AONM Professional Training

The Facts About Lyme Disease Testing

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COLLEGE of AMERICAN PATHOLOGISTS

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Agenda

- **Downsides of using the CDC approach**
- The importance of **cellular tests**
- The unique qualities of the **Tickplex** test
- Patients rarely have “just“ Lyme – **multiple infections/**the **ArminLabs checklists**
- **Correlation with scientific articles** related to the patient‘s s diagnosis
- **Accreditations**

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Borrelia/Lyme Disease – shortcomings of NHS testing, which is based on the CDC system

GOV.UK

Home > Health and social care > Public health > Health protection > Infectious diseases

Guidance

Lyme disease services

Diagnostic and advisory services for Lyme disease.

From: UK Health Security Agency

Published 1 July 2014

Last updated 14 April 2022 — See all updates

Diagnostic services

First line laboratory testing for suspected Lyme disease may be available through local NHS service providers. Where this is not available, and for all confirmatory testing, the UK Health Security Agency (UKHSA) Rare and Imported Pathogens Laboratory (RIPL) at Porton Down, provides a Lyme disease diagnostic service.

Lyme disease is usually diagnosed by serology. RIPL uses a modified 2-tier testing approach. The initial screening test is a combined IgG and IgM ELISA that detects antibodies against 2 Borrelia burgdorferi antigens – VlsE1 and pepC10. For positive or indeterminate results this is followed by separate IgG and IgM confirmatory assays using ViraChip microarray immunoblots.

PCR is also available and may be useful in testing joint fluid and biopsies of skin rashes. It has poor sensitivity on CSF and antibody detection is the preferred first line test on CSF. PCR is not usually performed on blood as the duration of bacteraemia is short.

See sample testing advice for information on the tests available, how to submit samples for Lyme disease testing, and guidance on test interpretation.

RIPL can also perform further tests for other tick-borne diseases. Please contact the laboratory to discuss.

Lyme disease test request form

Collection

Rare and imported pathogens laboratory (RIPL)

Lyme disease: resources and guidance

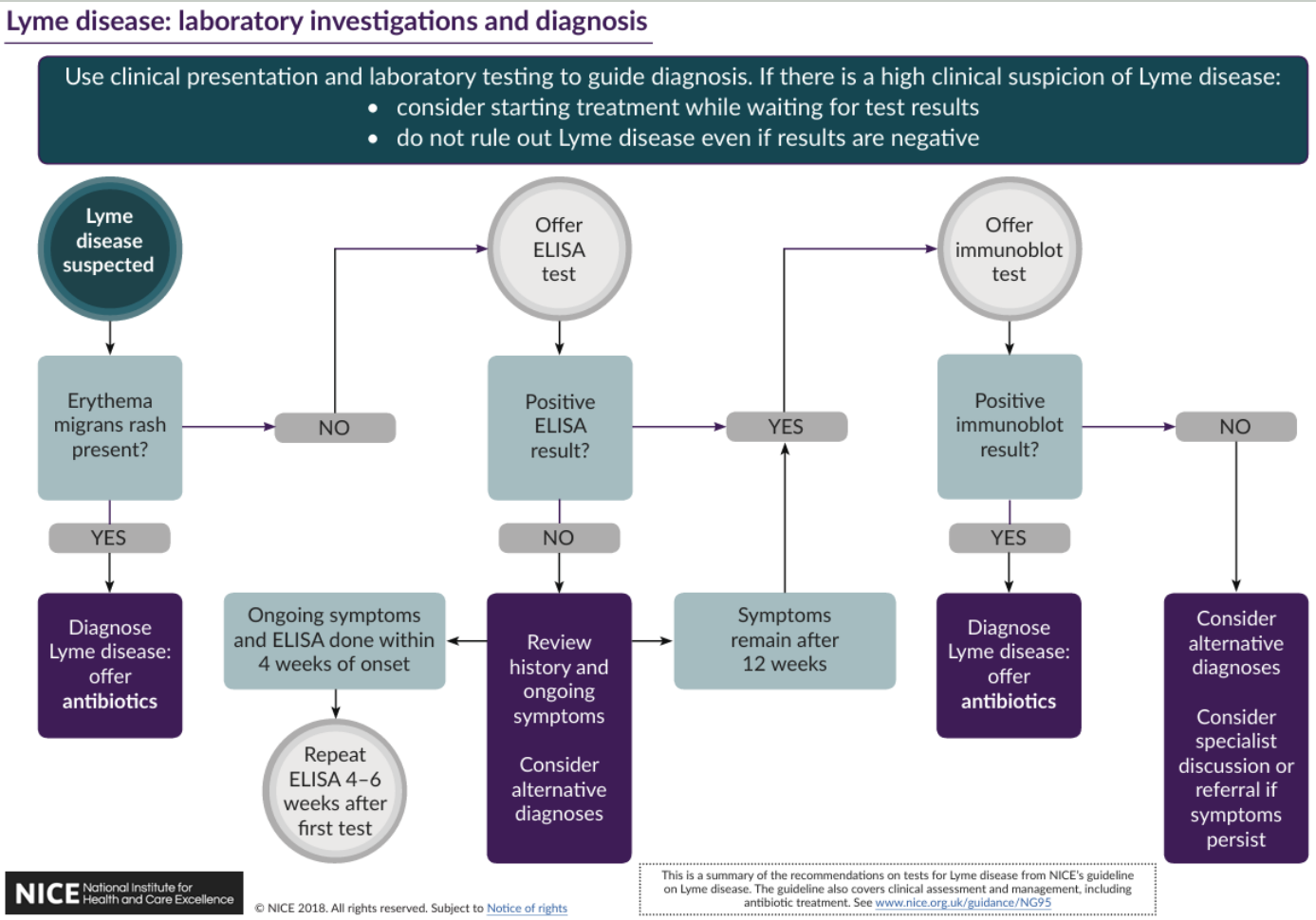
Still using the "two-tier" testing system established at a conference in Dearborn, Michigan, 1994

No distinction between IgG and IgM in the first-tier test

2nd-tier test only carried out only if the 1st is +ve or "indeterminate"

Source: https://assets.publishing.service.gov.uk/media/672ceffc3b601d048796addc/RIPL-user-manual-November-2024-version-29.pdf

Two-tier testing offered by the NHS – the CDC system



UK's "National Institute for Health and Care Excellence"

1

The initial test offered by the NHS is called an **ELISA** test which is usually performed at your local hospital laboratory … It can produce false positive and false negative results. If the ELISA test is positive or equivocal, the blood sample is usually sent to the **National Reference Laboratory** at Porton Down in England or the **NHS Highland National Lyme Borreliosis Testing Laboratory** at Raigmore Hospital in Scotland. The Western blot (sometimes called an Immunoblot) is then performed. This test **may still miss cases for various reasons.** It's important to be aware that a **negative result cannot rule out Lyme disease**, especially as it can take up to 4-6 weeks after being infected by the bacteria for antibodies to develop, if at all.

