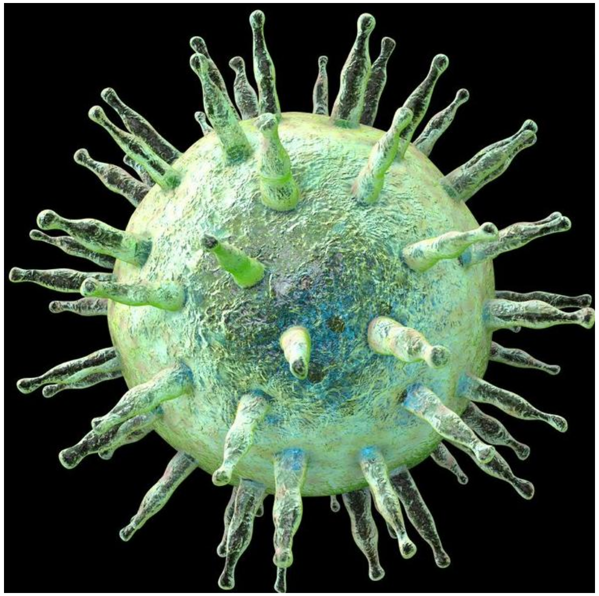

# Testing for Infections: Principles to keep in mind

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# Learning objectives

**Learning objectives**

- To understand the limitations of conventional testing
  for stealth infections, especially when chronic
- To gain insight into principles that can be helpful in deciding which
  infection-related tests to use for your patients

# What types of infection are the focus in this presentation?

**Vector-borne infections**
Borrelia
Babesia
Bartonella
Ehrlichia/Anaplasma
Rickettsia

**Primarily chronic rather than acute**

**Opportunistic infections**
**Bacteria:**
Chlamydia
Mycoplasma
Yersinia
Campylobacter

**Viruses:**
Herpes viruses: EBV, CMV, HSV 1/2, VZV, HHV6. HHV7, HHV8
Enteroviruses
SARS-CoV-2

# Agenda

- **Limitations of conventional testing for infections, especially when chronic: example Lyme Disease**

- Principles for selecting helpful tests
  1. In-depth history
  2. Use a questionnaire/checklist driven by an evidence-based algorithm
  3. Correlate your choice with references if possible; refer to resources linking the p/t's diagnosis to infections
  4. Choose tests where you either have IgA available ...
  5. ... or T-cell tests (and ideally both)
  6. Consider using an immune panel alongside the viral and bacterial axes
  7. Make sure the laboratory is fully accredited

- Resources

# Borrelia/Lyme Disease – shortcomings of NHS testing

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**

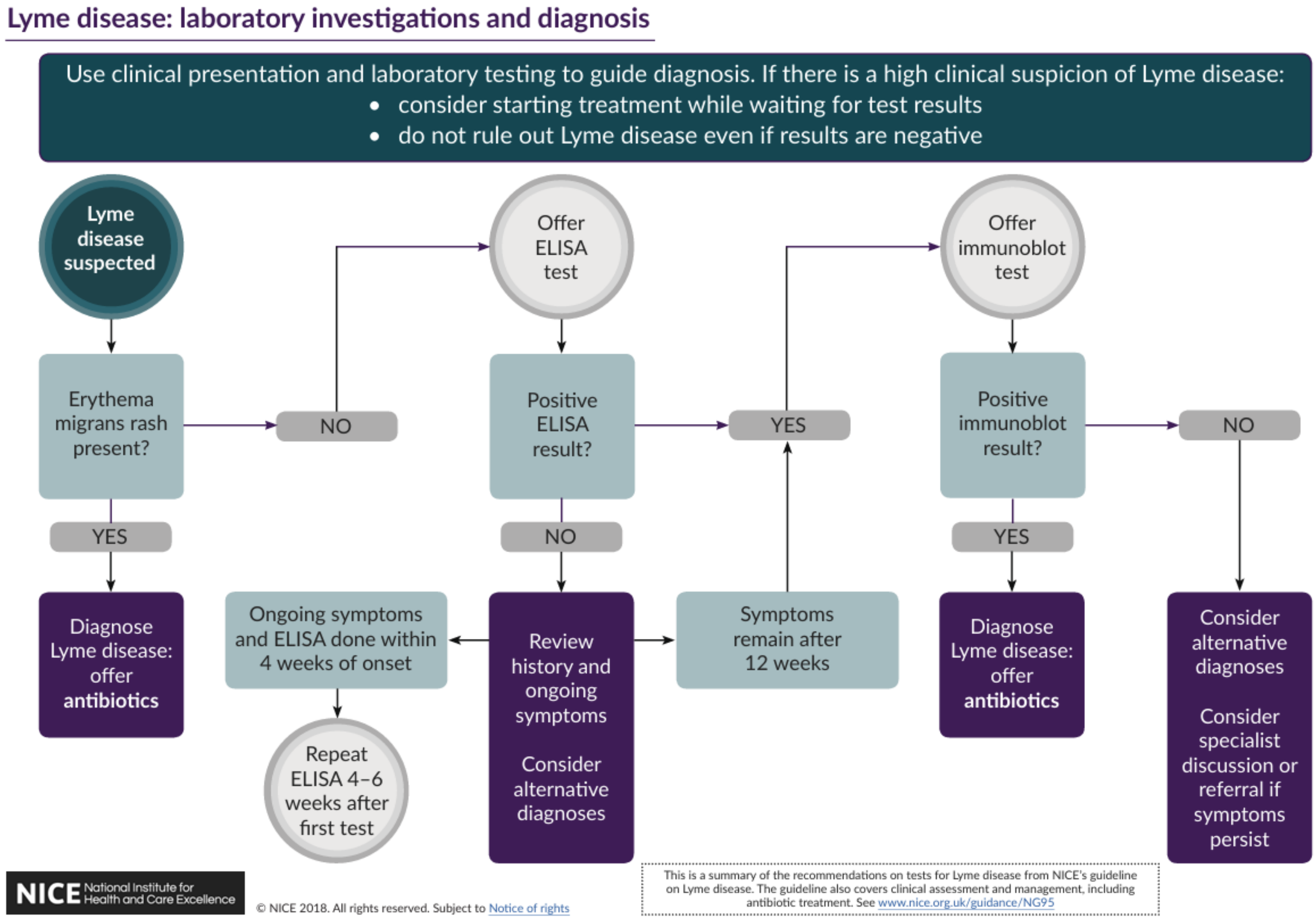
**Still using the "two-tier" testing system established at a conference in Dearborn, Michigan, 1994**

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

2$^{nd}$-tier test only carried out if the 1$^{st}$ 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




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=3337718872023121492215;
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*

# The Tier 1 ELISA detects “early, acute Lyme disease“

**GOV.UK**

Blog

## UK Health Security Agency

Organisations: UK Health Security Agency

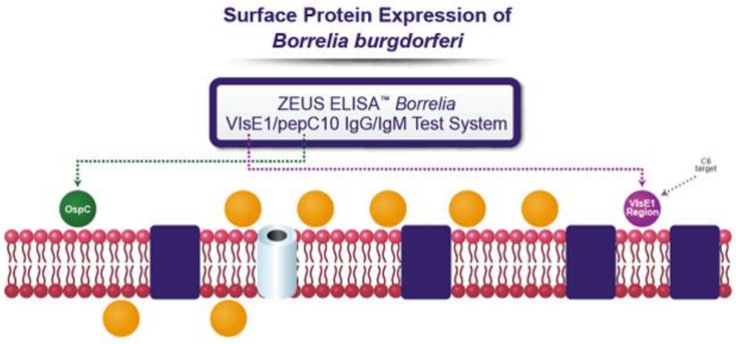
## What is Lyme disease and why do we need to be tick-aware?

