XV International Conference on Lyme Disease & Other Tick-Borne Disorders

Diagnosis, Treatment & Research Updates

April 6-7, 2002 Farmington, CT, USA

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DISEASE

PREVENTION & BASIC SCIENCE
- — Ecology, Entomology
- — Vaccine
- — Animal models
- — Microbiology
- — Pathogenesis
- — Pathology
- — Other:

PATIENT MANAGEMENT
- X Clinical manifestations
- X Laboratory diagnosis
- — Early disease management
- — Late disease management
- X Chronic disease management
- — Other:

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RECOVERY OF BORRELIA BURGDORFERI BY PCR DNA ANALYSIS IN UTERINE SURGICAL PATHOLOGY.

Authors: Gregory Bach,D.O.,FAAIM, Private/research practice – Colmar, PA.
Dr. Harold Smith, Private practice, Bloomfield, PA

OBJECTIVES: The author reports a patient with Lyme Disease and a complex of gynecological problems including miscarriage, uterine bleeding, carcinoma of the cervix and ultimately hysterectomy. The case report draws attention to physicians who are caring for patients with obstetric/gynecological problems to diligently pursue multiple diagnostic tests investigating the possibility of Lyme Disease as a contributory factor in their illness so that appropriate intervention can begin in a timely fashion.

METHODOLOGY: The literature is reviewed regarding Lyme Disease and pregnancy and a patient thoroughly investigated by testing with serology, PCR DNA amplification, culturing, and surgical pathology. The patient is reported to and accepted by the CDC as Lyme Disease.

RESULTS: A 43 year old woman was diagnosed in 6/99 with disseminated Lyme Disease after suffering a multitude of diagnostically supportive symptoms and signs such as joint swelling, chills, and night sweats, urinary bladder dysfunction, GERD, muscle fasciculations, photophobia and the triad of pain syndromes, cognitive-affective disorders, and profound fatigue. In addition, she had chronic pelvic pain, unexplained menstrual irregularity, and problems with conception. Testing for co-infections was also positive for Babesia and Ehrlichia. The patient was treated with long term protocols of cefuroxine (as Axetil), doxycycline, atovaquone and proguanil HCl for Babesiosis, and IM LA Bicillin when symptoms and signs continued and GERD impaired oral regimens. Over the next 26 months whenever the patient stopped antimicrobials the symptoms and signs of illness returned despite consultation and care with psychiatric and gastrointestinal modalities. Repeat PCR DNA positivity and IgM/IgG serological responses confirmed and supported ongoing active persistent infection. At this time the patient had a miscarriage and Pap smear/cone biopsy diagnosed precancerous cervical pathology. Due to extensive uterine bleeding the gynecologist performed a hysterectomy. Surgical pathology revealed chronic endocervicitis, granulation tissue and giant cell reaction suggestive of large microbe chronic infection and absence of any human papilloma virus effect. Tissue for PCR DNA analysis of Borrelia Burgdorferi was positive.

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CONCLUSION: Concerns the Borrelia Burgdorferi may be as serious a pathogen as syphilis for the obstetrical course of infected women has perturbed both patients and physicians. There have been multiple medical literature reports and investigations of this likely association. Tessa Gardner, M.D., has published extensively on obstetrical outcomes citing an overall 11% incidence of fetal death for any trimester with earlier trimesters exposure have worse outcomes (63%). On the contrary, Strobino in 1993, using serological probes, concluded there was little risk of bad outcomes for pregnancies in a Lyme endemic area. However, this study does not that diagnosis greater than one year before pregnancy and tick bites in a three year window prior to pregnancy were associated with a high 14.3% major defects in offspring and were "significantly associated with congenital malformations but this could have reflected reporting differences between exposed and unexposed women". The author reports this case to highlight his own experience that up to two-thirds of women in his practice with Lyme Disease also have serious gynecological and obstetrical problems across a wide range of manifestations. The case points out that physicians need to diligently pursue investigative measures to diagnosis disseminated Lyme Disease as early as possible so that treatment may prevent bad outcomes and diminish the impact of this disease. The case calls for more attention to investigating obstetrical and gynecological surgical specimens for Borrelia burgdorferi on a much more common basis.

References: Gardner, T. Lyme Disease. (Pat Coyle) 1995. Chapter 23. Strobino B. Lyme Disease and pregnancy outcome: A prospective Study of Two Thousand Prenatal Patients. AJOG, 1993, Aug. 169(sPt1):367-374.

Special thanks for peer review to: Dr. Robert Bransfield, M.D., and Dr. E. E. Yost, M.D.

