

# Lyme Disease: Prevalence, symptoms and testing

#### **Armin Schwarzbach MD, PhD**

Medical Doctor and Specialist for Laboratory Medicine

And

Gilian Crowther (MA Oxon), Dip ND/NT, mANP

AONM Director of Research





#### **Agenda**

## **Origins and prevalence**

### **Symptoms**

## **Testing**

Two-tier testing system
EliSpot (LTT-Interferon Gamma Release Assay)
Other tests for Borrelia
Coinfections

CD3/CD57 assay

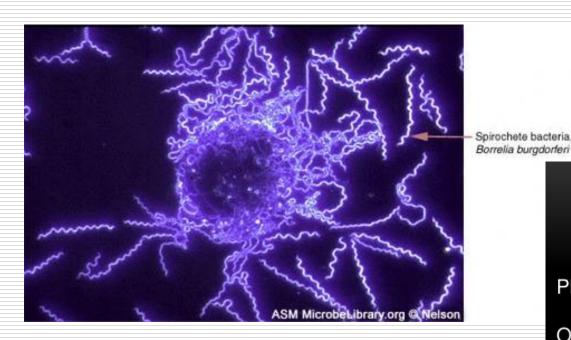
How to decide what to test for

- Checklists
- Tailored testing protocols





## A bacterium uniquely able to burrow its way through tissue



Spiral-shaped bacteria able to penetrate the tissue of other organisms by boring their way through like a corkscrew. Motility due to their strong flagella. Avg. 20-30 µm in length and 0.2-0.5 µm in width.

## **TAXONOMY**

Super kingdom: Bacteria

Phylum: spirochaetes

Order: spirochaetales

Family: spirochaelaceae

Genus: Borrelia

Species: borrelia burgdorferi

DR.T.V.RAO MD



Science News from research organizations

Lyme Disease Bacterium Came From Europe Before Ice Age

June 30, 2008 Date:

Wellcome Trust Source:

The bacterium responsible for Lyme disease, Borrelia burgdorferi, originated in America, Summary:

or so researchers thought. Now, however, scientists has shown that this bug in fact

came from Europe, originating from before the Ice Age.









RELATED TOPICS

**FULL STORY** 

WILD NATURE

#### Bacteria found in 15 million-year-old amber similar to Lyme disease

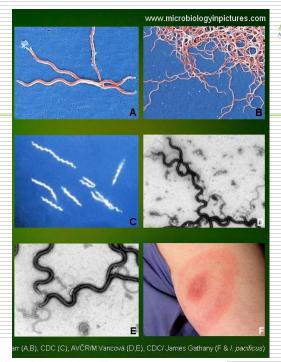
Published May 30, 2014 Associated Press

GRANTS PASS, Ore. - Fossilized bacteria found inside a tick encased in 15 million-year-old amber indicates the bacteria that cause Lyme disease were likely around long before there were humans to get the disease.



... and in 5,300 year-old "Iceman" Ötzi/

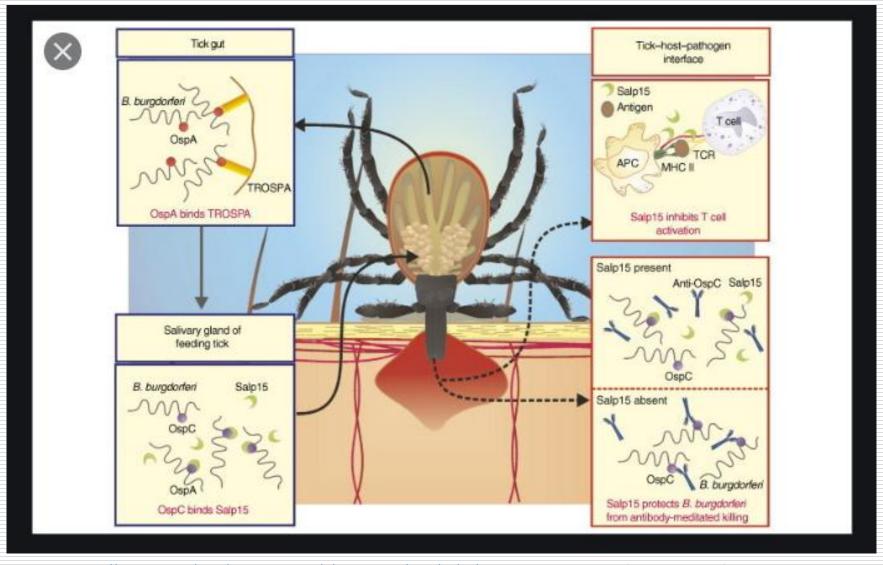
"Zink's team found almost two-thirds of the genome of Borrelia burgdorferi, a bacterium that causes Lyme disease."



This document is intellectual property of Armin Schwarzbach MD PhD. Reproduction only with permission. Please note the copyright.



## The microscopic bacteria settle in the mid gut of a tick



Source: https://www.scoop.it/topic/amazing-science/p/4032773469/2014/12/01/design-and-development-of-a-novel-vaccine-for-protection-against-lyme-disease-borreliosis

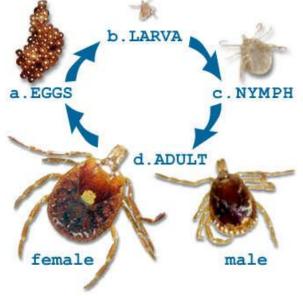


#### Lyme Disease: How is it transmitted?

Lyme Disease is caused by the pathogen transmitted (primarily) by infected ticks, when they bite and feed from a host.

~ 900 species exist.¹ Larvae, nymphs, and adults are highly skilled at detecting carbon dioxide, host odours, vibrations, and warm, moist air currents. Nymphs are tiny, but they can be more virulent than adult ticks



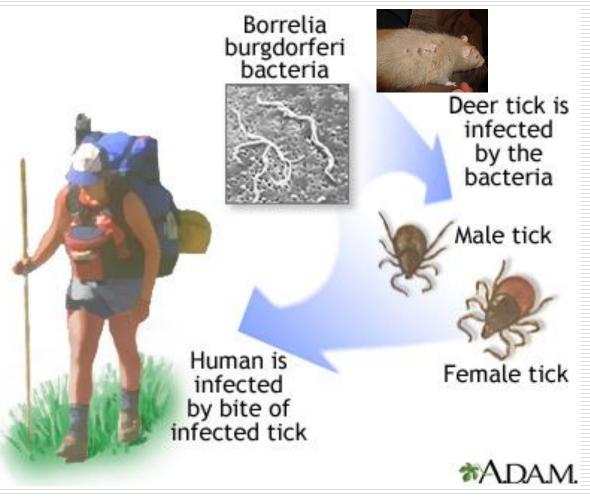








### The infectious agent is a bacterium: "Borrelia"



- □ Development:Larvae → Nymph →Adult
- Each stage of development needs one blood feed
- Ticks can survive9 years
- 1 female tick has around 9,000 progeny

https://medlineplus.gov/ency/article/001319.htm



## Many natural reservoirs of ticks and Borrelia

- Mice, rats
- Birds
- Foxes
- Deer
- Rabbits
- Hedgehogs
- Snakes

## Domestic animals:

- Dogs
- Cats
- Cows
- □ Goats
- Horses
- Guinea pigs













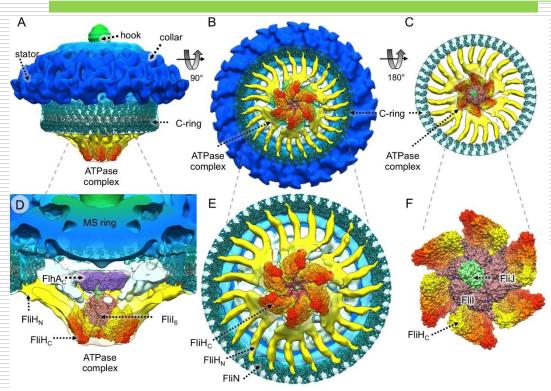




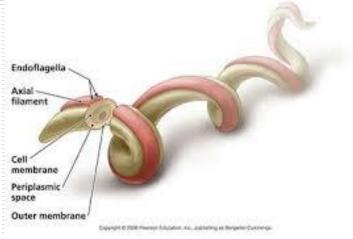


This document is intellectual property of Armin Schwarzbach MD PhD. Reproduction only with permission. Please note the copyright.

# Its flagellum needs vast amounts of energy for propulsion – which it gets from its host's blood and metabolites



Huge need for ATP – which it steals from its hosts, and particularly from mammals, in the form of purines (ATP)



AMERICAN SOCIETY FOR MICROBIOLOGY

Infection and Immunity®

Infect Immun. 2012 Sep; 80(9): 3086–3093. doi: 10.1128/IAI.00514-12 PMCID: PMC3418744 PMID: 22710875

Borrelia burgdorferi Harbors a Transport System Essential for Purine Salvage and Mammalian Infection

Sunny Jain, <sup>a</sup> Selina Sutchu, <sup>a</sup> Patricia A. Rosa, <sup>b</sup> Rebecca Byram, <sup>b</sup> and Mollie W. Jewett Ma

R. P. Morrison, Editor

➤ Author information ➤ Article notes ➤ Copyright and License information Disclaimer

This article has been cited by other articles in PMC

#### ABSTRACT

Go to: ☑

Borrelia burgdorferi is the tick-borne bacterium that causes the multistage inflammatory disease Lyme disease. B. burgdorferi has a reduced genome and lacks the enzymes required for de novo synthesis of purines for synthesis of RNA and DNA. Therefore, this obligate pathogen is dependent upon the tick vector and mammalian host environments for salvage of purine bases for nucleic acid biosynthesis. This pathway is vital for B. burgdorferi survival throughout its infectious cycle, as key enzymes in the purine salvage

Source: https://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.3000050



#### How the name "Lyme Disease" originated

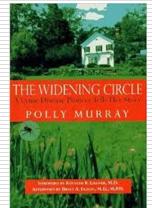
1975: a cluster of children and adults in the Lyme, Connecticut area experienced uncommon arthritic symptoms. Murray and her family were plagued by a range of baffling and debilitating symptoms in the 1960s and early '70s; the doctors they consulted were mostly unhelpful, uninterested.