2

Source: 1. https://www.nice.org.uk/guidance/ng95/resources/visual-summary-pdf-4792272301?UID=333718872023121492215;
2. https://lymediseaseuk.com/lyme-disease-testing/

IgM antibodies generally = recent exposure, but dissipate swiftly; IgG antibodies only show past exposure

"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."[2]

IgM Antibody Functions and its Role in Disease

During infection, innate or "natural immunity" is provided by poly-reactive IgM antibody made by (B1a) B cells. IgM antibody acts to quickly recognize and initiate an immune response by directly neutralizing pathogens or clearing novel antigens. The three components of the IgM antibody-mediated immune response are activation of complement (C1qR and Fcα/μR), recruitment of phagocytic cells, and opsonization. Current research suggests that B1b B cells which make IgM antibodies may provide memory to certain pathogens and support T-cell independent immune responses. IgM antibody also acts as an educator of the immune system by transporting antigens to lymph tissues where memory is induced. Read more »

"The time required for the development of IgG antibodies following HSV infection varies from 21 to over 42 days with most individuals having detectable IgG 21–28 days after exposure to the infection and probably lasting for life.[7–,9] **IgM antibodies are usually detectable 9–10 days after exposure and last 7–14 days**, although they may remain detectable for up to 6 weeks in a minority of individuals.[9–,11] IgM antibodies may be detectable during recurrences of the infection, particularly with some of the commercial ELISAs."[2]

Source: 1. https://www.labtestsonline.org.au/learning/test-index/antibody-tests; 2. https://www.genscript.com/IgM-antibody.html

STTT: CDC's suggested result reporting and interpretation: nowhere is there mention of chronic, ongoing activity

STTT = Standard two-tier testing

Table 1a. Suggested Guidance for Reporting Results from the Standard Two-Tiered Lyme Disease Serologic Testing Using a Total Ig Immunoassay as a First Tier Assay

Test Sequence			Interpretation for Laboratories	Interpretation for Providers	Comments/Further Actions (may be included on the laboratory report)
Tier 1: Total Ig Immunoassay	Tier 2a: IgM Immunoblot[a,b]	Tier 2b: IgG Immunoblot[c]			
Negative	Testing Not Indicated[d]	Testing Not Indicated[d]	Negative for antibodies to *B. burgdorferi* (Lyme disease).	No laboratory evidence of infection with *B. burgdorferi* (Lyme disease).	Negative results may occur in patients recently infected (≤14 days) with *B. burgdorferi*. If recent infection is suspected, repeat testing on a new sample collected in 7-14 days is recommended.
Positive/ Equivocal	Negative	Negative[e]	Antibodies to *B. burgdorferi* (Lyme disease) not confirmed.	No laboratory evidence of infection with *B. burgdorferi* (Lyme disease).	Negative results may occur in patients recently infected (≤14 days) with *B. burgdorferi*. If recent infection is suspected, repeat testing on a new sample collected in 7-14 days is recommended.
Positive/ Equivocal	Positive[e]	Negative[e]	IgM-class antibodies to *B. burgdorferi* (Lyme disease) detected.	Results are consistent with acute or recent infection with *B. burgdorferi* (Lyme disease).	IgM immunoblot results should only be considered as indicative of recent infection in patients presenting within 30 days of symptom onset. Consideration of IgM immunoblot results in patients with symptoms lasting >30 days is discouraged due to the risk of false positive IgM immunoblot results or prolonged IgM seropositivity following disease resolution. Testing of a new specimen collected in 7-14 days to demonstrate IgG seroconversion may be considered to confirm infection.
Positive/ Equivocal	Negative	Positive[e]	IgG-class antibodies to *B. burgdorferi* (Lyme disease) detected.	Results are consistent with *B. burgdorferi* infection (Lyme disease) in the recent or remote past. IgG-class antibodies may remain detectable for months to years following resolution of infection.	Results should not be used to monitor or establish adequate response to therapy. Response to therapy is confirmed through resolution of clinical symptoms; additional laboratory testing should not be performed.
Positive/ Equivocal	Positive[e]	Positive[e]	IgM- and IgG-class antibodies to *B. burgdorferi* (Lyme disease) detected.	Results are consistent with *B. burgdorferi* infection (Lyme disease) in the recent or remote past. Antibodies may remain detectable for months to years following resolution of infection.	Results should not be used to monitor or establish adequate response to therapy. Response to therapy is confirmed through resolution of clinical symptoms; additional laboratory testing should not be performed.

a Immunoblots for IgM antibodies to *B. burgdorferi* are interpreted as "negative" if <2 *B. burgdorferi*-specific proteins are detected. Conversely, if ≥2 out of a possible 3 *B. burgdorferi*-specific proteins are detected, the immunoblot is interpreted as "positive" for IgM-class antibodies to *B. burgdorferi*. The *B. burgdorferi*-specific proteins that may be detected include: p23, p39, p41.
b Testing for IgM antibodies to *B. burgdorferi* is not indicated in patients presenting >30 days post-symptom onset.
c Immunoblots for IgG antibodies to *B. burgdorferi* are interpreted as "negative" if <5 B. burgdorferi-specific proteins are detected. Conversely, if ≥ 5 out of a possible 10 *B. burgdorferi*-specific proteins are detected, the immunoblot is interpreted as "positive" for IgG-class antibodies to *B. burgdorferi*. The *B. burgdorferi*-specific proteins that may be detected include: p18, p23, p28, p30, p39, p41, p45, p58, p66, p93.
d In accordance with the current standard two-tiered testing algorithm, testing by the IgM and IgG blots is not indicated due to negative initial screening immunoassay.
e Laboratories may choose to report individual bands when the overall test is positive and individual IgG bands when the overall test result is negative. Reporting of individual IgM bands when the overall test is negative is not recommended.

"IgG is produced in a delayed response to an infection and can be retained in the body for a long time …. Detection of IgG usually indicates a prior infection or vaccination."[2]

"IgG-class antibodies may remain detectable for months to years following resolution of infection."

Source: https://www.cdc.gov/lyme/media/pdfs/2024/05/Standard_Two_Tiered_Testing_Suggested_Results_Reporting_Interpretation.pdf;
2. *https://www.thermofisher.com/uk/en/home/life-science/antibodies/antibodies-learning-center/antibodies-resource-library/antibody-methods/immunoglobulin-igg-class.html#:~:text=IgG%20is%20produced%20in%20a,a%20prior%20infection%20or%20vaccination.*

Tier 1: If it does come up +ve, it has sensitivity for detecting “early, acute Lyme disease“

GOV.UK

Blog

UK Health Security Agency

Organisations: UK Health Security Agency

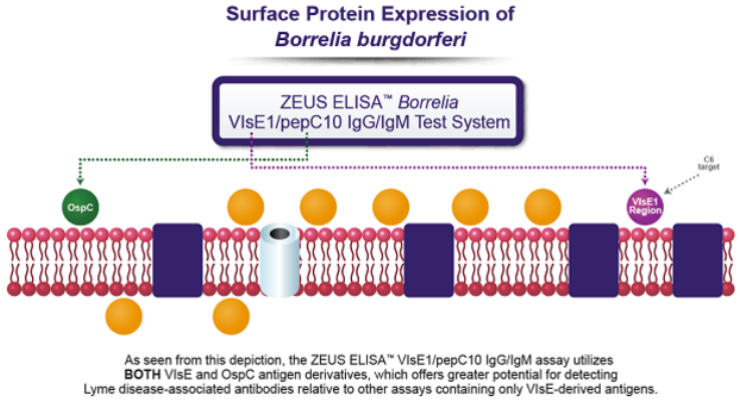
What is Lyme disease and why do we need to be tick-aware?

Blog Editor, 21 March 2024 - Health Protection

If you have a classic bullseye rash, then you should be treated for Lyme disease without the need for a test. If you have a recent tick exposure and symptoms of Lyme disease (but no bullseye rash), guidance to NHS doctors in England is to take a blood sample and send it for testing at an NHS or UKHSA laboratory.

The tests work by looking for antibodies that a person infected with Lyme disease would produce.

The antibodies take some time to reach levels that can be detected, therefore, tests carried out within the first 4 weeks of infection may be negative and may need to be repeated on a fresh blood sample taken 4 to 6 weeks after the first test.