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HOW MANY DAYS OF URINE COLLECTION ARE NECESSARY FOR DETECTION OF LYME ANTIGENS IN URINE?

Nick Harris*, Gina Remollo and Jyotsna Shah, IGeneX Inc., 797 San Antonio Road, Palo Alto, CA 94303

Lyme disease is a tick borne infection. The most dangerous time for Lyme disease is from April to September, a period when people are more apt to be outdoors where they may encounter the infected ticks. Often a characteristic "bulls eye" rash appears at the site of the tick bite, sometimes followed by a flu-like illness. There may be no apparent immune response for several weeks to several years, depending on B cell antibody production. B cell activity may be detected as an IgM response in two to four weeks or as late as one to two years or as an IgG response in four to six weeks. However, the presence of antibody does not prove active Lyme disease. It is documented in literature that *Borrelia burgdorferi*, the causative agent of Lyme disease, is found in the bladder, and its antigens are shed in the urine. IGeneX recently developed a second generation of the "Lyme Urine Antigen Immunoassay" referred to as Lyme Dot Blot Assay (LDA). The LDA is designed for the qualitative detection of *Borrelia burgdorferi specific antigens* in human urine. The assay detects *B. burgdorferi* specific antigens 23-25kD, 31kD, 34kD, 39kD, and 93kD. The limit of detection of B. burgdorferi antigens in urine is 12.5ng/ml .

We have observed that Lyme patients with active disease may be positive in the Lyme Dot Blot Assay (LDA). First morning urine specimens are used because the first urine that is voided after 6-8 hours of sleep usually contains the highest amount of Lyme antigen and therefore are more likely to be positive, if the Lyme antigens are present. Some patients are positive most of the time they are tested, but the majority of patients are only sporadically positive. In order to determine the best balance between the number of samples to be tested, and the detection of Lyme antigen in urine, we analyzed the LDA results of urine samples received between April and December 2001.

Between April and December 2001, 3 urine samples were received from over 1000 patients for testing by the LDA. Of these, over 300 patients with 3 urine samples submitted were positive by the LDA. The results were analyzed by statistical experts to find the best balance between the number of samples to be tested, and the detection of Lyme antigen in urine by positive LDA. The preliminary analysis of these 300+ LDA positive patients submitting 3 samples, collected on 3 different days, indicates that the chance of finding a positive LDA in this population of positive LDA patients with 2 samples is 85-90%, and with one sample is 55-60%. These results demonstrate that while not every sample may be positive by LDA, three first morning samples are the most cost-effective compromise for obtaining a single positive result.

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PCR ASSAY FOR DIRECT DETECTION OF BABESIA-WA1 IN WHOLE BLOOD

Jyotsna Shah*, Mike Mao, Suzanne Ward, Princess Mariano and Nick Harris
IGeneX Inc., 797 San Antonio Road, Palo Alto, CA 94303

Babesiosis is a tick-borne disease caused by an intraerythrocytic parasite. At present, four species of Babesia are known to infect man. They are *Babesia microti*, *Babesia divergens*, *Babesia MOI* and *Babesia-WA1*. *B. microti* was thought to be present in NE Coastal regions of the US. but recently cases have been reported from Switzerland too. *B. divergens* is found in Europe. *Babesia MOI*, which is very closely related to *B. divergens*, is found in Missouri, US. Several cases of *Babesia-WA1* have been reported from the West Coastal regions of the US.

The Giemsa-staining of a whole blood smear is the method of choice 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-WA1*. The diagnostic assay is a four step PCR assay that detects *Babesia-WA1*, directly from blood samples. It is highly specific and sensitive. The first step of the assay specifically removes "common PCR inhibitors" from blood and at the same time selects and purifies the DNA. In the second step, the purified *Babesia* specific fragment is PCR amplified with *Babesia-WA1* primers. In the third step, the PCR amplified *Babesia-WA1* DNA fragment is detected by agarose gel electrophoresis. The fourth step, Southern Blot analysis, is a confirmation step for the *Babesia-WA1*. The combination of the four steps provide very high specificity (see below) and sensitivity.

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 *Babesia-WA1* DNA sequence currently in the GENEBANK, but not to non-*Babesia* parasites such as *Plasmodium* species, *Trypanosomes*, and human DNA sequences. Based on the sequence information, the *Babesia-WA1* probe only "hybridizes" or "binds" to *Babesia-WA1* PCR product. Thus, any of the "right size" amplified product detected by agarose gel electrophoresis and positive by Southern Blot analysis with *Babesia-WA1* probe would be *Babesia-WA1* specific.

