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LDUK Q&A – Answers from ArminLabs

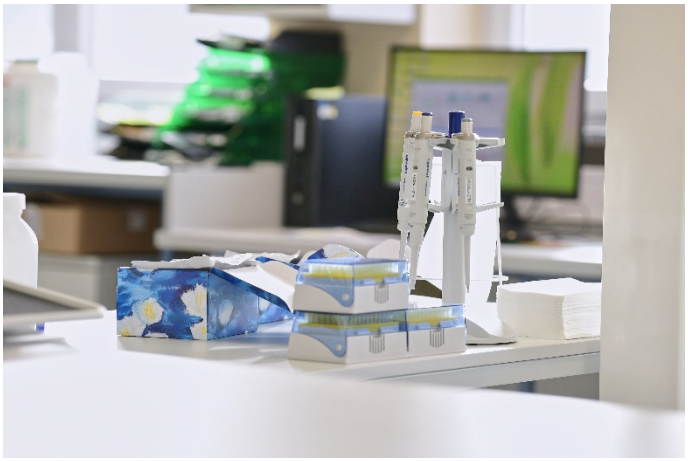
1. Please give details of your laboratory accreditations, including which body has awarded them, and which tests they apply to.

ArminLabs is certified by the German Accreditation Board DAkks, international accreditation no. DIN EN ISO 15189:2014. The Certificate is attached.
We do not carry out any in-house tests of our own design. Every test we perform is commercially available, certified and validated internally by ArminLabs and externally by the test producers. For every test a positive and a negative control is always being performed to check for influencing factors.

2. Please list the tests you do for Borrelia infection. For each test, please give an analysis of the limitations of the test and the strengths of the test.

Tests	Limitations	Strengths
Tickplex Basic	- Sensitivity: 95% - Specificity: 98% - Best to perform with an additional parameter, like CD3/CD57 or an EliSpot for the current activity - Possible seronegativities	- Screening for Borrelia antibodies **including persister forms** (roundbodies) - **Accredited**
Borrelia Elispot	- Sensitivity: 84% - Specificity: 82-100% - Requires interpretation by a specialized health professional - Preanalytic handling critical (transit time)	- Tests T-cell activity against Borrelia, **reflects current activity** for the previous 6-8 weeks - **No seronegativity issues, as this is not an antibody test** - Cytokine production can still be measured during a downregulated humoral immune response
Borrelia SeraSpot®	- Sensitivity: 97.2% - 98.2% - Specificity 98.6% - 100% - No detection of persister forms - Possible seronegativities	- Tests B-cell immune response (modern Western Blot) - **Lower susceptibility to errors** due to predefined points/spots with specific antigens - **Accredited**
Borrelia ImmunoBlot	- Poor sensitivity observed in routine use - High specificity - No detection of persister forms - Possible seronegativities	- Highly standardized, tests a pattern of antibodies against a variety of antigens of Borrelia - **Accredited**
Borrelia ELISA	- Poor sensitivity observed in routine use - Poor specificity observed in routine use - No detection of persister forms - Possible seronegativities	- Highly standardized, semi-quantitative B-cell activity - **Accredited**
Borrelia C6 ELISA	- Poor sensitivity observed in routine use - Poor specificity observed in routine use - No detection of persister forms - Possible seronegativities	- Tests part of the B- cell immune response against Borrelia - **Accredited**

Certain limitations apply to all antibody testing. In general antibody tests are highly sensitive and specific. But they are sometimes not the ideal tests to perform for several reasons (attached is some information about seronegativity). Even if the test itself has a high sensitivity and specificity, the overall outcome might end, due to possible seronegativity and other influencing factors, in a poor sensitivity/specificity. ArminLabs recommends an extended anamnesis (detailed history) to decide which test should be performed at which stage of the disease, to choose the best test at the time being performed.



Where there are limitations, please explain how patients/doctors are

a) advised of the limitations

If requested, the AONM Team as ArminLabs distributor in the UK explains the handling of the specimens/blood samples. The methods are explained on request and the limitations, depending on the concerns. Attached is the preanalytics information. In the case of the time-critical Elispots, cells are coloured to monitor whether the lymphocytes are still alive. AONM has numerous presentations that explain the issues around the different testing methods, and users can download these very easily. The same presentations are also available as recordings, and can be viewed on a dedicated Youtube channel.

b) what other tests or actions might correct or improve the limitations

ArminLabs has developed checklists for Lyme and co-infections to offer a free preanalytic tool for patients and health specialists/physicians. These checklists help to decide which tests are necessary. This enables users to increase the outcome and reduce costs.
In the event of a complicated medical history the patient or his health professional are invited to discuss with us whether it is advisable to add further tests relating to immunology, rheumatology and co-infections.
ArminLabs does not run any in-house tests of its own design, and is in constant exchange with the test producers on improving test performance and reducing any test limitations.

c) Which geno-species of Borrelia are the tests sensitive for? Which species may not be picked up?

The serology tests cover B. burgdorferi, B. afzelii, B. garinii.
The Elispot covers B. burgdorferi, B. afzelii, B. garinii and Borrelia miyamotoi (as an individual test). There is no evidence of any cross-reactions among the species.

3. Please answer as many of the following questions on particular tests as are relevant for your lab. If you are unable or unwilling to give answers, please just simply state that.

Indirect tests looking for the reaction of the body to the infection:

Serology/Antibody tests:

a. ELISA/EIA/C6-ELISA

a.i. What precise test kit do you use? Please give the manufacturer's name, test name and manufacturer's information for the test. Is the test CE marked?

ELISA:

- Euroimmun test kit
- Manufacturer's information: http://www.euroimmun.com/products/indications/infektions-serologie/ak-gegen-borrelien-fsme-viren.html
- The test is CE marked

a.ii. What sensitivity and specificity data are given for the test?

ELISA for IgM and IgG (combined)

- Sensitivity: 100 %
- Specificity: 90.2 % - 96,4 %

a.iii. Please reference the research data that is the evidence for these figures.

