different manifestations of the disease arise from infections with different species of *Leishmania*. This parasite belongs to the genus *Leishmania* and the Trypanosomatidae family. This disease, due to its zoonotic nature can affect to humans and dogs, being transmitted by wild animals as asymptomatic reservoirs. Leishmaniosis causes different clinical manifestations ranging from self-healing cutaneous lesions (CL), mucosal lesions (MCL) to fatal visceral infections (VL). Visceral leishmaniasis predominates in Africa, America and South-East Asia regions. Cutaneous leishmaniasis predominates in the Eastern Mediterranean and American regions; and mucocutaneous leishmaniasis occurs mainly in the region of the Americas (figure 1).

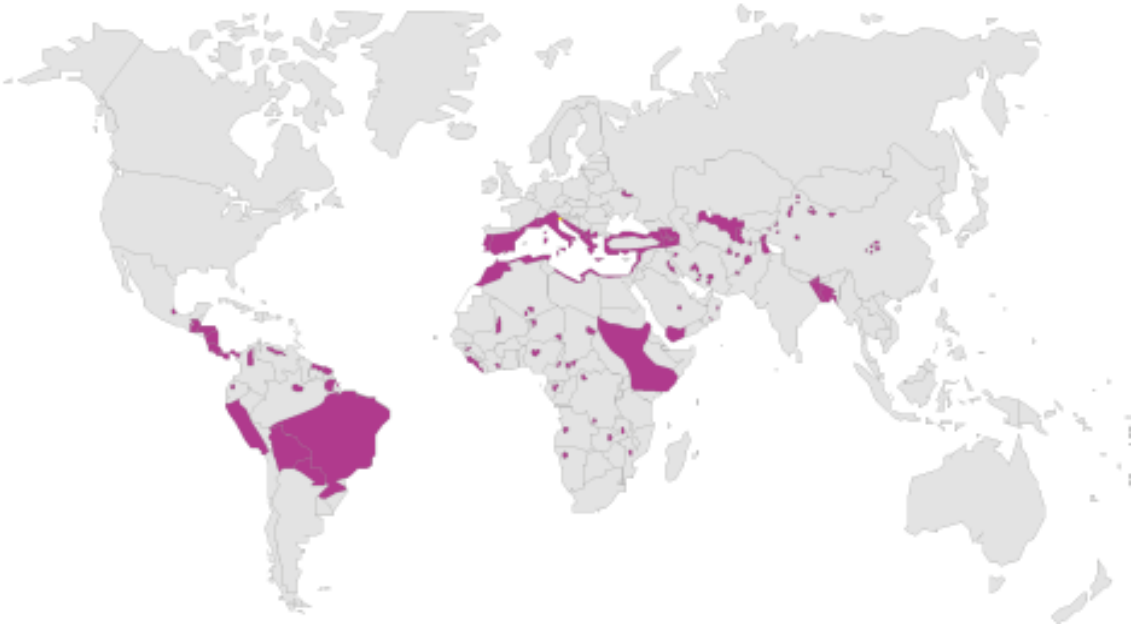

Figure 1. Geographical distribution of cutaneous and mucocutaneous leishmaniasis in the Old and New World.

Adapted from WHO: http://www.who.int/leishmaniasis/leishmaniasis_maps/en/index.html

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The protein **KMP-11** is a kinetoplastid membrane protein of 11 kDa. It has been described that the mayority of individuals with leishmania infection without disease did not have antibodies to KMP-11, therefore serological tests with these recombinant antigen may be helpful as tools to determine therapeutic responses for VL, being the detection of antibodies to KMP11 helpful to differentiate subclinical Leishmania infection from active VL (Passos *et al.*, 2005).

The protein **K39** is a repetitive immunodominant epitope in a kinesin-related protein that is highly conserved among viscerotropic leishmania species. It has been described that a rK39 ELISA is sensitive and specific for serodiagnosis of human and canine VL (Scalone *et al.*, 2002).

Both antigens were produced as recombinant proteins by Rekom Biotech, showing a high purity and integrity as it is show in figure 2 and 3. Both recombinant antigens can also be obtained biotinylated in order to bind them to streptavidine binded-nanoparticule or to other plastic surfaces.