Seventy-nine EDTA whole blood samples from patients with Babesiosis-like symptoms from Northern California were tested by the *Babesia-WA1* PCR assay. Of the 79 samples tested, three were positive for *Babesia-WA1*. Two have been already confirmed as *Babesia-WA1* by sequencing. None of the *B. microti* positive samples gave a positive result. The limit of detection of the assay is between 10-100 copies of the ribosomal DNA (rDNA) fragment 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.

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PCR ASSAY FOR DIRECT DETECTION OF BARTONELLA HENSELAE IN WHOLE BLOOD

Jyotsna Shah, Mike Mao, Suzanne Ward, Princess Mariano and Nick Harris
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Bartonella henselae is commonly associated with Cat Scratch Disease (CSD) and Bacillary Angiomatosis (BA) in immunocompromised patients. Recently Escow 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 these patients 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* 16S rDNA fragment. The assay is highly sensitive and has no cross-reaction to *Bartonella quinta.*

The diagnostic assay for the detection of *B. henselae* is a four step PCR procedure that detects *B. henselae* in blood samples. The first step of the assay specifically removes the "common PCR inhibitors" from blood, and at the same time selects, and purifies the DNA fragment of interest. In the second step, the purified *Bartonella* specific fragment is PCR amplified with *B. henselae* specific primers. In the third step, the PCR amplified *B. henselae* specific DNA fragment is detected by agarose gel electrophoresis. The fourth step, Southern Blot analysis, is a confirmation step for the *B. henselae.* Combinations of the four steps provide very high specificity (see below) and sensitivity.

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 *B. henselae* DNA sequence, currently in the GENEBANK, but not to *B. quinta*, other tick-borne pathogens, blood-borne parasites and human DNA sequences. Based on the sequence information, and confirmed by DNA sequencing, the *B. henseale* probe only "hybridizes" or "binds" to *B. henselae* PCR product. Thus, any "right size" amplified product detected by agarose gel electrophoresis and positive by Southern Blot analysis with *B. henselae* probe is *B. henselae* specific.
A study was done on over 30 EDTA whole blood samples. This included whole blood samples from patients suspected of tick-borne diseases and *B. henselae* and *B. quinta* spiked EDTA whole blood samples. All the *B. henselae* spiked samples were positive. The limit of detection in the spiked EDTA whole blood samples was one *B. henselae*. There was no cross-hybridization to *B. quinta* positive samples.

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TWO CASES OF LYME DISEASE-ASSOCIATED OBSESSIVE COMPULSIVE DISORDER (OCD) ARE DESCRIBED. OBSESSIONS MAY BE TERMINATED SUDDENLY AND PERMANENTLY BY THE FIRST INJECTION OF IM PENICILLIN **Abstract by Virginia T. Sherr, M.D.***

Known, but more often unsuspected, streptococcal infections have been revealed to be capable of creating obsessive compulsive symptoms that are in perfect agreement with the criteria of the **Diagnostic and Statistical Manual, Edition IV–American Psychiatric Association.** [1] In addition, these symptoms have cleared up following aggressive treatment by appropriate antibiotics. In relationship to another infection, **chronic neuroborreliosis** (chronic neuro-Lyme disease), the author finds there is much evidence that the spirochetal microbe, *Borrelia burgdorferi*, the cause of Lyme disease (LD), may likewise negatively affect parts of the brain that control OCD. In the case of the streptococcal and now spirochetal infections, it has been shown that antibiotics can be effective treatments of the psychiatric disorder. The disorder itself is one of the few wherein treatment improvements can be monitored and actually visualized by PET and more recently devised scans following therapy. Disordered dopamine and serotonin chemistry at the neuron level may create a kind of circular effect of thoughts or behavioral rituals. Some areas of the brain that are affected are the orbital frontal cortex, the caudate nuclei and the anterior cingulate regions. The following cases represent 2 patients who experienced the onset of severe obsessive and/or compulsive symptoms with onset of LD and how their treatment with antibiotics led to the defeat or the amelioration of those symptoms.

Patient 1–Obsessions. A circumspect 50 y-o woman developed constant, intrusive, sexual fantasies about a man whom she actively disliked. She felt helpless in the face of this unwanted, total preoccupation and was contemplating suicide when her LD was diagnosed and treated. Obsessions continued to torture her despite use of a variety of antibiotics and psychotropics until she received **IM Bicillin 2.4 million units.** Within 24 hours of the first dose her mind was cleared of the ideation. She was grateful that this burden had been lifted from her. The obsessions have not returned in the past 5 months. Now it takes great mental effort for her to think of the man at all.

