

Tick-borne pathogens...it's complicated!



From website: http://well.blogs.nytimes.com/2012/07/30/another-tick-borne-disease-to-ward-off-instant/?_r=1&_type=blogs&_r=0

IPRL-NCSU Testing on Tick or Other Vector Samples.

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In the comprehensive study of vector borne diseases, detection and risk assessment can be made at several ecological levels. The VBDDL, of course, is aiming its efforts at detection of pathogens in blood of individual host animals for the diagnosis and management of vector borne disease.(A below) It is also worth knowing precisely which blood sucking vectors are present in a region as each species is known to carry specific pathogens.(B below) The study of vector transmission may require analysis of the contents of a vector's blood meal in order to determine which host animals may be reservoirs or targets of pathogens.(C,D below) Identification of pathogens in **ticks, fleas or mosquitos** that are in contact with animal and human beings gives surveillance data to allow the medical profession to be prepared in the event of shifts in vector populations due to climate change, population changes or even intentional bioterrorist activities.(A,E,F)

The Intracellular Pathogens Research Laboratory (IPRL), at the College of Veterinary Medicine, North Carolina State University offers molecular testing services for vector-borne pathogens in different samples including blood, tissues and in various vectors (ticks, fleas, mosquitos and other blood sucking arthropods) in collaboration with outside research colleagues or agencies. Most agreements will take the form of a Testing Service Agreement under direction of NCSU Office of SPARCS. **Vector testing by the IPRL is for research only, not diagnostic purposes, and collections are expected to be in quantities of 10 or more. No individual ticks!**

The services available for molecular screening include (see **Table on last page** for details on species in each genus):

- A. Detection and identification (by DNA sequencing) of vector-borne pathogens (VBP) of the genera *Anaplasma*, *Babesia*, *Bartonella*, *Borrelia*, *Ehrlichia*, *Francisella*, *Mycoplasma* (hemotropic group), *Neorickettsia*, *Rickettsia*, *Theilaria*, and *Wolbachia*.
- B. Identification of vector species (for ticks) by mitochondrial 16SrRNA sequencing.
- C. Identification of host blood source in engorged vectors.
- D. Quantification of blood meal in vectors (fleas)
- E. Quantification of VBP in samples by qPCR.

- F. Flavivirus detection by RT-PCR: West Nile, Dengue virus (serotype 1 to 4), tick-borne encephalitis virus, powassan virus, and yellow fever virus

The budget detailed below represents the estimated costs for each service. DNA extraction and a primary PCR amplification is the minimum required testing for each vector sample. Primary target is defined as the amplification and sequence identification of either (B) vector species, (C) host (by blood DNA analysis), or (A) pathogen species as a single genus target. Any additional (“secondary”) target or screening will be charged independently, but at slightly lower cost as the extraction process is already completed. Identification to the species level (“speciation”) is carried out to the extent possible for each positive genus result. Flavivirus testing (includes 5 viral groups) will be considered as a single testing and is budgeted independently due to differences in genetic material extraction and testing (RNA).

Budget

Description	Price/vector
DNA extraction and PCR screening of a primary target	\$36.75
Additional secondary PCR screening (species or genus)	\$21.52
Flavivirus amplification and identification (by cDNA sequencing)	\$63.00

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- Samples should be in individually labeled vials, preserved in 75% ethyl alcohol and packaged in accordance with all shipping regulations.
- Package should include paperwork describing testing request and inventory.
- Results will be reported on an electronic spreadsheet with results for each sample and each pathogen, as requested.

Ship to:

Intracellular Pathogens Research Lab
 1060 William Moore Dr. RB 462A
 CVM-NCSU
 Raleigh, N.C. 27607

Insights gained from IPRL associated research.

