

NEW PCR ASSAYS FOR DIRECT DETECTION OF:

- **BARTONELLA HENSELAE**
- **BABESIA WA-1**

PCR Assay

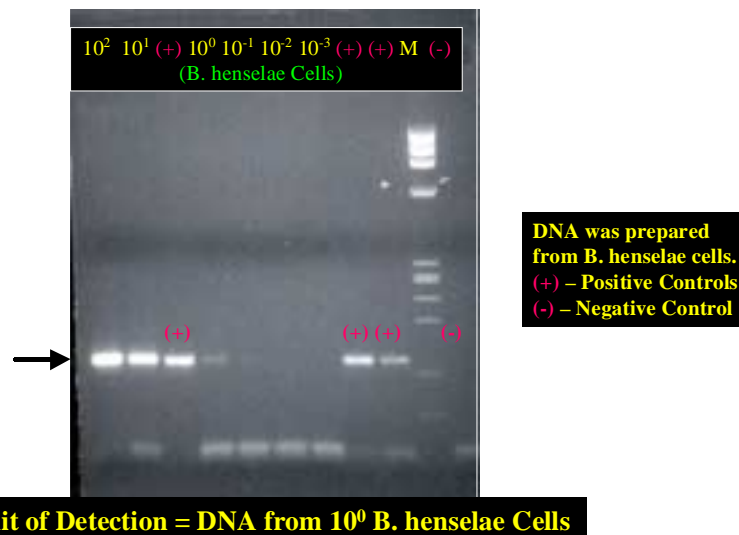
The diagnostic assay for the detection of *B. henselae* or the Babesia WA-1 is a four step PCR procedure that detects the organism in whole blood samples. The first step of the assay specifically removes the “common PCR inhibitors” from whole blood, and at the same time selects and purifies the DNA fragment of interest. In the second step, the purified fragment is PCR amplified with the specific primers. In the third step, the PCR amplified DNA fragment is detected by agarose gel electrophoresis. The fourth step, Southern Blot and/or Dot Blot analysis, is a confirmation step for the organism. Combinations of the four steps provide very high specificity and sensitivity.

Bartonella henselae by PCR

Bartonella henselae is commonly associated with Cat Scratch Disease (CSD) and Bacillary Angiomatosis (BA) in immunocompromised patients. Recently, Eskow et al (2001) presented evidence that *B. henselae* is also a potential human tick-borne pathogen and that it can be a co-infecting agent of the central nervous system in the presence of neuroborreliosis. They found elevated levels of *B. henselae*-specific antibodies in patients with neuroborreliosis using the immunofluorescent assay. *B. henselae*-specific DNA was detected in their blood by PCR. We have developed a PCR assay that detects *B. henselae* specific rDNA fragment. The assay is highly sensitive and has no cross-reaction to *Bartonella quintana*.

Limit of Detection: The Limit of Detection of purified *B. henselae* DNA is one *B. henselae* bacterium. This corresponds to approximately 100 *B. henselae* organisms per ml of EDTA whole blood.

Bartonella henselae – Limit of Detection



Bartonella henselae by PCR (continued)

Specificity: The assay is highly specific against non-*B. henselae* Bartonella species (including *B. quintana*), other tick-borne and blood borne pathogens.

The high specificity is provided by: (1) one of the PCR amplification primers, and (2) the probe used for Southern Blot analysis. This PCR primer has 100% homology to *Bartonella* DNA sequence currently in the GENE BANK, but not to non-*Bartonella* bacteria and human DNA sequences. Based on the sequence information, the *B. henselae* probe only “hybridizes” or “binds” to *B. henselae* PCR product.

Bartonella henselae PCR Specificity Study

DNA Tested	Result	DNA Tested	Result
Human Monocytic Ehrlichia	Negative	Coxiella brunetii	Negative
Leshmania donovani	Negative	Ehrlichia sennetsu	Negative
Trypanosoma cruzi	Negative	Orientia tsutsugamushi	Negative
Trypanosoma brucei	Negative	Rickettsia prowazekii	Negative
Toxoplasma gondii	Negative	West Nile Virus	Negative
Plasmodium falciparum	Negative	Ehrlichia equi	Negative
Rickettsia rickettsii	Negative	Babesia WA-1	Negative
Rickettsia typhi	Negative	Babesia microti	Negative
Rickettsia conorii	Negative	Bartonella henselae Positive Control	Positive
Bartonella quinta	Negative	Bartonella henselae Negative Control	Negative

The PCR Primers are specific for Bartonella henselae

Reproducibility: The assay is very reproducible. *Bartonella henselae* spiked whole blood samples were tested by the *B. henselae* PCR protocol on three different days. On all three days the Limit of Detection was 100 *B. henselae* organisms per ml of sample.