1. Burgdorfer W, Barbour AG, Hayes SF, Benach JL, Grunwaldt E, Davis JP, Lyme disease-a tickborne Spirochetosis? Science 216 (1982) 1317-1319.
2. Christova I. Enzyml-linkend immunosorbent assay, immunofluorescent assay, and recombinant immunoblotting in the serodiagnosis of early Lyme borreliosis. Int J Immunopathol Pharmacol 16 (2003) 261-268.
3.EUROIMMUN AG. Komorowski L., Janssen A, Stöcker W, Probst C. Dimersation of recom-binant OspC leads to an antigen with enhancend potential for active vaccina-tion. 12. Inter-national Conference on Lyme Borreliosis and other Tick-Borne Dis-eases, Ljubljana, Slowenien (2010).
4. EUROIMMUN AG. Komorowski L, Probst C, Janssen A, Stöcker W. Polypeptide und Verfahren zur spezifischen Detektion von Antikörpern bei Patienten mit einer Borrelien-infektion. Europäische Patenanmeldung EP2199303 (2008).
5. EUROIMMUIN AG. Meyer W, Janssen A, Scheper T, Schlumberger W, Stöcker W, Lyme borreliosis: Prevalence of antibodies against non-proteinic (lipid) antigens. Int J Med Microbiol 298S2 (Suppl.45) (2008) 39.
6. EUROIMMUN AG. Probst C, Ott A, Scheper T, Meyer W, Stöcker W, Komorowski L. N-Terminal disulfide-bridging of Borrelia outer surface protein C increases ist diag-nostic and vaccine potentials. Ticks and Tick-Borne Dieseases 3 (2012) 1-7.
7. EUROIMMIN AG. Meyer W, Scheper T, Schlumberger W, Stöcker W. Antibodies Against the Newly Identified Major Antigen VlsE: A milestone in the Serological Di-agnosis of Lyme Borreliosis. Poster zum EUREGIO. Congress of Clinical Chemistry and Laboratory Meicine, Aachen (2003).
8. EUROIMMUN AG. Steinhagen K, Schlumberger W, Stöcker W. Nachweis einer spezifischen intrathekalen Antikörpersynthese mit modernen ELISA-Testsystemen: Hohe Trefferquote bei Miltipler Sklerose und Neuroborreliose. J Lab Med 25 (2001) 135-149.
9. EUROIMMUN AG. Meyer W, Janssen A, Kühn B, Scheper T, Suer W, Komorowski L, Probst C, Schlumberger W, Stöcker W. EUROLINE Borrelia RN.AT: Marriage of authentic recombinant and native borrelia antigens in a line immunoassay format. International Journal of Medical Microbiology 298S2 (Suppl.45): 39-40 (2008).
10. goettner G, Schulte-Spechtel U, Hillermann R, Liegl G, Wilske B, Fingerle V. Im-provement of Lyme borreliosis serodiagnosic by a newly developed recombinant immunoglobulin G (IgG) and IgM line immunoblot assay and addition of VlsE and DbpA homologues. J Clin Microbiol 43 (2005) 3602-3609.
11. Hauser U, Lehnert G, Lobentanzer R, Wilske B. Interpretation Criteria for Stand-ardized WesternBlots for Three European Species of Borrelia burgdorferi Sensu Lato. J Clin Microbiol 35 (1997) 1433-1444.
12. Johnson R, Schmid GP, Hyde FW, Steigerwalt AG, Brenner DJ. Borrelia burgdorferi sp. Nov: Etiologic agent of lyme disease. Int J Syst Bacteriol 34 (1984) 496-497.
13. Oehme R, Hartelt K, Backe H, Brockmann S, Kimmig P. Foci of tick-borne dis-eases in southwest Germany. Int J Med Microbiol 291 (2002) 22-29.
14. Robert-Koch-Institut. Surveillance der Lyme-Borreliose am Beispiel des Bundes-landes Brandenburg. Epidemiologisches Bulletin 14 (1998) 93-97.
15. Schulte-Spechtel U, Lehnert G, Liegl G, Fingerle V, Heimerl C, Johnson BJ, Wilske B. Significant improvement oft he recombinant Borrelia-specific immunglobu-lin G immuno-blot test by addition of VlsE and a DbpA homologue derived from Bor-relia garinii for dieagnosis of early neuroborreliosis. J Clin Microbiol 41 (2003) 1299-1303.
16. Tilton RC, Ryan RW. The laboratory diagnosis of Lyme disease. J Clin Immuno-assay 16 (1993) 208-214.
17. Wilske b, Fingerle V, Schulte-Spechtel U. Microbiological and serological of Lyme borreliosis. FEMS Immunol Med Microbiol 49 (2007) 13-21.
18. Wildke B. Epidemiologiy and diagnosis of Lyme borreliosis. Ann Med 37 (2005) 568-579.
19. Wilske B, Zöller L, Brade V, Eiffert H, göbel UB, stanek G, Pfister HW, MiQ 12/2000 Qualitätsstandards in der mikrobiologisch-infektiologischen Diagnostik: Lyme-Borreliose. Urban & Fischer Verlag München Jena (2000).
20. Zhang JR, Hardham JM, Barbour AG, Norris SJ. Antigenic Variation in Lyme Disease Borreliae by Promiscuous Recombination of VMP-like Sequence Cas-settes. Cell 89 (1997) 275-285.

a.iv. If you use an in-house test, please give comparable detail.

ArminLabs does not use any in-house tests.

a.v. Does the patient need to stop taking antibiotics, other medicines or supplements before the test is performed, and if so, how far in advance of testing?

The decision depends on the recommendation and advice of your health professionals. Furthermore, there are no studies, nor are there any references from test manufacturers, that the test can be impaired by antibiotics or other medications.[1]

[1] Massive, high dosed chemotherapeutics, immune suppressants or interferon gamma might affect the test results. ArminLabs recommends a consultation with the attending health professional in such cases.

b. Western Blot/Immunoblot

b.i. What precise kit do you use? Please give the manufacturer's name, test name and manufacturer's information for the test.

Immunoblot:

- Euroimmun test kit
- Manufacturer's information: http://www.euroimmun.com/products/indications/infektions-serologie/ak-gegen-borrelien-fsme-viren.html#c487
- The test is CE marked

b.ii. Which bands are reported on and what weight are the different bands given in interpretation?

- IgG: p18, p19, p20, p21, p58, OspC (p25), p39, p41, p83, LBb, LBa, VlsE- Bg, VlsE- Bb und VlsE- Ba
- IgM: OspC- Bg nativ, OspC- Bb nativ, OspC- Ba nativ, p39, p41, VlsE- Bb
- The most important antigen contained in the test is covalently bound, dimeric OspC advanced (European patent EP 2 199 303 A1) from four human pathogenic Borrelia strains (B. afzelii, B. burgdorferi, B. garinii, B. spielmanii). OspC advanced is over 30% more specific than conventional recombinant OspC antigens.

b.iii. Given that the subject of which bands are used is so historically controversial, how much flexibility do you have or take in deciding which bands you report on?

The interpretation of the Immunoblot is according to the manufacturer's recommendations. ArminLabs don't diverge from the manufacturer's guidelines regarding the interpretation.

b.iv. What sensitivity and specificity data are given for the test?