Armed with dozens of case histories of other sufferers, which she had been compiling to convince the state of Connecticut that something serious was happening in Lyme, she was finally able, in 1975, to interest a Yale researcher into studying what was by then called Lyme arthritis.

In 1979, doctors at Yale identified the tick that transmits the disease

In 1982 the entomologist William Burgdorfer and colleagues isolated the infectious agent that causes Lyme Disease and now bears his name, *Borrelia burgdorferi*, in hundreds of *Ixodes* ticks that they analysed.







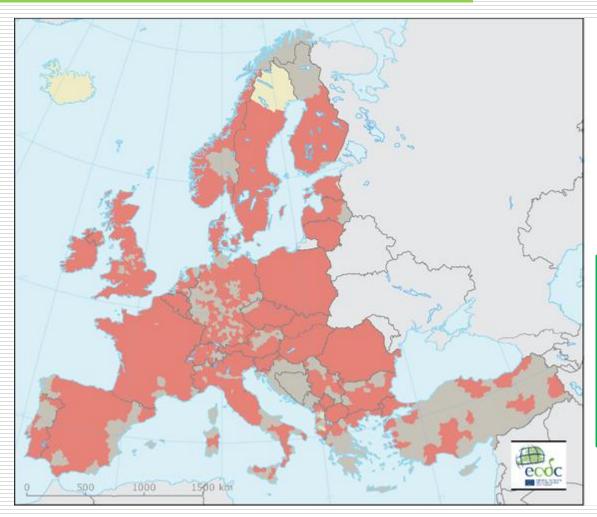
## Fastest-growing vector-borne disease in the UK, Europe and USA<sup>1,2</sup>

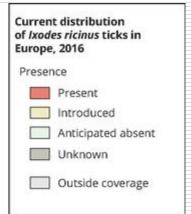
- US: The Global Lyme Alliance says most recent estimates are of 427,000 new cases of Lyme in the US every year, and that cases of Lyme continue to outpace other infectious diseases in the US by significant margins<sup>3</sup>
- UK: Medlock et al (2018) in a major study published in the International Journal of Environmental Research and Public Health stated that "Lyme borreliosis is already a significant and growing public health concern for the UK", with the records of Ixodes ricinus having more than doubled between 2010 and 2017.<sup>4</sup>
- Recent draft guidelines from NICE (the UK's <u>National Institute for Health and Care Excellence</u>) state: "Infected ticks are found throughout the UK and Ireland ... particularly high-risk areas are the south of England and Scottish Highlands, but <u>infection can occur in many areas</u>"5
- A 2017 study by scientists in Edinburgh affirms: "Lyme Borreliosis is ... the most common zoonotic infection in Western Europe, approaching endemic proportions in many European countries."

Source: 1. https://www.ncbi.nlm.nih.gov/books/NBK52945/ 2. http://www.ticktalkireland.org/whatislyme.html 3. https://globallymealliance.org/about-lyme/; 4. Assessment of the Public Health Threats Posed by Vector-Borne Disease in the United Kingdom (UK). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6210260/pdf/ijerph-15-02145.pdf; 5. https://www.nice.org.uk/guidance/indevelopment/gid-ng10007/documents; 6. https://www.ncbi.nlm.nih.gov/pubmed/26966194



#### **European distribution of Ixodus ricinus ticks**





An official European
Economic Union map
of the European
distribution of Ixodes
ricinus ticks reveals
them across almost
the entire EEU

Source: https://www.eea.europa.eu/data-and-maps/figures/european-distribution-of-borrelia-burgdorferi-1/map4.13 hh07

#### Abundant especially in Scotland, in parks ...





#### Tick-borne Illness Campaign Scotland

Campaigning for better treatment for Lyme Disease and related co-infections

**Our Petition** 

Background Patient Stories Useful Links

Home > Background

#### Background

- Incidence
- Issues with Testing and **Diagnosis**
- Issues with Treatment

#### Incidence of Lyme Disease in Scotland

Incidence of Lyme Disease in Scotland

On average, 5% of ticks in Scotland are infected with Borrelia, and the number of cases of Lyme Disease in humans is has been higher in the last decade: in 1996 there were fewer than 30 new cases in Scotland; in 2014 there were around 230. However, GPs estimate that only 20-40% of cases are referred, and so the numbers are likely to be much higher. Analyses are very rough because the real figures are unknown. Dr. Darrel Ho-Yen, previous head of the national Lyme Disease Testing Service in Scotland, believed that the known number of proven cases should be multiplied by 10 "to take account of wrongly-diagnosed cases, tests giving false results, sufferers who weren't tested, people who are infected but not showing symptoms, failures to notify and infected individuals who don't consult a doctor". A study in 2019 concluded that the incidence rate in 2010–2012 combined was 37.3 per 100,000 persons per year in Scotland, which equates to 2023 cases per year.

Tests in donated blood have concluded that 4.2% of blood donors have positive Borrelia serology. With the estimated 2015 mid-year population of Scotland standing at 5.373 million, that equates to 225,666 blood donors of Scotland having been infected. The number of infected people is likely to be higher as those who are ill are less likely to give blood. Not everyone who is infected has current symptoms. The prevalence of positive serology amongst blood donors was even higher in the Highlands, being 8.6% around Inverness.

Note: "Positive serology among blood donors ... 8.6% around Inverness"

Source: http://www.ticscotland.org.uk/incidence#:~:text=A%20study% 20in%202019%20concluded,to%202023%20cases%20per%20vear.

"The abundance of ticks and the acarological risk, particularly at Richmond Park, highlight the need for appropriate communication of the associated risk to the general public frequenting these recreational areas."

> Med Vet Entomol. 2015 Dec;29(4):448-52. doi: 10.1111/mve.12137. Epub 2015 Sep 24.

#### Tick Abundances in South London Parks and the Potential Risk for Lyme Borreliosis to the General Public

C Nelson <sup>1</sup>, S Banks <sup>1</sup>, C L Jeffries <sup>1</sup>, T Walker <sup>1</sup>, J G Logan <sup>1</sup>

Affiliations + expand

PMID: 26400641 DOI: 10.1111/mve.12137

#### Abstract

Tick abundances and prevalences of infection with Borrelia burgdorferi sensu lato, the causative agent of Lyme disease, were investigated in four South London parks. A total of 360 transects were sampled using three methods of collection (blanket, leggings and flags) simultaneously. No ticks were found on Wimbledon Common or at Hampton Court, but 1118 Ixodes ricinus (Ixodida: Ixodidae) ticks were collected at Richmond and Bushy Parks. At Richmond Park, lower canopy humidity [odds ratio (OR)

This document is intellectual pro Reproduction only with permission. Please note the copyright.



#### ... in Ireland



RECOMMENDED VACCINATIONS

GENERAL HEALTH RISKS

Hepatitis A

Lyme Disease

Ireland General Health Risks: Lyme Disease

#### **COUNTRY RISK**

Risk of Lyme disease is present throughout Ireland.

#### Description

Lyme Disease is caused by bacteria belonging to the genus Borrelia transmitted through the bite of infected ticks belonging to the Ixodes genus. Borrelia burgdorferils the predominant cause of the illness in North America, and Borrelia afzelii and Borrelia garinii in Europe and Asia. Ticks get infected when they feed on deer, birds, and rodents who are reservoirs for the bacteria and spread it to humans typically by nymphs (immature ticks). Due to climate change, tick populations are moving further north in latitude.

Ireland

#### Risk

Lyme Disease is present in North America, Europe, and Asia. Travellers involved in outdoor activities in forested areas are at risk, including campers, hikers, and hunters. Brushing against vegetation or walking in city parks known to have infected ticks can also

International Association for Medical Assistance warns "Risk of Lyme Disease is present throughout Ireland"

#### THE IRISH TIMES

NEWS SPORT BUSINESS OPINION LIFE & STYLE CULTURE
Politics > Election 2020 | Oireachtas | Poll | Elections

Sun, Jul 12, 2020

Tech that means business

#### State's first Lyme disease resource centre to open

Mater hospital unit to meet growing demand as the number of suspected cases increases

@ Mon, Nov 5, 2018, 00:05

Paul Cullen



.vme disease is a bacterial infection that is spread to humans by infected ticks.

Prof. Jack Lambert, who set up Ireland's first Lyme disease resource centre in Dublin in Nov. 2018, believes the number of cases is being underestimated, as many are being misdiagnosed as other disorders.

"It's more common than we're aware of. I think there may be thousands of cases of Lyme in Ireland," he says, adding that he sees about five new or chronic cases of the disease each week.



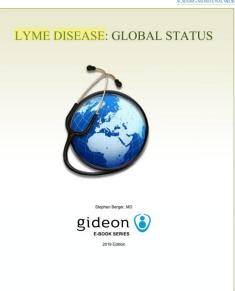
## Prevalence of infection high in Europe (Gideon 2019)

"Overall mean prevalence of infection among ticks in Europe 13.7% - 18.6% of adults and 10.1% of nymphs"





Lyme disease: Global Status

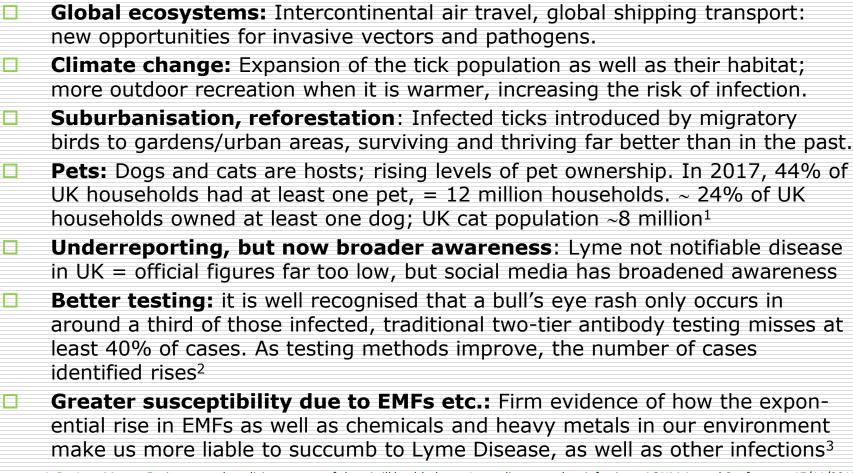


- The overall mean prevalence of Borrelia infection among ticks in Europe is 13.7% 18.6% of adults and 10.1% of nymphs.
- 22.3% of questing adult Ixodes ricinus ticks from humans in Central Europe were found to carry spirochetes
   (2012) 57
- Borrelia spp. were found in 18% of cat-ticks and 1% of dog-ticks in Europe (Hungary, France, Italy, Belgium and Germany). (2018 publication) <sup>58</sup>
- Rates among ticks are highest among nymphs (>11%) and adults (>20%) in Central Europe (Austria, Czech Republic, Germany, Switzerland, Slovenia and Slovakia) - with peak prevalence between 5 °E and 25 °E longitude (2011 publication).
- A meta-analysis of tick carriage rates among 24 European countries (2017 publication) see reference
   updated meta-analysis see reference
- A survey of tick (*Dermacentor reticularis*) carriage rates in Belgium, Germany, The Netherlands, and Great Britain (2019 publication) - see reference <sup>62</sup>