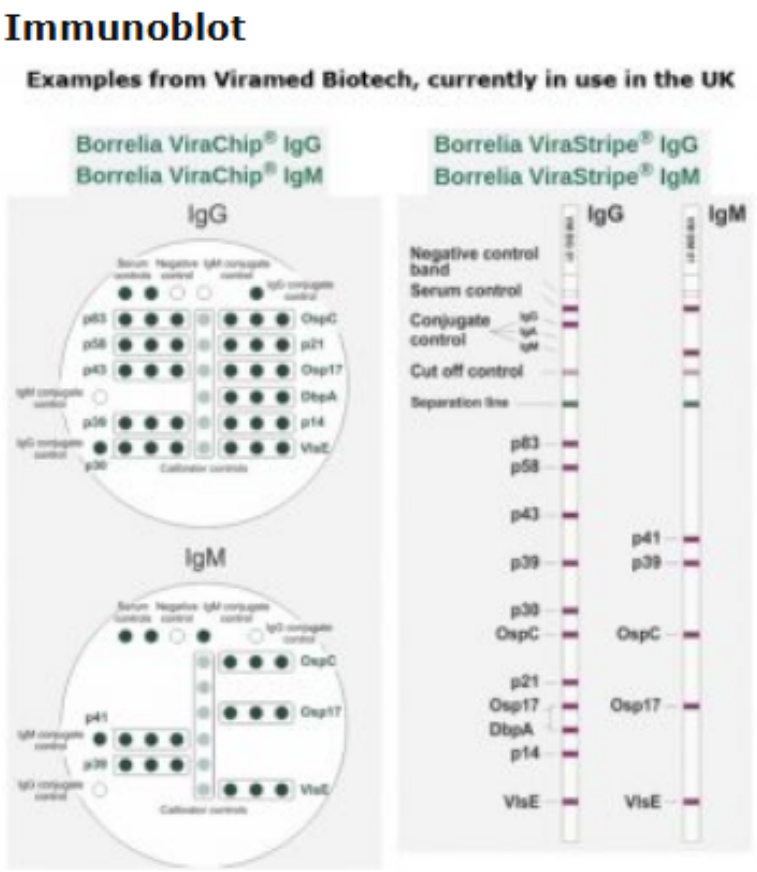


Other Description

- Achieves superior clinical sensitivity for detecting early, acute Lyme disease
- Achieves superior clinical specificity, reducing false positive results
- Yields excellent assay reproducibility using highly purified synthetic, recombinant antigens

Source: https://ukhsa.blog.gov.uk/2024/03/21/what-is-lyme-disease-and-why-do-we-need-to-be-tick-aware/

Tier 2: The Immunoblot – Even if IgG is positive, you can't detect current ongoing Lyme Disease from that



"CDC criteria for positive IgM immunoblots require the presence of at least two of the following three bands: 23-, 39-, and 41-kDa.

CDC criteria for positive IgG immunoblots require at least five of the following ten bands: 18-, 23-, 28-, 30-, 39-, 41-, 45-, 58-, 66-, and 93-kDa."[2]

1

So you have a system based on only IgG and IgM. If IgM shows up, it is most likely to be recent. If IgG shows up, you can't say it confirms chronicity. IgG is considered to be past/protective.

Source: 1. https://www.lymediseaseaction.org.uk/resources/glossary/; 2. https://pmc.ncbi.nlm.nih.gov/articles/PMC10674374/pdf/pathogens-12-01282.pdf

"...inadequate for the diagnosis of the disease"

International Journal of General Medicine

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ORIGINAL RESEARCH

Commercial test kits for detection of Lyme borreliosis: a meta-analysis of test accuracy

This article was published in the following Dove Press journal:
International Journal of General Medicine
18 November 2016
Number of times this article has been viewed

Michael J Cook[1]
Basant K Puri[2]

[1]Independent researcher, Dorset, UK; [2]Department of Medicine, Hammersmith Hospital, Imperial College London, London, UK

Abstract: The clinical diagnosis of Lyme borreliosis can be supported by various test methodologies; test kits are available from many manufacturers. Literature searches were carried out to identify studies that reported characteristics of the test kits. Of 50 searched studies, 18 were included where the tests were commercially available and samples were proven to be positive using serology testing, evidence of an erythema migrans rash, and/or culture. Additional requirements were a test specificity of ≥85% and publication in the last 20 years. The weighted mean sensitivity for all tests and for all samples was 59.5%. Individual study means varied from 30.6% to 86.2%. Sensitivity for each test technology varied from 62.4% for Western blot kits, and 62.3% for enzyme-linked immunosorbent assay tests, to 53.9% for synthetic C6 peptide ELISA tests and 53.7% when the two-tier methodology was used. Test sensitivity increased as dissemination of the pathogen affected different organs; however, the absence of data on the time from infection to serological testing and the lack of standard definitions for "early" and "late" disease prevented analysis of test sensitivity versus time of infection. The lack of standardization of the definitions of disease stage and the possibility of retrospective selection bias prevented clear evaluation of test sensitivity by "stage". The sensitivity for samples classified as acute disease was 35.4%, with a corresponding sensitivity of 64.5% for samples from patients defined as convalescent. Regression analysis demonstrated an improvement of 4% in test sensitivity over the 20-year study period. The studies did not provide data to indicate the

"A meta-analysis of Lyme test accuracy published by Prof B. Puri and M. Cook in November 2016 concluded that the weighted mean sensitivity of all ELISA tests (over a 20-year period) was 62.3%, and 62.4% for the Western Blot. With a mean sensitivity (the probability that a positive sample will be defined as positive by the test) of only 53.9% for synthetic C6 peptide ELISAs according to the meta-analysis above, 46% of cases are being missed and not even being referred for the confirmatory Western Blot, where a further 37.5% (on average) remain undetected.
Puri and Cook concluded: "These results lend support to the recently published conclusion of Stricker and Johnson to the effect that **'FDA-cleared commercial serological testing for Lyme disease is inadequate for the diagnosis of the disease'**."

Source: Cook MJ, Puri BK. Commercial test kits for detection of Lyme borreliosis: a meta-analysis of test accuracy. Int J Gen Med. 2016 Nov 18;9:427-440; https://aonm.org/wp-content/uploads/2017/02/CAM-Mag-Feb-2017-.pdf

Seronegativity also a huge issue – “Sero“ meaning serology = B cells = immunoglobulin testing

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE | ARCHIVE

Seronegative Lyme Disease

Authors: 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. Author Info & Affiliations

Published December 1, 1988 | N Engl J Med 1988;319:1441-1446 | DOI: 10.1056/NEJM198812013192203

VOL. 319 NO. 22

Abstract

The diagnosis of Lyme disease often depends on the measurement of serum antibodies to *Borrelia burgdorferi*, the spirochete that causes this disorder. Although prompt treatment with antibiotics may abrogate the antibody response to the infection, symptoms persist in some patients.

We studied 17 patients who had presented with acute Lyme disease and received prompt treatment with oral antibiotics, but in whom chronic Lyme disease subsequently developed. Although these patients had clinically active disease, none had diagnostic levels of antibodies

“We conclude that the presence of chronic Lyme disease cannot be excluded by the absence of antibodies against *B. burgdorferi* and that **a specific T-cell** blastogenic **response** to *B. burgdorferi* **is evidence of infection** in sero-negative patients with clinical indications of chronic Lyme disease.”

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

1. **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."

2. **Tylewska-Wierzbanowska S; Chmielewski T;** 2002 **Limiation 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.** 2001 **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."

4. **Brunner M.** 2001 **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."

