# The Challenges of Diagnosing and Curing Lyme Disease

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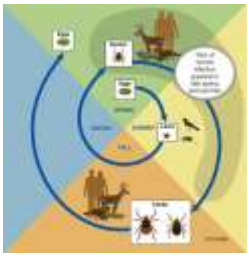
## What is Lyme Disease?

**Lyme disease is a zoonotic disease caused by *Borrelia burgdorferi* spirochetal bacteria.**

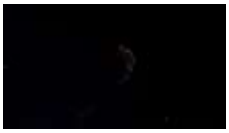
**The Lyme disease-causing bacteria are transmitted to humans by the bite of an *Ixodes* spp. tick (deer tick).**

Image showing appearance and relative sizes of adult male and female, nymph and larval ticks including deer ticks (Ixodes scapularis), Lone star ticks (Amblyomma americanum), and dog ticks (Dermacentor variabilis). Of those pictured, only the Ixodes scapularis ticks are known to transmit Lyme disease.



## Spirochetes:

- A diverse group of bacteria found in soil, deep marine sediments
- Commensal in the gut of arthropods
- Obligate parasites of vertebrates



*Borrelia burgdorferi* are helical shaped bacteria about 10-25µm long.

***Borrelia burgdorferi* pathogenic spirochetes:**

**-multiple plasmids/some indispensable**

**-large variety of lipoproteins**

**-characteristic diminution of lipoprotein expression concurrent with host antibody response**

(Liang, F. T., F. K. Nelson, and E. Fikrig. 2002. Molecular adaptation of Borrelia burgdorferi in the murine host. Journal of Experimental Medicine 196:275-80.)

**Lyme Borreliosis:**

Transmitted by ticks (*Ixodes*); nymph stage responsible for transmission in 90% of cases; feed for 48 hours or more

| | |
|---|---|
| **STAGE I.**<br>**Acute illness (weeks)** | **site of tick bite— erythema migrans**<br>**headache, fatigue, malaise**<br>**arthralgia, myalgia** |
| **STAGE II.**<br>**Dissemination**<br>**(weeks to months)** | **Facial palsy**<br>**Cardiac: heart block, pericarditis**<br>**myocarditis**<br>**CNS: meningoencephalitis**<br>**cranial neuritis** |
| **STAGE III.**<br>**Late chronic form** | **destructive chronic arthritis (U.S.)**<br>**acrodermatitis atrophicans (Eur)**<br>**neuropathy, cognitive impairment** |

## History of Lyme Disease

**Early in the 20th century, European physicians observed patients erythema migrans (EM), associated this rash with the bite of ticks, and postulated that it was caused by a bacterium.**

**Physicians observe clusters of children with arthritis in and around Lyme, Connecticut.**

**Conclusive evidence that *B. burgdorferi* caused Lyme disease came when spirochetes were cultured from patients with the Lyme (EM) rash.**

1900 — 1970 — 1982 — 1984

**Spirochetes identified in the midgut of the adult deer tick, *Ixodes dammini* and given the name *Borrelia burgdorferi*.**

•Healthline News →
•No, You Do Not Have Chronic Lyme Disease

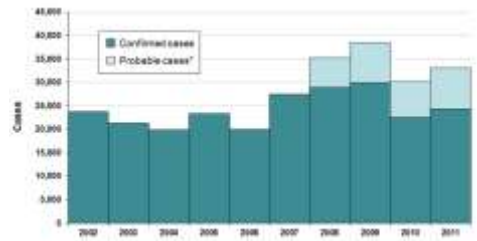
**No, You Do Not Have Chronic Lyme Disease**
Written by David Hello | Published on September 10, 2014

Infectious disease specialists agree that "chronic Lyme disease" does not exist, and that Lyme disease from a tick bite can be cured with a short course of antibiotics.
Add "chronic Lyme disease" to the list of conditions doctors say are nothing more than hype.

Lyme disease is a bacterial illness carried by ticks. The Infectious Diseases Society of America (IDSA) maintains that it is easily diagnosed and usually curable with a short course of antibiotics. The IDSA represents 9,000 U.S. doctors.