Source: Lyme disease: Global Status: 2019 edition, Gideon Infomatics



#### Why the growing prevalence?



Sources: 1. Dr. Jean Monro, Environmental medicine aspects of chronic ill health due to Lyme disease and co-infections, AONM Annual Conference, 17/11/2019, <a href="https://aonm.org/wp-content/uploads/2019/11/Monro.pdf">https://aonm.org/wp-content/uploads/2019/11/Monro.pdf</a>; 2. Lyme Disease: Finding a Path Through the Labyrinth. <a href="https://aonm.org/wp-content/uploads/2019/11/AONM-Newsletter-vp-content/uploads/2019-1.pdf">https://aonm.org/wp-content/uploads/2019/11/AONM-Newsletter-vp-content/uploads/2019/11/AONM-Newsletter-vp-content/uploads/2019-1.pdf</a>



#### Can be transmitted by other arthropods, too



HOME ABOUT US LYME 101 SUPPORT DARE 2B TICK AWARE RESOURCES VIDEOS MEDIA CONTACT



FIND OUT MORE O

Lyme disease is only transmitted by deer ticks

Lyme disease can be transmitted by many species of ticks and other arthropods including black fly, sand flea, biting flea and mosquito http://cassia.org/library/N Engl J Med 1990 Jun 14,322(24),1752.htm Lyme disease transmitted by biting fly

http://www.lymefight.info/wp-content/uploads/2013/11/Handbook-Transmission-Studies-1-page.doc\_Lyme transmitted by many types of vectors

http://www.lymeneteurope.org/forum/viewtopic.php?f=5&t=1489 Borrelia burgdorferi in other insects and ticks

http://www.txlda.com/transmission.php -Lyme can be transmitted by many types of ticks

http://www.stopthelymelies.com/lyme-disease-101/transmission-controversies Lyme is transmitted by sand flea, mosquito, flea, biting flies, mites, other arthropods

http://lymediseaseresource.com/wordpress/sandflies-in-iraq-infect-americans-who-bring-home-lyme-disease-and-multiple-co-infections/ - transmitted by sand fleas in Iraq

http://www.ncbi.nlm.nih.gov/pubmed/4075471 Lyme disease transmitted by mosquito or biting fly

Source: https://palyme.org/wp-content/uploads/2018/04/Lyme-Disease-Myth-vs-Fact.pdf



#### **Agenda**

## Origins and prevalence

### **Symptoms**

### **Testing**

Two-tier testing system
EliSpot (LTT-Interferon Gamma Release Assay)
Other tests for Borrelia
Coinfections

CD3/CD57 assay

How to decide what to test for

- Checklists
- Tailored testing protocols



#### **EM** rash – but only appears 30 - 40% of the time









One sign of infection that sometimes occurs is a gradually expanding lesion called erythema migrans ("EM rash"), which may appear 5 - 7 days after a tick bite (latest 7 - 10 weeks later). Only 30 - 40% of Lyme patients actually develop this rash (and only 30 - 40% remember being bitten).<sup>1</sup>

1. Dr. Armin Schwarzbach, Parliamentary Meeting on Lyme Disease at the Houses of Parliament, 19<sup>th</sup> January 2015, <a href="http://lymediseaseuk.com/wp-content/uploads/2015/03/arminschwarzbachpresentationlondonarmin19thjan-2015.pdf">http://lymediseaseuk.com/wp-content/uploads/2015/03/arminschwarzbachpresentationlondonarmin19thjan-2015.pdf</a>

#### What are the symptoms of Lyme disease?

ACADEMY'S NUTRITIONAL MEDICINE

#### Lyme Disease is most often categorised into three stages.

#### Stage One of Lyme Disease:

- Flu-like symptoms;
- Rash (that may look like a "bull's-eye");
- High temperature;
- Fever:
- Chills;
- General feeling of illness;
- Neck stiffness (a stiff neck);
- · Joint pain;
- Muscle pain;
- Swollen lymph glands;
- Fatigue.

#### Stage Two of Lyme Disease:

- Joint pain;
- · Headache:
- Disturbed vision;
- · Blurry vision:
- Swelling of the joints (swollen joints);
- · Tingling sensation in the hands and feet;
- Numbness of the skin (a numbing sensation);
- Problems with concentrating (lack of concentration);
- Short-term memory loss;
- Forgetfulness;
- Heart rhythm disturbances (a difference to the rhythm of the heart, i.e. fast heartbeat or slower heartbeat), heart palpitations.

More serious symptoms may develop if Lyme disease is left untreated or is not treated early on, as the bacteria spreads to other parts of the body.

#### Stage Three of Lyme Disease

#### Later disease symptoms may include:

- Arthritis of the large joints: knees, hips, etc.
- Severe headaches:
- Heart arrhythmia;
- Brain disorders and brain disturbance, including: memory loss, mood disturbances and sleep interruption;
- Difficulty concentrating;
- Mental 'fogginess';
- Mood changes/mood swings;
- Aggression;
- Panic attacks:
- Anxiety;
- Temporary amnesia / forgetfulness;
- Difficulty following conversations;
- Difficulty speaking;
- Numbness or tingling in limbs: arms, legs, feet or hands;
- Extreme fatigue/tiredness;
- Temporary paralysis of facial muscles ('facial palsy', where there is a drooping or loss of muscle tone in the face);
- Peripheral nerve dysfunction.

#### Sources:

- 1. Textbook of Clinical Neuropsychiatry and Behavioral Neuroscience, D. Moore and B. Puri, 2012. Hodder Arnold
- 2. <a href="https://www.nhsinform.scot/illnesses-and-conditions/infections-and-poisoning/lyme-disease">https://www.nhsinform.scot/illnesses-and-conditions/infections-and-poisoning/lyme-disease</a>

## Lyme Borreliosis can underlie a vast number of neuropsychiatric disorders too ...



Journal List > Healthcare (Basel) > v.6(3); 2018 Sep > PMC6165408





Healthcare (Basel). 2018 Sep; 6(3): 104

Published online 2018 Aug 25. doi: 10.3390/healthcare6030104

PMCID: PMC6165408 PMID: 30149626

Neuropsychiatric Lyme Borreliosis: An Overview with a Focus on a Specialty Psychiatrist's Clinical Practice

Robert C. Bransfield

Author information ▶ Article notes ▶ Copyright and License information ▶ Disclaimer

Abstract Go to: ♥

There is increasing evidence and recognition that Lyme borreliosis (LB) causes mental symptoms. This article draws from databases, search engines and clinical experience to require search engines and clinical experience to require search engines.

article draws from databases, search engines LB causes immune and metabolic effects that symptoms, usually presenting with significant autism spectrum disorders, schizoaffective disorder, social anxiety disorder, generalized symptoms), eating disorders, decreased libid impairments, dementia, seizure disorders, su episodes, derealization and other impairment comprehensive psychiatric clinical exam, revenuely physical exam relevant to the patient's compand knowledgeable interpretation of laborate may help improve functioning and prevent for between LB and neuropsychiatric impairment conditions can improve understanding of the

Source: Bransfield RC. Neuropsychiatric Lyme a Focus on a Specialty Psychiatrist's Clinical F "LB causes immune and metabolic effects that result in a gradually developing spectrum of neuropsychiatric symptoms, usually presenting with significant comorbidity which may include developmental disorders, autism spectrum disorders, schizoaffective disorders, bipolar disorder, depression, anxiety disorders (panic disorder, social anxiety disorder, generalized anxiety disorder, posttraumatic stress disorder, intrusive symptoms), eating disorders, decreased libido, sleep disorders, addiction, opioid addiction, cognitive impairments, dementia, seizure disorders, suicide, violence, anhedonia, depersonalization, dissociative episodes, derealization and other impairments."