- In collaboration with the NC Dept of Natural Resources, PCR testing of individual *I. affinis* (n=155) and *I. scapularis* (n= 298) ticks for the presence of ***Borrelia* DNA** found that in *I. affinis*, *Borrelia* DNA was detected in 63.2% ticks while in *I. scapularis*, no *Borrelia* DNA was found. This study highlighted the potential importance of *I. affinis* in the maintenance of the enzootic transmission cycle of *B. burgdorferi* s.l. in North Carolina. The lack of *Borrelia* DNA in *I. scapularis* highlights the need for additional studies defining the transmission cycle for *B. burgdorferi* s.s. in the southeastern USA, specifically in the state of North Carolina which seems to be a transitional region for Lyme Disease. Published article: Maggi RG, Reichelt S, Toliver M, Engber B. *Borrelia* species in *Ixodes affinis* and *Ixodes scapularis* ticks collected from the coastal plain of North Carolina. *Ticks & Tick-borne Diseases* 2010;1:168-171.
- In collaboration with the Dept of Entomology at NCSU, a total of 331 **bed bugs** from NC were screened for *Bartonella* spp. DNA. *Bartonella* DNA was not amplified from any

bed bug, although five bed bugs contained DNA sequences corresponding to *Burkholderia multivorans*, a pathogen in nosocomial infections not previously linked to an arthropod vector. Published article: Saenz VL, Maggi RG, Breitschwerdt EB, Kim J, Vargo EL, et al. (2013) Survey of *Bartonella* spp. in U.S. Bed Bugs Detects *Burkholderia multivorans* but Not *Bartonella*. PLoS ONE 8(9): e73661. doi:10.1371/journal.pone.0073661

- An ongoing study is establishing a more sensitive and specific quantitative PCR protocol for **Rickettsial spp** as found in *Rhipicephalus sanguineus* ticks collected in NC by C.Apperson of the NCSU Dept of Entomology. Once optimized and validated, the method will be utilized by the VBDDL in diagnostic testing of companion animals for Rickettsia.
- A case of Rocky Mountain spotted fever after a bite of an *Amblyomma americanum* tick was documented by PCR performed on the patient's blood and the tick. When the *ompA* and 17-kDa sequences from the tick and the *ompA* sequences from the patient's blood were compared with those from *R. amblyommii*, *R. parkeri*, and *R. rickettsii*, sequences matched ***R. rickettsii***. Published article: Breitschwerdt EB, Hegarty BC, Maggi RG, Lantos PM, Aslett DM, Bradley JM. *Rickettsia rickettsii* transmission by a lone star tick, North Carolina. EID. 2011;17:873-5.

Table: Species detected by PCR testing:

1. *Anaplasma* genus:

- *A. platys*
- *A. phagocytophilum*

2. *Ehrlichia* genus:

- *E. canis*
- *E. chaffeensis*
- *E. ewingii*
- *E. muris*
- *E. ruminantium*
- Panola Mountain *Ehrlichia*

3. *Babesia* genus:

- *B. canis*
- *B. canis canis*
- *B. canis rossi*
- *B. canis vogeli*
- *B. gibsoni*
- *B. microti*
- *B. divergens*

4. *Bartonella* genus:

- *B. henselae* (including 8 strains)
- *B. vinsonii berkhoffii* (including 4 genotypes)
- *B. quintana*
- *B. clarridgeiae*
- *B. bacilliformis*
- *B. koehlerae*
- *B. bovis*
- *B. elizabethae*
- *B. washoensis*

5. *Borrelia* genus:

- *B. burgdorferi*
- *B. afzelii*
- *B. garinii*
- *B. lonestari*
- *B. valaisiana*
- *B. lusitaniae*
- *B. spielmanii*
- *B. myamotoi*/*B. andersonii*
- *B. parkeri*
- *B. hermsii*

- *B. coriaceae*
- *B. turicatae*
- *B. bissettii*

6. *Francisella* genus

- 7. *F. tularensis** Positive results will be automatically reported to health officials.

8. *Mycoplasma* genus:

- *M. fermentans*
- *M. haemocanis*
- *M. haematoparvum*

9. *Neorickettsia* genus:

- *N. risticii*
- *N. helminthoeca*
- *N. sennetsu*

10. *Rickettsia* genus:

- *R. rickettsii*
- *R. parkeri*
- *R. amblyommi*
- *R. felis*
- *R. conorii*
- *R. africae*
- *R. massiliae*
- *R. canadensis*
- *R. typhi*

11. *Theilaria* genus

- *T. annae*
- *T. annulata*
- *T. bicornis*
- *T. buffeli*
- *T. cervi*
- *T. equi*
- *T. microti*
- *T. parva*
- *T. sergenti*

12. *Wolbachia* genus

- *Wolbachia* spp (for indirect detection of *Dirofilaria immitis*)