But a growing number of Americans are saying their Lyme disease symptoms persist well beyond the three-week course of antibiotic treatment. They complain of muscle aches, headaches, and fatigue. They have found doctors to treat them with more antibiotics, but not without controversy.

**Reported Cases of Lyme Disease by Year, United States, 2002-2011**




CDC Case definition

**Confirmed:** a) a case of EM with a known exposure, or b) a case of EM with laboratory evidence of infection and without a known exposure or c) a case with at least one late manifestation that has laboratory evidence of infection.
**Probable:** any other case of physician-diagnosed Lyme disease that has laboratory evidence of infection

**Content source:** Centers for Disease Control and Prevention
National Center for Emerging and Zoonotic Infectious Diseases (NCEZID)
Division of Vector-Borne Diseases (DVBD)

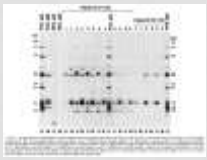
## CDC Estimates 300 000 US Cases of Lyme Disease Annually

# Lyme Borreliosis Research

- **Diagnosis**
  - Longitudinal assessment of antibody responses
  - Development of a Luminex®-based diagnostic test
  - Examination of responses of individuals with clinical cure vs. those with PTLDS
- **Treatment**
  - Effects of bacteriostatic antibiotics (doxycycline, ceftriaxone) on *B. burgdorferi*
  - Efficacy of antibiotic treatment in rhesus macaques

## DIAGNOSIS OF LYME DISEASE

Serology-early stage
IFA, ELISA (Whole cell lysate or C6 peptide), western blots
PCR
Late stage
CSF, urine, blood testing for both antigen and antibodies
--difficult to culture from blood, skin biopsy



**Inadequacies:**
-The two-tier and C6 tests not specific or sensitive enough to diagnose all patients at all phases of disease.
-WCL or recombinant proteins from one species/strain/isolate used, despite antigenic variability that can preclude antibody recognition.
-The western blot misses antibodies that target conformational epitopes.
-Potential for patient serum cross-reactivity to shared bacterial antigens has led to stringent diagnostic criteria that hinder accurate diagnosis

## Project: A Multiplex Platform for Lyme disease Diagnosis and Treatment Response

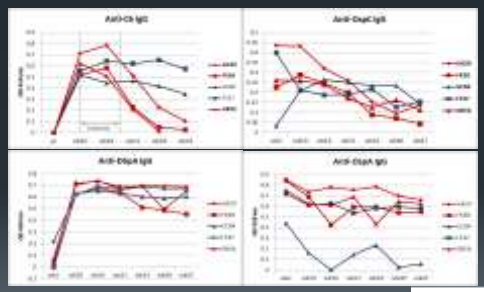
- Longitudinal assessment of antibody responses
- Development of a Luminex®-based diagnostic test

## How do immune responses change over the course of infection and post-treatment?

A longitudinal assessment of antibody responses to multiple diagnostic antigens following experimental infection and treatment had not previously been reported.

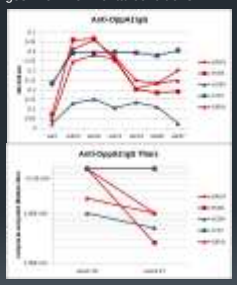
Our goal was to identify a combination of antigens that could indicate infection at all phases of disease and response to antibiotic treatment.

Anti-C6, OspC, DbpA and OspA IgG antibody responses from 5 monkeys: 3 treated at 4 months post-infection and 2 untreated.



OppA-2 transcript was detected in heart base tissue of mice post-antibiotic treatment. It belongs to an oligopeptide permease operon induced during changes in environmental conditions.

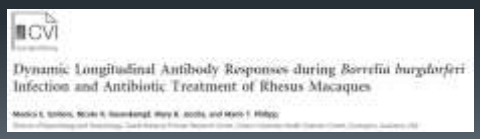
..and it's antigenic



..and it declines with antibiotic treatment (few animals tested)

## Conclusions:

- The results of this study indicate that the antigens OspA, OspC, DbpA and OppA-2 may offer distinct benefits when combined with the C6 peptide into a multi-antigen diagnostic test.
- Observation of the dynamic longitudinal responses to various antigens over time can provide insight for optimal design of diagnostic tests based upon the multiplex platform.