#### Lyme Disease is a chameleon ...

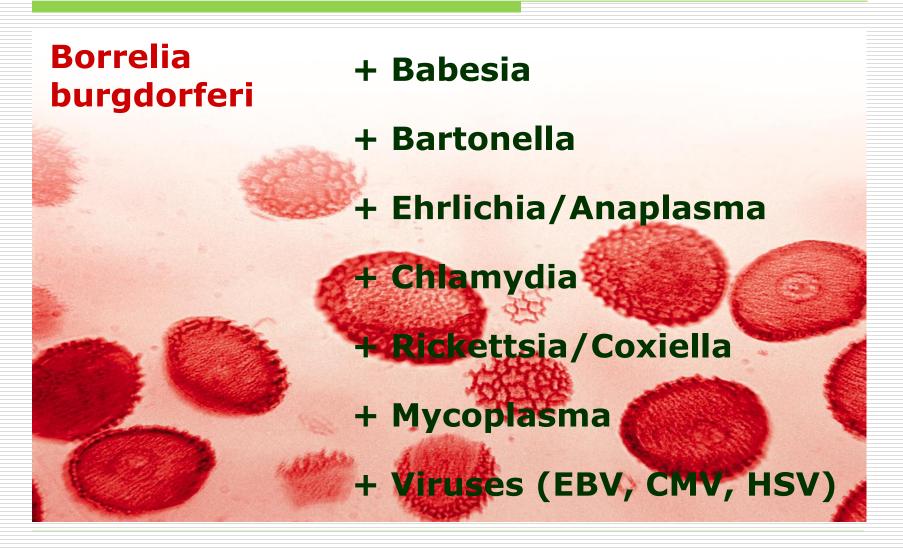
- CFS/MEFibromyalgia
- Chronic EBV
- Rheumatoid arthritis
- Polymyalgia rheumatica
- Polymyositis/dermatomy ositis
- Lupus
- Multiple Chemical Sensitivity
- Bipolar disorder
- Schizoaffective disorder
- MS



- ALS (Amyotrophic lateral sclerosis
- Alzheimer's disease
- Parkinson's Disease
- Thyroid disease
- Addison's disease
- Hyperparathyroidism
- ADHD
- Autism
- □ Etc. .... ~ 300 diseases



## Worth remembering though that Lyme Disease rarely occurs alone .... Ticks are "nature's dirty needle"





## Babesia haemangiomata





### **Clinical presentation of Babesia**





- Headaches
- Night sweats
- Fevers
- Dry cough
- Air hunger
- Easy bruising
- Tinnitus
- Rage
- Despair
- Chills
- Flushing
- Sleep disturbance
- Vivid or violent dreams

- Dysphagia
- Psychic phenomena
- Severe neurological illnesses
- □ Thirst/Polydipsia
- Fatigue
- Rheumatoid arthritis
- Nausea (severe)
- Malaise
- Anaemia, thrombocytosis, thrombocytopenia
- Abdominal pain



### Striae/stretch marks characteristic of Bartonella





The Lyme Disease Association thanks Dr. Martin Fried, Jersey Shore University Medical Center, for this picture of a Bartonella rash.



#### **Clinical presentation of Bartonella**

Brain Fog Psychiatric issues Fever Plantar pain, costal margin pain Headaches (ice pick) Rapid mood shifts Photophobia Abdominal tenderness Tachycardia Subcutaneous nodules Bowel problems IBS>>>IBD Anxiety Swollen glands Swollen joints OCD behavior Swollen lymph nodes Anxiety Purple non-blanching **Endocarditis** abdominal striae Retinitis Peripheral neuropathy



#### Mycoplasma: vectors/nutrition

Spread by biting insects (ticks, mosquitoes, fleas, biting flies), contaminated food, and human to human via airborne droplets.

Mycoplasma have lost almost all the genes required for making nucleotides, amino acids, etc., so they scavenge everything they need for survival from their host (vitamins, minerals, fats, carbohydrates, and amino acids).

To gain access to needed resources, mycoplasma generate inflammation by manipulating the signalling mechanisms of the immune system (cytokines). Inflammation breaks down tissues and allows the bacteria to gain access to the host's resources.



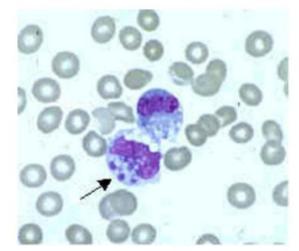
Drawn to ciliated structures: Most mycoplasmal symptoms come from infection and damage of cilia. Mycoplasma use inflammation to make epithelial and endothelial structures more porous, penetrating to deeper cilia, giving access even to the mitochondria.



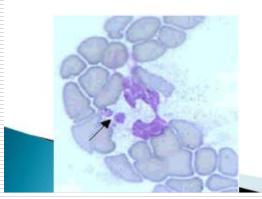
## Ehrlichia/Anaplasma: rod shaped bacteria that live inside white blood cells

Small intracellular gram-negative bacteria that cannot exist outside host cells.

Ehrlichia prefers mononuclear phagocytes, while Anaplasma prefers neutrophils



Ehrlichia chaffeensis
primarily infects
mononuclear leukocytes
(predominantly monocytes
and macrophages),



The pathogen that causes human granulocytic ehrlichiosis (HGE) (Anaplasmosis) primarily infects granulocytes (neutrophils and rarely eosinophils).

Source: <a href="https://en.wikipedia.org/wiki/Ehrlichia\_chaffeensis">https://en.wikipedia.org/wiki/Ehrlichia\_chaffeensis</a>;



#### Symptom checklists can be helpful



Name, first name.



#### Short Symptom Checklist for Lyme Borreliosis

•	Actual and former symptoms: Please mark with a cross	X			
1	Former or recent tick bite				
2	Former or recent bull 's eye rash				
3	Summer flu after tick bite				
4	Fatique/Malaise/Lethargy				
5	Loss of physical/mental capacity, general weakness				
6	Neck-pain, neck stiffness				
7	Headache				
8	Painful joints, swollen joints				
9	General aches and pains, tendon problems				
10	Muscle pain, muscle weakness				
11	Fever, feverish feeling, shivering				
12	Ears: intermittent red, swollen earlap				
13	Heart problems, disturbance of cardiac rhythm				
14	Cough, expectoration, breathlessness				
15	Night sweat				
16	Sleeplessness, waking up aroundp.m				
17	Tinnitus				
18	Swollen lymph nodes				
19	Numbness of the skin				
20	"Burning" or "pins and needles" skin sensations, painful sole or foot				
21	Back pain, back stiffness				
22	Occasional muscle twitching in the face, arms, legs				
23	Shivering, chill				
24	Blurred, foggy, cloudy, flickering, double vision				
25	Aggressiveness, drowsiness, panic attacks, anxiety, mood swings				

Concentration problems, short-term memory loss, forgetfulness

Total number of symptoms for Lyme Borreliosis

Skin partly thin, paper-like, transparent, dry

Link to symptom lists for coinfections is on the webpage:

https://aonm.org/symptoms-list-oflyme-disease-and-co-infections/

in Schwarzbach MD PhD. te the copyright.



#### **Agenda**

## Origins and prevalence

### **Symptoms**

## **Testing**

Two-tier testing system
EliSpot (LTT-Interferon Gamma Release Assay)
Other tests for Borrelia
Coinfections

CD3/CD57 assay

How to decide what to test for

- Checklists
- Tailored testing protocols



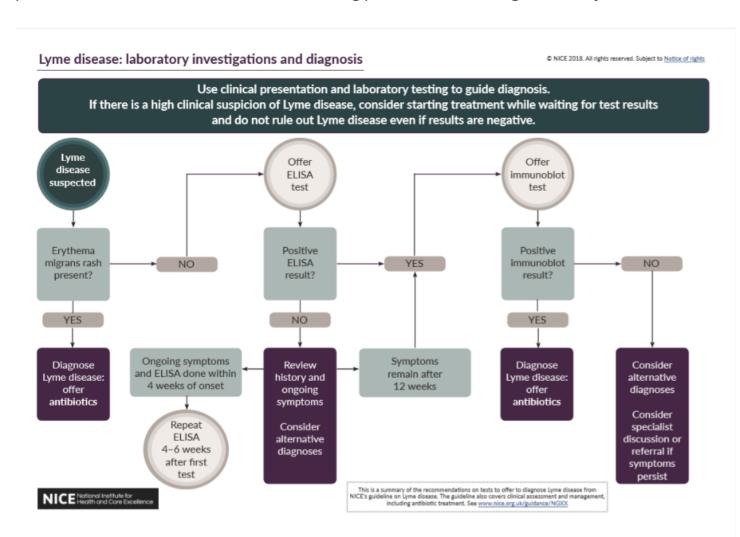
### **Agenda**

## Pitfalls of the traditional two-tier testing system for Lyme



## Traditional "two-tier" testing, developed by the CDC and still used worldwide

process that doctors should use when assessing patients and ordering tests for Lyme disease.





# Specificity and sensitivity of Borrelia antibodies by ELISA and Western Blot

Year Author/Literature

(2010) Schwarzbach A. (unpublished)

	Specificity/Sensitivity
(1993) Schmitz et al. Eur J Clin Microbiol Infect Dis 12,419.424	100% / 66%
(1995) Engstrom SM, Shoop E et al. J Clin Microbiol 33, 419-27.	96% / 55%
(1996) Ledue TB, Collins MF, Craig WY J Clin Microbiol 34, 2343-50	. 100% / 44%
(1999) Trevejo RT, Krause PJ et al. J Infect Dis 179, 931-8.	100% / 29%
(2001) Nowakiwski et al. Clin Infect Dis 33, 2023-2027	99% / 66%
(2003) Bacon RM, Biggerstaff BJ et al. J Infect Dis 187, 1187-99.	99% / 67%
(2005) Coulter P, Lema C et al. J Clin Microbiol. 43(10), 5080-5084	l / 25%
(2008) Steere AC, McHugh G et al. Clin Infect Dis 47,188-95.	99% / 18%
(2008) Binnicker MJ, Jespersen DJ et al. J Clin Microbiol 46, 2216-2	21. 100% / 49%
(2009) Klemann W, Huismans BD.	

- /32-42% ELISA

- / 60%

92% / 60% Blot

□ Average ~99% / ~43%

Umwelt-Medizin-Gesellschaft; 22(2) 132-138



## Example of a Borrelia IgG/IgM antibody test; the difficulties of serology

Borrelien-Ak recomBead Test recomBead Borrelien-IgG Ergebnis pl00 VlsE p58 p39 OspA OspC (B. sensu stricto)	negativ 0.0 0.0 0.0 0.0 0.0	negativ COI COI COI COI COI						
Untersuchung	Ergebnis	Dimension Referenz	pereich					
OspC (B. afzelii) OspC (B. garinii) p18 (B. sensu stricto) p18 (B. afzelii) p18 (B. garinii 1) p18 (B. garinii 2) p18 (B. spielmanii) recomBead Borrelien-IgM Ergebnis p100 VlsE p58 p39 OspA OspC (B. sensu stricto) OspC (B. afzelii) OspC (B. garinii) p18 (B. sensu stricto)	0.0 0.0 0.2 0.0 0.0 0.0 0.0 negativ 0.0 0.1 0.0 0.0	COI	"Detection of IgM antibodies tends to indicate a recent initial exposure to an antigen, whereas detection of total or IgG antibodies indicates exposure some time ago."1					
p18 (B. afzelii) p18 (B. garinii 1) p18 (B. garinii 2) p18 (B. spielmanii) Interpretation Borrelien	0.0 0.0 0.0 0.0	COI	ce: 1 https://www.genscript.com/lgM-antibody.html					
Im quantitativen Borrelien-Blot zeigen sich keine Borrelien- spezifischen IgG- und IgM-Antikörper. Aus serologischer Sicht spricht der Befund gegen eine stattgefundene Infektion mit Borrelien. Zu beachten ist allerdings, dass in der Früh- phase einer Infektion (4 - 6 Wochen) Borrelien-spezifische Antikörper fehlen können und dass auch in späteren Krank- heitsstadien seronegative Verläufe möglich sind. Reproduction omy with permission. Rease note die copyright.								



