



# NATIONAL VETERINARY LABORATORY

P.O. Box 239, 1Tice Road  
Franklin Lakes, NJ 07417  
877-NVL-LABS (877-685-5227)  
[www.natvetlab.com](http://www.natvetlab.com)

## NEWSLETTER

### What is the Best Test for Detection of *Bartonella* Infection?©

Evelyn E. Zuckerman, Editor

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#### In This Issue:

The Summer, 2009 issue of the NVL Newsletter will discuss the various tests that are now available for detection of *Bartonella* infection. It is imperative that veterinarians have an accurate, practical (blood test), and affordable test.

#### Diagnostic Test Concepts:

In 2006 a WHO Diagnostics Evaluation Expert Panel met and published a major review on evaluating diagnostics.<sup>1</sup> They concluded that diagnostic tests are crucial for the management of patients and for the control of infectious diseases.<sup>1</sup> Accurate diagnostic tests enable patient management and aid in clinical decisions, particularly when clinical signs are not specific enough to allow diagnosis of a particular infection, which often occurs in veterinary medicine. This is especially true for cats with varied inflammatory diseases caused by *Bartonella*. Screening for asymptomatic infection is another major role for accurate diagnostic tests. Many infectious pathogens cause non-specific signs or no signs at all. Undetected and untreated infections often lead to serious long-term complications and may enable the chain of transmission. Surveillance, including verification of the pathogen elimination, is the aim of successful disease control or elimination programs. This was accomplished by the FeLV test and control programs introduced by us in the 1970s and by the FeBart® *Bartonella* WB test and therapy program that we began in 1999.<sup>2,3</sup> Finally, accurate diagnostic tests for pathogens enable epidemiological studies to determine the method of spread and the control of various infectious agents.

There are 2 basic methods, direct or indirect, of detecting any pathogen in animals or humans. Direct methods detect the whole or parts of a pathogen directly in the blood or tissues whereas indirect methods detect the reaction of the animal or human to a pathogen.

#### Direct Detection Methods:

1. Isolation in culture.
2. Detection of antigens of the pathogen.
3. PCR- detection of the nucleic acid (DNA).
4. Visualization by special stains.

#### Indirect Detection Methods:

1. Serology- detection of antibodies against the pathogen.
2. Detection of an immune cellular response against the pathogen- TB & *Bartonella* skin tests.

Good examples of the direct method of detection of a feline pathogen are the FeLV IFA and ELISA tests. In 1972 we developed the first FeLV test, the IFA test, which directly detects viral antigens in leukocytes and platelets.<sup>2</sup> In 1988, while doing research in the Infectious Disease Service at Memorial Sloan Kettering Cancer Center, we developed the first applied blood test for the human retrovirus, HTLV-I, and were part of the scientific team that demonstrated that this newly discovered human retrovirus was transmitted by blood transfusion.<sup>4</sup> These HTLV-I IFA screening and confirmatory western blot tests, for detection of antibodies to the virus, are indirect detection methods.

#### What is the Most Accurate, Practical and Affordable *Bartonella* Screening Test?

Presently there are 5 blood tests available to detect *Bartonella spp.*: Serology- 1-3) western blot, ELISA, and IFA tests, 4) PCR DNA detection, and 5) culture. However, it must be emphasized that *Bartonella* often are only intermittently present in the peripheral blood (bacteremia) of infected animals.<sup>5,6</sup>

#### Culture of Blood?

*Bartonella* are very slow growing and fastidious, requiring special media for growth (Figure 1).<sup>5</sup> Some recommend blood culture as the most reliable test but state that several consecutive cultures are needed since *Bartonella* only circulate intermittently.<sup>5</sup> Isolation is proof of infection (bacteremia) whereas a negative culture may simply have been taken at a time when the organism was not circulating. Cultures can take as long as 6 weeks and are more expensive than serology which makes this technique impractical and not very accurate for practitioners, since weeks in culture often grow contaminants before *Bartonella*. Positive cultures must be confirmed to be *Bartonella* by PCR or antigen analysis which increases the cost significantly.

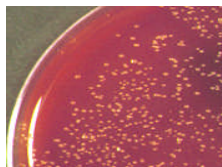


Figure 1. *Bartonella* isolated (tan rounded colonies) from the blood of a 6-month-old kitten. >1,000 *Bartonella/ml* was isolated after 35 days in blood agar culture. Isolation is not a very sensitive assay for detection of *Bartonella* infections.