Patient 2–Compulsive hand washing. A medical student had such a severe hand-washing ritual that he confined himself to his own room. He feared vague but horrific contaminations that might, through him, somehow harm his family. He described himself as having "magical thinking" that led to washing his hands raw. Debilitated, he thought that he was doomed. "When diagnosed and treated with antibiotics for LD, I felt much better and lost the magical thinking but the hand-washing continued." Luvox was added to his medications. This controlled the ritual washing enough to allow him to enroll in a different graduate program, living independently. In similar cases, usual OCD treatments such as high dose SSRI medications do not work well until treatment with antibiotics is undertaken.

1. Perlmutter SJ, Garvey MA, Castellanos X, et al. **A case of pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections.** Am J Psychiatry 155:11. November 1998. (Re: PANDAS)

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A CAROUSEL OF MICROBES IN THE TICK-BORNE MENAGERIE– PATHOGEN MICROBES MAY ROTATE IN TURN AS THEIR CO-INFECTORS WAX AND WANE

Abstract by Virginia T. Sherr, M.D.

It is generally assumed that if a prominent tick-borne microbe is treated aggressively, any hidden co-infections will stay at a steady level unless they, too, have been actively addressed whereupon they are expected to maintain a permanent back seat. When this assumption is tested in the clinical setting, it is discovered that surprise exacerbations of previously unknown or treated co-infections are likely to occur and, if not suspected and tested for, they likely will confound the success of the physician's treatment plan. For example, in 1998 a patient who previously had tested negative for ehrlichiosis, Mrs. P, was nearing completion of 8 months of IV antibiotic treatment for her chronic neuroborreliosis (chronic Lyme disease of the nervous system). Her symptoms, which had been subsiding, returned at nearly full force. At first, the increase of her symptoms was blamed on the idea that the IV cefotaxime (Claforin) had lost its power to eradicate spirochetes. As the syndrome progressed, her symptoms coalesced into clusters, then into waves of sweats, chills, itching, burning, hot and cold spells, blurred vision, irritability and neurological changes such as clumsiness. The periodicity of the mostly afternoon waves made it reasonable to test for babesiosis, which is a malaria-like tick-borne disease (TBD) caused by an intra-red blood cell protozoa. Testing showed an IFA titer of >1:512—a significantly positive test for active babesiosis. Atovaquone (Mepron) immediately was used to treat this parasite and once again the symptoms subsided. The course of treatment included several oral antimicrobials combining to destroy the menagerie within Mrs. P. Then, in 1999, after a period of improvement, a serious flare of symptoms recurred. The cornucopia of tick-borne organisms was tested for once more and a previously quiescent ehrlichiosis—called by some "Spotless Rocky Mountain Fever" and by others, "the Rodney Dangerfield of TBDs for it gets no respect" was revealed as now virulent in this patient. There was a positive HGE (Human Granulocytic) antibody titer of 1:160 that helped to explain why the patient was weak and otherwise symptomatic. This recurrent, rickettsial, intra-white cell bacteria had been tested for early on when the Lyme and then the babesiosis were flaring and had been thought to be absent. Mrs. P's condition has required on-going treatment with antibiotics and this familiar pattern is noted to be circular. In recent years, attempts to eliminate any cystic spirochetal forms by using metronidazole combined with appropriate antibiotics, led to rounds of symptom flares that, in turn, led to further testing including that for the likely-to-be-lurking ehrlichiosis. Once again, this patient who had avoided the out-of-doors, who has no pets, and has not been re-exposed to tick-bites, has a titer of 1:160 but now for antibodies to HME (Human Monocytic Ehrlichiosis). She has experienced the spinning manifestations of a variety of microbes in the carnival that has become her health. Seemingly, the animals on the "carousel" take turns at becoming the dominant player, dependent upon which of the associates are resilient enough to take charge when the latest dominant alpha microbe is diminished and is beaten into submission.

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APRIL 5-6, 2002 Farmington, CT, USA

Poster Presenter Registration: YTBD Incl. presentations, poster displays, 2 lunches, 4 breaks, book of proceedings, 1 Dinner reception

Room: YTBD single/double **Reception:** 4/5/02 **Presentations:** 4/5, 4/6 (4/5, 4/6 incl. breakfast)

Abstract Form

Poster Submission Deadline: YTBD - Space limited, apply early.

Abstracts must be in English and you should use a separate form for each submission.

Type your abstract within the area shown. Type the title in capital letters, list authors, affiliation and location where research was done, and use an asterisk to indicate the poster presenter. Additional pages are allowed, but remember to use a left hand 1" border.