For IgG:

- Sensitivity – 88.5%
- Specificity – 99.2 %

For IgM

- Sensitivity – 93.0 %
- Specificity – 94.9 %

b.v. Please reference the research data that is the evidence for these figures.

1. Kaiser R, Fingerle V, Hofmann H, Krause A. Topical aspects of Lyme Borreliosis (Lyme disease).
2. https://cdc.gov/lyme/stats/tables.html
3. Centers for Disease Control and Prevention (CDC). Recommendations for test performance and interpretation from the Second National Conference on serologic Diagnosis of Lyme Disease. MMWR Morb Mortal Wkly Rep. (1995) 44, 590-591.
4. Barclay SS, Melia MT, Auwaerter PG. Misdiagnosis of late-onset Lyme arthritis by inapprobriate use of borrelia burgdorferi immunoblot testing with synovial fluid. Clin Vaccine Immunol. (2012) 19, 1806-1809.
5. Marques AR. Laboratory diagnosis of Lyme disease:advances and challenges. Infect Dis Clin North Am. (2015) 29, 295-307.
6. Lexikon der Infektionskrankheiten des Menschen. 3.auflage, 2009, Springer Medizin Verlag Heidelberg.

b.vi. What information or guidance do you give doctors on the interpretation of IgM versus IgG results?

- Stage 1[2]:

Serological detection of IgM antibodies against Borrelia burgdorferi is possible in 50% to 90% of Stage I patients. The prevalence of specific IgG antibodies is significantly lower. At this stage of the disease, however, serological evidence is often negative. If the corresponding symptoms are present, a follow-up in the following weeks is recommended.

- Stage 2[2]:

Antibodies are detectable in Stage II in 50% to 90% of patients. In the early phase of this stage, antibodies of the IgM class are predominantly still positive, in the late phase only antibodies of the IgG class often occur. However, IgM class antibodies can also persist for a long time.

- Stage 3[2]:

At this stage, IgG class antibodies for persister forms are significantly elevated in 90% to 100% of patients, while IgM class antibodies are rarely detectable.

[2] As mentioned e.g. in the "Textbook of Clinical Neuropsychiatry and Behavioral Neuroscience", D. Moore and B. Puri, 2012. Hodder Arnold, p. 573

b.vii. Does the patient need to stop taking antibiotics, other medicines or supplements before the test is performed, and if so, how far in advance of testing?

The decision depends on the recommendation and advice of your health professionals. Furthermore, there are no studies, nor are there any references from test manufacturers, that the test can be impaired by antibiotics or other medications. [3]

Indirect tests looking for other immune reactions to infection:

c. LTT/Elispot/other T-cell tests (where more than one type of LTT is offered please deal with each separately)

c.i. What type of test is this, and what change is monitored as the indication of T-cell reactivity?

ArminLabs uses the Elispot method. Due to the stimulation, the Th1-cells secrete the cytokine interferon-gamma, which indicates the T-cell reactivity.

c.ii. What precise kit(s) do you use? Please give the manufacturer's name, test name and manufacturer's information for the test.

- EliSpot Interferon Gamma Release Assay Kit for Borrelia
- The test is CE marked

c.iii. If you are not able to give the information in (ii) because you use an in-house test, please explain this and/or give all the components of the test you perform in terms of hardware, reagents, antigens, readers and protocols.

ArminLabs does not use any in-house tests.

c.iv. What antigens and antigen mixes are used?

- Borrelia full antigen
- Mix of OspA peptides, native OspC and recombinant DbpA
- LFA-1 Peptide Mix

c.v. What sensitivity and specificity data apply to this test?

- Sensitivity – 84%
- Specificity – 94%

c.vi. Please reference fully the research data that is the evidence for these figures. If this material is behind a paywall, please quote the relevant portion of the reference and share the full text with the LDUK admins so that they can verify it applies to the test given.

1.Jin, C.; Roen, D.R.; Lehmann, P.V.; Kellermann, G.H. An Enhanced ELISPOT Assay for Sensitive Detection of Antigen-Specific T Cell Responses to Borrelia burgdorferi. Cells 2013
2. Nordberg, M.; Forsberg, P.; Nyman, D.; Skogman, B.H.; Nyberg, C.; Ernerudh, J.; Eliasson, I.; Ekerfelt, C. Can ELISPOT Be Applied to A Clinical Setting as A Diagnostic Utility for Neuroborreliosis? Cells 2012
3. IgG subclasses in Lyme borreliosis: a study of specific IgG subclass distribution in an interferon-γ-predominated disease. Widhe, Mona; Ekerfelt, Christina; Forsberg, Pia; Bergström, S.; 1998
4. Lehman PV et al.: Unique Strengths of ELISPOT for T Cell Diagnostics in: Kalyuzhny AE. Handbook of ELISPOT: Methods and Protocols, Methods in Molecular Biology, Vol. 792. 2nd Ed: Springer; 2012; also Handbook of ELISPOT 3rd edition, Humana Press, July 2018.

c.vii. What are the cut-off thresholds for these tests? Please ensure that the information given in (v) and (vi) corresponds to the cut-off thresholds quoted in patient test results.

- Negative: 0-1 SI (stimulation index)
- Limit value/weak positive SI =2-3
- Positive: SI >3

The cut off values are according to the manufacturer's recommendations

c.viii. Does the patient need to stop taking antibiotics, other medicines or supplements before the test is performed, and if so, how far in advance of testing?

The decision depends on the recommendation and advice of your health professionals. Furthermore, there are no studies, nor are there any references from test manufacturers, that the test can be impaired by antibiotics or other medications. [4]

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[3] Massive, high dosed chemotherapeutics, immune suppressants or interferon gamma might affect the test results. ArminLabs recommends a consultation with the attending physician for the final decision.
[4] Massive, high dosed chemotherapeutics, immune suppressants or interferon gamma might affect the test results. ArminLabs recommends a

d. CD57 / NK-cells CD

d.i. What thresholds do you use and what interpretations do you place on the values of CD57 derived from your tests?

Reference ranges (mean/range)

Chronic immune suppression:	0 /ul - 100 /ul
Borderline:	101 /ul - 130 /ul
Healthy:	131 /ul - 360 /ul

ArminLabs gives a short interpretation in the final report that low or borderline CD57 result, which indicates chronic immune suppression, may be associated with Lyme disease (it can also be caused by other bacteria such as Chlamydia pneumoniae or Mycoplasma pneumoniae). Decreased CD57 cells are indicative of chronic conditions of this kind (symptoms > 1 year), they do not indicate a fresh infection.