#### PCR of Blood?

PCR is a very sensitive DNA amplification test for the presence of *Bartonella* DNA but because the bacteria only intermittently circulate, the test does not offer much advantage over culture. Numerous PCR assays have been developed for direct detection of *Bartonella spp.* in cats, dogs and people (Figure 2). A gene fragment specific for either the riboflavin citrate synthase (*ribC*) or a heat shock protein (*htrA*) is demonstrable by PCR in the majority of *Bartonella* infections. DNA is extracted from blood, followed by real-time PCR.<sup>7</sup> A DNA internal control should be multiplexed into each assay to monitor the nucleic acid extraction and the PCR processes for inhibition. A negative result does not rule out the presence of *Bartonella* DNA in quantities below the sensitivity of this assay or the possibility of PCR inhibitors in samples.

#### PCR *Bartonella* Test



Figure 2. PCR test developed in our laboratory to compare our different *Bartonella* isolates. Only 64% of people with cat scratch disease are PCR positive whereas 84% are antibody positive.

There have been many studies with varying results regarding the interpretation of PCR tests for detection of *Bartonella* infection.<sup>8,9,10</sup> Unfortunately, there have been few studies comparing culture, PCR and serology in the same patients. One such comparative study of human patients by an internationally recognized group concluded that:

“The most effective tool for the diagnosis of endocarditis and CSD is specific serology. For patients with endocarditis, the serology had a sensitivity of 97%. Furthermore, 95% of these patients had an antibody titer against *Bartonella sp.* of  $\geq 1:1,600$ . The sensitivity was 90% for patients with *B. henselae* CSD. We found, however, that serology has no value in the diagnosis of BA (bacillary angiomatosis), as only one of seven was positive. This result is probably due to the immunocompromised status of these patients.”<sup>5</sup>

Their findings are summarized in Table 1.

**TABLE 1. Sensitivities of techniques used for the diagnosis of *Bartonella* infections.<sup>5</sup>**

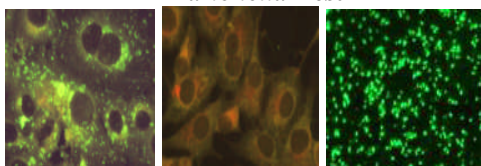
Disease (n)	Result by:			No. of patients
	Culture	PCR	Serology	
BA (7)	+	NT*	--	2
	+	NT	+	1
	--	+	--	4
Endocarditis (38)	+	+	+	18
	+	NT	+	1
	--	NT	+	5
	--	--	+	6
	--	+	+	7
CSD (78)	--	+	--	1
	+	+	+	1
	+	+	--	3
	+	--	--	1
	--	--	+	53
	--	+	+	15
	--	+	--	5

\* NT. not tested.

## Serology?

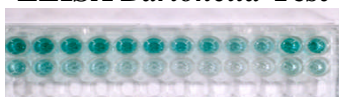
Serologic methods for detection of antibodies directed against *Bartonella* have been employed more than any other technique in the literature for detection of *Bartonella* infections in cats, dogs and people. In 1999, after 5 years of research comparing culture isolation with serology, our data showed that the most accurate and reproducible test for detection of *Bartonella* infection in cats is the serologic detection of antibodies to the bacteria using the western blot method (WB).<sup>3</sup> ELISA and IFA antibody tests result in an all or none color development (Figures 3 & 4) whereas WB tests result in a specific multiple antibody profile (Figures 5 & 6). Multiple studies have shown that the WB is the most specific and sensitive serologic assay. Since the introduction of our FeBart<sup>®</sup> *Bartonella* WB test, various groups and laboratories have recommended different tests. Some state that culture is the "Gold Standard" test while others recommend PCR. Any test has its strengths and weaknesses but large, well designed, comparative studies of serology and PCR for *Bartonella* detection in cats have not been performed. Several veterinary laboratories now offer IFA and ELISA antibody and PCR tests. However, none of them offer therapy evaluation tests.