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.




As seen from this depiction, the ZEUS ELISA™ VlsE1/pepC10 IgG/IgM assay utilizes **BOTH** VlsE and OspC antigen derivatives, which offers greater potential for detecting Lyme disease-associated antibodies relative to other assays containing only VlsE-derived antigens.

### 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/

# Often such a long meandering process that even early, acute cases are missed

NICE National Institute for Health and Care Excellence

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Home > NICE Guidance > Conditions and diseases > Infections > Bites and stings

## Lyme disease

NICE guideline [NG95] Published: 11 April 2018 Last updated: 17 October 2018

Guidance | Tools and resources | Information for the public | Evidence | History

- Overview
- Recommendations
- Recommendations for research
- Rationale and impact

### Guidance

### Recommendations

1.2.15 If the ELISA is positive or equivocal:

- perform an immunoblot test for Lyme disease **and**
- consider starting treatment with antibiotics while waiting for the results if there is a high clinical suspicion of Lyme disease.

1.2.16 If the ELISA for Lyme disease is negative and the person still has symptoms, review their history and symptoms, and think about the possibility of an alternative diagnosis.

1.2.17 If Lyme disease is still suspected in people with a negative ELISA who were tested within 4 weeks from symptom onset, repeat the ELISA 4 to 6 weeks after the first ELISA test.

1.2.18 If Lyme disease is still suspected in people with a negative ELISA who have had symptoms for 12 weeks or more, perform an immunoblot test.

1.2.19 Diagnose Lyme disease in people with symptoms of Lyme disease and a positive immunoblot test.

1.2.20 If the immunoblot test for Lyme disease is negative (regardless of the ELISA result) but symptoms persist, consider a discussion with or referral to a specialist, to:

- review whether further tests may be needed for suspected Lyme disease, for example, synovial fluid aspirate or biopsy, or lumbar puncture for cerebrospinal fluid analysis **or**
- consider alternative diagnoses (both infectious, including other tick-borne diseases, and non-infectious diseases).

Choose a specialist appropriate for the person's history or symptoms, for example, an adult or paediatric infection specialist, rheumatologist or neurologist.

1.2.21 If the immunoblot test for Lyme disease is negative and symptoms have resolved, explain to the person that no treatment is required.

*Source:* https://www.nice.org.uk/guidance/ng95/chapter/recommendations

# “Could indicate early infection if there has been recent exposure”

**LYME DISEASE STUDIES**
Specimen: BLOOD (CLOT)
Provider Specimen Comments:

| Investigation | Normality | Result |
|---|---|---|
| LYME DISEASE STUDIES | | |
| BORRELIA IGG/IGM (EIA ZEUS BOR | | POSITIVE |
| LABORATORY COMMENT | | Taken together, this positive EIA and single OspC IgM band may be a non-specific reaction (due to cross reaction with, for example, EBV) but could indicate early infection if there has been recent exposure. Note that erythema migrans should always be treated on clinical suspicion |

**LYME DISEASE STUDIES**
Specimen: BLOOD (CLOT)
Provider Specimen Comments:

| Investigation | Normality | Result |
|---|---|---|
| LYME DISEASE STUDIES | | |
| IGG TO BORRELIA P83 ANTIGEN | | Negative |
| IGG TO BORRELIA P58 ANTIGEN | | Negative |
| IGG TO BORRELIA P43 ANTIGEN | | Negative |
| IGG TO BORRELIA P39 ANTIGEN | | Negative |
| IGG TO BORRELIA P30 ANTIGEN | | Negative |
| IGG TO BORRELIA OSPC ANTIGEN | | Negative |
| IGG TO BORRELIA P21 ANTIGEN | | Negative |
| IGG TO BORRELIA OSP17 ANTIGEN | | Negative |
| IGG TO BORRELIA DBPA ANTIGEN | | Negative |

# “...inadequate for the diagnosis of the disease“

International Journal of General Medicine

Dovepress
open access to scientific and medical research

Open Access Full Text Article

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

# Other real examples: How possible chronic cases fall between the cracks

**In chronic disease, IgG may be there, but will be discounted as "past"; IgM probably will not be present**

ENDOCRINOLOGY

| | | |
|---|---|---|
| Cytomegalovirus Ab(IgG) | 183.0 | AU/ml |
| | < 6.0 AU/mL is considered non-reactive<br>>=6.0 AU/mL is considered reactive | |
| Cytomegalovirus Ab(IgM) | Negative | |
| Comment | Result suggestive of previous CMV infection. | |

IMMUNOLOGY

| | | | |
|---|---|---|---|
| Epstein-Barr virus screen<br>EBNA IgG antibody | * 36 | U/ml | (< 5 U/ml Negative) |
| EBV Early Ag ab.(IgG) | <5 | U/ml | (<10 U/ml Negative) |
| EBV VCA ab.(IgM) | <10 | U/ml | (<20 U/ml Negative) |
| Comment | Results suggestive of past ( latent ) EBV infection. | | |

**"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."**

*Source:* [http://www.microbiologybook.org/mayer/Ab%20formation2000.htm](http://www.microbiologybook.org/mayer/Ab%20formation2000.htm)

# Agenda

- **Limitations of conventional testing for infections, especially when chronic: example Lyme Disease**

- Principles for selecting helpful tests

  1. In-depth history
  2. Use a questionnaire/checklist driven by an evidence-based algorithm
  3. Correlate your choice with references if possible; refer to resources linking the p/t‘s diagnosis to infections
  4. Choose tests where you either have IgA available ...
  5. ... or T-cell tests (and ideally both)
  6. Consider using an immune panel alongside the viral and bacterial axes
  7. Make sure the laboratory is fully accredited

- Resources

# 1. In-depth history may well suggest possible pathogen triggers

Grew up on a farm?
Hiking in long grass, the hills, woods?
Camping?
Cats/dogs?
Horse riding? (when young?)
Foreign travel: which countries exactly?
Around young children?
COVID?