Seronegativity has always been recognised

Author	Year	Title	Journal
39. Pachner A.	1995	Early disseminated Lyme disease.	Am J Med, 98 (suppl 4A):4A-30S-51S – Discussion.
		"The correlation between a positive Western blot and Lyme arthritis is probably the best of almost any Western blot and any Lyme disease manifestation. With neurologic disease, I have had a lot of patients who don't have a positive Western blot; they just have not developed a peripheral antibody response, for whatever reason."	
40. Coyle PK; Schutzer SE; Deng Z; Krupp LB; Belman MD; Benach JL; Luft BJ.	1995	Detection of Borrelia burgdorferi-specific antigen in antibody negative cerebrospinal fluid in neurologic Lyme disease.	Neurology, 45:2010-2014
		[From the abstract:] " RESULTS: Of the 35 of 83 (42%) patients who were positive for OspA antigen in their CSF, 15 (43%) were antigen positive despite being antibody-negative in CSF. Seven of these 15 (47%) had otherwise normal routine CSF analyses. Six of these 15 (40%) patients met strict CDC surveillance criteria for Lyme disease: four (27%) patients had seroconversion coincident with new neurologic problems; and three (20%) with characteristic syndromes for Lyme disease were seronegative, but had complexed antibody to B. burgdorferi. The final two patients (13%) were seropositive and had unexplained neurologic problems not characteristic of Lyme disease. CONCLUSIONS: B. burgdorferi antigen can be detected in CSF that is otherwise normal by conventional methodology, and can be present without positive CSF antibody. Since CSF antigen implies intrathecal seeding of the infection, the diagnosis of neurologic infection by B. burgdorferi should not be excluded solely on the basis of normal routine CSF or negative CSF antibody analyses."	
		[From the article:] "Prompt and precise diagnosis is difficult because basic microbiologic tests such as culture and staining have not been useful, on a broad scale, to document the presence of the spirochete in a body fluid. Instead, detection of specific antibodies to B burgdorferi in blood and CSF is commonly used to support or refute a clinical suspicion of infection. Many of the commercially available assays have been plagued by lack of sensitivity, specificity, and reproducibility. Furthermore, the absence of free antibodies to B burgdorferi components has been documented in well-characterized erythema-migrans-positive cases of Lyme disease, including those with prominent neurologic involvement."	
41. Karma A; Seppala I; Mikkila H; Kaakkola S; Viljanen M; Tarkkanen A.	1995	Diagnosis and clinical characteristics of ocular Lyme borreliosis.	American Journal of Ophthalmology, 119(2):127-35
		[From the abstract:] "Results of ELISA disclosed that five patients [out of ten] were seropositive, two patients showed borderline reactivity, and three patients were seronegative. Four of the five patients with borderline or negative results by ELISA had a positive result by western blot analysis. ... CONCLUSIONS: Late-phase ocular Lyme borreliosis is probably underdiagnosed because of weak seropositivity or seronegativity in ELISA assays."	
42. Lawrence C; Lipton RB; Lowy FD; Coyle PK.	1995	Seronegative chronic relapsing neuroborreliosis.	European Neurology, 35(2):113-7
		[From the abstract:] This article reports a Lyme disease patient "who experienced repeated neurologic relapses despite aggressive antibiotic therapy." The patient was seronegative. "Although the patient never had detectable free antibodies to B. burgdorferi in serum or spinal fluid, the CSF was positive on multiple occasions for complexed anti-B. burgdorferi antibodies, B. burgdorferi nucleic acids and free antigen."	
43. Millner M.	1995	Neurologic manifestations of Lyme borreliosis in children.	Wiener Medizinische Wochenschrift,145(7-8):178-82
		"Our own observations in children which suffered from an acute neuroborreliosis (NB) showed the following:... Indeed, there is a seronegative NB also in children."	

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- The importance of **T-cell testing – the cellular response**
- The unique qualities of the Tickplex test
- Patients rarely have “just“ Lyme – **multiple infections/**the **ArminLabs checklists**
- **Correlation with scientific articles** related to the patient‘s s diagnosis
- **Accreditations**

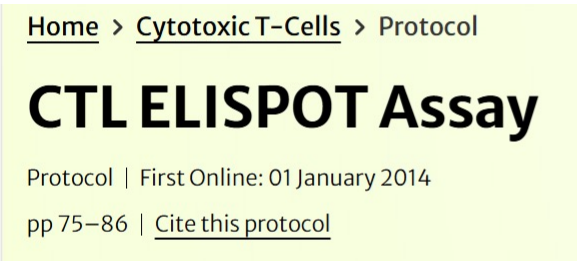
So then the question is: what test/s to use for chronic conditions, as we have seen how patients can fall between the cracks with standard (NHS) tests, and IgG tests only show past infection?

There is also the T cell arm of the immune system: tests of cellular immunity

There is another arm to the immune system that can be tested, too: not just B cells, but T cells. Tests of cellular T-cell immunity are called EliSpots (enzyme-linked immunosorbent spot).

Using T-cells to show a cellular response against antigens is much more sensitive, and is more likely to indicate active infection in contrast to IgG antibodies, which can remain for months or years long after an infection is gone, and IgM a/bs, which generally do not persist very long. EliSpot technology quantifies T-cells that secrete signature proteins (such as a given cytokine) against a specific antigen by evaluating the number of spot-forming units using a stimulation index (SI). This is a type of lymphocyte transformation test using an Interferon Gamma Release Assay.

“Accuracy, sensitivity, reproducibility, and robustness – a gold standard”



SPRINGER NATURE

“Enzyme-linked immune absorbent spot (Elispot) is a quantitative method for measuring relevant parameters of T cell activation. The sensitivity of Elispot allows the detection of low-frequency antigen-specific T cells that secrete cytokines and effector molecules, such as granzyme B and perforin. Cytotoxic T cell (CTL) studies have taken advantage with this high-throughput technology by providing insights into quantity and immune kinetics. Accuracy, sensitivity, reproducibility, and robustness of Elispot resulted in a wide range of applications in research as well as in the diagnostic field. Actually, CTL monitoring by Elispot is a gold standard for the evaluation of antigen-specific T cell immunity in clinical trials and vaccine candidates where the ability to detect rare antigen-specific T cells is of relevance for immune diagnostic.”

Source: Ranieri E, Popescu I, Gigante M. CTL ELISPOT assay. *Methods Mol Biol.* 2014;1186:75-86.

New "Springer Protocols" book (2024) with a chapter on EliSpots

Book | © 2024

Chapter 6

Adaptive Immune Response Investigation in Lyme Borreliosis

Mihail Pruteanu, Armin Schwarzbach, and Markus Berger

Abstract

To diagnose Lyme Borreliosis, it is advised to use an enzyme-linked immunosorbent test to check for serum antibodies specific for Lyme and all tests with positive or ambiguous enzyme-linked immunosorbent assay (ELISA) results being confirmed by immunoblot. This method of measuring the humoral immunity in human fluids (e.g., by ELISA) has provided robust and reproducible results for decades and similar assays have been validated for monitoring of B cell immunity. These immunological tests that detect antibodies to *Borrelia burgdorferi* are useful in the diagnosis of Borreliosis on a routine basis. The variety of different *Borrelia* species and their different geographic distributions are the main reasons why standards and recommendations are not identical across all geographic regions of the world. In contrast to humoral immunity, the T cell reaction or cellular immunity to the *Borrelia* infection has not been well elucidated, but over time with more studies a novel T cell-based assay (EliSpot) has been developed and validated for the sensitive detection of antigen-specific T cell responses to *B. burgdorferi*. The EliSpot Lyme assay can be used to study the T cell response elicited by *Borrelia* infections, which bridges the gap between the ability to detect humoral immunity and cellular immunity in Lyme disease. In addition, detecting cellular immunity may be a helpful laboratory diagnostic test for Lyme disease, especially for seronegative Lyme patients. Since serodiagnostic methods of the *Borrelia* infection frequently provide false positive and negative results, this T cell-based diagnostic test (cellular assay) may help in confirming a Lyme diagnosis. Many clinical laboratories are convinced that the cellular assay is superior to the Western Blot assay in terms of sensitivity for detecting the underlying *Borrelia* infection. Research also suggests that there is a dissociation between the magnitude of the humoral and the T cell-mediated cellular immune responses in the *Borrelia* infection. Lastly, the data implies that the EliSpot Lyme assay may be helpful to identify *Borrelia* infected individuals when the serology-based diagnostic fails to do so. Here in this chapter the pairing of humoral and cellular immunity is employed to evaluate the adaptive response in patients.

