

## Enzyme-Linked Immunosorbent Assay for IgG Serological Diagnosis of Human Babesiosis

### Introduction

Human babesiosis is an emerging tick-borne disease that may be life-threatening. Laboratory tests for babesiosis include blood smear examination, inoculation of hamsters with patient blood, and immunofluorescent antibody assays (IFA). These methods are either relatively insensitive, time-consuming, labor-intensive, or expensive. Our laboratory has optimized a PCR reaction for the direct detection of the genetic material of *Babesia microti* in clinical specimens. The objective of the present study was to develop an ELISA for the detection of the long lasting human immune system response, or IgG antibodies, to *Babesia* antigen.

### Materials and Methods

*Babesia* antigens were harvested from hamster blood experimentally infected with *Babesia microti*, lysed, and used as a coating antigen in microtiter plates. The coating concentration, serum dilution, and conjugate antibody dilution were determined by checkerboard tests for optimal differentiation of patient sera from normal control sera. A panel of 23 well characterized serum samples determined to be positive for Babesia exposure by multiple methods including a combination of blood smear, PCR, IFA, and clinical evaluation along with a panel of 23 negative serum samples from healthy donors were used to evaluate the performance of the established ELISA method.

Microtiter plates were coated with *Babesia microti* antigen and reacted with human sera and peroxidase-conjugated goat anti-human IgG ( $\gamma$ -chain). The specific immune sandwich was detected by addition of the peroxidase substrate tetramethyl benzidine (TMB). The absorbance value of each well was measured at 450 nm utilizing a microtiter plate spectrophotometer. Two reference wells containing all reagents except serum were included in each plate as blank controls. The ELISA index of each test serum was calculated as the absorbance value of sample serum divided by the absorbance value of calibrator control.

Table 1: ELISA IgG Result Interpretation

ELISA Index	Interpretation
< 0.9	Negative
0.9 - 1.1	Equivocal
> 1.1	Positive

Receiver Operating Characteristic (ROC) curve analysis was used to evaluate the performance of the established methods and determine the optimum cutoff point of the ELISA index. The relative sensitivity of the ELISA assay was calculated as the percentage of the well characterized positive serum samples that were positive in our ELISA assay. The relative specificity of the ELISA assay was calculated as the percentage of negative serum samples that were negative in ELISA assay. The confidence intervals (CI) for the area under the ROC curve, sensitivity, and specificity were calculated using the binormal distribution at a level of 95%.

### References

1. Benach et al. (1985). *J Infect Dis* **152**, 473-7.
2. Eskow et al. (2001). *Arch Neurol* **58**, 1357-63.
3. Krause et al. (1992). *Pediatrics* **89**, 1045-8.
4. Krause et al. (1996). *Jama* **275**, 1657-60.
5. Adelson (2003). Conference on Lyme Disease and other Tick-Borne Disorders.(1992).

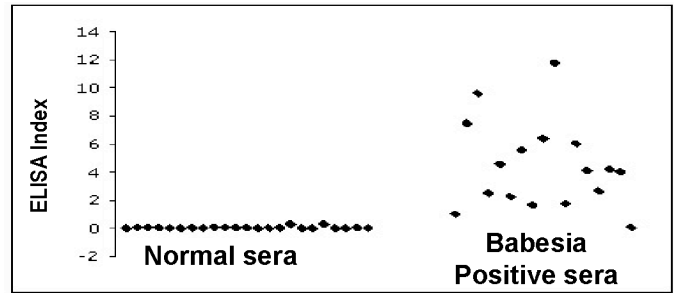


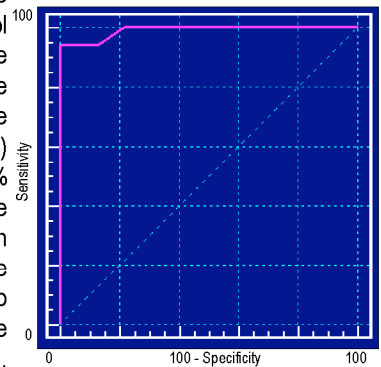
Figure 1: Distribution of ELISA indices

### Results and Discussion

The distribution of ELISA indices of serum samples from the well characterized *Babesia* positive (PC) and negative (NC) control groups is shown in Figure 1.

The **sensitivity** and **specificity** of the ELISA relative to IFA test were **94.1%** (95% CI, 71.2% - 99.0%) and **100%** (95% CI, 100% - 100%), respectively. The agreement of results between ELISA and the *Babesia* positive sera was 97.5%. According to the ROC curve analysis (Figure 2), the AUC was 0.990 (95% CI, 0.892 – 0.990). Serum samples from patients with *Borrelia burgdorferi*, *Bartonella henselae*, *Bartonella quintana*, Dengue virus, or West Nile virus antibodies were tested for cross-reactivity in the *Babesia* ELISA.

Figure 2



One out of the five Lyme Disease samples and one out of the eight

Table 2: Cross Reactivity

Category	ELISA Negative
Lyme Disease	4 / 5
<i>B. henselae</i>	5 / 5
<i>B. quintana</i>	2 / 3
Dengue Virus	3 / 3
West Nile Virus	2 / 2

*Bartonella* samples had ELISA index values above the cutoff. It has been estimated that coinfection with *B. microti* in Lyme Disease patients could be as high as 13% (1,3,4). Coinfection of *Bartonella* and *Borrelia burgdorferi* had been reported by our laboratory in humans (2) and in *Ixodes scapularis* (5).

### Conclusion

Results suggest that the developed *Babesia* ELISA can provide an accurate assessment for the detection of specific antibodies in patients exposed to *Babesia microti*. The development of this new immunological assay has been submitted for publication to a peer-review scientific journal. An IgM ELISA is currently under development.