# 2. Use questionnaires/checklists to home in on the most likely infections

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

# 2 cont.: Checklist for potential reactivation of infections post Covid can also indicate the most likely tests to perform

Name, first name XXXXXX Date (DD/MM/YYYY) XXXXX

| | Your current and former symptoms<br>Please click on the boxes next to the symptoms that you suffer from | X |
|---|---|---|
| 1 | Stomach ache, gut problems | X |
| 2 | Anaemia | |
| 3 | Diarhoea intermittent, intestinal crampings/pain | |
| 4 | Fever or feverish feeling | X |
| 5 | Lack of concentration, memory loss, forgetfulness | X |
| 6 | Encephalitis/Inflammation of the brain | |
| 7 | Yellowish colour of the skin/eyes | |
| 8 | Painful joints or swollen joints | X |
| 9 | General aches and pains, tendon problems | X |
| 10 | Flu-like symptoms | X |
| 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 | X |
| 16 | Impaired liver function/ liver laboratory values | |
| 17 | Pneumonia, bronchitis | |
| 18 | Swollen lymph nodes | X |
| 19 | Enlargement of the spleen | |
| 20 | Fatigue / exhaustion, intermittent or chronic CFS | X |
| 21 | Muscle pain, muscle weakness | X |
| 22 | Shivering, chill | X |
| 23 | Blurred, foggy, cloudy, flickering, double vision | X |
| 24 | Nausea, vomiting | |
| 25 | Dark urine | |
| 26 | Itching or pain when urinating | |
| 27 | Tingling, numbness, "burning" sensations | X |
| 28 | Neck pain, neck stiffness | |
| 29 | Shoulder pain | |
| 30 | Back pain, pelvic pain | |
| 31 | Sleeplessness | |
| 32 | Night sweat, sometimes between 2 and 4 a.m. | |
| 33 | Sore throat, throat pain | X |
| 34 | Tinnitus, hearing loss | |
| 35 | Dry skin | |
| 36 | Conjunctivitis, inflammation of the eyes | |
| 37 | Panic attacks, depression, psychosis, mood swings | |
| 38 | Seizures, tremors | |
| 39 | Sinusitis | |

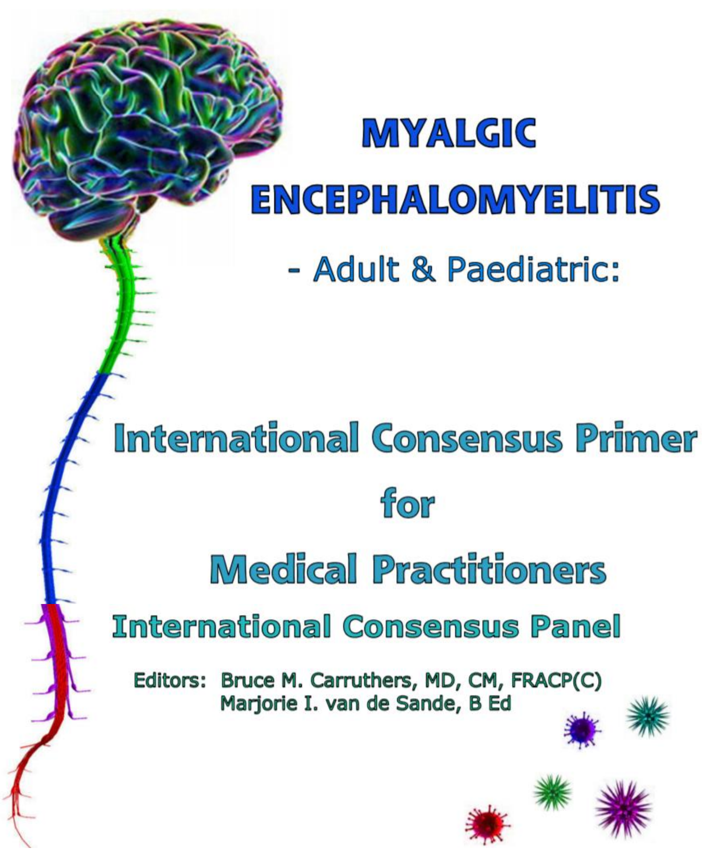
Below you'll find the number of the symptoms for each of the infections that we test for and the ranking, in which order you should test for them

| Ranking of the infections | No. of symptoms | Rank |
|---|---|---|
| Chlamydia pneumoniae | 12 | 1 |
| Mycoplasma pneumoniae | 12 | 1 |
| Yersinia | 6 | 5 |
| Campylobacter | 6 | 5 |
| HSV 1/2 | 8 | 3 |
| EBV | 9 | 2 |
| CMV | 9 | 2 |
| VZV | 7 | 4 |
| HHV 6 | 9 | 2 |
| Parvovirus | 9 | 2 |
| Coxsackie-Virus | 12 | 1 |
| Echovirus | 8 | 3 |

We pinpoint the cause

# 3. Correlate your choice with references if possible

**Refer to resources linking the patient's diagnosis to infections – does the lab have any? Or can you do a search linking the most likely stealth pathogens to his/her condition?**




**Example: ME/CFS – International Consensus Primer for Medical Practitioners lists infections under "Causal Factors" – well referenced:**

ON • Causal Factors • Phases

MYALGIC ENCEPHALOMYELITIS – Adult & Paediatric:

● blood transfusions ● anaesthetics ● toxic chemicals ● heavy metals ● severe physical trauma: whiplash/spinal injury/surgery ● undue psychological stress[17-23]

**Precipitating Events and Causal Factors:** Most patients enjoyed healthy, active lifestyles prior to the onset of ME. Widely dispersed epidemics support an infectious cause. Symptoms at onset are usually consistent with an infectious process.

1. **Infectious agents associated with ME**

   *Viruses:* ● Enterovirus[24-26] ● Epstein Barr virus (EBV)[27] ● Human herpes virus (HHV 6 and 7)[28, 29] ● Cytomegalovirus[30] ● Parvovirus B19[31]

   *Bacteria:* Chlamydophila pneumonia[32] ● Mycoplasma[33] ● Coxiella burnettii[27]

   It is unclear whether these infectious agents initiated ME or are opportunistic and developed due to an impaired immune system. No one virus has been universally implicated for all patients. A prospective study reported that six months following acute infections of Epstein-Barr virus, Coxiella Burnetii, or Ross River virus, 11% of the patients had CFS.[34] This supports the presence of ME subtypes. Antibody testing for a number of viruses revealed subtype-specific relationships for Epstein Barr virus and enterovirus, two of the most common infectious triggers for ME.[27]

2. **Possible etiological process:** A growing body of evidence suggests that a primary cause of ME is neuropathic viruses that may infect neurological and immune cells and damage the capillaries and micro-arteries in the CNS bed causing diffuse brain injury. The initial infection may cause profound dysregulation of immune system pathways that may become chronic or cause autoimmunity even when the level of the infectious agent is reduced.[35]

**Onset Survey**
1,000+ patients
**75.6%: infection alone or infection + 1 or more factors:**
environmental exposure,
physical trauma,
vaccinations,
other stressors
*Vernon SD. CFIDS of America*

https://www.investinme.org/Documents/Guidelines/Myalgic%20Encephalomyelitis%20International%20Consensus%20Primer%20-2012-11-26.pdf

# Example: Type 1 Diabetes and Cytomegalovirus

Pak CY, Eun HM, McArthur RG, Yoon JW. Association of cytomegalovirus infection with autoimmune type 1 diabetes. Lancet. 1988 Jul 2;2(8601):1-4.

Osame K, Takahashi Y, Takasawa H, Watanabe S, Kishimoto M, Yasuda K, Kaburagi Y, Nakanishi K, Kajio H, Noda M. Rapid-onset type 1 diabetes associated with cytomegalovirus infection and islet autoantibody synthesis. Intern Med. 2007;46(12):873-7.

Aarnisalo J, Veijola R, Vainionpää R, Simell O, Knip M, Ilonen J. Cytomegalovirus infection in early infancy: risk of induction and progression of autoimmunity associated with type 1 diabetes. Diabetologia. 2008 May;51(5):769-72.