Cf. 3 pages of references for these T-cell tests at the end of the presentation

"The EliSpot Lyme assay can be used to study the T cell response elicited by Borrelia infections, which bridges the gap between the ability to detect humoral immunity and cellular immunity in Lyme disease. **Many clinical laboratories are convinced that the cellular assay is superior to the Western Blot assay in terms of sensitivity for detecting the underlying Borrelia infection..** Research also suggests that there is a dissociation between the magnitude of the humoral and the T cell-mediated cellular immune responses in the Borrelia infection."

Three parameters for Borrelia in the T-cell test – LFA-1 is a marker of autoimmune activity

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

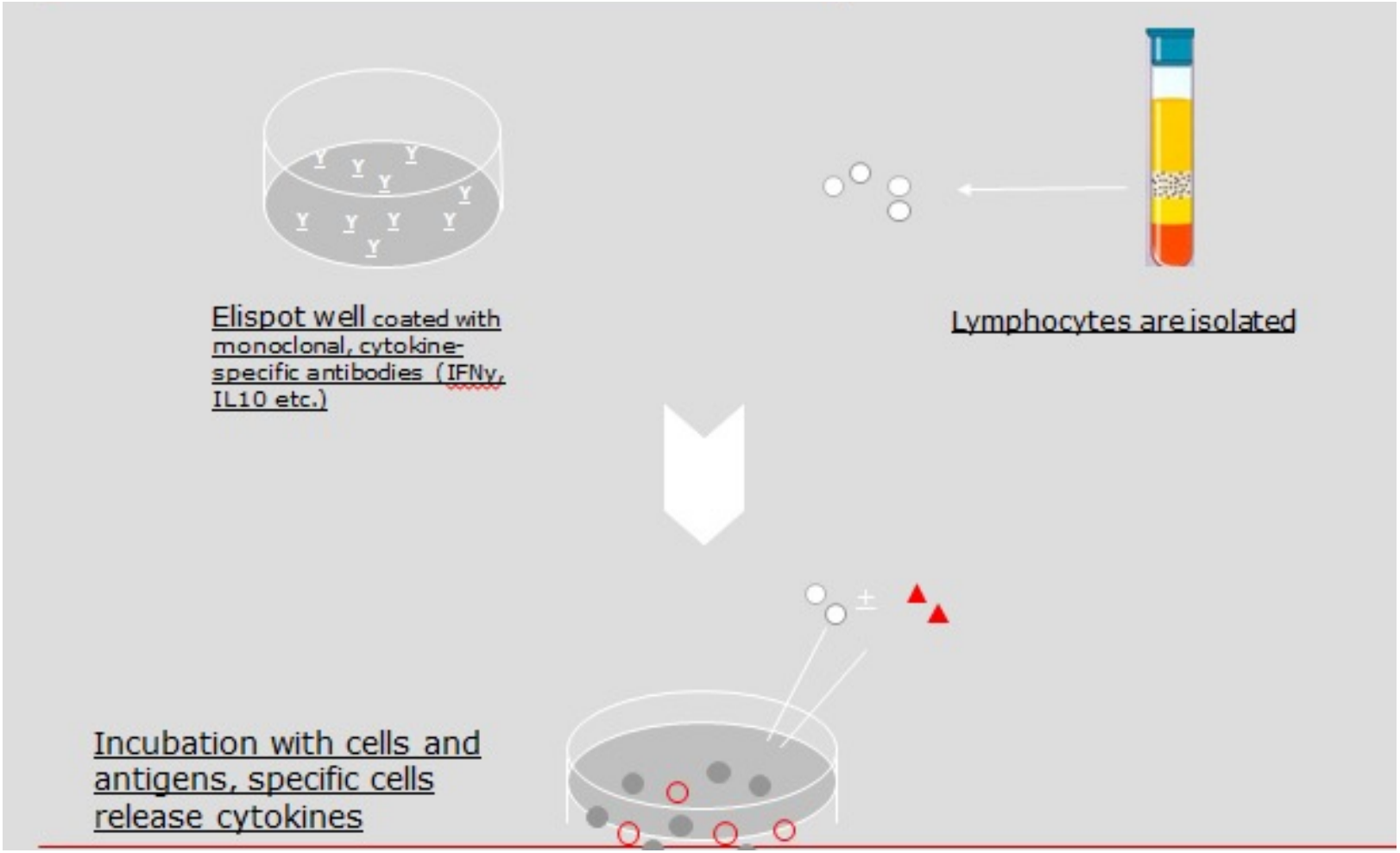
```
1 Borrelia burgdorferi LFA-1     !          7 SI
      0-1    = negative
      2-3    = weak positive
      > 3    = positive
```

Borrelia-burgdorferi **LFA-1 (Lymphocyte Function Antigen 1)**
Own body protein + Borrelia burgdorferi sensu stricto (shared epitope). LFA1 can be associated with autoimmune diseases: collagenosis, Rheumatoid Arthritis, vasculitis. If positive or borderline positive look at: ANA, CCP-antibodies, ANCA

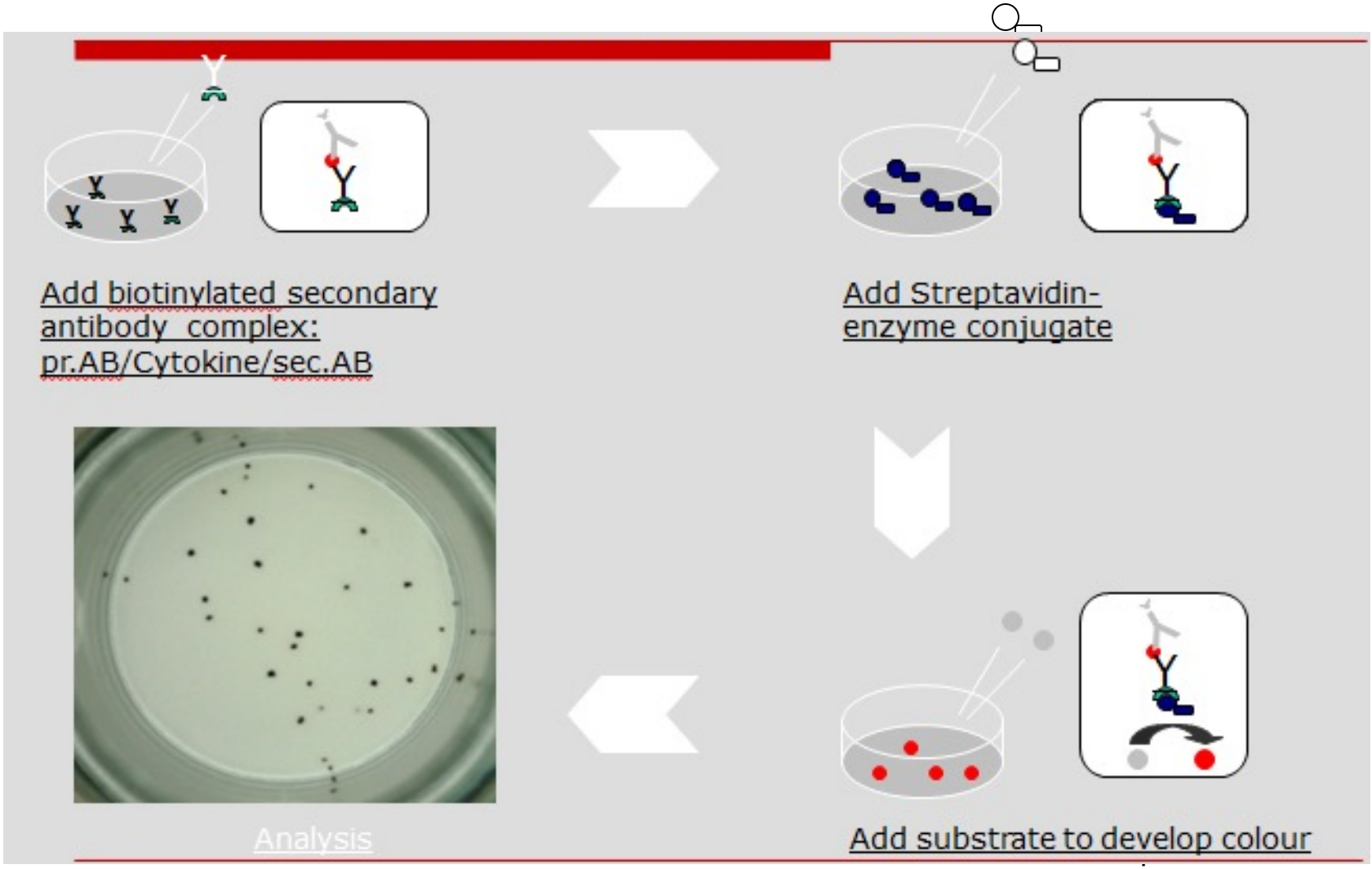
Example: “Borrelia burgdorferi has been shown to have protein homology with TSH receptor and therefore plays a role as an antigenic trigger for autoimmune thyroid disease”*