1. Stricker RB, Winger EE. Normalization of the CD57 natural killer cell subset associated with prolonged antibiotic therapy in patients with chronic Lyme disease. Clin Immunol (2002)
2. Stricker RB, Winder EE. Decreased CD57 lymphocyte subset in patients with chronic Lyme disease. Immunology Letters 76 (2001)
3. Stricker RB, Burrascano JJ, Winger EE. Longterm decrease in the CD57 lymphocyte subset in a patient with chronic Lyme disease. Ann Agric Environ Med 2002
4. Sincovics JG, Horvarth JC., Human natural killer cells: A comprehensive review. International Journal of Oncology (2005)
5. Focosi D, Petrini M. CD57 Expression on Lymphoma Microenvironment As a New Prognostic Marker Related to Immune Dysfunction Journal of Clinical Oncology, (2007)
6. Focosi D., Bestagno M., Burrone O., Petrini M . (2010) CD57 T lymphocytes and functional immune deficiency . Journal of Leukocyte Biology Volume 87

d.ii. How does the CD57 inform your interpretation of other tests?

The CD57+ cells document the extent of the immune suppression of chronic Lyme disease or other bacterial infections (see above). Based on the current literature, CD57+ cells provide prognostic laboratory parameters during and after the treatment of chronic Lyme disease. Clinical research studies and case studies have shown that chronic Lyme infections are often accompanied by changes in the cellular immune defense. Evidence for this is a decreased number of natural killer cells (NK/CD3-CD56+), in particular, a decreased absolute number of activated NK cells (CD3-CD56+CD57+). While acute Borrelia burgdorferi infections and other diseases show normal CD57+ parameters, chronic Lyme patients often have less than 100 CD57+ cells/µl. According to scientific studies, a suppressed absolute number of CD57+ cells has mainly been observed in patients whose nervous system had been affected, rather than in patients whose tissue or skeleton system has been affected. A decrease of CD57+ cells persists until an improvement in symptoms has been achieved with the use antibiotics and other forms of therapy. In reverse, a decreased CD57+ parameter is seen as a measurable signal of an active chronic Borrelia infection (or of other bacterial infections such as Chlamydia pneumoniae or Mycoplasma pneumoniae), and can be a possible indicator of successful therapy as it rises. In the event of a negative Lyme test, we recommend more intensive history taking, and if necessary recommend further testing of other bacteria that can suppress TH1 cytokine production and trigger similar symptoms.

d.iii. What other populations of NK killer cells do you test for and what information do they give?

- CD3+ cells - population of T-lymphocytes
- CD56+/ CD3- cells - non-activated functional NK cells
- CD45+ cells - specific marker to classify the NK cells

We can perform almost any other form of immunological test on request.

d.iv. Does the patient need to stop taking antibiotics, other medicines or supplements before the test is performed, and if so, how far in advance of testing?

The decision depends on the recommendation and advice of your health professionals. Furthermore, there are no studies, nor are there any references from test manufacturers, that the test can be impaired by antibiotics or other medications.[4]

Direct testing methods

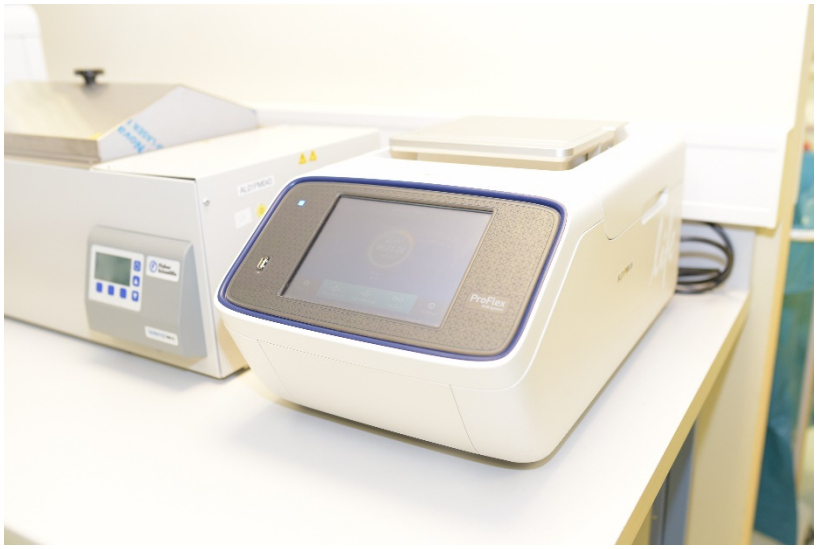
e. PCR

e.i. What materials/samples do you perform Borrelia PCR tests on?

Human Samples: blood, CSF, biopsies, tissue, body fluids, joint fluid
Animal samples: blood, biopsies, body fluids
Other material can be processed on request

e.ii. Can you perform PCR testing on ticks?

Yes, PCR testing on ticks can be performed by our partner laboratory ArminLabs PreAnalytics GmbH. The test system used is commercially available and certified. ArminLabs PreAnalytics GmbH offers the **tick PCR for all medically relevant Borrelia species**, including **Borrelia miyamotoi, TBE, Anaplasma phagocytophilum, Rickettsia, Babesia, Bartonella**. First a multiplex PCR is performed with a PN-Mix using DNA isolated from the tick. The respective biotinylated amplicons are characterized by a hybridization reaction with SSOPs for the pathogens and controls immobilized on nitrocellulose membranes in a distinctive line format.



e.iii. What are the benefits and limitations of PCR testing?

The Polymerase chain reaction (PCR) is a method for direct detection of a pathogen. Checking for the presence of a unique gene of the pathogen gives you undeniable proof of the causative pathogen of the disease if it's found in the human body.

Benefits of PCR testing: If the pathogen is detected in the human body, this is **undeniable proof of the disease**. Tick PCRs are a cost-effective method of screening for pathogens, and in the event of symptoms, a good preanalytic tool for the physician to decide which treatment might be necessary. Some accident insurance companies also cover the treatment costs if they have evidence of the "accident" (tick bite and a tick that has tested positive).
Limitations of the PCR are the sample type and stage of the disease. In-house PCR tests without accreditation cannot be used as undeniable evidence of the disease.

e.iv. What are the specificity and sensitivity values for PCR tests on different samples?

Specificity - 100% (depending on primer sequences and accreditation of the PCR test)
Sensitivity - varies depending on the sample type and stage of the disease
Below are some examples listed from a state institute for externally certified and accredited PCR testing methods, depending on the sample type:

Examination material	Sensitivity
Skin (Erythema migrans, ACA)	50 – 70 %
Cerebrospinal fluid (Neuroborreliosis II)	10 – 20 %
Joint punctate (Lyme arthritis)	50 – 70 %

PCR from urine and blood cannot be recommended for diagnostic purposes according to the current state of knowledge.

e.v. Does the patient need to stop taking antibiotics, other medicines or supplements before the test is performed, and if so, how far in advance of testing?