#### Seronegativity in Lyme borreliosis and Other Spirochetal Infections

## 103 articles in this collection alone

## "If false results are to be feared, it is the false negative result which holds the greatest peril for the patient."

Gestational Lyme borreliosis. Implications for the fetus. MacDonald AB. Rheum Dis Clin North Am, 15(4):657-77. 1989.

Author Year Title Journal

#### Borrelia burgdorferi

2002

2001

2001

 Dejmkova H; Hulinska D; Tegzova D; Pavelka K; Gatterova J; Vavrik P. 2002 Seronegative Lyme arthritis caused by Borrelia garinii.

Clinical Rheumatology, 21(4):330-4

[From the abstract:] "A case of a female patient suffering from Lyme arthritis (LA) without elevated antibody levels to Borrelia burgdorferi sensu lato is reported. Seronegative Lyme arthritis was diagnosed based on the classic clinical manifestations and DNA-detected Borrelia garinii in blood and synovial fluid of the patient, after all other possible causes of the disease had been ruled out. The disease was resistant to the first treatment with antibacterial agents. Six months after the therapy, arthritis still persisted and DNA of Borrelia garinii was repeatedly detected in the synovial fluid and the tissue of the patient. At the same time, antigens or parts of spirochaetes were detected by electron microscopy in the synovial fluid, the tissue and the blood of the patient. The patient was then repeatedly treated by antibiotics and synovectomy has been performed."

Tylewska-Wierzbanowska S; Chmielewski T; Limitation of serologic testing for Lyme borreliosis: evaluation of ELISA and western blot in comparison with PCR and culture methods.

Wien Klin Wochenschr, 114(13-14):601-5

[From the abstract:] "No correlation was found between levels of specific B. burgdorferi antibodies detected with a recombinant antigen ELISA and the number of protein fractions developed with these antibodies by immunoblot. Moreover, Lyme borreliosis patients who have live spirochetes in body fluids have low or negative levels of borrelial antibodies in their sera. This indicates that an efficient diagnosis of Lyme borreliosis has to be based on a combination of various techniques such as serology, PCR and culture, not solely on serology." [Testing was performed on samples from 90 patients.]

3. Breier F; Khanakah G; Stanek G; Kunz G; Aberer E; Schmidt B; Tappeiner G. Isolation and polymerase chain reaction typing of Borrelia afzelii from a skin lesion in a seronegative patient with generalized ulcerating bullous lichen sclerosus et atrophicus.

Br J Dermatol, 144(2):387-392

[From the abstract:] "Spirochaetes were isolated from skin cultures obtained from enlarging LSA lesions. These spirochaetes were identified as Borrelia afzelii by sodium dodecyl sulphate-polyacrylamide gel electrophoresis and polymerase chain reaction (PCR) analyses. However, serology for B. burgdorferi sensu lato was repeatedly negative."

Brunner M.

New method for detection of Borrelia burgdorferi antigen complexed to antibody in seronegative Lyme disease.

J Immunol Methods, 249(1-2):185-190

[From the abstract:] "...serologic tests for early Lyme disease can be falsely negative due to lack of sensitivity of ELISAs and Western blots. Most routine antibody tests are designed to detect free antibodies, and in early, active disease, circulating antibodies may not be free in serum but sequestered in complexes with the antigens which originally triggered their production. This difficulty may be overcome by first isolating immune complexes (IC) from the serum and using this fraction for testing. Free Borrelia-specific antibodies can then be liberated from the immune complexes which may enhance test sensitivity in patients with active disease. We developed a technique that captures the antibody component of IC on immunobeads, and subsequently releases the antigen component of IC. Immunoblotting with monoclonal antibody detected at least one antigen to be OspA, thus definitively demonstrating a Borrelia-specific antigen in circulating IC in early Lyme disease. This test is also useful in demonstrating Bb antigen in otherwise seronegative Lyme disease patients."

### **Agenda**

## **EliSpot tests (LTT-Interferon Gamma Release Assay)**

#### Testing the other arm of the immune system: T-cells

Using T-cells to show a cellular response against antigens is much more sensitive, and indicates active infection (in contrast to antibodies, which can remain for months or years long after an infection is gone). EliSpot (enzyme-linked immunosorbent spot) technology has long been used in Germany to do exactly this: it quantifies T-cells that secrete signature proteins (such as a given cytokine) against a specific antigen. The Borrelia EliSpot evaluates the number of spot-forming units using a stimulation index (SI) based on IGRA (Interferon Gamma Release Assay).

## The Elispot technique reflects the current T-cellular activity of bacteria and viruses

### **Chapter 1**

#### **Unique Strengths of ELISPOT for T Cell Diagnostics**

Paul V. Lehmann and Wenji Zhang

#### **Abstract**

The T cell system plays an essential role in infections, allergic reactions, tumor and transplant rejection, as well as autoimmune diseases. It does so by the selective engagement of different antigen-specific effector cell lineages that differentially secrete cytokines and other effector molecules. These T cell subsets may or may not have cytolytic activity, can preferentially migrate to different tissues, and display variable capabilities to expand clonally. The quest of T cell immune diagnostics is to understand which specific effector function and T cell lineage is associated with a given clinical outcome, be it positive or adverse. No single assay can measure all of the relevant parameters. In this chapter, we review the unique contributions that ELISPOT assays can make toward understanding T cell-mediated immunity. ELISPOT assays have an unsurpassed sensitivity in detecting low frequency antigen-specific T cells that secrete effector molecules, including granzyme and perforin. They provide robust, highly reproducible data—

even by first time users. Because cytometry, ELISPOT is ideally su ditions. These include defining (a establishing the fine–specificity of concentrations of the antigen in se secretory products released by T because T cells survive ELISPOT

Handbook of ELISPOT

Methods and Protocols
Third Edition

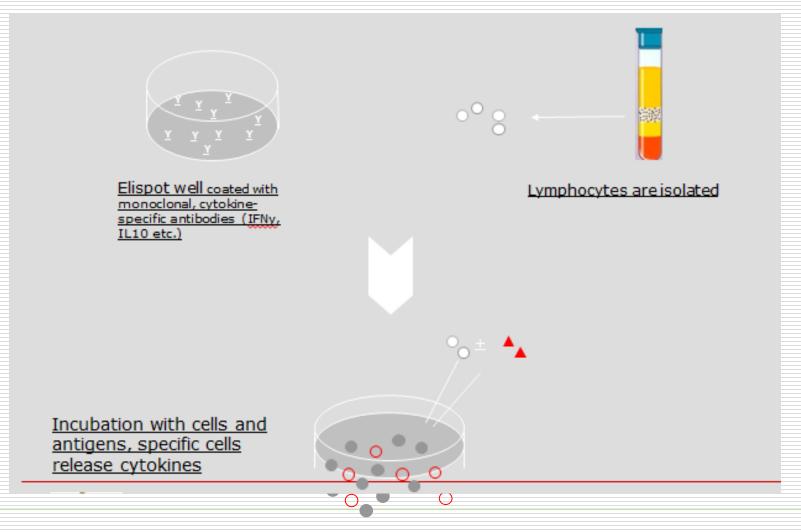
Humana Press

**Springer Protocols** 

"The quantification of single cell interferon-gamma (IFN-γ) release for assessing cellular immune responses using the Enzyme-linked immunospot (ELISPOT) assay is an invaluable technique in immunology."

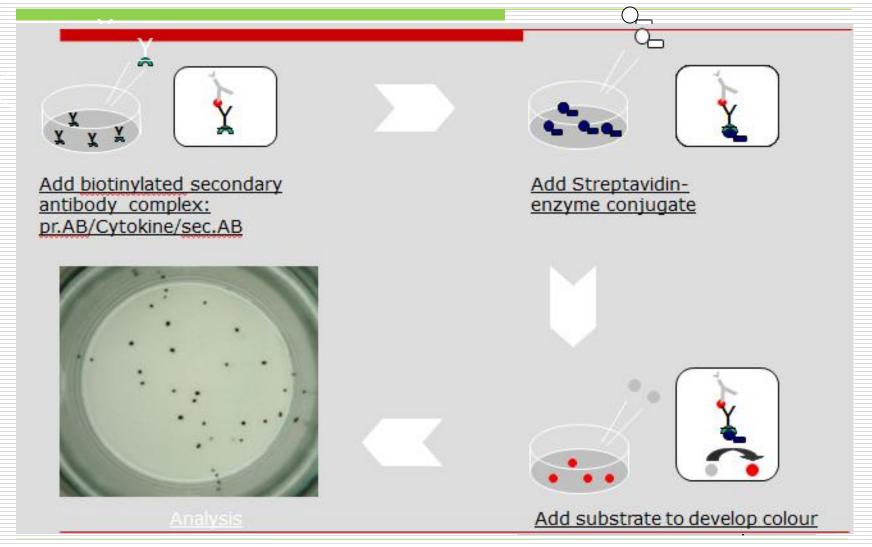
Source: 1 <u>Sedegah M</u>. The Ex Vivo IFN-y Enzyme-Linked Immunospot (ELISpot) Assay <u>Methods Mol Biol.</u> 2015;1325:197-205; <u>Humana Press; 3rd ed. 2018</u> edition (14 July 2018)

### **Elispot LTT: Methodology (I)**





### Elispot LTT: Methodology (II)





### **Example: Borrelia Elispot**

#### Borrelia burgdorferi Elispot

Borrelia burgdorferi Full Antigen + 32 SI
Borrelia b. OSP-Mix (OSPA/OSPC/DbpA) + 29 SI
Borrelia burgdorferi LFA-1 (+) 2 SI

>3 = positive

2-3 = weak positive

<2 = negative

The results of the EliSpot-Tests indicate current cellular activity against Borrelia burgdorferi.