* Kharrazian D, Herbert M, Vojdani A. Immunological Reactivity Using Monoclonal and Polyclonal Antibodies of Autoimmune Thyroid Target Sites with Dietary Proteins. J Thyroid Res. 2017;2017:4354723.

Methodology of the EliSpot T-cell test (1/2)



Methodology of the EliSpot T-cell test (2/2)



Next generation EliSpot = Lyme iSpot (1/2)

AUTOIMMUN DIAGNOSTIKA GMBH

Next generation antigens for cellular immune response against Lyme coinfections in routine diagnostics

Next generation EliSpot = Lyme iSpot (1/2)

AUTOIMMUN DIAGNOSTIKA GMBH

Interpretation

IFNy negative
IL-2 positive
→ Latent or cured state of Borrelia Infection
No indication for treatment, Monitoring if clinical symptoms remain

Balance between
IL-2 and IFNy positive cells
→ Persistent state of Borrelia Infection
Diagnostic verification and monitoring if clinical Symptoms remain

IFNy positive
IL-2 negative
→ Active Immune answer to Borrelia Infection
Indication for Treatment, follow-up after treatment

IFNy negative
IL-2 negative
→ No Borrelia Infection
No Treatment

Borrelia iSpot – INF gamma and IL2

Borrelia iSpot

1	Borr.iSpot INF gamma Full Ag.*	4	SI
1	Borr.iSpot INF gamma OSP-Mix	0	SI
1	Borr.iSpot INF gamma LFA-1	0	SI
1	Borr.iSpot IL2 Full antigen *	2	SI
1	Borr.iSpot IL2 OSP-Mix	0	SI
1	Borr.iSpot IL2 LFA-1	0	SI

SI = Stimulation Index

0-1 = negative
2-3 = weak positive
> 3 = positive

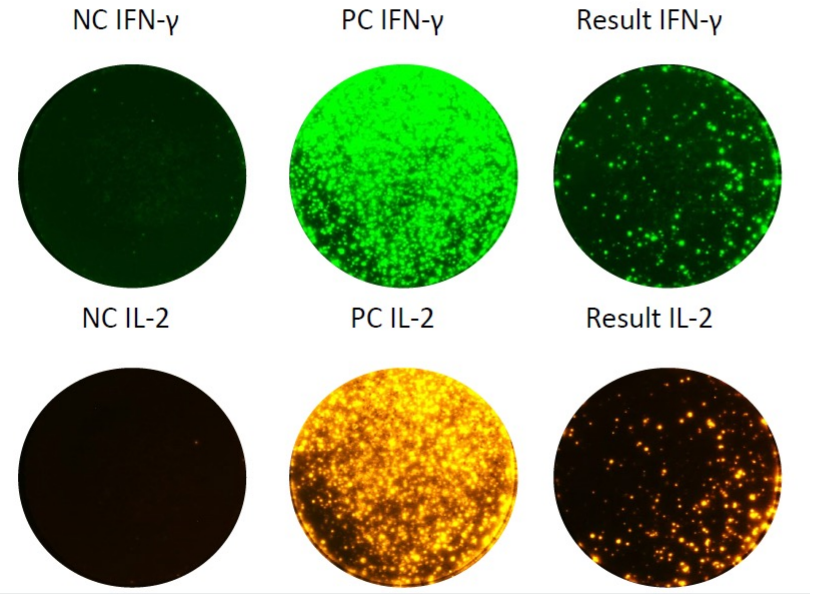
The result of the Borrelia iSpot test indicates positive cellular activity against Borrelia burgdorferi.

Explanation of antigens:
Borrelia-burgdorferi Full Antigen: Borrelia burgdorferi B31

Both positive and negative sample controls are used

Positive and Negative Sample Controls in EliSpot and iSpot

- Negative control to avoid false positive results
- Positive control to avoid false negative results
- Both controls are used to exclude cross-reactivity
- Both controls are obligatory and mandatory for the accreditation of the laboratory



References for the Elispot (T-cell testing): examples (1/3)

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References for the Elispot (T-cell testing): examples (1/3)

Clinical Infectious Diseases

MAJOR ARTICLE

Detection of IFN-γ Secretion by T Cells Collected Before and After Successful Treatment of Early Lyme Disease

Steven M. Callister,[1] Dean A. Jobe,[1] Aleksandra Stuparic-Stancic,[2] Misato Miyamasu,[3] Jeff Boyle,[3] Raymond J. Dattwyler,[4,5] and Paul M. Arnaboldi[4,5]

[1]Microbiology Research and Molecular Diagnostics Laboratory, and [2]Department of Urgent Care, Gundersen Health System, La Crosse, Wisconsin; [3]Qiagen, Inc, Germantown, Maryland; [4]Biopeptides Corporation, East Setauket, and [5]Department of Microbiology and Immunology, New York Medical College, Valhalla, New York

Background. Current serodiagnostics for Lyme disease lack sensitivity during early disease, and cannot determine treatment response. We evaluated an assay based on QuantiFERON technology utilizing peptide antigens derived from *Borrelia burgdorferi* to stimulate interferon-gamma (IFN-γ) release as an alternative to serodiagnosis for the laboratory detection of Lyme disease.

Methods. Blood was obtained from patients with erythema migrans before (n = 29) and 2 months after (n = 27) antibiotic therapy. IFN-γ release was measured by enzyme-linked immunosorbent assay (ELISA) following overnight stimulation of whole blood with the peptide antigens, and compared to the results of standard serological assays (C6, ELISA, and Western blot).

Results. IFN-γ release was observed in pretreatment blood of 20 of 29 (69%) patients with Lyme disease. Following antibiotic treatment, IFN-γ was significantly reduced ($P = .0002$), and was detectable in only 4 of 20 (20%) initially positive patients. By contrast, anti-C6 antibodies were detected in pretreatment sera from 17 of 29 (59%) subjects, whereas only 5 of 29 (17%) patients had positive Western blot seroreactivity. Antibody responses persisted and expanded following treatment.

Conclusions. Our findings suggest that measurement of IFN-γ after incubating blood with *Borrelia* antigens could be useful in the laboratory diagnosis of early Lyme disease. Also, after antibiotic treatment, this response appears to be short lived.