Hjelmesaeth J, Sagedal S, Hartmann A, Rollag H, Egeland T, Hagen M, Nordal KP, Jenssen T. Asymptomatic cytomegalovirus infection is associated with increased risk of new-onset diabetes mellitus and impaired insulin release after renal transplantation. Diabetologia. 2004 Sep;47(9):1550-6.

Zanone MM, Favaro E, Quadri R, Miceli I, Giaretta F, Romagnoli R, David E, Perin PC, Salizzoni M, Camussi G. Association of cytomegalovirus infections with recurrence of humoral and cellular autoimmunity to islet autoantigens and of type 1 diabetes in a pancreas transplanted patient. Transpl Int. 2010 Mar 1;23(3):333-7.

Ekman I, Vuorinen T, Knip M. Early childhood CMV infection may decelerate tne progression to clinical type 1 diabetes. Pediatr Diabetes. 2019;20:73-77.

Batchy AA et al. Human Cytomegalovirus Infection Associated with Low Insulin Secretion in a Type 1 Diabetic Population in Pointe Noire . Med Clin Res Open Access. 2022; 3(1):1-5.

# Example: Type 1 Diabetes and Enteroviruses

Isaacs SR, Roy A, Dance B, Ward EJ, Foskett DB, Maxwell AJ, Rawlinson WD, Kim KW, Craig ME. Enteroviruses and risk of islet autoimmunity or type 1 diabetes: systematic review and meta-analysis of controlled observational studies detecting viral nucleic acids and proteins. Lancet Diabetes Endocrinol. 2023 Aug;11(8):578-592.

King ML, Shaikh A, Bidwell D, Voller A, Banatvala JE. Coxsackie-B-virus-specific IgM responses in children with insulin-dependent (juvenile-onset; type I) diabetes mellitus. Lancet. 1983 Jun 25;1(8339):1397-9.

Krogvold L, Genoni A, Puggioni A, Campani D, Richardson SJ, Flaxman CS, Edwin B, Buanes T, Dahl-Jørgensen K, Toniolo A. Live enteroviruses, but not other viruses, detected in human pancreas at the onset of type 1 diabetes in the DiViD study. Diabetologia. 2022 Dec;65(12):2108-2120.

Yeung WC, Rawlinson WD, Craig ME. Enterovirus infection and type 1 diabetes mellitus: systematic review and meta-analysis of observational molecular studies. BMJ. 2011 Feb 3;342:d35.
Diaz-Horta, Oscar & Sarmiento, Luis & Baj, Andreina & Cabrera-Rode, Eduardo & Toniolo, Antonio. (2011). Echovirus Epidemics, Autoimmunity, and Type 1 Diabetes.

Carré A, Vecchio F, Flodström-Tullberg M, You S, Mallone R. Coxsackievirus and Type 1 Diabetes: Diabetogenic Mechanisms and Implications for Prevention. Endocr Rev. 2023 Jul 11;44(4):737-751.

Diaz-Horta, Oscar & Sarmiento, Luis & Baj, Andreina & Cabrera-Rode, Eduardo & Toniolo, Antonio. (2011). Echovirus Epidemics, Autoimmunity, and Type 1 Diabetes.

T1D

# Diabetes Type 1: Possible pathogen involvement

**From searches of the scientific literature:**

1. CMV
2. Coxsackie B Virus
3. Echovirus
4. SARS-CoV-2

Selection | MS

# Example: Multiple sclerosis and viruses (1/2)


Bjornevik K et al. Longitudinal analysis reveals high prevalence of Epstein-Barr virus associated with multiple sclerosis. Science. 2022 Jan 21;375(6578):296-301.

Gilden DH. Infectious causes of multiple sclerosis. Lancet Neurol. 2005 Mar;4(3):195-202.

Kus, Sumeyye et al. (2024). Enterovirus Radiculomyelitis in a Relapsing-remitting Multiple Sclerosis Patient (P3-6.002). Neurology. 102.

Lundström W et al. Human Herpesvirus 6A Is a Risk Factor for Multiple Sclerosis. Front Immunol. 2022 Feb 10;13:840753.

Voumvourakis KI et al. Human herpesvirus 6 infection as a trigger of multiple sclerosis: an update of recent literature. BMC Neurol. 2022 Feb 15;22(1):57.

Pumphrey CM et al. Acute Presentation of Newly Diagnosed Multiple Sclerosis Associated With Polymerase Chain Reaction-Proven Human Herpesvirus 6 Central Nervous System Infection. Cureus. 2022 Apr 20;14(4):e24319.

Lucas RM et al and the Autoimmune Investigator Group. Risk of a first clinical diagnosis of central nervous system demyelination in relation to human herpesviruses in the context of Epstein-Barr virus. Eur J Neurol. 2023 Sep;30(9):2752-2760.

Grut V et al. Human herpesvirus 6A and axonal injury before the clinical onset of multiple sclerosis. Brain. 2024 Jan 4;147(1):177-185.

Cermelli C, Jacobson S. Viruses and multiple sclerosis. Viral Immunol. 2000;13(3):255-67.

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Jeanne Billioux B et al. HHV-6 and Multiple Sclerosis. Human Herpesviruses HHV-6A, HHV-6B & HHV-7. 2014:123–42.

Santiago O et al. Relation between Epstein-Barr virus and multiple sclerosis: analytic study of scientific production. Eur J Clin Microbiol Infect Dis. 2010;29:857–66

https://hhv-6foundation.org/multiple-sclerosis/hhv-6a-can-travel-through-the-nose-to-the-brain

# Example: MS and viruses (2/2)

Najafi S, Ghane M, Poortahmasebi V, Jazayeri SM, Yousefzadeh-Chabok S. Prevalence of Cytomegalovirus in Patients With Multiple Sclerosis: A Case-Control Study in Northern Iran. Jundishapur J Microbiol. 2016 Jul 3;9(7):e36582.
Forteza, Francisco & Quinaz, C.. (2023). Post-COVID-19 multiple sclerosis with concomitant herpes simplex virus type 1 meningoencephalitis. Neurology Perspectives. 4.
MacDougall M, El-Hajj Sleiman J, Beauchemin P, Rangachari M. SARS-CoV-2 and Multiple Sclerosis: Potential for Disease Exacerbation. Front Immunol. 2022 Apr 22;13:871276.
Bellucci G, Rinaldi V, Buscarinu MC, Reniè R, Bigi R, Pellicciari G, Morena E, Romano C, Marrone A, Mechelli R, Salvetti M, Ristori G. Multiple Sclerosis and SARS-CoV-2: Has the Interplay Started? Front Immunol. 2021 Sep 27;12:755333.
Lake CM, Breen JJ. Sequence similarity between SARS-CoV-2 nucleocapsid and multiple sclerosis-associated proteins provides insight into viral neuropathogenesis following infection. Sci Rep. 2023 Jan 8;13(1):389.
Sarwar S, Rogers S, Mohamed AS, Ogula E, Ayantayo RA, Ahmed A, Shahzadi I, Kataria S, Singh R. Multiple Sclerosis Following SARS-CoV-2 Infection: A Case Report and Literature Review. Cureus. 2021 Oct 25;13(10):e19036.
Shalaby NM, Shehata HS. Could SARS-CoV-2 herald a surge of multiple sclerosis? Egypt J Neurol Psychiatr Neurosurg. 2021;57(1):22.
Palao M, Fernández-Díaz E, Gracia-Gil J, Romero-Sánchez CM, Díaz-Maroto I, Segura T. Multiple sclerosis following SARS-CoV-2 infection. Mult Scler Relat Disord. 2020 Oct;45:102377.
Langer-Gould A, Wu J, Lucas R, Smith J, Gonzales E, Amezcua L, Haraszti S, Chen LH, Quach H, James JA, Barcellos LF, Xiang AH. Epstein-Barr virus, cytomegalovirus, and multiple sclerosis susceptibility: A multiethnic study. Neurology. 2017 Sep 26;89(13):1330-1337.