# Immunodominant proteins: OSP = outer surface protein DbpA = decorin-binding protein A LFA = Lymphocyte Function Antigen 1 SI = stimulation index

#### Borrelia EliSpot \*

1 Borrelia b. Full Antigen ! 15 SI

0-1 = negative

2-3 = weak positive

> 3 = positive

1 Borrelia b. OSP-Mix ! 15 SI

0-1 = negative

2-3 = weak positive

> 3 = positive

1 Borrelia burgdorferi LFA-1 0 SI

0-1 = negative

2-3 = weak positive

> 3 = positive

The results of the EliSpot tests indicate current cellular activities against Borrelia burgdorferi.

This document is intellectual prop activities as Reproduction only with permission. Please note the copyright.



#### References for the Elispot: examples

- Navarrete MA ELISpot and DC-ELISpot Assay to Measure Frequency of Antigen-Specific IFNγ-Secreting Cells, in Hnasko R (Editor), Elisa Methods and Protocols 2015.
- Navarrete MA, Bertinetti-Lapatki C, Michelfelder I et al (2013) Usage of standardized antigen-presenting cells improves ELISpot performance for complex protein antigens. J Immunol Methods 391:146–153
- Czerkinsky CC, Nilsson LA, Nygren H et al (1983) A solid-phase enzyme-linked immunospot (ELISPOT) assay for enumeration of specific antibody-secreting cells. J Immunol Methods 65:109–121
- Nordberg et al.: Can ELISPOT be applied to a clinical setting as a diagnostic utility for Neuroborreliosis?, Cells 2012, I, 153-167
- Jin, Chenggang & Roen, Diana & Lehmann, Paul & Kellermann, Gottfried. (2013). An Enhanced ELISPOT Assay for Sensitive Detection of Antigen-Specific T Cell Responses to Borrelia burgdorferi. Cells. 2. 607-20. 10.3390/cells2030607.
- Forsberg, P., Ernerudh, J., Ekerfelt, C., Roberg, M., Vrethem, M., & Bergström, S. (1995). The outer surface proteins of Lyme disease borrelia spirochetes stimulate T cells to secrete interferongamma (IFN-gamma): diagnostic and pathogenic implications. *Clinical and experimental immunology*, 101(3), 453–460.
- Callister, Steven & Jobe, Dean & Stuparic-Stancic, Aleksandra & Miyamasu, Misato & Boyle, Jeff & Dattwyler, Raymond & Arnaboldi, Paul. (2016). Detection of IFN-γ Secretion by T Cells Collected Before and After Successful Treatment of Early Lyme Disease. Clinical Infectious Diseases. 62. ciw112. 10.1093/cid/ciw112.
- Schoor, F. & Baarsma, Ewoud & Gauw, Stefanie & Joosten, Leo & Kullberg, B. & Wijngaard, Cees & Hovius, J.. (2019). Validation of cellular tests for Lyme borreliosis (VICTORY) study. BMC Infectious Diseases. 19. 10.1186/s12879-019-4323-6.
- Raymond J. Dattwyler, M.D., David J. Volkman, M.D., Ph.D., Benjamin J. Luft, M.D., John J. Halperin, M.D., Josephine Thomas, B.S., and Marc G. Golightly, Ph.D. N Engl J Med (1988). Seronegative Lyme Disease. NEJM. 319:14411446

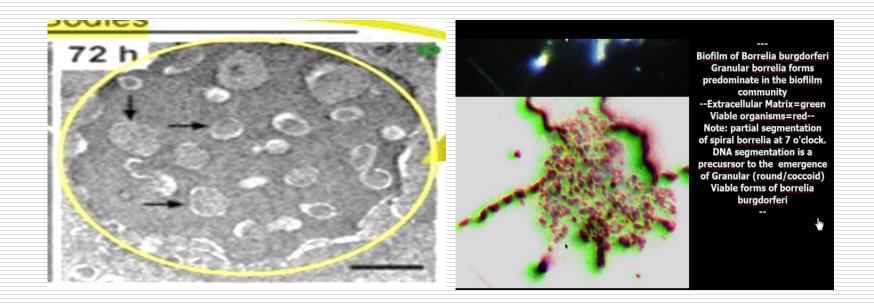


#### Elispot references (contd.)

- Moller I, Michel K, Frech N et al (2008) Dendritic cell maturation with poly(I:C)-based versus PGE2-based cytokine combinations results in differential functional characteristics relevant to clinical application. J Immunother 31:506–519
- Warncke M, Dodero A, Dierbach H et al (2006) Murine dendritic cells generated under serumfree conditions have a mature phenotype and efficiently induce primary immune responses. J Immunol Methods 310:1–1
- Malyguine A, Strobl SL, Shafer-Weaver KA et al (2004) A modifi ed human ELISPOT assay to detect specifi c responses to primary tumor cell targets. J Transl Med 2:9
- Moodie Z, Price L, Gouttefangeas C et al (2010) Response definition criteria for ELISPOT assays revisited. Cancer Immunol Immunother 59: 1489–1501
- Janetzki, S. & Britten, C.M. The impact of harmonization on ELISPOT assay performance. *Methods Mol. Biol.* **792**, 25–36 (2012)
- Zhang, W. & Lehmann, P. Objective, user-independent ELISPOT data analysis based on scientifically validated principles. *Methods Mol. Biol.* **792**, 155–171 (2012)
- <u>Calarota SA</u>. Enumeration and characterization of human memory T cells by enzyme-linked immunospot assays. <u>Clin Dev Immunol</u>. 2013;2013:637649
- Keilholz U, Weber J, Finke JH et al (2002) Immunologic monitoring of cancer vaccine therapy: results of a workshop sponsored by the Society for Biological Therapy. J Immunother 25:97–138
- Scheibenbogen C, Lee KH, Mayer S et al (1997) A sensitive ELISPOT assay for detection of CD8+ T lymphocytes specific for HLA class I-binding peptide epitopes derived from infl uenza proteins in the blood of healthy donors and melanoma patients. Clin Cancer Res 3:221–226
- <u>Sedegah M</u>. The Ex Vivo IFN-γ Enzyme-Linked Immunospot (ELISpot) Assay Methods Mol Biol. 2015;1325:197
- Nehete PN, Gambhira R, Nehete BP et al (2003) Dendritic cells enhance detection of antigenspecifi c cellular immune responses by lymphocytes from rhesus macaques immunized with an HIV envelope peptide cocktail vaccine. J Med Primatol 32:67–73



## Round bodies (pleomorphic forms) and biofilm-like colonies of Borrelia burgdorferi in vitro: Antibodies?

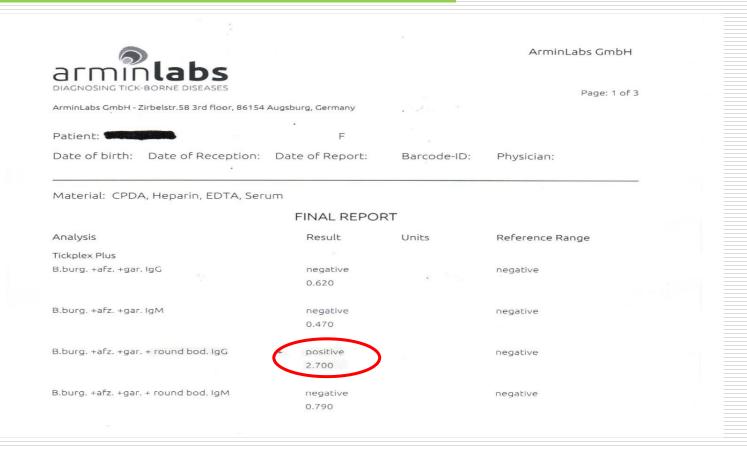


...pleomorphic B. burgdorferi should be taken into consideration as being clinically relevant and influence the development of novel diagnostics and treatment protocols...

Merilainen L., Herranen A., Schwarzbach A., Gilbert L. Morphological and biochemical features of B.b. pleomorphic forms, Microbiology, published online ahead of print January 6, 2015, doi: 10/mic.0.000027



## Tickplex: Highly sensitive multiplex methodology – only test in the world that can identify "round bodies"





## More sensitive "Western Blot" equivalent available - can also request Western Blot if you need it

```
Analysis
                                     Result Unit
                                                   Reference Range
                                                                       Chart
 Borrelia IgG-/IgM-SeraSpot:
                                 positive
1 Borr. burgdorferi SeraSpot IgG
1 Borr. SeraSpot VlsE (afzelii)
                                 positive
1 Borr. SeraSpot p39 (afzelii)
                                 negative
                                 negative
1 Borr. SeraSpot p58 (garinii)
1 Borr. SeraSpot p100 (garinii)
                                 negative
                                                VIsE = Variable major protein-like
                                 negative
1 Borr. SeraSpot OspC (afzelii)
                                                sequence expressed
                                 negative
1 Borr. SeraSpot OspC (garinii)
1 Borr. SeraSpot OspC (s.s.)
                                 negative
                                                OSP = outer surface protein
                                 positive
1 Borr. SeraSpot dbpA (afzelii)
                                                DbpA = decorin-binding protein A
1 Borr. SeraSpot dbpA (garinii)
                                 negative
1 Borr. SeraSpot dbpA (s.s.)
                                 negative
1 Borr. burgdorferi SeraSpot IgM
                                positive
1 Borr. SeraSpot VlsE (afzelii)
                                 negative
                                 negative
1 Borr. SeraSpot p39 (afzelii)
1 Borr. SeraSpot p58 (garinii)
                                 negative
1 Borr. SeraSpot p100 (garinii)
                                 negative
1 Borr. SeraSpot OspC (afzelii)
                                 negative
1 Borr. SeraSpot OspC (garinii)
                                 positive
                                 negative
1 Borr. SeraSpot OspC (s.s.)
1 Borr. SeraSpot dbpA (afzelii)
                                 negative
1 Borr. SeraSpot dbpA (garinii)
                                 negative
1 Borr. SeraSpot dbpA (s.s.)
                                 negative
     The specific Borrelia burgdorferi-IgG/IgM-antibodies by
     SeraSpot (modern Borrelia Westernblot) indicate humoral
     immune response against Borrelia burgdorferi.
     Please look at the Borrelia-EliSpot and the CD57-positive
     NK-cells. Take into consideration the clinical symptoms and
     the differential diagnosis (co-infections).
```

## Coinfections: Babesia, Bartonella and Ehrlichia/Anaplasma



Babesien EliSpot \*

1 Babesia microti EliSpot ! 5 SI 0-1 = negative

2-3 = weak positive

> 3 = positive

The result of the EliSpot test indicates current cellular activity against Babesia microti.