Keywords. *Borrelia burgdorferi*; IFN-γ; Lyme disease; T cell; cytokine release assay.

The detection of antibodies to *Borrelia burgdorferi* is the standard method for the laboratory diagnosis of Lyme disease. Whether using lysates of whole *Borrelia* species, mixtures of recombinant proteins, or specific peptide antigens (eg, C6, PepC10) as assay targets [1–6], current serological assays rarely exceed a sensitivity of 50% in the positive detection of antibody in early disease. In addition, these antibody detection assays do not provide accurate information concerning treatment response, as antibody levels often remain elevated for years after the infection has been cleared [5–7]. New approaches are therefore needed to overcome the shortcomings of current serologic assays.

Antigen-specific T-cell activation is typically initiated shortly after infection. The expanding cell population secretes cytokines that, among other activities, drives the development of a mature adjunct to traditional serologic testing methods, especially because the results may provide more accurate information on the presence of active infection compared to antibody responses.

Early attempts to evaluate the utility of monitoring T-cell responses in patients with Lyme disease yielded inconclusive results [11–14]. However, these studies relied prominently on T-cell proliferation as a measurement of T-cell activity, and this approach can suffer from a significant lack of specificity [13]. Furthermore, cytokines, including interferon gamma (IFN-γ), have been shown to inhibit T-cell proliferation under certain conditions [14], which would in turn reduce the usefulness of proliferation as a marker of infection. On the other hand, antigen-induced cytokine release may be a more reliable (albeit indirect) method to confirm T-cell activation [15, 16].

Callister SM, Jobe DA, Stuparic-Stancic A, Miyamasu M, Boyle J, Dattwyler RJ, Arnaboldi PM. Detection of IFN-γ Secretion by T Cells Collected Before and After Successful Treatment of Early Lyme Disease. Clin Infect Dis. 2016 May 15;62(10):1235-1241, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4845790/

Agenda

- **Downsides of using the CDC approach**
- The importance of **cellular tests**
- The unique qualities of the **Tickplex** test
- Patients rarely have “just“ Lyme – **multiple infections/**the **ArminLabs checklists**
- **Correlation with scientific articles** related to the patient‘s s diagnosis
- **Accreditations**

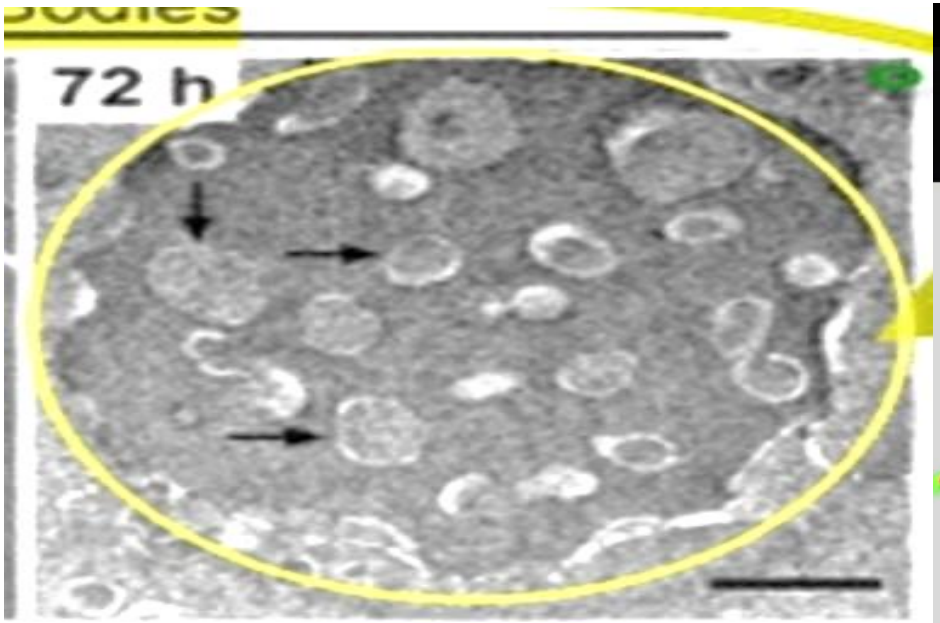
TickPlex is a very sensitive serological test that can also detect round bodies (the cyst form of Borrelia)

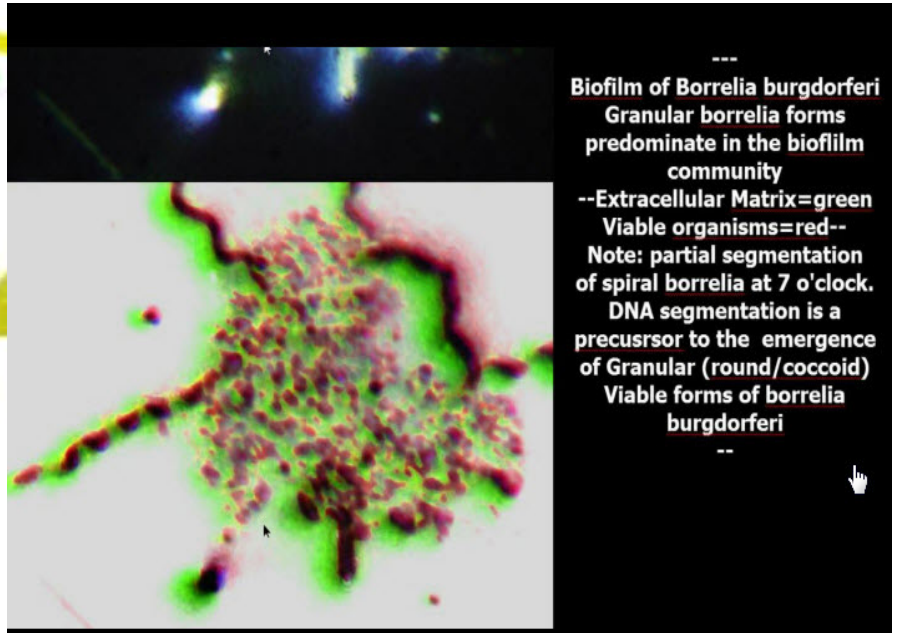
Basic Test/Tickplex Plus	**Result**	**Reference range**
Basic Test (neu)		
B.burg.+afz.+gar.IgG	**positive**	**negative**
	! 1,507 Ratio	
Ratio 0,01 - 0,89	= negative	
Ratio 0,90 - 0,99	= weak	
Ratio >= 1,00	= positive	
B.burg.+afz.+gar.IgM	**positive**	**negative**
	! 1,403 Ratio	
Ratio 0,01 - 0,89	= negative	
Ratio 0,90 - 0,99	= weak	
Ratio >= 1,00	= positive	
B.burg.+afz.+gar+round bod.IgG	negative	negative
	0,706 Ratio	
Ratio 0,01 - 0,89	= negative	
Ratio 0,90 - 0,99	= weak	
Ratio >= 1,00	= positive	
B.burg.+afz.+gar+round bod.IgM	**positive**	**negative**
	! 1,565 Ratio	
Ratio 0,01 - 0,89	= negative	
Ratio 0,90 - 0,99	= weak	
Ratio >= 1,00	= positive	

The antibodies indicate humoral immune responses against Borrelia burgdorferi.

Please look at the results of the EliSpots and the CD57-positive NK-cells.

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





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

Sources: 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; Miklossy J, Kasas S, Zurn AD, McCall S, Yu S, McGeer PL. Persisting atypical and cystic forms of Borrelia burgdorferi and local inflammation in Lyme neuroborreliosis. J Neuroinflammation. 2008 Sep 25;5:40.

References for the persister forms of *Borrelia burgdorferi* and chronicity, including in its "round-body" (cystic) form (1/2)

At least three morphologic forms of persistent *B. burgdorferi* have been observed in experimental studies, these being: spirochete, spheroplast (or L-form), and cystic or round-body forms. These persistent forms have been found to be highly resistant to conventional antibiotic treatment.