The patient should stop taking antibacterial medications prior to PCR testing (minimum one week, but it depends on the half-life of the medication) for samples with blood flow, urine or cerebrospinal fluid (it's not necessary, for example, to stop prior to a joint punctate).

Other Tests

Please describe in terms of methodology and accuracy any other tests for Borrelia infection that are carried out in your lab.

TickPlex - First immunoassay for persister forms

The TickPlex assay is performed on the basis of an ELISA. However, compared to other ELISA assays, this test contains a new antigen for persister forms of Borrelia. TickPlex is an accredited test.

TickPlex Plus allows the simultaneous determination of multiple pathogens in the complexity of tick-borne infections with a high sensitivity of approximately 95% and a specificity of 98%. TickPlex Plus detects IgM and IgG antibodies of various bacterial and viral pathogens at the same time. This article covers the test methodology and substantiation extensively:

https://www.nature.com/articles/s41598-018-34393-9

We distinguish two tests that examine the following viruses and bacteria, including Borrelia round bodies:

TickPlex Basic	TickPlex Plus
Borrelia burgdorferi sensu stricto	Borrelia burgdorferi sensu stricto
B. burgdorferi afzelii	B. burgdorferi afzelii
B. burgdorferi garinii	B. burgdorferi garinii
	Babesia microti
	Bartonella henselae
	Ehrlichia chaffeensis
	Rickettsia akari
	Coxsackievirus Type A16
	Epstein-Barr virus
	Human parvovirus B19
	Mycoplasma fermentans & pneumoniae

Material required: 1 x serum/SST tube

4. Which tests would you recommend to patients who suspect a) acute and b) chronic Lyme disease? Are there any tests you would recommend for very early disease, within a few weeks of being bitten? How would you advise patients with limited financial resources?

a) Acute Lyme

- Anamnesis (detailed history taking)
- Tick available → Tick PCR
  - Positive for Lyme or co-infections → start treatment (to be on the safe side)
  - Negative for Lyme and co-infections → monitor your body and well being
- No tick available
  - Symptoms develop → start treatment (not all symptoms are Lyme related, some might be related to other infections)
  - No symptoms develop → monitor your body and well being
- To be on the safe side, Elispot can be done after 7-14 days
- **NO CD3-/CD57+** cells needed, since these cannot be used as a parameter in a fresh Borrelia infection; this parameter can only be used after approx. 1 year

b) Chronic Lyme

- Extended anamnesis (including Lyme & co-infection checklist - attached)
- TickPlex basic (detection of persister forms)
- Elispot (current activity)
- CD3-/CD57+ (immune status)

Early disease

- Elispot can be done after 7-14 days

Limited resources

- Extended anamnesis (including Lyme & co-infection checklist - attached) to decide whether further testing is necessary and which tests should be done, depending on the ranking
- AONM can advise on these steps

5. What tests for co-infections are carried out in your labs? Please list the infections, with the tests you use to detect them and any data on accuracy that is available.

Co-infections

Ehrlichia & Anaplasma
- EliSpot (Specificity - upon request, Sensitivity - upon request) - **accredited**

Anaplasma phagocytophilum
- Anaplasma phagocytophilum IgG (Specificity - 100%, Sensitivity - upon request) - **accredited**
- Anaplasma phagocytophilum IgM (Specificity - upon request, Sensitivity - upon request) - **accredited**
- Anaplasma phagocytophilum DNA-PCR

Ehrlichia chaffeensis
- Ehrlichia chaffeensis IgG (Specificity - upon request, Sensitivity - upon request) - **accredited**
- Ehrlichia chaffeensis IgM (Specificity - upon request, Sensitivity - upon request) - **accredited**

Bartonella
- Bartonella henselae EliSpot (Specificity - upon request, Sensitivity - upon request) - **currently undergoing accreditation**
- Bartonella (henselae + quintana) IgG (Specificity - 89.5%, Sensitivity - 96.7%) - **accredited**
- Bartonella (henselae + quintana) IgM (Specificity - 75-98%, Sensitivity - 42%) - **accredited**
- Bartonella DNA-PCR

Babesia
- Babesia microti EliSpot (Specificity - upon request, Sensitivity - upon request) - **currently undergoing accreditation**
- Babesia microti IgG (Specificity - upon request, Sensitivity - upon request) - **accredited**
- Babesia microti IgM (Specificity - upon request, Sensitivity - upon request) - **accredited**
- Babesia sp. DNA-PCR

Chlamydia
- Chlamydia pneumoniae EliSpot (Specificity - upon request, Sensitivity - upon request) - **accredited**
- Chlamydia pneumoniae IgG (Specificity - 95,1%, Sensitivity - 100%) - **accredited**
- Chlamydia pneumoniae IgA (Specificity - 94,6%, Sensitivity - 96,8%) - **accredited**
- Chlamydia trachomatis EliSpot (Specificity ~ 94%, Sensitivity ~ 84%) - **accredited**
- Chlamydia trachomatis IgG (Specificity - 97%, Sensitivity - 100%) - **accredited**
- Chlamydia trachomatis IgA (Specificity - 97,4%, Sensitivity - 100%) - **accredited**

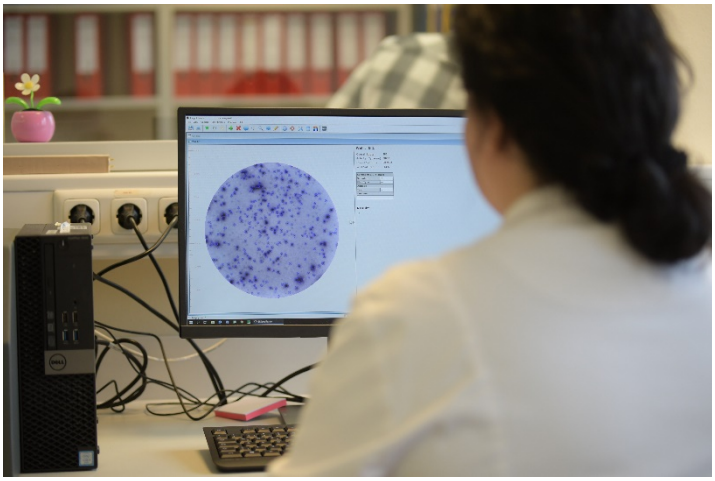
Mycoplasma
- Mycoplasma pneumoniae EliSpot (Specificity - upon request, Sensitivity - upon request) - **accredited**
- Mycoplasma pneum. IgG (Specificity - 100%, Sensitivity - 100%) - **accredited**
- Mycoplasma pneum. IgA (Specificity - 100%, Sensitivity - 100%) - **accredited**
- Mycoplasma fermentans IgG and IgM (Specificity - upon request, Sensitivity - upon request) - **accredited**