Bartonella EliSpot \*

1 Bart.henselae EliSpot ! 10 SI

0-1 = negative

2-3 = weak positive

> 3 = positive

The result of the EliSpot test indicates current cellular activity against Bartonella henselae.

Ehrlichia/Anaplasma EliSpot

Ehrlichia/Anaplasma-EliSpot + 4 SI

>3 = positive

2-3 = weak positive

<2 = negative



### Chlamydia pneumoniae/Mycoplasma

```
Analysis
                                          Result Units
                                                            Reference Range
  Chlamydia pneumoniae EliSpot
1 Chlamydia pneumoniae-EliSpot!
                                         15 SI
    0-1 = negative
    2-3 = weak positive
    > 3 = positive
    The result of the EliSpot test indicates current celluar
    activity against Chlamydia pneumoniae.
  Chlamydia pneum. IgG-/IgA-AB
4 Chlam.pneum. IgG-AB (ELISA)
                                 positive
                                                          negative
                                      1,570 Ratio
    Ratio < 0.8
                         = negative
    Ratio 0,8 - 1,1
                         = weak
    Ratio >= 1,1
                         = positive
4 Chlam.pneum. IgA-AB (ELISA)
                                 positive
                                                          negative
                                      1,150 Ratio
    Ratio < 0,8
                         = negative
    Ratio 0,8 - 1,1
                         = weak
    Ratio >= 1,1
                         = positive
1 Mycoplasma pneum. EliSpot
                                         22 SI
    0-1 = negative
    2-3 = weak positive
    > 3 = positive
    The result of the EliSpot test indicates current cellular
    activity against Mycoplasma pneumoniae.
 Mycoplasma pneum. IgG-/IgA-AB
4 Mycopl.pneum. IgG-AB (ELISA)
                                 positive
                                                          negative
```





Rickettsia EliSpot

Rickettsia-EliSpot + 5

>3 = positive

2-3 = weak positive

<2 = negative

The result of the EliSpot-Test indicates current cellular activity against Rickettsia helvetica/conorii/rickettsii.

Yersinia EliSpot

1 Yersinia EliSpot

!

9 SI

SI

Toxoplasma IgG-/IgM-antibodies

7 Toxopl. gondii IgG-AB (ELISA) positive

negative

! 2,534 Ratio

Ratio < 0,8

= negative

Ratio 0,8 - 1,1

= weak

Ratio >= 1,1

= positive



#### **Enteroviruses**

#### Coxsackie-Virus antibodies

Coxsackie-Virus-IgG Type A7 (IFT)	+	1:3200	Titer	< 1:100
Coxsackie-Virus-IgG Type B1 (IFT)	+	1:3200	Titer	< 1:100
Coxsackie-Virus-IgA Type A7 (IFT)	+	1:320	Titer	< 1:10
Coxsackie-Virus-IgA Type B1 (IFT)	+	1:320	Titer	< 1:10

#### Echo IgG-/IgA-antibodies

```
6 ECHO IgG-antibodies (IFT) + 1:100 < 1:100 [ ...... *> 6 ECHO IgA-antibodies (IFT) + 1:100 < 1:10 [ ...... *>
```

The specific positive ECHO-virus-IgG/IgA-antibodies indicate current humoral immune responses against ECHO-virus (recent infection with ECHO-virus?).





Analysis		Result	Units	Reference	Range
EBV EliSpot (lytic+latent)					
1 EBV Elispot (lytic)  0-1 = negative  2-3 = weak positive  > 3 = positive	ı	16 SI			
1 EBV EliSpot (latent)  0-1 = negative  2-3 = weak positive  > 3 = positive	ı	8 SI			
The result of the EliSpot to activity against Epstein-Bar			ent cellua	r	
Explanation of EBV antigens: EBV-lytic antigen: sign for virions EBV-latent antigen: sign for of infectious EBV virions	replicat				itive ak positive pative
Cytomegalo Virus EliSpot					
CMV-EliSpot	+ 289		SI		
				>3 = pos	itive
				2-3 = we	ak positive
				<2 = neg	jative
The result of the EliSpot-Test is an ind	ication for a	current cellu	ılar activity ag	ainst Cytomega	lo-Virus.



#### **EliSpot** is available for:

- Borrelia burgdorferi (3 subspecies: B.b. sensu stricto + B.b. garinii + B.b. afzelii)
   Borrelia myamotoi
- Bartonella
- Babesia
- Chlamydia pneumoniae
- Chlamydia trachomatis
- Mycoplasma pneumoniae
- Ehrlichia/Anaplasma
- Yersinia enterocolitica
- Epstein Barr Virus (EBV)
- Cytomegalovirus (CMV)
- ☐ Herpes Simplex Virus 1 / 2
- Varicella Zoster Virus (VZV)
- HHV-6, HHV-7

Also:

Candida

Aspergillus niger



#### **Agenda**

### **Origins and prevalence**

### **Symptoms**

### **Testing**

Two-tier testing system
EliSpot (LTT-Interferon Gamma Release Assay)
Other tests for Borrelia
Coinfections

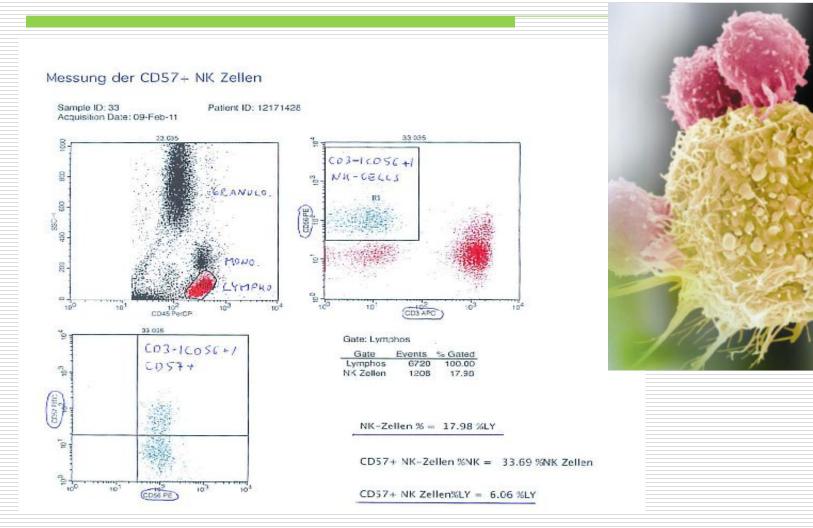
#### CD3/CD57 assay

How to decide what to test for

- Checklists
- Tailored testing protocols



## CD57 is a stable marker of human natural killer (NK) cell subsets



Source: Differential activation of CD57-defined natural killer cell subsets during recall responses to vaccine antigens. White MJ, Nielsen CM, McGregor RH, Riley EH, Goodier MR - Immunology (2014)



#### A low CD57+ indicates chronic immune suppression

CD3-/CD57+ Cells

```
5 CD3-/CD56+ Flow Cytometry
5 T cells CD3+ (%)
                             82,18 %
                                       62,00 - 80,00 [ ......
                                           900 - 1900 [ ..*.... ]
5 T cells CD3+ (absolute)
                             1225 /ul
5 NK cells CD56+ CD3- (%) -
                                        4,75 %
                                                      [ *.....
5 NK cells CD56+ CD3- (absolute)
                                71 /ul
                                           60 - 700
                             18,27 % 2,00 - 77,00 [ .*..... ]
5 CD57+ NK-cells (%)
                                           100 - 360
5 CD57+ NK-cells (absolute)
                                13 /ul
```

The result of the CD57-cell count indicates chronic immune-suppression, which can be caused by Borrelia burgdorferi or other bacteria like Chlamydia pneumoniae or Mycoplasma pneumoniae.



Suppression = generally bacterial causes: Borrelia, Chlamydia pneumoniae, Mycoplasma

Source (partly, rest Dr. Schwarzbach): Ginger Saveley PhD, http://www.publichealthalert.org/everything-you-always-wanted-to-know-about-the-cd-57-test-but-were-too-sick-to-ask.html



## A low CD57<sup>+</sup> count has particularly been observed in patients with neurological symptoms



#### Immunology Letters

Volume 76, Issue 1, 1 February 2001, Pages 43-48



Decreased CD57 lymphocyte subset in patients with chronic Lyme disease

Raphael B. Stricker a A M, Edward E. Winger b

https://doi.org/10.1016/S0165-2478(00)00316-3

Get rights and content

ities

have not

te subset

loskeletal

ed duration

gic tests

patients

sults: All

"Patients with chronic LD and predominant neurologic symptoms had significantly lower mean CD57 levels than patients with predominant musculoskeletal symptoms (30±21 vs. 58±37 cells per µl, P=0.002). CD57 levels increased in chronic LD patients whose symptoms improved, while patients with refractory disease had persistently low CD57 counts."

*P*<0.001). Nineteen of 37 patients (51%) who were tested after initiating antibiotic therapy had decreased CD57 levels (mean, 66±39 cells per μl), and all five patients tested after completing antibiotic treatment had normal CD57 counts (mean, 173±98 cells per μl). In contrast, all 10 patients with acute LD and 82% of AIDS patients had normal CD57 levels, and the difference between these groups and the pre-treatment patients with chronic LD was

"Conclusions: A decrease in the CD57 lymphocyte subset may be an important marker of chronic LD. Changes in the CD57 subset may be useful to monitor the response to therapy in this disease" ....

"The CD57 lymphocyte subset appears to be a useful marker of long-term infection with the Lyme disease spirochete."
(Stricker, Burrascano, 2002)



## CD57+ numbers tend to rise in patients with viral burdens ...



Front Immunol. 2013; 4: 422.