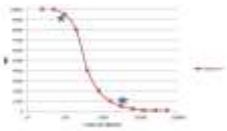



## A Multiplex Platform for Lyme Disease Diagnosis and Treatment Response

*Combine antigens with the C6 peptide such that the range of detection will cover all phases of infection and provide valuation of treatment outcome.*

Depiction of the indirect antibody detection method. The antigen of interest (blue) is coupled to fluorometric beads (green). Binding of specific antibodies from serum samples is detected by a secondary antibody that is conjugated to PE (red).

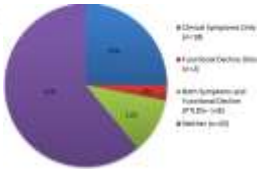
*This application of the technology can provide the ability to quantify serum antibody levels --an attribute essential for measuring the decline in anti-C6 (and possibly anti-OppA-2) antibodies following antibiotic treatment.*

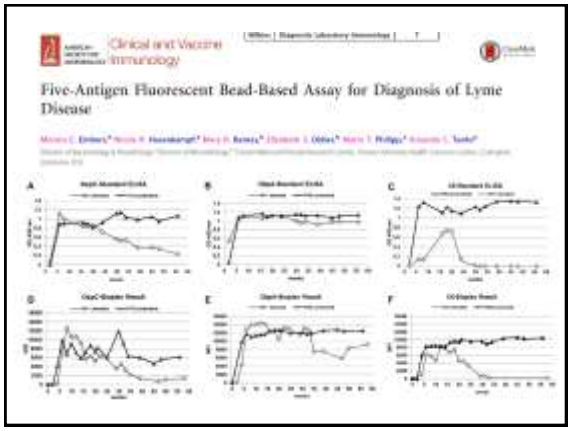


## Objectives:

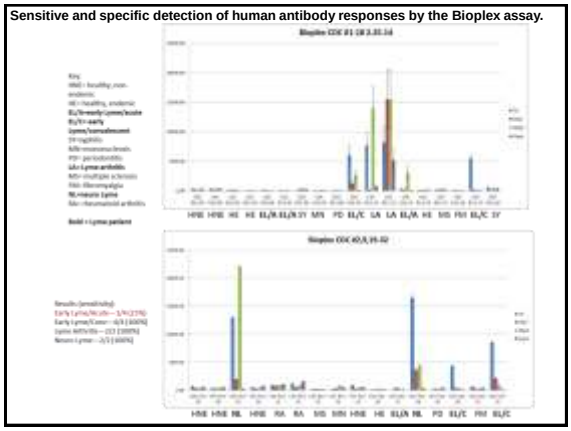
**1. Test multiplex assay against a CDC panel of Lyme patients for sensitivity/specificity in comparison to C6**

**2. Test for differences in specific antibody among PTLDS patients**





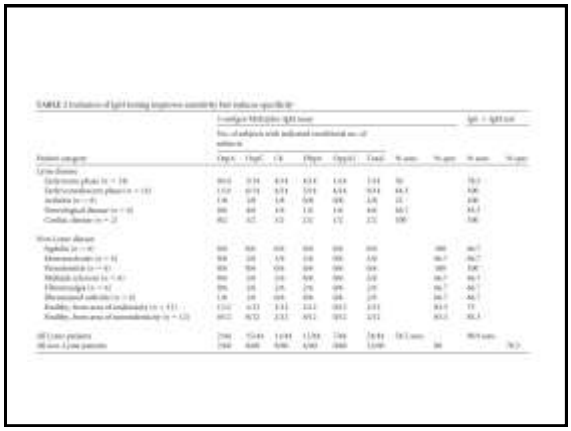

**Sensitive and specific detection of human antibody responses by the Bioplex assay.**



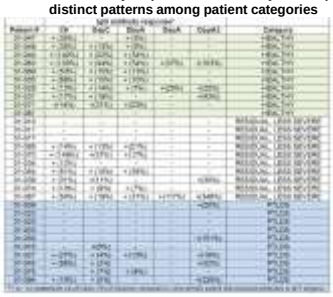
**Comparison of the 5-antigen Multiplex assay to the EIA and 2-tier test shows improved sensitivity while maintaining specificity**