Each abstract should contain: objectives of the research, methodology, result, and conclusion. A conference committee member will contact you upon acceptance. Abstracts are published in the Conference Compendium, which is distributed to all conference registrants. *Abstracts are printed as submitted.* The space below is 5 1/2" tall and 7 1/4" wide.

DISEASE _____

PREVENTION & BASIC SCIENCE
- __ Ecology, Entomology
- __ Vaccine
- __ Animal models
- __ Microbiology
- __ Pathogenesis
- __ Pathology
- __ Other:

PATIENT MANAGEMENT
- __ Clinical manifestations
- __ Laboratory diagnosis
- __ Early disease management
- __ Late disease management
- __ Chronic disease management
- __ Other:

LYME WESTERN BLOTS: IMPACT OF PRODUCTION VARIABLES, VACCINATION AND INTERPRETIVE CRITERIA.

Kathleen M. Gibney, B.S.*, Megan J. Mills, B.S., Paul T. Fawcett, Ph.D.
Alfred I. duPont Hospital for Children, Wilmington Delaware

Western blotting remains the gold standard for serologic tests used to aid in diagnosing Lyme borreliosis. In contrast to ELISA and IFA methods using whole spirochetes as substrate sources, WB allows one to detect and assess the binding of antibodies to individual components of the spirochetes, not just antibody binding to all components. ELISA and dot blot assays which use recombinant proteins, allow one to detect binding to specific components as with WB however the array of components available as substrate is limited to a single component (ELISA) or just a few (dot blot). Only WB prepared by electrophoretic separation of whole spirochetes allows one to assess an individual's immune response to essentially all of the spirochete's protein components simultaneously.

Our laboratory has been using WB to test for antibodies to *B. burgdorferi* since 1989 with an assay developed and manufactured in-house. It was designed and tested to provide high specificity on pediatric samples from individuals living in an endemic area. We have compared our assay with two commercially available WBs and have tested its performance on serum from Lyme vaccine recipients and on disease (non-Lyme) control sera.

Here we report on the results of comparing our in-house WB with commercially available WB and use of our interpretive criteria versus the CDC/Dearborn criteria for pediatric Lyme patients. Comparison with commercial blots indicate that our in-house WB had greater specificity without significantly less sensitivity. The three WB methods evaluated demonstrated variations in total band counts, band identification, and overall interpretation.

We conclude that the variables in manufacture of WB can cause significant differences in performance and that use of appropriate disease controls (non-Lyme) is a valuable tool in establishing performance of WB and interpretation criteria.

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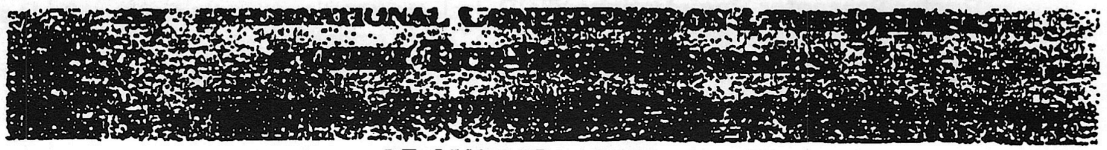

April 6-7, 2002 Farmington, CT, USA

Poster Presenter Registration: $200. Incl. scientific sessions, poster displays, Proceedings/Compendium, breaks, and reception dinner.

Hotel: Hartford Marriott 800-228-9290. Rooms ($89 single/double) incl. breakfast.

Reception: 4/6/02 Presentations & Poster Displays: April 6 & 7, 2002.

Abstract Form

Poster Submission Deadline: March 20, 2002. Space limited, apply early.

Abstracts must be in English and you should use a separate form for each submission.

Type your abstract within the area shown. Type the title in capital letters. List authors, affiliation and location where research was done, and use an asterisk to indicate the poster presenter. Additional pages are allowed, but remember to use a left hand 1" border.

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ASSOCIATION OF LYME DISEASE WITH MONOCLONAL GAMMOPATHIES
3 CASE STUDIES

In our practice of rheumatology we have observed 3 patients treated for chronic Lyme disease who have developed monoclonal gammopathies. Patient 1 developed IgG kappa Multiple Myeloma while patients 2 and 3 developed IgG lambda monoclonal gammopathies. Chronic Lyme Disease is an infectious disease causing a chronic systemic inflammatory condition. We propose that chronic Lyme disease and IgG monoclonal gammopathy occuring concomittantly in our three patients implies a possible pathogenetic relationship.

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