# Example: Multiple sclerosis and bacteria (1/2)

**Borrelia**

Fritzsche M. Chronic Lyme borreliosis at the root of multiple sclerosis--is a cure with antibiotics attainable? Med Hypotheses. 2005;64(3):438-48.
Marshall V. Multiple sclerosis is a chronic central nervous system infection by a spirochetal agent. Med Hypotheses. 1988 Feb;25(2):89-92.
Murray R, Morawetz R, Kepes J, el Gammal T, LeDoux M. Lyme neuroborreliosis manifesting as an intracranial mass lesion. Neurosurgery. 1992 May;30(5):769-73. PMID: 1584393
Kurtz SK. Relapsing fever/Lyme disease. Multiple sclerosis. Med Hypotheses. 1986 Nov;21(3):335-43.
2000 (Poland): Lyme borreliosis and Multiple sclerosis: Any Connection? A Seroepidemic study. Ann Agric Environ Med. issue 7, 141-143
2001 (Norway): Association between Multiple sclerosis and Cystic Structures in Cerebrospinal Fluid. Infect 29:315
2004 (Switzerland): Chronic Lyme borreliosis at the root of Multiple sclerosis – is a cure with antibiotics attainable?
Meier, C., F. Grahmann, A. Engelhardt, and M. Dumas. 1989. Peripheral nerve disorders in Lyme-borreliosis: nerve biopsy studies from eight cases. Acta Neuropathol. 79:271–278;
Sigal, L. H., and A. H. Tatum. 1988. Lyme disease patients’ serum contains IgM antibodies to Borrelia burgdorferi that cross-react with neuronal antigens. Neurology 38:1439–1442;
Garcia-Monco JC, Coleman JL, Benach JL (1988) Antibodies to myelin basic protein in Lyme disease. J Infect Dis 158 : 667- 668

# Example: MS and bacteria (2/2)

**Mycoplasma**
Libbey JE et al. Role of pathogens in multiple sclerosis. Int Rev Immunol. 2014 Jul-Aug;33(4):266-83.
Maida E. Immunological reactions against Mycoplasma pneumoniae in multiple sclerosis: preliminary findings. J Neurol. 1983;229(2):103-11.
Cossu D, Yokoyama K, Hattori N. Bacteria-Host Interactions in Multiple Sclerosis. Front Microbiol. 2018 Dec 4;9:2966.
“Autoimmune diseases caused by mycoplasmas” Harold W. Clark, PhD, Mycoplasma Research Institute Florida
“Detection of Mycoplasma pneumoniae and Chlamydia pneumoniae in ruptured atherosclerotic plaques”; Higuchi ML, et.al., Braz J Med Biol Res. 2000 Sep;33(9):1023-6
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Taylor L.N.D. Mycoplasma – Stealth Pathogens. Amer Soc Microbiol. 182, 5238-5250 (2001)
**Chlamydia pneumoniae**
Detection of Mycoplasma pneumoniae and Chlamydia pneumoniae in ruptured atherosclerotic plaques”; Higuchi ML, et.al., Braz J Med Biol Res. 2000 Sep;33(9):1023-6
Contini C et al. Chlamydophila pneumoniae Infection and Its Role in Neurological Disorders. Interdiscip Perspect Infect Dis. 2010;2010:273573.
**Bartonella**
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2546763/,
https://www.lymeneteurope.org/forum/viewtopic.php?t=2445

# Multiple Sclerosis: Possible pathogen involvement

**From searches of the scientific literature:**

1. EBV
2. HHV6
3. SARS-CoV-2
4. Coxsackie Virus
5. Echovirus
6. Borrelia
7. Chlamydia pneumoniae
8. Mycoplasma

# Example: Crohn's/Ulcerative Colitis and viruses (1/2)

Selection IBD

Nyström N et al. Human enterovirus species B in ileocecal Crohn's disease. Clin Transl Gastroenterol. 2013 Jun 27;4(6):e38.
Asadzadeh Aghdaei H et al. Virus in the pathogenesis of inflammatory bowel disease: role of Toll-like receptor 7/8/3. Gastroenterol Hepatol Bed Bench. 2021 Fall;14(4):295-303. PMID: 34659656; PMCID: PMC8514217.
Adiliaghdam F et al. Human enteric viruses autonomously shape inflammatory bowel disease phenotype through divergent innate immunomodulation. Sci Immunol. 2022 Apr 8;7(70):eabn6660.
Dehghani T et al. Association Between Inflammatory Bowel Disease and Viral Infections. Curr Microbiol. 2023 Apr 27;80(6):195.
Chia, J. et al. (2019) Echovirus 18 Infection Is Associated with Crohn's Disease. Open Journal of Gastroenterology, 9, 174-183.
Riemann JF. Viral agents in Crohn's disease. Acta Hepatogastroenterol (Stuttg). 1977 Dec;24(6):403-4. PMID: 602615
Craviotto V et al. Viral infections in inflammatory bowel disease: Tips and tricks for correct management. World J Gastroenterol. 2021 Jul 21;27(27):4276-4297.
https://www.sciencedaily.com/releases/2022/10/221005111916.htm
Matsuzawa-Ishimoto Y et al. The γδ IEL effector API5 masks genetic susceptibility to Paneth cell death. Nature. 2022 Oct;610(7932):547-554.
Mehrabani-Khasraghi S, Ameli M, Khalily F. Investigation of Ulcerative Colitis for Herpes Simplex Virus and Cytomegalovirus Genomic Sequences by the Polymerase Chain Reaction. Gene Cell Tissue. 2015;2(4):e32846.