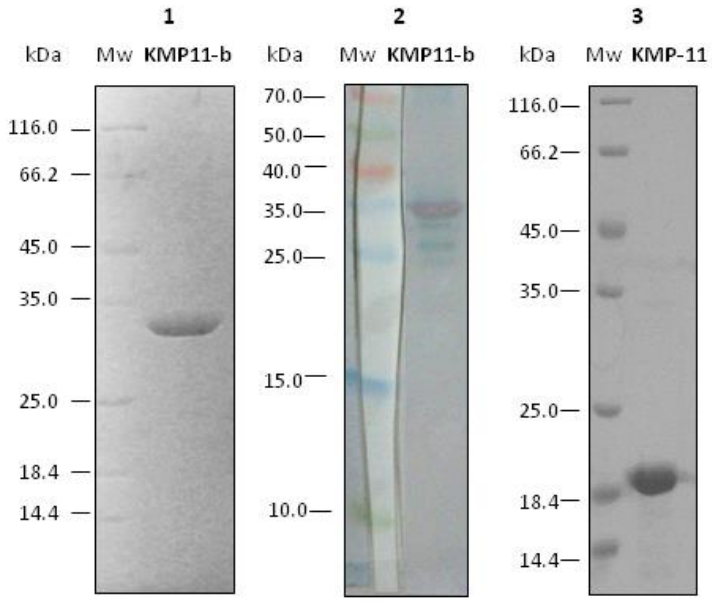

Figure 2. SDS-PAGE and western blot analysis of the recombinant antigen KMP-11 of Leishmania: 5 µl of the biotinylated recombinant antigen RAG0026 in a SDS-PAGE (1) and in WB with HRP-conjugated streptavidine (2); 5 µl of the non-biotinylated recombinant antigen RAG0038 in a SDS-PAGE (3).

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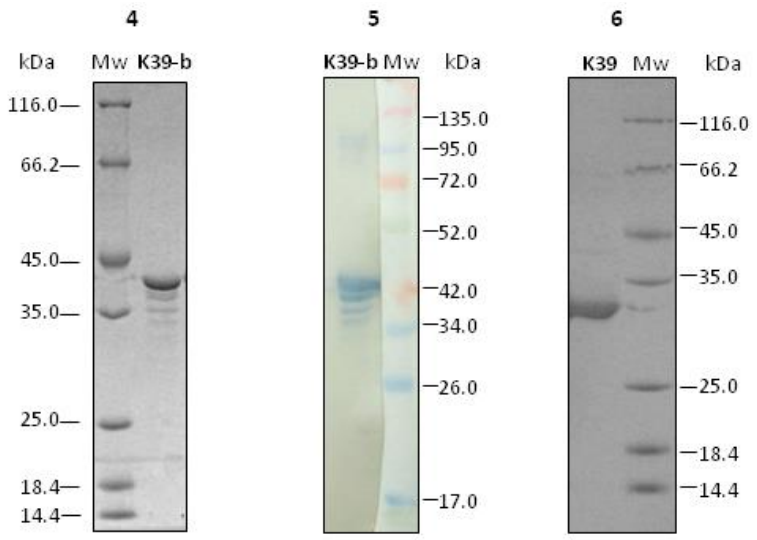

Figure 3. SDS-PAGE and western blot analysis of the recombinant antigen K39 of Leishmania: 1 µl of the biotinylated recombinant antigen RAG0039 in a SDS-PAGE (4) and in WB with HRP-conjugated streptavidine (5); 3 µl of the non-biotinylated recombinant antigen RAG0061 in a SDS-PAGE (6).

Rekom Biotech´s Leishmania antigens, non-biotinylated K39 (RAG0061) and non-biotinylated KMP11 (RAG0038) were evaluated together by an "in house" ELISA assay performed by Dr. V I Bishor (Ubio Biotechnology Systems Pvt Ltd) with the following specifications:

- Coating: KMP11 and K39 together, 250 ng of each antigen per well (total 500 ng/well) in carbonate/bicarbonate buffer.
- Specimen sera are diluted 1:100 using serum diluent buffer.
- The detection is performed by using anti Human IgG HRP (Jackson Immunoresearch, USA), 1:20,000 or 1: 50,000 in propietary conjugate diluent.
- Substrate is TMB followed by stop solution. The readings were taken in Biorad Plate Reader. The Mean OD values of 8 positive and 9 negative sera were given below.