Yersinia
- Yersinia enterocolitica EliSpot (Specificity - upon request, Sensitivity - upon request) - **accredited**
- Yersinia IgG (enterocolitica) (Specificity - 100%, Sensitivity - 77,8%) - **accredited**
- Yersinia IgA (enterocolitica) (Specificity - 100%, Sensitivity - 77,8%) - **accredited**

Rickettsia
- Rickettsia EliSpot - currently undergoing accreditation
- Rickettsia rickettsii+typhi IgG (Specificity - upon request, Sensitivity - upon request) - **accredited**
- Rickettsia rickettsii+typhi IgM (Specificity - upon request, Sensitivity - upon request) - **accredited**
- Rickettsia akari IgG (Specificity - upon request, Sensitivity - upon request) - **accredited**
- Ricksettsia akari IgM (Specificity - upon request, Sensitivity - upon request) - **accredited**
- Rickettsia sp. DNA-PCR

Campylobacter jejuni
- Campylobacter jejuni IgG (Specificity - upon request, Sensitivity - upon request) - **accredited**
- Campylobacter jejuni IgA (Specificity - upon request, Sensitivity - upon request) - **accredited**

EBV
- EBV EliSpot (2 antigens: lytic+latent) (Specificity ~ 94%, Sensitivity ~ 84%) - **accredited**
- EBV IgG (Specificity - upon request, Sensitivity - upon request) - **accredited**



- EBV IgM (Specificity - upon request, Sensitivity - upon request) - **accredited**
- EBV EBNA (Specificity - upon request, Sensitivity - upon request) - **accredited**

CMV
- CMV EliSpot (Specificity - upon request, Sensitivity - upon request) - **accredited**
- CMV IgG (Specificity – 99.6%, Sensitivity - 100%) - **accredited**
- CMV IgM (Specificity - 100%, Sensitivity - 99,2%) - **accredited**

VZV
- VZV EliSpot (Specificity - upon request, Sensitivity - upon request) - **accredited**
- VZV IgG (Specificity - 100%, Sensitivity - 100%) - **accredited**
- VZV IgA (Specificity - 100%, Sensitivity - 100%) - **accredited**
- VZV IgM (Specificity - 100%, Sensitivity - 100%) - **accredited**
- VZV DNA-PCR

HHV
- HHV 6-IgG (Specificity - 100%, Sensitivity - 95%) - **accredited**
- HHV 6 IgM (Specificity - 100%, Sensitivity - 100%) - **accredited**
- HHV 6 DNA-PCR
- HHV 7 IgG (Specificity - upon request, Sensitivity - upon request) - **accredited**
- HHV 8 IgG (Specificity - upon request, Sensitivity - upon request) - **accredited**
- HHV 8 DNA-PCR

HSV 1/2
- HSV 1 EliSpot (Specificity ~ 94%, Sensitivity ~ 84%) - **accredited**
- HSV 2 EliSpot (Specificity ~ 94%, Sensitivity ~ 84%) - **accredited**
- HSV 1/2 IgG (Specificity - 100%, Sensitivity - 100%) - **accredited**
- HSV 1/2 IgA (Specificity – 93.1%, Sensitivity – 95.7%) - **accredited**
- HSV 1/2 IgM (Specificity – 93.1%, Sensitivity – 95.7%) - **accredited**

Coxsackievirus
- Coxsackievirus A7, B1 and A16 IgG (Specificity - cross-reactivities within enteroviruses are known, Sensitivity - 98%) - **accredited**
- Coxsackievirus A7 and B1 IgA (Specificity - cross-reactivities within enteroviruses are known, Sensitivity - 98%) - **accredited**
- Coxsackievirus A16 IgM - **accredited**

Tick-borne encephalitis
- TBE IgG (Specificity - 100%, Sensitivity - 100%) - **accredited**
- TBE IgM (Specificity - 100%, Sensitivity - 100%) - **accredited**

Parvovirus B19
- Parvovirus B19 IgG (Specificity - 100%, Sensitivity - 100%) - **accredited**
- Parvovirus B19 IgM (Specificity - upon request, Sensitivity - upon request) - **accredited**

Enterovirus
- Enterovirus IgG (Specificity - 100%, Sensitivity - 96%) - **accredited**
- Enterovirus IgA (Specificity - 100%, Sensitivity - 95,2%) - **accredited**

Echovirus type 7
- Echovirus type 7 IgG (Specificity - cross-reactivities within enteroviruses are known, Sensitivity - 100%) - **accredited**
- Echovirus type 7 IgA (Specificity - cross-reactivities within enteroviruses are known, Sensitivity - 100%) - **accredited**

Candida albicans
- Candida albicans EliSpot (Specificity - upon request, Sensitivity - upon request) - **currently undergoing accreditation**
- Candida albicans IgG (Specificity - 96%, Sensitivity - 100%) - **accredited**
- Candida albicans IgM (Specificity - 89,2%, Sensitivity - 100%) - **accredited**
- Candida albicans IgA (Specificity - 100%, Sensitivity - 100%) - **accredited**

Aspergillus Mix Peptide 1 & 2 EliSpot (Specificity - upon request, Sensitivity - upon request) - **currently undergoing accreditation**

Toxoplasma gondii

- Toxoplasma gondii IgG (Specificity - 100%, Sensitivity - 100%) - **accredited**
- Toxoplasma gondii IgM (Specificity - 95,3%, Sensitivity - 100%) - **accredited**

Echinococcus multilocularis + granulosus IgG (Specificity - 93,8%, Sensitivity - 90,6%) - **accredited**

Entamoeba histolytica IgG (Specificity - 100%, Sensitivity - 100%) - **accredited**

Leishmania infantum IgG (Specificity - 100%, Sensitivity - 100%) - **accredited**

Taenia solium IgG (Specificity - 100%, Sensitivity - 97,3%) - **accredited**

Toxocara canis IgG (Specificity - 100%, Sensitivity - 100%) - **accredited**

Trichinella spiralis IgG (Specificity - 100%, Sensitivity - 94,81%) - **accredited**

It should be noted that Arminlabs can perform many other tests of other possible coinfections, too, that may not be on the order form, on request.