# Example: Crohn’s/UC and viruses (2/2)

Mourad FH et al. Ulcerative Colitis and Cytomegalovirus Infection: From A to Z. J Crohns Colitis. 2020 Sep 7;14(8):1162-1171.
Pillet S et al. Cytomegalovirus and ulcerative colitis: Place of antiviral therapy. World J Gastroenterol. 2016 Feb 14;22(6):2030-45.
Jentzer A et al. Cytomegalovirus and Inflammatory Bowel Diseases (IBD) with a Special Focus on the Link with Ulcerative Colitis (UC). Microorganisms. 2020 Jul 20;8(7):1078.
Garrido E et al. Clinical significance of cytomegalovirus infection in patients with inflammatory bowel disease. World J Gastroenterol. 2013 Jan 7;19(1):17-25.
Nissen LH et al. Epstein-Barr virus in inflammatory bowel disease: the spectrum of intestinal lymphoproliferative disorders. J Crohns Colitis. 2015 May;9(5):398-403.
Zhang H et al. Impact of Epstein-Barr virus infection in patients with inflammatory bowel disease. Front Immunol. 2022 Oct 28;13:1001055.
Jena A et al. Cytomegalovirus in ulcerative colitis: an evidence-based approach to diagnosis and treatment. Expert Rev Gastroenterol Hepatol. 2022 Feb;16(2):109-120.
Schreiner P et al. Varicella zoster virus in inflammatory bowel disease patients: what every gastroenterologist should know. J Crohns Colitis. 2020 Jun 27:jjaa132.
Ham M et al. Varicella zoster virus infection in patients with inflammatory bowel disease. Gastroenterol Hepatol (N Y). 2013 Jan;9(1):56-8. PMID: 24707238; PMCID: PMC3975982.
Wang, Lin. (2016). Crohn’s disease with recurrent herpes zoster: A case report. World Chinese Journal of Digestology. 24. 1618. 10.11569/wcjd.v24.i10.1618.
Zou M et al. Causal association between inflammatory bowel disease and herpes virus infections: a two-sample bidirectional Mendelian randomization study. Front Immunol. 2023 Jul 3;14:1203707.

# IBD: Possible pathogen involvement

**From searches of the scientific literature:**

1. Coxsackievirus
2. Echovirus
3. Norovirus
4. EBV
5. CMV
6. VZV
7. Campylobacter

**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?**

# 4. The most useful antibody in a chronic infection is Immunoglobulin A

## IgA is an excellent immunoglobulin as it indicates ongoing infection (whether recent or chronic), as well as reactivation/reinfection

"IgA antibody is the most abundant antibody class in human serum and has a unique role in mediating immunity. IgA is a polyvalent antibody that is translocated to mucosal surfaces as the first line of defense against infections. Most of the secreted IgA lines the mucosal surfaces including respiratory, digestive and genitorurinary tracts to protect against pathogens while maintaining gut homeostasis."

**The persistence of IgA antibodies in Yersinia, as an example** →

JOURNAL ARTICLE

### Persistence of IgM, IgG, and IgA Antibodies to *Yersinia* in Yersinia Arthritis

Get access ›

Kaisa Granfors ✉, Matti Viljanen, Anja Tiilikainen, Auli Toivanen

*The Journal of Infectious Diseases*, Volume 141, Issue 4, April 1980, Pages 424–429, https://doi.org/10.1093/infdis/141.4.424

**Published:** 01 April 1980 **Article history** ▾

Cite Permissions Share ▾

**Abstract**

IgA antibodies to *Yersinia enterocolitica* were demonstrated in the sera of 13 patients with severe yersinia arthritis who were studied six to eight months after an acute infection with *Yersinia*. Four of the patients were monitored for two to three years, and they continued to demonstrate these antibodies. Only one of 12 control patients (individuals with yersinia infection without arthritis) had IgA antibodies specific to *Yersinia* six to eight months after the acute infection. The persistence of IgO antibodies was also in direct correlation to the occurrence of arthritis, but not as clearly as was the persistence of IgA antibodies. Antibodies of the IgM class persisted in most cases for only one to three months and always disappeared during the first six months after the onset of the infection. Thus, the demonstration of IgA antibodies to *Yersinia* is important in the diagnosis of yersinia arthritis, and the occurrence of IgM

*Source:* *https://www.genscript.com/IgA-antibody.html*; Granfors K, Viljanen M, Tiilikainen A, Toivanen A. Persistence of IgM, IgG, and IgA antibodies to Yersinia in yersinia arthritis. J Infect Dis. 1980 Apr;141(4):424-9.

# 4 cont.: IgA antibodies very helpful as they indicate active infection along the mucosal membranes

**VZV IgG-/IgA-/IgM-antibodies**

4 VZV IgG antibodies (ELISA) positive
4643,8 IE/l

| | |
|---|---|
| <80 IE/l | negative |
| >80 – < 110 IE/l | weak |
| >110 IE/l | positive |

4 VZV IgA antibodies (ELISA) positive
1,287 Ratio

| | |
|---|---|
| Ratio < 0,8 | = negative |
| Ratio 0,8 – 1,1 | = weak |
| Ratio >= 1,1 | = positive |

4 VZV IgM antibodies (ELISA) negative
0,306 Ratio

| | |
|---|---|
| Ratio < 0,8 | = negative |
| Ratio 0,8 – 1,1 | = weak |
| Ratio >= 1,1 | = positive |

**Chlamydia pneum. IgG-/IgA-AB**

**4 Chlam.pneum. IgG-AB (ELISA) positive**
**! 1,525 Ratio**

| | |
|---|---|
| Ratio < 0,8 | = negative |
| Ratio 0,8 – 1,1 | = weak |
| Ratio >= 1,1 | = positive |

**4 Chlam.pneum. IgA-AB (ELISA) positive**
**! 1,628 Ratio**

| | |
|---|---|
| Ratio < 0,8 | = negative |
| Ratio 0,8 – 1,1 | = weak |
| Ratio >= 1,1 | = positive |

| Analysis | | Result Units | Reference Range | Chart |
|---|---|---|---|---|
| **Coxsackie IgG-/IgA-antibodies** | | | | |
| **4 Coxsackie-IgG Typ A7 (IFT)** | **+** | **1:100** | **< 1:100** | **[ ........ *>** |
| **4 Coxsackie-IgG Typ B1 (IFT)** | **+** | **1:1000** | **< 1:100** | **[ ........ *>** |
| **4 Coxsackie-IgA Typ A7 (IFT)** | **+** | **1:100** | **< 1:10** | **[ ........ *>** |
| **4 Coxsackie-IgA Typ B1 (IFT)** | **+** | **1:10** | **< 1:10** | **[ ........ *>** |

The specific positive Coxsackie-Virus Type A7/B1-IgG-/IgA-antibodies indicate current humoral immune responses against Coxsackie-Virus Type A7 and Coxsackie-Virus Type B1 (recent infection with Coxsackie-Virus Type A7/B1?).

| Analysis | | Result Units | Reference Range | Chart |
|---|---|---|---|---|
| **7 ECHO IgG-antibodies (IFT)** | **+** | **1:1000** | **< 1:100** | **[ ........ *>** |
| **7 ECHO IgA-antibodies (IFT)** | **+** | **1:10** | **< 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 |
|---|---|---|
| **SARS-CoV2 virus IgG/IgA Ab** | | |
| **3 SARS CoV2 IgG-Ab** | **positive** | **negative** |
| | **! 5,021 Ratio** | |

| | |
|---|---|
| Ratio < 0,8 | = negative |
| Ratio 0,8 – 1,1 | = weak |
| Ratio >= 1,1 | = positive |

| | | |
|---|---|---|
| **3 SARS CoV2 IgA-Ab** | **positive** | **negative** |
| | **! 1,140 Ratio** | |

| | |
|---|---|
| Ratio < 0,8 | = negative |
| Ratio 0,8 – 1,1 | = weak |
| Ratio >= 1,1 | = positive |

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

Immunoglobulin A is not available when the infection does not live in the mucosal membranes: EBV (Epstein Barr Virus, glandular fever), CMV (Cytomegalovirus), Parvo Virus B19, etc.

So how to test chronic infection in infections where there is no IgA available?