| | 5-antigen Multiplex-IgG | | | | | | | EIA[a] | | 2-tier[a,b] | | C6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Patient Category | OspA | OspC | C6 | DbpA | OppA2 | total | % sens | total | % sens | total | % sens | total | % sens |
| Lyme | | | | | | | | | | | | | |
| Early/acute (14) | 0/14 | 1/14 | 3/14 | 3/14 | 5/14 | 7/14 | 50% | 4/12* | 33.3% | 1/14 | 7.1% | 6/14 | 42.8% |
| Early/conv (14) | 0/14 | 3/14 | 12/14 | 6/14 | 11/14 | 13/14 | 92.9% | 13/14 | 92.9% | 11/14 | 78.6% | 12/14 | 85.7% |
| Arthritis (8) | 3/8 | 4/8 | 8/8 | 8/8 | 8/8 | 8/8 | 100% | 8/8 | 100% | 8/8 | 100% | 8/8 | 100% |
| Neuro (6) | 0/6 | 2/6 | 5/5* | 3/6 | 4/6 | 5/6 | 83.3% | 5/6 | 83.3% | 5/6 | 83.3% | 6/6 | 100% |
| Cardio (2) | 0/2 | 0/2 | 2/2 | 2/2 | 2/2 | 2/2 | 100% | 2/2 | 100% | 2/2 | 100% | 2/2 | 100% |
| Non-Lyme | | | | | | | % spec | | % spec | | % spec | | % spec |
| Syphilis (6) | 0/6 | 1/6 | 2/5* | 1/6 | 1/6 | 2/6 | 66.7% | 5/6 | 16.7% | 1/6 | 83.3% | 0/5* | 100% |
| Mononucleosis (6) | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 100% | 2/5* | 60% | 0/6 | 100% | 1/6 | 83.3% |
| Periodontitis (6) | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 100% | 0/6 | 100% | 0/6 | 100% | 0/5* | 100% |
| Multiple sclerosis (6) | 0/6 | 1/6 | 0/6 | 0/6 | 0/6 | 1/6 | 83.3% | 1/6 | 83.3% | 0/6 | 100% | 0/6 | 100% |
| Fibromyalgia (6) | 0/6 | 0/6 | 0/6 | 0/6 | 1/6 | 1/6 | 83.3% | 0/6 | 100% | 0/6 | 100% | 0/6 | 100% |
| Rheum arthritis (6) | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 100% | 2/6 | 66.7% | 1/6 | 83.3% | 0/6 | 100% |
| Healthy endemic (12) | 0/12 | 0/12 | 0/12 | 1/12 | 1/12 | 1/12 | 91.7% | 3/11* | 72.7% | 0/12 | 100% | 0/12 | 100% |
| Healthy non-end (12) | 0/12 | 0/12 | 0/11* | 0/12 | 0/12 | 0/12 | 100% | 1/11* | 90.9% | 0/12 | 100% | 1/12 | 91.6% |
| | | | | | | | | | | | | | |
| All Lyme patients | 3/44 | 10/44 | 30/44 | 22/44 | 30/44 | 35/44 | **79.5% sens** | 32/42 | 76.1% sens | 27/44 | 61.4% sens | 34/44 | 77.2% sens |
| All non-Lyme patients | 0/60 | 2/60 | 2/60 | 2/60 | 3/60 | 5/60 | **91.7% spec** | 14/57 | 75.4% spec | 2/60 | 96.7% spec | 2/58 | 96.6% spec |

*Equivocal results excluded
[a]EIA Lyme IgM and IgG polyvalent assay by bioMerieux, Inc.
[b]IgM and IgG immunoblotting assays by Marsha Diagnostics, Inc



**Differences in serum antibody responses detected by the Bioplex assay show distinct patterns among patient categories**



- **9/10 patients that returned to health generated strong IgG (13-140% of Lyme Arthritis patient control) responses to two or more antigens in the Bioplex assay.**
- **All (10/10) patients categorized as having PTLDS either showed: (1) weak responses (<10%) to all antigens; or (2) produced an anti-OppA2 titer that did not decline.**