6. Do you offer advice to doctors regarding interpretation of test results?

AONM as local partner from ArminLabs offers free consultations and advice to doctors/therapists on the interpretation of the results and on complicated clinical situations of patients. AONM also holds Arminlabs interpretation/training sessions every second Tuesday at 6.30 pm.

7. Is the sensitivity of any of the Lyme tests you perform affected by antibiotic or steroid use? Please give details.

The decision depends on the recommendation and advice of your health professionals. Furthermore, there are no studies, nor are there any references from test manufacturers, that the test can be impaired by antibiotics or other medications.

8. Do UK patients need a doctor's signature to order tests from you?

Patients do not need to have a doctor's signature to send blood. Nevertheless, we recommend a consultation with a health professional first or discussion with AONM as local partner for ArminLabs to determine which tests it would be best to perform.

9. How should UK patients order your tests? If appropriate please give a link.

Patients from the UK & Northern Ireland can directly contact our distributor AONM to order the tests.
https://aonm.org/patient-advice-for-ordering-tests/
AONM supports patients and health professionals in choosing the right and necessary tests.

10. For patients who have questions which have not been posed here, do you have an email address where they might ask questions, and can your staff deal with questions in English?

Patients can get in direct contact with AONM through the contact form on website https://aonm.org/contact-aonm/, through e-mail info@aonm.org or phone +44 3331 210 305. Staff at AONM as well as ArminLabs can deal with questions in English.

11. Where can patients find a list of your tests, with prices?

Patients can either contact AONM directly to receive a list of the tests ArminLabs offers in the UK, including prices, or patients can check it online at the AONM website https://aonm.org/arminlabs-price-list-and-order-forms/ .

arminlabs
DIAGNOSING TICK-BORNE DISEASES

Questions for ArminLabs:

Please could you comment on the fact that a significant number of people who gain negative results on NHS 2-tier testing show positive results on the Lyme Elispot offered by Arminlabs? What is the reason for this?

The serological diagnosis of Lyme borreliosis is complicated. There are different reasons why people may have positive results on the EliSpot while having negative results on NHS 2-tier testing.

Fresh infection	
NHS	ArminLabs
First performs an ELISA test, and if this is positive a Western Blot (or Immunoblot) test is conducted as confirmation	Elispot
However, it can take our immune system up to 6 weeks to develop IgG and IgM antibodies	T-cell activity increases within 7-14 days
Formation of IgM antibodies can be completely absent	A pathogen always produces cytokines

For chronic infections there are even more reasons for a negative NHS 2-tier test and a positive EliSpot. It is known that Borrelia bacteria can change into pleomorphic forms. The immune system produces different antibodies to those pleomorphic forms than to the spirochetes. So you need to have different antibody tests, such as the TickPlex. IgM antibodies may be completely absent in advanced disease. Cross-reactions with other microorganisms have been observed: bacterial diseases such as herpes virus infections (especially EBV or CMV) play an important role. False positive antibody responses may also occur in the presence of autoimmune antibodies. And last but not least the Borrelia bacteria can downregulate the humoral immune response. All these factors do not influence EliSpots.

1. Please could you comment on the bandings of the Elispot results?

a) What do the numbers actually refer to?

The EliSpot method offers an opportunity to gain insight into this protective immune defense. It detects the IFN-gamma reaction of pathogen-specific activated T cells. The EliSpot is a quantitative evaluation and the numbers refer to the number of activated T cells (within the sample) due to their cytokine release (each reaction triggers a spot). The results are expressed in SI units (SI=Stimulation index).

b) What does the band “0-1 = negative” mean? How does a positive number give a negative result?

0-1 is the stimulation index, which equals the negative control. 1 spot is, according to the manufacturer, not significant for an infection. Nevertheless, EliSpot results have to be interpreted within the patient’s overall clinical picture. A negative result does not completely exclude the exposure to the pathogen or infection being tested. Preanalytic influencing factors which can lead to an unspecific result of cytokine release, can be asked at ArminLabs.

c) What does the band “2-3 = weak positive” mean? Is it a positive result or not?

The infection fluctuates in the human body, the activated T cells do too. A weak positive result of between 2-3 SI units is a borderline result, which is not necessarily positive. Again, the interpretation of the results of the EliSpot assay may only be performed taking into account the overall clinical picture.

d) What does the band “>3 = positive” mean? With what degree of certainty is this a positive?

A negative and positive control is performed for each patient. If the controls are not in order, the test cannot be evaluated. With a typical test result, no or very few spots (<10) are to be expected in the negative control. The positive control should be >20 spots. In the event of strongly pre-stimulated samples, ArminLabs informs the patient/therapist that the test should be repeated. A new test kit will be sent upon request. The result >3 refers to a high number of SI units. If the positive and negative control are in order, this result evidences a high cytokine production against Borrelia.

2. Please could you explain the 3 parts of the Elispot? What is the significance of the different antigen mixes and what parts of the infection picture do they each give us?

a) Borrelia b Full antigen

Borrelia burgdorferi B31 reference strain (Borrelia burgdorferi sensu stricto) lysate of Borrelia burgdorferi contains many different antigens capable of evidencing cellular activity

b) Borrelia b OSP-mix

Borrelia burgorferi peptide mix: OspA of Borrelia b. sensu stricto, Borrelia afzelii, Borrelia garinii + OspC native + DbpA recombinant - special antigens with higher specificity than the lysate, due to less interfering factors; the antigens of the Borrelia burgdorferi OSP-mix are the most specific antigens from the lysate and lead to an overall higher significance

c) Borrelia burgdorferi LFA-1

Borrelia burgdorferi LFA-1 (Lymphocyte Function Antigen 1): Protein of the body + Borrelia burgdorferi sensu stricto (shared epitope). LFA-1 is frequently associated with autoimmune diseases like Collagenoses, rheumatoid arthritis, vasculitis (ANA, CCP antibodies, ANCA). It helps to decide whether the patient is suffering from an active infection or an autoimmune disease.

3. In terms of past or recent/current infections:

a) Can the Lyme EliSpot show positive for past infections even if you don't have a recent/current infection?

No, the Lyme EliSpot can't show a positive result for past infections if you don't have a recent/current infection. Lymphocytes have a life cycle of maximum 6 weeks. That's why the Lyme EliSpot can be used to monitor treatment progress and success. IgG antibodies can be used to detect past infections.

b) Does the test measure IFN-gamma from both memory and effector cells?

The EliSpot measures IFN-gamma secretion from TH1 effector cells.

c) What effect does that have on what the test can tell the clinician?