ORDERING INFORMATION:

TEST NO: 280
Bartonella henselae—PCR
 Specimen Requirement: 1 ml of whole blood—EDTA

TEST NO: 290
 Tick test for *Bartonella henselae* by PCR
 Please contact the office for instructions.

Sample Type	N	SUMMARY	
		B. henselae POS	NEG
Spiked Samples	9	9	0
Whole Blood	9	9	0
Controls	3	0	3
Whole Blood	3	0	3
Bartonella Species	6	4	2
<i>B. quintana</i>	1	0	1
<i>B. clarridggiae</i>	1	0	1
<i>B. elizabethae</i>	1	1	0
<i>B. henselae</i>	3	3	0
Non Bartonella Organisms	17	0	17
Total	35	13	22

BABESIA WA-1 by PCR

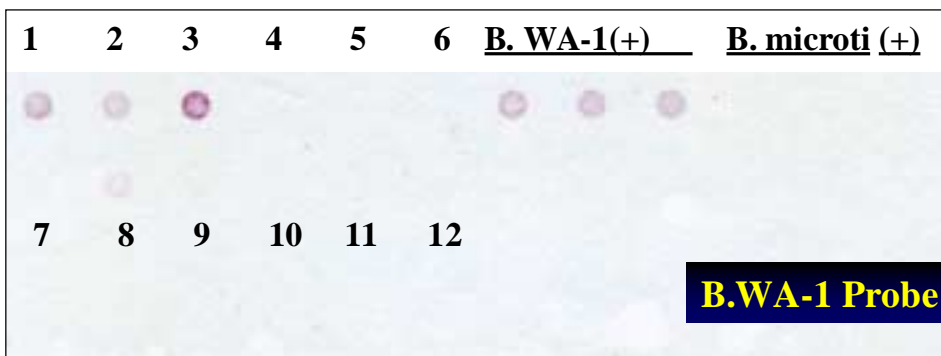
Babesiosis is a tick-borne disease caused by an intraerythrocytic parasite. Currently, four species of *Babesia* are known to infect man. They are *Babesia microti*, *Babesia divergens*, *Babesia MO-1* and *Babesia WA-1*. *B. microti* was thought to be primarily present in Midwest and Eastern regions of the US, but recently cases have been reported from Switzerland. *B. divergens* is found in Europe. *Babesia MO-1*, which is very closely related to *B. divergens*, is found in Missouri. Several cases of *Babesia WA-1* have been reported from the West Coastal regions of the US.

The Giemsa-staining of a whole blood smear is the clinical standard for diagnosing Babesiosis. However, it is neither sensitive nor specific. Currently, IGeneX offers *Babesia* PCR. We have recently developed a highly specific and sensitive PCR based test for detection of *Babesia WA-1*. It is a four step PCR assay that detects *Babesia WA-1* directly from blood samples. It is highly specific and sensitive.

Specificity: The assay is highly specific. There is no cross reaction to *Babesia microti*, *Plasmodium falciparum*, other tick-borne pathogens, or whole blood.

The high specificity is provided by: (1) one of the PCR amplification primers, and (2) the probe used for Dot Bot analysis. This PCR primer has 100% homology to *Babesia WA-1* DNA sequence currently in the GENE BANK, but not to non-*Babesia WA-1* bacteria and human DNA sequences. Based on the sequence information, the *Babesia WA-1* probe only “hybridizes” or “binds” to *Babesia WA-1* PCR product.

Babesia WA-1 PCR Assay Clinical Study



Clinical Study: A set of 12 EDTA whole blood samples from patients were tested by *Babesia WA-1* PCR assay. The set included three *Babesia WA-1* positive samples (#1–3) three *Babesia* negative samples (#4–6), and six *Babesia microti* positive samples (#7–12).

As shown, the *Babesia WA-1* probe only hybridized to samples #1–3, the *Babesia WA-1* positive samples, but not to the *B. microti* or Negative samples.

Limit of Detection: The Limit of Detection of the assay is between 10–100 copies of the ribosomal DNA (rDNA) fragments spiked into EDTA whole blood. Assuming that there are between 100–200 copies of rDNA fragments per parasite, this corresponds to less than one organism per sample tested.

Reproducibility: The assay is very reproducible. *Babesia WA-1* spiked blood samples were tested by the *Babesia WA-1* PCR protocol on three different days. On all three days, the Limit of Detection was 10² rDNA fragments.