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”

Home > Cytotoxic T-Cells > Protocol

## CTL ELISPOT Assay

Protocol | First Online: 01 January 2014

pp 75–86 | Cite this protocol

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

## 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**


Book | © 2024

"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.

# Examples: Epstein Barr virus/Mycoplasma

**EBV EliSpot (lytic+latent)**

**1 EBV EliSpot (lytic) ! 657 SI**

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

**1 EBV EliSpot (latent) ! 65 SI**

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

The result of the EliSpot test indicates current celluar activity against Epstein-Barr-Virus (EBV).

Explanation of EBV antigens:
EBV-lytic antigen: sign for replication of infectious EBV virions
EBV-latent antigen: sign for EBV latency with no production of infectious EBV virions

**Mycoplasma pneum.EliSpot**

**1 Mycoplasma pneum. EliSpot ! 7 SI**

SI = Stimulation Index

The result of the EliSpot test indicates current cellular activity against Mycoplasma pneumoniae.

# T-cell tests (EliSpots) for Epstein Barr Virus and Cytomegalovirus show both lytic and latent values

```
  CMV EliSpot

1 CMV lytic                    !        355 SI
     0-1    = negative
     2-3    = weak positive
     > 3    = positive

1 CMV Latent                   !        106 SI
     0-1    = negative
     2-3    = weak positive
     > 3    = positive

     The result of the EliSpot test indicates current celluar
     activity against Cytomegalo Virus (CMV).

     Explanation of CMV antigens:
     CMV-lytic antigen: sign for replication of infectious CMV
     virions
     CMV-latent antigen: sign for CMV latency with no production
     of infectious CMV virions
```

**Lytic = currently replicating**

**Latent = dormat, but suppressing immunity, and can unfold again with any new assault to the immune system**

# What type of testing to select for these “stealth infections“ in (likely) chronic conditions?

**Vector-borne infections**
Borrelia: T-cell test,
and/or very sensitive IgG/IgM that can detect the “round body” (different signature)
Babesia: T-cell test
Bartonella: T-cell test
Ehrlichia/Anaplasma: T-cell test
Rickettsia: T-cell test

**Opportunistic infections**
**Bacteria:**
Chlamydia pneumoniae/trachomatis: IgG/IgA, T-cell test
Mycoplasma: IgG/IgA, T-cell test
Yersinia: IgG/IgA, T-cell test
Campylobacter: IgG/IgA

**Viruses:**
Herpes viruses: EBV, CMV, HSV 1/2, VZV, HHV6. HHV7, HHV8 – differentiated (see next page)
Enteroviruses: IgG/IgA
SARS-CoV-2: IgG/IgA, iSpot

# Immunoarrays for EBV very useful if they have the full array of markers

**9 markers including viral capsid antigen (VCA), early antigen (EA), & Epstein-Barr Nuclear Antigen (EBNA)**

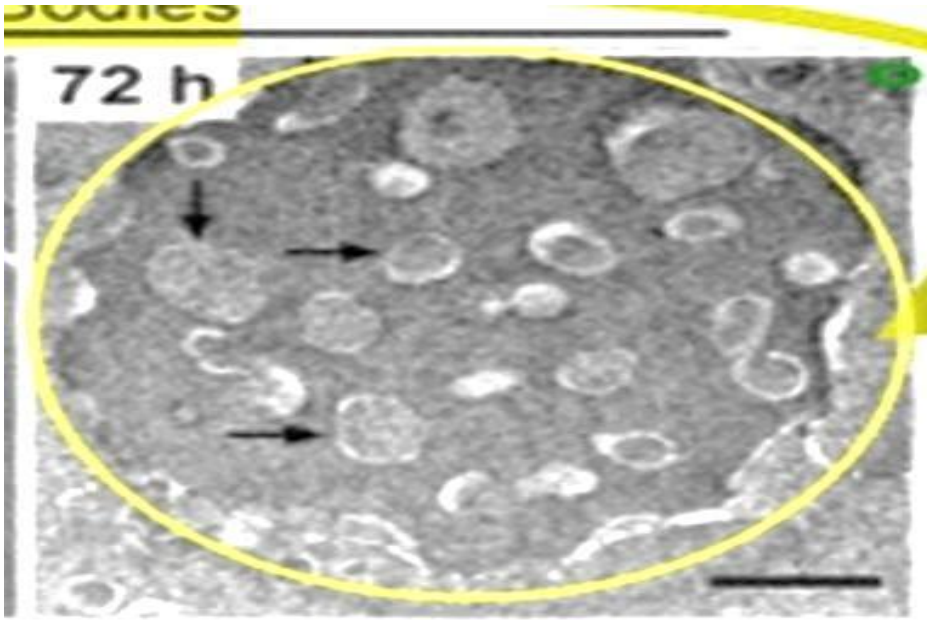
**Epstein-Barr-Virus Immuno-Array**

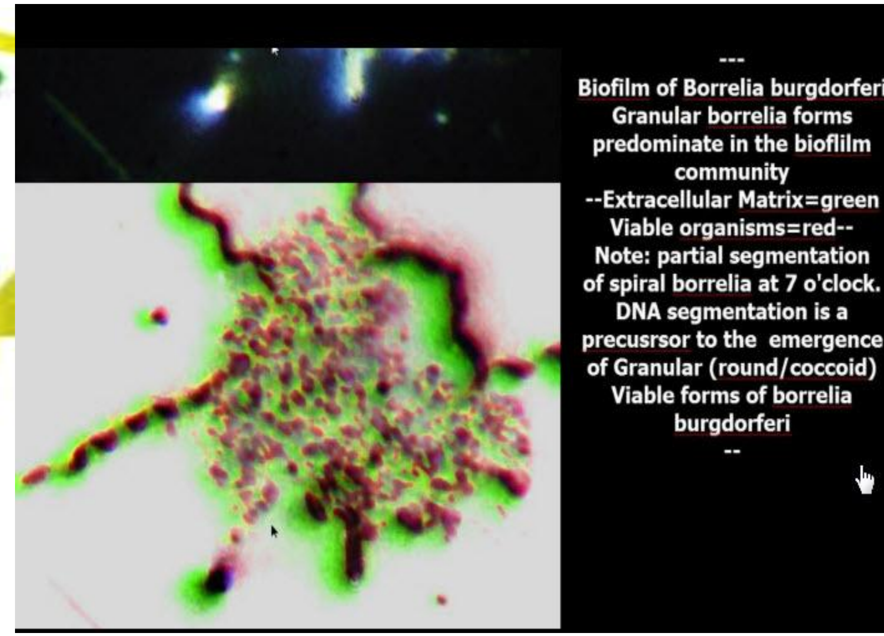
| | | | |
|---|---|---|---|
| EBV VCA p18 IgG | + | positive | negative |
| EBV VCA p23 IgG | + | positive | negative |
| EBV EA p54 IgG | | negative | negative |
| EBV EA p138 | + | positive | negative |
| EBV EBNA-1 IgG | + | positive | negative |
| EBV VCA p18 IgM | | negative | negative |
| EBV VCA p23 IgM | | negative | negative |
| EBV EA p54 IgM | + | positive | negative |
| EBV EA p138 IgM | | negative | negative |

The specific EBV-IgG/IgM-, EBV-Early Antigen-antibodies and EBV-EBNA-antibodies indicate humoral immune response against Epstein Barr Virus (former or reactivated or EBV-infection in convalescence?).