Published online 2013 Dec 9. doi: 10.3389/fimmu.2013.00422

Functional Significance of CD57 Expression on Human NK Cells Relevance to Disease

Carolyn M. Nielsen, Matthew J. White, Martin R. Goodier, and Eleanor M. Riley, American R. Goodier, and Eleanor M. Riley, R. Goodier, Matthew J. White, and Eleanor M. Riley, Martin R. Goodier, and Eleanor M. Riley, and E

<u>Author information</u> ► <u>Article notes</u> ► <u>Copyright and License information</u> ►

This article has been cited by other articles in PMC.

outline areas for further research.

"Chronic viral infections such as HCMV (104), human immunodeficiency virus (HIV) (105), hepatitis C virus (106), and Epstein-Barr virus (EBV) (107) infections offer some of the clearest examples of expansion of CD57+CD8+ T cells, presumably as a result of persistent antigenic stimulation"

Abstract

Go to: ☑

PMCID:

Historically, human NK cells have been identified as CD3<sup>-</sup>CD56<sup>+</sup>CD16<sup>±</sup> lymphocytes. More recently it has been established that CD57 expression defines functionally discrete sub-populations of NK cell cells, CD57 expression has been regarded as a marker of terminal differentiation and (perhaps wron anergy and senescence. Similarly, CD57 expression seems to identify the final stages of peripheral maturation; its expression increases with age and is associated with chronic infections, particularly cytomegalovirus infection. However, CD57<sup>+</sup> NK cells are highly cytotoxic and their presence seem beneficial in a number of non-communicable diseases. The purpose of this article is to review our cunderstanding of CD57 expression as a marker of NK cell function and disease prognosis, as well as to

**Keywords:** CD57, NK cells, HCMV infection, ageing, chronic infection, cancer, autoimmune diseases, T cells

Source: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3856678/

"Similar skewing of NK cells toward the CD57+ phenotype is now reported in a variety of viral infections"



#### ... whereas the CD3+ count tends to be low

CD:	57 F	low	Cvto	metry

T cells CD3 + (%)	76.58	%	62-80
T cells CD3 + (absolute)	- 659	/ul	900-1900
NK cells CD56+CD3- (%)	16.91	%	6-29
NK cells CD56+CD3- (absolute)	146	/ul	60-700

ArminLabs GmbH - CEO: Armin Schwarzbach MD PhD
Zirbelstraße 58, 2nd floor · 86154 Augsburg · Germany · Phone: 0049 821 780 931 50 www.arminlabs.com
Email: info@arminlabs.com · VATReg-No.: DE815543871 · Amtsgericht Augsburg HRB 29350

There is also one sign for a viral infection, this can be seen with the low CD3+cells, which can be analysed using the CMV Elispot, EBV Elispot, HSV 1/2 Elispot, VZV antibodies, Coxsackie Virus antibodies.



#### **Agenda**

### Origins and prevalence

### **Symptoms**

### **Testing**

Two-tier testing system
EliSpot (LTT-Interferon Gamma Release Assay)
Coinfections

CD3/CD57 assay

#### How to decide what to test for

- Checklists
- Tailored testing protocols



## Checklists help decide which infections to test for; history and physical signs/symptoms also vital (1/2)

•	Actual and former symptoms: Please mark with a cross	Χ
1	Former or recent tick bite	X
2	Former or recent bull's eye rash	$\overline{\times}$
3	Summer flu after tick bite	
4	Fatique/Malaise/Lethargy	$\overline{\times}$
5	Loss of physical/mental capacity, general weakness	$\overline{\times}$
6	Neck-pain, neck stiffness	X
7	Headache	$\overline{\times}$
8	Painful joints, swollen joints	$\times$
9	General aches and pains, tendon problems	$\times$
10	Muscle pain, muscle weakness	$\times$
11	Fever, feverish feeling, shivering	
12	Ears: intermittent red, swollen earlap	
13	Heart problems, disturbance of cardiac rhythm	$\times$
14	Cough, expectoration, breathlessness	
15	Night sweat	
16	Sleeplessness, waking up around 3 p.m.	$\overline{\times}$
17	Tinnitus	
18	Swollen lymph nodes	$\times$
19	Numbness of the skin	
20	"Burning" or "pins and needles" skin sensations, painful sole or foot	$\times$
21	Back pain, back stiffness	$\times$
22	Occasional muscle twitching in the face, arms, legs	
23	Shivering, chill	$\times$
24	Blurred, foggy, cloudy, flickering, double vision	$\times$
25	Aggressiveness, drowsiness, panic attacks, anxiety, mood swings	
26	Concentration problems, short-term memory loss, forgetfulness	
27	Skin partly thin, paper-like, transparent, dry	
	Total number of symptoms for Lyme Borreliosis	16
ntibioti	cs? When? Which one(s)? How long?	

Reproduction only with permission. Please note the copyright.



## Checklists help decide which infections to test for; history and physical signs/symptoms also vital (2/2)

#### Coinfections-Checklist

	Actual and former symptoms Please mark with a cross	X	Score-Points (filled in by physician/naturopath)	Ranking
1	Stomach ache, gut problems	$\times$	Ehrlichia&Anaplasma.5	4
2	Anaemia		Babesia: 4	5
3	Diarhoea intermittent		Rickettsia:4	. 5
4	Fever or feverish feeling	$\times$	Bartonella:7	2
5	Lack of concentration, memory disturbance, forgetfulness	$\boxtimes$	Chl.pneumoniae:6	3
6	Encephalitis/Inflammation of the brain (NMR)		Chl.trachomatis:2	
7	Yellowish colour of the skin/eyes		Yersinia: 3	. 6
8	Painful joints, swollen joints		Mycoplasma:5	4
9	General aches and pains, tendon problems		Coxsackie-/Echo-Virus: 8	1
10	Flu-like symptoms intermittent	$\times$	EBV/CMV/HSV/VZV: 8	. 1
11	Rash(es)	$\times$		
12	Small red/purple spots of the skin			1
13	Heart problems, disturbance of cardiac rhythm	$\times$		
14	Cough, expectoration			
15	Headache	$\times$		
16	Impaired liver function/ liver laboratory values	$\boxtimes$		
17	Pneumonia, bronchitis			
18	Swollen lymph nodes	$\times$		
19	Tonsilitis	$\times$		
20	Enlargement of the spleen			





#### Coinfections-Checklist

Name	, first name	Dat	e (DD/MM/YYYY)		
	Actual and former symptoms Please mark with a cross	X	Score-Poin (filled in by physician/nature	1	Ranking
1	Stomach ache, gut problems	$\times$	Ehrlichia:	7	3
2	Anaemia		Babesia:	4	6
3	Diarhoea intermittent		Rickettsia:		4
4	Fever or feverish feeling	$\times$	Bartonella:		2
5	Lack of concentration, memory disturbance, forgetfulness	$\boxtimes$	Chl.pneumoniae:	9	1
6	Encephalitis/Inflammation of the brain (NMR)		Chl.trachomatis:	5	5
7	Yellowish colour of the skin/eyes		Yersinia:	5	5
8	Painful joints, swollen joints	$\times$	Mycoplasma:	7.	3
9	General aches and pains, tendon problems	$\times$	Coxsackie-Virus: .		1
10	Flu-like symptoms intermittent	$\times$	EBV/CMV/HSV:	7	3
11	Rash(es)	X			
12	Small red/purple spots of the skin				
13	Heart problems, disturbance of cardiac rhythm	$\times$			
14	Cough, expectoration				
15	Headache	$\times$			
16	Impaired liver function/ liver laboratory values				
17	Pneumonia, bronchitis				
18	Swollen lymph nodes	$\times$			
19	Tonsilitis				
20	Enlargement of the spleen				
21	Fatigue / exhaustion, intermittent or chronic CFS	$\overline{\mathbb{X}}$			
22	Muscle pain, muscle weakness	$\overline{\boxtimes}$			
23	Shivering, chill				
24	Blurred, foggy, cloudy, flickering, double vision	$\times$			
25	Nausea, vomiting	$\boxtimes$			
26	Dark urine				
27	Itching or pain when urinating				

Ranked in order of priority – draw for first place here: Chlamydia pneumoniae (CPN) and Coxsackie

# IHCAN summary of Dr. Bransfield's AONM interview on the links between Lyme Disease, COVID-19, and psychiatric disease





### A tale of two panderlics: exploring the links between Lyme and COVID

Psychiatrist **Dr ROBERT BRANSFIELD**, MD, has been helping Lyme patients for more than 30 years.

His ground-breaking research revealing how Lyme creates psychiatric illness has led to a comprehensive system of patient assessment. Here he also provides clinical insights from new research showing that patients under active treatment for Lyme seem to be protected from COVID-19.

've been treating Lyme patients for more than 30 years, and now we're suddenly dealing with COVID-19. We have to ask: how are they similar, how are they different?

Both are zoonotic diseases, both have multiple strains that show different manifestations, both can be avoided by wearing protection. Both are global diseases, both have no symptoms in some and severe symptoms in others. Both have some scientific support suggesting that you can get reactivated, both can be relapsing, both have a spectrum of very different symptoms in different patients, both are very complicated and difficult to understand, both are associated with very complicated immune reactions including cytokine storms. They're

A Tale of Two Pandemics:
Lyme & COVID-19

Robert C Bransfield, MD, DLFAPA
AONIA, London, England
May, 2020
This article is edited and extracted from a transcript
provided by AONIA (The Academy of Nutritional
Medicine). De Bransfield by presentation is one of
a competing (and engine) AONIA waking review
on COVID-19, full details—and Dr Bransfield's hall
webinar—at: https://aorm.org.wobman.

multi-system illnesses; they have their mental manifestations, neurological manifestations; you see fatigue, brain fog, you see poor quality testing – and they can be financially catastrophic – although some people can make money from

There's a gap between frontline physician observations and healthcare bureaucratic policies. In both cases there's a focus on public health and vaccines, and a lack of focus on effective treatment. And in both cases, there are regulations that sometimes impede effective treatment. There's misinformation in both cases, there are theories about biological manupulation or wartare that may have started these infections, and there's dogma interfering with forward progress. And with both there's this debate about on-label versus off-label

18 lhean-mag-eom I JULY 2020

"His groundbreaking research reveals how Lyme Disease causes psychiatric disease"