T-cell activity is already increased after 2-3 days (while the IgM antibody titer may still be negative), and is already clearly decreasing after approx. 6 - 8 weeks after the end of successful therapy (IgM/IgG antibodies often only decrease after 6 - 12 months). So the EliSpot can be used for swift and targeted monitoring of how therapies are working.

4. How is the Seraspot different to and better than the conventional Western Blot?

The Seraspot is a modern Western Blot. It is a solid-phase immunoassay for the qualitative in vitro determination of human antibodies of the immunoglobulin class IgG and IgM against Borrelia antigens from serum or plasma. The wells of the microtiter plate are coated with recombinant anti genes. The test is quantifiable and allows a more accurate evaluation and lower susceptibility to errors due to the predefined points/spots with specific antigens. The Seraspot also has some different bands to the Western Blot, for example it does not include the p41 band.

Extra questions - free-style!

Please give here any additional information you would like to share with patients.
You might like to tell us about the history of your laboratory, your plans and hopes for the future, your perspectives on the situation of Lyme patients in the UK, such as trends or differences from other countries' experience, your views on the current difficulties and possible future developments in Lyme testing.

ArminLabs was established 2015 by Dr. Armin Schwarzbach. After 12 years working and analyzing patients with chronic infections, Dr. Schwarzbach founded his independent laboratory in Augsburg/Germany. ArminLabs is specialized in diagnosing tick-borne infections like Lyme, Babesia, Chlamydia and other co-infections. The laboratory is internationally ISO DIN EN ISO 15189:2014 accredited in immunology, microbiology and virology. ArminLabs uses only externally certified and externally validated tests and offers clear and easy to read test result reports. Test results are reported within 7 days of specimen arrival.
ArminLabs offers the new and innovative TickPlex tests, which improve the sensitivity and specificity in comparison to Western Blot technology for Lyme testing. TickPlex can provide extra support in diagnosing chronic Lyme as it includes an antigen for pleomorphic forms of Borrelia.
The EliSpot tests assess T-cell activity against many pathogens, like Borrelia, Ehrlichia/Anaplasma, Bartonella, Babesia, Chlamydia, Mycoplasma, Rickettsia, Yersinia, EBV, HSV, VZV, CMV, Candida and Aspergillus. ArminLabs also offers advanced viral, yeast, mould and parasite testing - including Parvovirus, HHV6, Coxsackie, Echovirus, Toxoplasma, Leishmania and more.
The founder and CEO, Dr. med Armin Schwarzbach guides therapists in choosing the appropriate tests, interpreting the results and supporting optimal treatment options.
Current research projects are ongoing with a rheumatology clinic in Russia, on fibromyalgia with a clinic in the UK, and on POCKit with Prof. Gilbert and her team in Finland.

Lyme disease triggers lots of controversies between medical professionals, patients and politics not just in the UK, but all over the world. Standardisation and data material/statistics and studies are most important for the future, to end these controversies. Collecting the data and comparing it is one of the biggest challenges. ArminLabs offers standardised and comparable testing at fair prices for the patient. The ideal would be to collect data material and to compare results with other clinics and universities.
Unfortunately, there exist a lot of different Lyme tests that are brewed in-house, not accredited and not standardised. Due to these different test methods it is difficult to end the controversies because we cannot compare the data material collected. Laboratories offering these non-researched tests often do not put the patient's wellbeing first. They are not interested in sharing their data material or making their tests comparable to those of other laboratories. This behaviour makes the Lyme community easy game for authorities, pharmaceutical companies and critics.

Below you find statements for not currently recommended tests such as the so-called "LymeSpot revised" and the use of VlsE and p41i antigen in the EliSpot.

> *"We have to be very careful in not overinterpreting such experimental test systems like the so-called "LymeSpot revised". We do not think it is really a fact that Interleukin 2 will tell us the answer for patients by an easy red-green light-system until there is some data about specificity and sensitivity for the standard use of Interleukin 2."*
> *(Statement of German Professor for Experimental Science)*
> ArminLabs is not offering the "LymeSpot revised" test because there is no validation or any study for the "revised" LymeSpot. For several experts it is an experimental test without actual data and validation behind it.
> Also, the specialists at ArminLabs, are not sure whether using just Interleukin 2 can really give a definite answer for "yes" or "no" for treatment (easily called by laboratories offering "LymeSpot revised" and interpreting as "red-green light traffic principle"). ArminLabs has received information from several scientific experts for cytokines that Interleukin 1, Interleukin 4, Interleukin 6, Interleukin 10 and especially Interleukin 8 also play a very important role in the activity of infections in general. So just for scientific purposes the "revised" LymeSpot test might give among all other mentioned cytokines an additional information, but not in routine laboratory use. It might be also very dangerous in correct treatments for patients to say:" Green symbol = treatment and Red

symbol and Red/Green = Don't know what to do or no treatment". Laboratory medicine is not that easy.
Therefore ArminLabs has decided to wait for the validation and the first study (which has been running for several years now according the test producers) of "LymeSpot revised".
ArminLabs will continue to use the established Elispot like all other big laboratories in Germany and Europe. The diagnostic approach of ArminLabs is not to change to an experimental test system until we have full clarity about the correct cytokine profiles in Lyme disease and other infections.
"[…] I totally agree. IL-2 is not a good parameter by itself. I am working on a paper with Elispot + versus - and cytokines."
(Statement of Belgium Professor for physiology)

The producer of all EliSpot tests did a study with around 400 samples to figure out whether the additional VlsE and p41i are good parameters for interferon gamma release assay. VlsE is a good marker for serological tests, but it turned out that there is almost no response in interferon gamma testing. p41i is also a good serological marker, but it's not a specific marker. p41i is also contained in the lysate of the Borrelia burgdorferi Full antigen. There is no scientific evidence for the higher accuracy of the so-called "Elispot 2" compared to the Elispot. In our opinion the higher price is not justified and does not provide any benefit to the patient.
[…] 409 patients suspected for or with reported tick bite(s) in the past were tested for reactive T-cells after stimulation with Borrelia antigens […]
For the evaluation, positive donors in different disease stages were tested with individual proteins / peptides for published or known epitopes. The amount of T-cells which secrete IFNg after stimulation with VlsE and Bmp is low or practically non-existent. […] Although the Borrelia proteins VlsE, Bmp and p41 are well established and show a good concordance with the clinical picture in serological tests like ELISA or Western Blot, their potential to activate specific T-cells in the EliSpot is relatively low. Based on our studies and collaborations with external diagnostic laboratories, the overall results show that beside the Borrelia lysate the native OspC and the recombinant DbpA used are the most efficient activators for Borrelia specific T-cells.
Manufacturer's statement