ORDERING INFORMATION:

TEST NO: 688

Babesia WA-1—PCR

Specimen Requirement: 1 ml of whole blood—EDTA

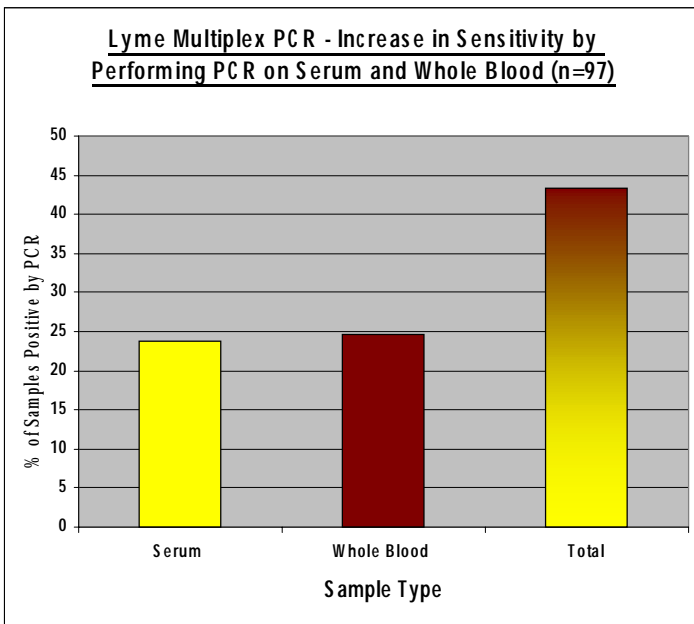
TEST NO: 675

Tick test for *Babesia WA-1* by PCR

Please contact the office for instructions.

Lyme Multiplex PCR—Increase in Assay Sensitivity by Performing Blood and Serum PCR

Lyme Multiplex PCR was performed on whole blood and serum samples of 97 patients suspected of Lyme Disease between February and April 2002. As shown below, 42 (43.3%) of the patients were Lyme PCR positive. Of these 42 positive patients, 24 (24.7%) patients’ whole blood samples were positive and 23 (23.7%) patients’ serum was positive. Only 5 patients had **both** blood and serum samples positive. Based on the data presented below, we recommend that for optimum results for patients suspected of Lyme Disease, the Lyme Multiplex PCR should be performed on both EDTA whole blood and serum samples.



Lyme ELISA: IGeneX vs. Other Laboratories

We have decided that we must be true to our beliefs and knowledge about Lyme disease. Other laboratories require a positive Lyme ELISA before performing the Western Blot. IGeneX has not required that, but we have allowed physicians and other laboratories to order an ELISA by itself. We have now instituted a policy that we will **ONLY** run a Lyme ELISA if a Lyme Western Blot is also requested. The studies by Bakken et.al. (J Clin Microbiol 1997; 35:537–543), clearly indicate the inadequate nature of the current screening tests for Lyme Disease. A screening test should have close to 95% sensitivity. The Western Blot approaches that percentage, while the ELISA does not. Until that changes, we cannot in good faith, run the ELISA test without running the Western Blot.

CNS Index

The CNS Index is a ratio of the CSF Lyme ELISA result divided by the serum Lyme ELISA result. Therefore, the above statement regarding the Lyme ELISA influences the concept and rationale of the CNS index. Because of this, the CNS Index will be discontinued effective July 1, 2002. We feel that the antibody test of choice for the Cerebral Spinal Fluid (CSF) is the Western Blot. Since the CSF has a lower protein concentration than serum, IGeneX always performs the CSF Western Blot using concentrated CSF.

MEDIA COVERAGE

IGeneX, Inc. will be featured in some upcoming media coverage nationwide. We are committed to educate patients, doctors, and the general public about the facts on Lyme Disease and other tick-borne diseases. Interviews with Dr. Harris and some of our valued colleagues, patients, and clients will be aired on various TV news and documentary channels.

PLEASE LET US KNOW WHAT YOU THINK! Your opinion is valuable to us.

It is important to remember that for a complete picture of the CSF, an antibody test (Western Blot) should be combined with the CSF Lyme Dot Antigen Assay and the CSF PCR.

ORDERING INFORMATION:

TEST NO:	Specimen
189— IgG Western Blot for CSF	2 ml CSF
188— IgM Western Blot on CSF	2 ml CSF
810— Lyme Dot Blot Assay on CSF	2 ml CSF
459— Lyme Multiplex PCR on CSF	2 ml CSF