### IFA *Bartonella* Test



**Figure 3. The IFA *Bartonella* test developed in our laboratory was not as accurate or as reproducible as our western blot test for detecting *Bartonella* infected cats. Left panel: antibody-positive test showing apple green fluorescence of *Bartonella* in infected cells. Middle panel: antibody-negative test- no fluorescing bacteria. Right panel: positive fluorescing bacterial preparation.**

### ELISA *Bartonella* Test

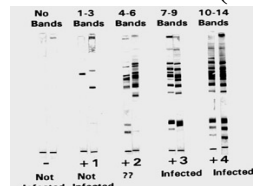


**Figure 4. Our *Bartonella* ELISA test was the least accurate and reproducible serological assay.**

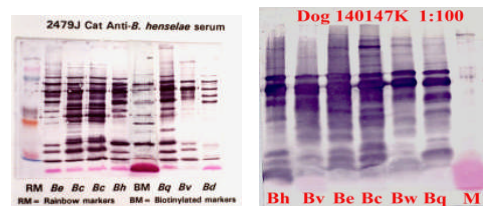
## Western Blot *Bartonella* Test:

WB detection of a profile of at least 6 antibodies against *Bartonella* proteins (Figure 5) insures the specificity of the technique.<sup>3</sup> We are able to detect infection with all 6 known species infecting cats and dogs since the sera of infected cats and dogs are cross-reactive for all infecting *Bartonella* species (Figure 6). In addition, cross-reacting antibodies to other bacteria are discounted by WB. As with other chronic pathogens such as FIV, HIV, and *Borrelia* (Lyme), antibodies to *Bartonella* co-exist with the organism and rarely are sterilizing of the infection. However, about 8% of *Bartonella* infected cats do not produce antibodies and are thus negative by WB. We also utilize the WB technique for dogs (Figures 5 & 6). WB tests are accurate, reproducible, require only 24 hours, and are cost effective.

## FeBart<sup>®</sup> Western Blot (WB) Test



**Figure 5. Grading system for the FeBart<sup>®</sup> Western Blot Test. - and +1: not infected, +2: 30% of cats infected, +3 & +4: infected.<sup>3</sup>**



**Figure 6 Left. FeBart<sup>®</sup> Western Blot test is able to detect all 6 feline *Bartonella* and is able to detect the cross-reacting proteins from other *Bartonella*. This figure shows the detection of proteins from *Be B. elizabethae*, *Bc B. clarridgeiae*, *Bb B. henselae*, *Bq B. quintana*, *Bv B. vinsonii*, and *Bd B. weissii* by an infected cat's serum.**

**Figure 6 Right. Seropositive dog showing cross-reactivity to 6 *Bartonella* species: *B. henselae*, *B. vinsonii*, *B. elizabethae*, *B. clarridgeiae*, *B. weissii* (bovis), and *B. quintana* (M= mol. wt. markers).**

## Therapy Evaluation Test:

The comparative WB titration test is the **ONLY** way to determine if therapy has eliminated *Bartonella* infection. The regular screening WB will remain positive, in about 90% of cats, for years even after elimination of infection because it is performed at a 1:100 serum dilution and infected cats can have very high antibody titers, some  $\geq 1:1,024,000$ . The titration test compares the titer of antibodies in the original sample with the post-therapy sample taken: **6 MONTHS OR LONGER AFTER THE END OF THERAPY.**

Therapy has been successful in eliminating *Bartonella* if there is a 4 fold or greater titer decrease.<sup>11</sup> During the past 10 years, 88% of the 9,358 cats, that we tested, have had titer reductions indicating the elimination of their *Bartonella* infections, whereas none of the 23 untreated infected cats, some observed for as long as 3 years, had titer reductions. For dogs, 104 of 121 (80%) treated dogs had titer reductions.

## Conclusions:

1. Detection of *Bartonella* antibodies (serology) has been used most often in the world's literature as the test for *Bartonella* infection.
2. WB is the most accurate serological test- it accounts for other bacterial cross-reacting antibodies.
3. Unlike culture and PCR of blood, WB serology does not rely on *Bartonella* being present in the blood.
4. WB is the most sensitive and specific serologic assay.
5. WB can be used to evaluate therapy- elimination of infection.
6. WB is rapid and economical.

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More *Bartonella* references can be obtained at: [www.nlm.gov/](http://www.nlm.gov/)