**Lab needs to provide interpretation guidelines for each marker**

# 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…"**

**See references at the end of the presentation for the existence of these**

Source: 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

# Test for “round bodies” (cyst form)

**Basic Test/Tickplex Plus**

7 Basic Test (new)

**7 B.burg.+afz.+gar.IgG** — **positive** — **negative**

! **1,135 Ratio**

Ratio 0,01 - 0,89 = negative
Ratio 0,90 - 0,99 = weak
Ratio >= 1,00 = positive

**7 B.burg.+afz.+gar.IgM** — **positive** — **negative**

! **1,525 Ratio**

Ratio 0,01 - 0,89 = negative
Ratio 0,90 - 0,99 = weak
Ratio >= 1,00 = positive

7 B.burg.+afz.+gar+round bod.IgG — negative — negative

0,456 Ratio

Ratio 0,01 - 0,89 = negative
Ratio 0,90 - 0,99 = weak
Ratio >= 1,00 = positive

**7 B.burg.+afz.+gar+round bod.IgM** — **positive** — **negative**

! **1,044 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 sensu stricto + B.b. afzelii + B.b. garinii + Borrelia burgdorferi round bodies.
The Tickplex Basic ELISA is a screening test for Borrelia pathogen-specific IgG and IgM antibodies and contains an antigen for persisted forms (round bodies) of Borrelia burgdorferi.
Please cross-reference this with the results of EliSpots/i-Spots tests and the CD57+ NK cell test that you may have done.

*Journal of Neuroinflammation* 2008, **5**:40 http://www.jneuroinflammation.com/content/5/1/40

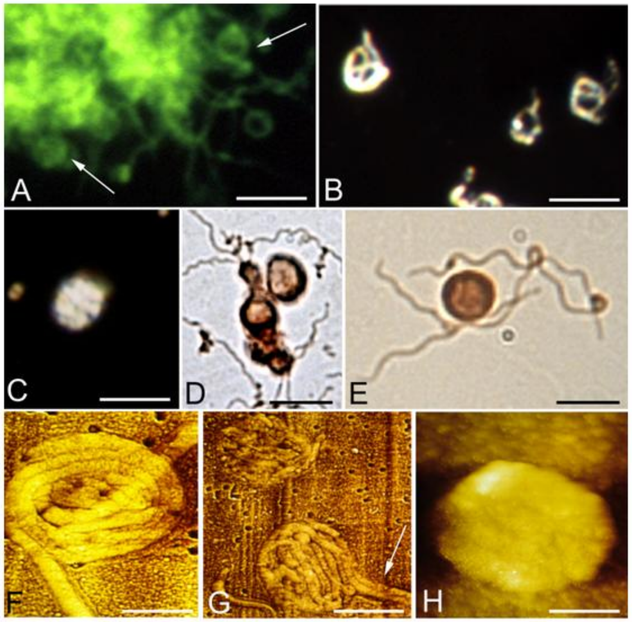



**Figure 3**
**Rolled and cystic forms of *Borrelia burgdoferi* spirochetes observed after one week of culture in medium to which Thioflavin S had been added.** A: Observation by Thioflavin S fluorescence. Arrows point to rolled cystic forms at the periphery of an agglomerated mass of spirochetes from strain B31. Rolled (B) and cystic (C) forms observed by dark field microscopy (strain B31). D and E: Cyst forms of *Borrelia burgdorferi* (strains ADB1 and B31, respectively) following immunostaining with the monoclonal anti-OspA antibody. F-H: Atomic force microscopy (AFM) images of Borrelia cysts. Rolled spirochetes are clearly visible in F (strain B31) and G (strain ADB1). Arrow in G shows that the cyst is formed by two spirochetes rolled together. H: The cystic form is entirely covered by a thickened external membrane masking the content of the cyst (strain B31). Bars: A-D = 6 µm; E = 5 µm; F = 1 µm; G = 2.5 µm; H = 0.5 µm.

1

Source: 1. 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.

# 6. Consider using an immune panel alongside the viral and bacterial axes

**Immunosuppression evident from the CD3+/57+ cells – both viral and bacterial**

**CD3-/CD57+ Cells**

| | | | | | |
|---|---|---|---|---|---|
| 2 CD3-/CD56+ Flow Cytometry | | | | | |
| **2 T cells CD3+ (%)** | **-** | **47,83 %** | **59,70 - 82,00** | **<*** | **........ ]** |
| **2 T cells CD3+ (absolute)** | **-** | **398 /ul** | **900 - 2600** | **<*** | **........ ]** |
| 2 NK cells CD56+ CD3- (%) | | 11,07 % | 5,40 - 30,90 | [ | .*...... ] |
| 2 NK cells CD56+ CD3- (absolute) | | 92 /ul | 77 - 427 | [ | *....... ] |
| 2 CD57+ NK-cells (%) | | 46,25 % | 2,00 - 77,00 | [ | ....*... ] |
| **2 CD57+ NK-cells (absolute)** | **-** | **43 /ul** | **100 - 360** | **<*** | **........ ]** |

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.

# 7. Accreditation

- If a German laboratory, make sure they have a “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.”
- Any European lab should have ISO 15189, and its test producers should have ISO 13485:2016

If a US laboratory, or also serves the USA, ensure that it is

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

# Agenda

- Limitations of conventional testing for infections, especially when chronic: example Lyme Disease

- Principles for selecting helpful tests

  1. In-depth history
  2. Use a questionnaire/checklist driven by an evidence-based algorithm
  3. Correlate your choice with references if possible; refer to resources linking the p/t‘s diagnosis to infections
  4. Choose tests where you either have IgA available ...
  5. ... or T-cell tests (and ideally both)
  6. Consider using an immune panel alongside the viral and bacterial axes
  7. Make sure the laboratory is fully accredited

- **Resources**

# 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/

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


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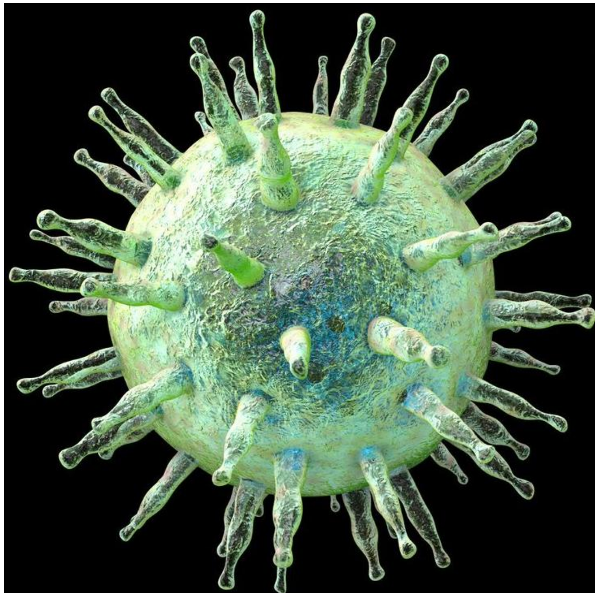
# References available for viral/bacterial associations with specific conditions

- 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.



# Thank you very much!

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