## SUMMARY

- Our 5-antigen Bioplex assay is more sensitive than currently used Lyme diagnostic tests and exhibits high specificity as well
- Low IgG titers to 4 of 5 antigens may be an indicator of treatment efficacy
- Incorporation of IgM into our assay significantly reduced specificity
- Using our test, changes in antibody titer may be quantified by the use of monoclonal antibody-based standard curves

# Lyme Borreliosis Research

- **Diagnosis**
  - Longitudinal assessment of antibody responses
  - Development of a Luminex®-based diagnostic test
  - Examination of responses of individuals with clinical cure vs. those with PTLDS
- **Treatment**
  - Effects of bacteriostatic antibiotics (doxycycline, ceftriaxone) on *B. burgdorferi*
  - Efficacy of antibiotic treatment in rhesus macaques

# Lyme disease treatment*

- Early or early disseminated phase patients who do not have neurological involvement : Doxycycline (100 mg twice daily) or amoxicillin (500 mg 3 times daily) for 14-21 days
- Patients with disseminated disease/arthritis: doxycycline or amoxicillin (same doses) for 28 days
- Patients with clinically evident neurological involvement: ceftriaxone (2 g once a day intravenous) for 2-4 weeks

*from the Guidelines from the Infectious Disease Society of America

- Despite generally effective treatment with antibiotics, a proportion of Lyme disease patients continue to experience symptoms after treatment, a phenomenon that can be labeled as Post-treatment Lyme Disease Syndrome (PTLDS).
- **The efficacy and accepted regimen of antibiotic treatment for human Borreliosis has been a very contentious issue.**


Persistence of *Borrelia burgdorferi* in Rhesus Macaques following Antibiotic Treatment of Disseminated Infection

-over 35,000 views

-Received "Faculty of 1000" recommended status

# POST-TREATMENT LYME DISEASE SYNDROME (PTLDS)

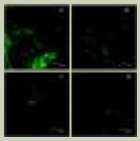
- **Potential causes include:**
  - **Induction of inflammatory responses by lingering dead spirochetes or spirochetal antigen**
  - **Continuation of active spirochetal infection**
  - **Irreversible sequelae from previous active infection (autoimmune)**

# THE RHESUS MACAQUE MODEL OF LYME DISEASE

- rhesus macaques most closely mimic the multi-organ character of the of human disease
- Unlike other animal models, disease hallmarks such as erythema migrans, carditis, arthritis, and neuropathy of the peripheral and central nervous systems are all observed in macaques.
- The spirochete burden in tissues following dissemination is very small, as in humans
- The advantages of this model are:
  - (1) compared to mice, the disease course, including duration and quantity of Bb in the blood more similar to that of humans;
  - (2) compared to human samples, the infection history (e.g. exact point of exposure, duration) is known. Also, tissues can be examined post-necropsy for the presence of Bb

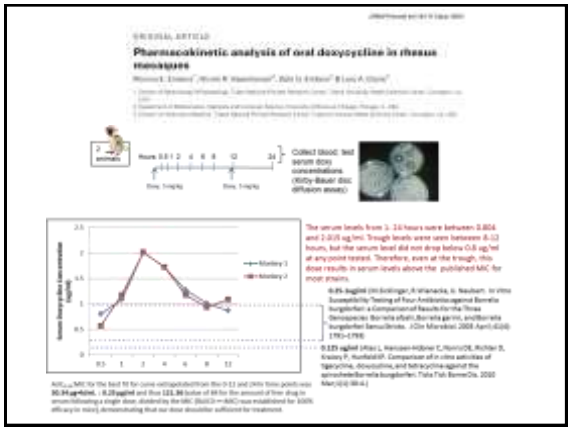
# CAVEATS AND OPEN QUESTIONS

- Lack of pharmacokinetic/pharmacodynamic data in rhesus macaques –this has been done and antibiotic levels used far exceeded that recommended for humans
- Did not use tick-mediated infection. Does initial inoculum affect treatment efficacy months later?
- What is the phenotype of persistent spirochetes? Are they viable/attenuated/dormant?
- Can spirochetes persist long-term, or are they eventually just cleared from the host?