Obtained experimental data (table I):

anti Human IgG HRP		
1/20,000	1/50,000	pre-validated sera
2.35	1.197	Positive
2.48	1.436	Positive
2.13	1.31	Positive
1.8	0.971	Positive
1.85	0.89	Positive
2.21	1.125	Positive
0.91	0.461	Positive

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anti Human IgG HRP		
1/20,000	1/50,000	pre-validated sera
0.98	0.482	Positive
0.42	0.222	Negative
0.25	0.125	Negative
0.4	0.21	Negative
0.43	0.22	Negative
0.42	0.21	Negative
0.41	0.201	Negative
0.39	0.182	Negative
0.385	0.195	Negative
0.39	0.203	Negative
0.06	0.054	BUFFER (0.2% BSA)

Table I. Optical density at 450/620 nm for positive and negative **IgG** sera. The "in house" ELISA assay was performed with plates coated with both recombinant antigens at the same time: KMP-11 and K39.

The resulted OD were plotted in order to analyze the results:

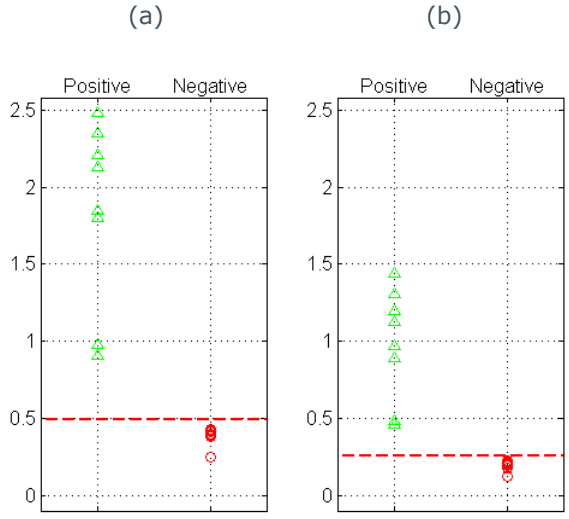

Figure 4. Scatter charts of absorbance values in sera from Leishmania infected patients and healthy controls for IgG antibodies. The test results were plotted as optical densities at 450/620 nm. The cutoff line for positive diagnosis is drawn at a value that equals the sum of the mean and two times the standard deviation for healthy samples, obtaining a value of 0.4946 for a dilution 1:20,000 of the anti-human IgG HRP (a) and a cut-off of 0.2542 for a dilution 1:50,000 of the anti-human IgG HRP (b).

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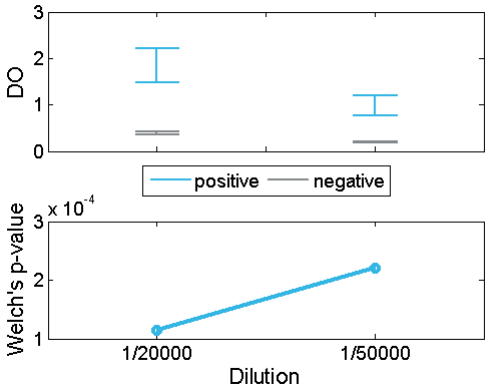

Figure 5. In this plot, the optical density (450/620 nm) for positive (blue) and negative (gray) sera is compared for each dilution of anti-human IgG HRP. An appropriate statistical test of significance for the comparison of means between both groups, the Welch's test, was employed. Eligible dilutions for the anti-human IgG HRP should present statistically significant differences between positive and negative sera. This happens when the intervals at the top do not overlap and, equivalently, when the p-value at the bottom is below 0.05. In the present figure, the two p-values are below 0.05 and thus the intervals do not overlap. Therefore, any of the dilutions of the conjugated IgG can be used to distinguish between positive and negative sera.

CONCLUSION

The ELISA method used in the present study was designed to evaluate the accuracy of both leishmania recombinant antigens working together in an immunoassay. We suggest that the combination of both antigens could be suitable for the identification of the disease.

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