The following references provide extensive evidence of the pleomorphism of *B. burgdorferi,* with frequent reference to the round-body or cystic form:

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Agenda

- **Downsides of using the CDC approach**
- The importance of **cellular tests**
- The unique qualities of the **Tickplex** test
- **Patients rarely have “just“ Lyme – multiple infections/the ArminLabs checklists**
- **Correlation with scientific articles** related to the patient‘s diagnosis
- **Accreditations**

ArminLabs has evidence-based questionnaires/checklists to home in on the most likely coinfections

Short Symptom Checklist for Lyme Borreliosis

Name, first name ..... Date: .....

►	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 around ..... p.m.	
17	Tinnitus	
18	Swollen lymph nodes	
19	Numbness of the skin	
20	"Burning" or "pins and needles" skin sensations, painful sole or f	
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	
26	Concentration problems, short-term memory loss, forgetfulness	
27	Skin partly thin, paper-like, transparent, dry	
	Total number of symptoms	

Antibiotics? When? Which one(s)? How long?

.....

Coinfections-Checklist

Name, first name ..... Date (DD/MM/YYYY) .....

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

The autofill checklists help decide which other infections to test for, as Lyme rarely occurs alone

Multiple Infection Checklist

arminlabs

Name, first name ..... Date (DD/MM/YYYY) .....

	Your current and former symptoms Please click on the boxes next to the symptoms that you suffer from	X
1	Stomach ache, gut problems	☐
2	Anaemia	☐
3	Diarhoea intermittent, intestinal crampings/pain	☐
4	Fever or feverish feeling	☐
5	Lack of concentration, memory loss, forgetfulness	☐
6	Encephalitis/Inflammation of the brain	☐
7	Yellowish colour of the skin/eyes	☐
8	Painful joints or swollen joints	☐
9	General aches and pains, tendon problems	☐
10	Flu-like symptoms	☐
11	Rash(es), striae, exanthema	☐
12	Small red/purple spots of the skin	☐
13	Heart problems, disturbed cardiac rhythm	☐
14	Cough, expectoration, "air-hunger"	☐
15	Headache, dizziness	☐
16	Impaired liver function/ liver laboratory values	☐
17	Pneumonia, bronchitis	☐
18	Swollen lymph nodes	☐
19	Enlargement of the spleen	☐
20	Fatigue / exhaustion, intermittent or chronic CFS	☐
21	Muscle pain, muscle weakness	☐
22	Shivering, chill	☐
23	Blurred, foggy, cloudy, flickering, double vision	☐
24	Nausea, vomiting	☐
25	Dark urine	☐
26	Itching or pain when urinating	☐
27	Tingling, numbness, "burning" sensations	☐
28	Neck pain, neck stiffness	☐
29	Shoulder pain	☐

Ranked in order of priority:
CPn, Mycoplasma and the Herpesviruses draw for
first place here ↓

Below you'll find the number of the symptoms for each of the infections that we test for and the ranking, in which order you should test for them

Ranking of the infections	No. of symptoms	Rank
Chlamydia pneumoniae	4	1
Mycoplasma pneumoniae	4	1
Yersinia	2	3
Campylobacter	2	3
HSV 1/2	4	1
EBV	4	1
CMV	4	1
VZV	3	2
HHV 6	4	1
Parvovirus	3	2
Coxsackie-Virus	3	2
Echovirus	2	3

Agenda

- **Downsides of using the CDC approach**
- The importance of **cellular tests**
- The unique qualities of the **Tickplex** test
- Patients rarely have “just“ Lyme – **multiple infections**
- Patients rarely have “just“ Lyme – **multiple infections/**the **ArminLabs checklists**
- **Correlation with scientific articles** related to the patient‘s s diagnosis
- **Accreditations**

References available for making correlations between different diagnoses and Borrelia, other bacteria, and viruses

- SARS-CoV-2
- Type 1 Diabetes
- Multiple Sclerosis
- Rheumatoid arthritis
- Hashimoto‘s/Graves
- IBD
- Sjögren’s Syndrome
- Myasthenia Gravis
- PANS/PANDAS
- ALS/Motor Neurone Disease
- Fibromyalgia
- M.E./CFS
- Parkinsonism
- Autism
- Alzheimer‘s/Dementia
- ...

Agenda

- **Downsides of using the CDC approach**
- The importance of **cellular tests**
- The unique qualities of the **Tickplex** test
- Patients rarely have “just“ Lyme – **multiple infections**
- Usefulness of **checklists**
- **Correlation with scientific articles** related to the patient‘s s diagnosis
- **Accreditations**

Example requirements for meeting ArminLabs' Accreditations

- Regular participation in interlaboratory comparisons for all tests offered
- Independent annual assessment of all processes and procedures in the laboratory
- Obligation to use only IVD-registered test methods, thus enabling independent verification of all results

ArminLabs does not perform any self-developed tests. Every test performed is commercially available and certified / validated internally by ArminLabs and externally by the test manufacturers, as well as accreditation bodies.

Stability Controls as a Requirement for Laboratory Accreditation

- Required to maintain the accreditation
- Short- and Long-Term Stability: Testing to confirm the stability of samples under different storage conditions (room temperature, refrigerated, frozen)
- Freeze-Thaw Stability: Assessing sample integrity after repeated freeze-thaw cycles, as may occur during transportation
- Cell vitality: Living lymphocytes are essential for cellular tests such as EliSpot and iSpot. Thus, stimulation comparisons of lymphocytes are also performed in the laboratory as a dependent variable on different transport times

RFID-Enhanced Tracking to Ensure Quality During Transportation

- Real-time Tracking of all samples, providing constant visibility into position and transport status
- Temperature Monitoring to confirm that samples are maintained within the specified temperature range at all times
- Alert Notifications for deviations, enabling immediate action to preserve sample integrity

This comprehensive control and documentation ensure that your samples arrive safely and in optimal condition, meeting the highest international standards.

The fullest accreditation possible, worldwide

- "DAkkS" certificate that is constantly renewed (DAkkS - https://www.dakks.de/en/home-en.html - the national accreditation authority of the Federal Republic of Germany)
- CE certification
- IVD (In-Vitro Diagnostics) registration
- Certificate of UKAS-equivalence: The UK accreditation system (UKAS) does not have the mandate to determine tests carried out in another country but "has confidence in the accreditation system operated by Deutsche Akkreditierungsstelle GmbH (DAkkS) and considers that the accreditation system operated by DAkkS is equivalent to UKAS' own accreditation system."
- ISO 15189, and ArminLabs test producers have ISO 13485:2016

For the US, also

- Accredited by the College of American Pathologists (CAP) and
- CLIA (Clinical Laboratory Improvement Amendments)

AONM Professional Training

The Facts About Lyme Disease Testing

Thank you very much –
Q&A

www.arminlabs.com

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DIAGNOSING TICK-BORNE DISEASES

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COLLEGE of AMERICAN PATHOLOGISTS

CERTIFICATE OF ACCREDITATION

Arminlabs I Medicum Bad Aibling MVZ GmbH
Clinical Laboratory
Augsburg, Germany
Armin Schwarzbach, MD,PhD

CAP#: 8458129
CLIA#: 99D2279264

The organization named above meets all applicable standards for accreditation and is hereby accredited by the College of American Pathologists' Laboratory Accreditation Program. Reinspection should occur prior to **August 08, 2026** to maintain accreditation.

Accreditation does not automatically survive a change in director, ownership, or location and assumes that all interim requirements are met.

Kathleen G. Beavis, MD
Chair, Accreditation Committee

Donald S. Karcher, MD, FCAP
President, College of American Pathologists

CAP ACCREDITED

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