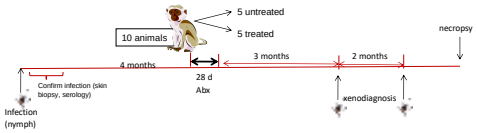


## Project: Defining "Persistence" in Post-treatment Lyme Disease

- Doxycycline pharmacokinetics in NHP
- Persistence following tick-mediated infection
- Infectivity of "persisters" in NHP (Koch's postulates)




**Tick-mediated infection, treatment, and evaluation for persistent infection**



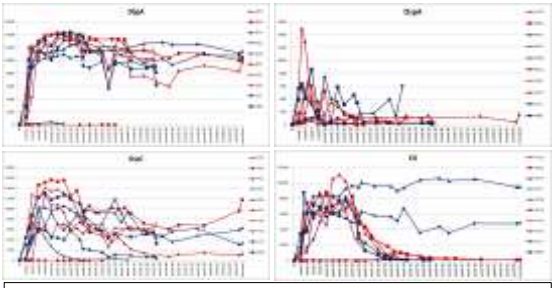

## Tick-mediated infection, treatment, and evaluation for persistent infection


-Only one of the ten animals developed a bona fide EM lesion, while others exhibited some diffuse erythema.

-Culture of skin biopsy tissue resulted in positive detection for 5 of 10 monkeys.

-Detection of Bb by DNA PCR was positive for 8 of 10 monkeys.

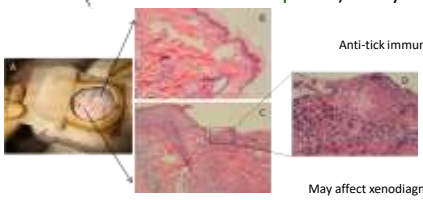
## Tick-mediated infection, treatment, and evaluation for persistent infection



**Antibody responses to four different Bb antigens over 60 weeks following infectious tick bite.** Antibody responses by untreated animals (dark blue) and animals treated with doxycycline between weeks 16-20 (red) are shown. All values shown are triplicate averages with the mean pre-immune value per individual animal subtracted.

## Results

Very few ticks positive for xenodiagnosis (stained with anti-Borrelia sp-FITC)—why?



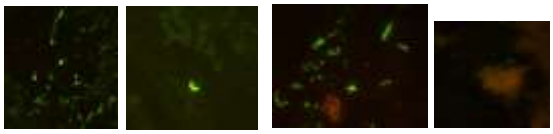

Anti-tick immunity

May affect xenodiagnosis and not transmission

# Results

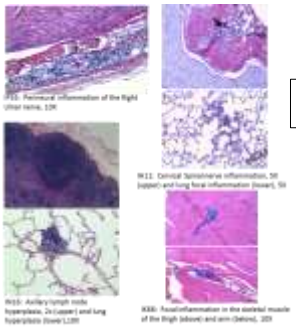
Some ticks positive for xenodiagnosis (stained with anti-OspA mAb)

Positive control



IP55-untreated animal | IL75-untreated animal | IK14-treated animal | IL09-treated animal

## Tick-mediated infection, treatment, and evaluation for persistent infection



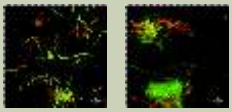
Histopathology images from infected monkeys. Descriptions for each per animal are below the image.

## SUMMARY

- **Tick-mediated infection/treatment study:**
  - EM was produced in 1/10 monkeys
  - All but one animal seroconverted
  - **Moderate pathology seen** in various tissues from both treated and untreated animals (9/10)
  - **Intact spirochetes detected** by IFA in xenodiagnostic ticks and affected tissues of treated and untreated animals
  - Bb transcripts were not detected in necropsy tissues analyzed
  - Persistence of live Bb confirmed by RT-PCR of heart tissue cultured "in vivo" and by xenodiagnostic tick staining using anti-OspA monoclonal antibody

## OTHER ONGOING PROJECTS

- Next generation sequencing (transcription profiling) of Bb treated with doxycycline, untreated, and treated/regrown
- Testing the use of therapeutic vaccination combined with antibiotic treatment for eradication of Bb



## MANY THANKS..

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