



LDUK Q&A – Answers by Innatoss Laboratories

14 November 2017

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

Innatoss has submitted the request for ISO 15189 accreditation. The application is currently under evaluation by the designated body in the Netherlands, the Raad voor Accreditatie. This accreditation applies to Lyme serology tests (ELISA's and immunoblots) and the cellular test for Q-fever, Q-detect™. Tests that are currently in a developmental phase or are used solely in research projects are not covered by ISO 15189 accreditation. As soon as they are verified they will be added to the scope of the accreditation.

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. Where there are limitations, please explain how patients/doctors are a) advised of the limitations and b) what other tests or actions might correct or improve the limitations.

Which genospecies of Borrelia are the tests sensitive for? Which species may not be picked up?

For Borrelia infections, we perform ELISA's (Euroimmun IgG, Euroimmun IgM, Immunetics C6) and immunoblots (Euroimmun RN-AT IgG, Euroimmun RN-AT IgM, Mikrogen recomLine IgG). On request, we also use the species-specific Euroimmun Western Blots. As with all serological tests for Lyme disease, limitations of these tests include:

1. A negative result does not exclude the possibility of infection with *B. burgdorferi sensu lato*. Patients in early stages of Lyme disease and those who have been treated with antibiotics may not have detectable antibody titers.
2. False positive results may be obtained with sera from patients with diseases other than Lyme disease including syphilis, periodontal disease, rheumatoid arthritis, systemic lupus erythematosus, and other autoimmune or infectious diseases.
3. A positive result does not always refer to the presence of active Lyme disease. Since IgG antibodies and some IgM antibodies persist longer, the presence of antibodies of a previous infection can be the cause. This is different from a 'false positive' test, in that there has been an infection with Borrelia to which the immune system responded.

The strength of these tests is that when they are used in combination, they have a high sensitivity to detect antibodies against *Borrelia*. The combination of test results shows whether there is/has been an infection and in many cases whether this is a recent or an older infection.

When test results are reported to patients, we always include an explanation stating that in Lyme disease it is important to include both clinical symptoms and laboratory test results when diagnosing patients. Patients are referred to their GP for advice on this. For some patients, we include an advice to have a follow-up serology test to provide more information on the stage of infection and/or the effectiveness of treatment.

The geno-species that our tests are sensitive to include: *Borrelia burgdorferi sensu stricto*, *B. afzelii*, and *B. garinii* (Euroimmun IgG, IgM ELISA & immunoblots, Immunetics C6 ELISA); *Borrelia burgdorferi sensu stricto*, *B. garinii*, *B. afzelii*, *B. bavariensis*, and *B. spielmanii* (Mikrogen immunoblot). Since we perform the ELISAs and immunoblots in parallel, infections with *B. spielmanii* will be identified even if the ELISA does not detect these.

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?

All ELISA kits that are used by Innatoss for Lyme serology testing are CE-certified tests. We perform IgG, IgM, and C6 ELISA's for our customers.

EUROIMMUN Anti-Borrelia plus VlsE ELISA (IgG)

The ELISA test kit provides an in vitro assay for human antibodies of the IgG class against *Borrelia* antigens in serum or plasma. The test kit contains micro-wells coated with a mixture of whole antigen extracts of *B. burgdorferi sensu stricto*, *B. afzelii*, *B. garinii* and recombinant VlsE of *B. burgdorferi*.

EUROIMMUN Anti-Borrelia ELISA (IgM)

The ELISA test kit provides an in vitro assay for human antibodies of the IgM class against *Borrelia* antigens in serum or plasma. The test kit contains micro-wells coated with antigen extracts of *B. burgdorferi sensu stricto*, *B. afzelii*, and *B. garinii*.

Immunetics® C6 Lyme ELISA

The antigen used in the Immunetics C6 Lyme ELISA kit is a synthetic peptide (C6 peptide) derived from the VlsE protein, which has been shown to be both specific and highly immunogenic. The peptide sequence is conserved and supposedly equally antigenic in humans infected with *Borrelia burgdorferi sensu stricto* or with European genospecies including *Borrelia afzelii* and *Borrelia garinii*. As the antigen represents a defined sequence within the protein, potential cross-reactivity with unrelated and partially related antigens found in other organisms is greatly reduced.

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

EUROIMMUN Anti-Borrelia plus VlsE ELISA (IgG)

Specificity and sensitivity: Sera of 165 patients with suspected borreliosis were analyzed using the EUROIMMUN Anti-Borrelia plus VlsE ELISA (IgG) and the EUROIMMUN Anti-Borrelia EUROLINE-Western blot (IgG) as a reference method. The test showed a specificity of 90.3% and a sensitivity of 100%.

EUROIMMUN Anti-Borrelia ELISA (IgM)

Specificity and sensitivity: Sera from 150 patients with suspected borreliosis were analyzed using the EUROIMMUN Anti-Borrelia ELISA (IgM) and the EUROIMMUN Anti-Borrelia EUROLINE-Western blot (IgM) as a reference method. The test showed a specificity of 96.4% and a sensitivity of 100%.

Immunetics® C6 Lyme ELISA

Specificity: Serum specimens were obtained from 1,842 normal blood donors, comprising 1,329 sera from individuals residing in regions endemic for Lyme disease (northeastern U.S.) and 513 sera from individuals residing in areas considered non-endemic for Lyme disease (southwestern U.S.). Sera were tested on the C6 Lyme ELISA and by Two-Tier protocol using a whole cell sonicate ELISA in the first step. The specificity of the C6 Lyme ELISA was equivalent to that of the Two-Tier protocol for both endemic and non-endemic healthy blood donors (99%).

Sensitivity: A total of 517 well-characterized serum samples were obtained from North American patients who were diagnosed with Lyme disease based on either (1) documented erythema migrans; (2) culture or PCR positive for *B. burgdorferi*; or (3) Lyme arthritis or other symptoms of disseminated Lyme disease. The C6 ELISA was evaluated in comparison with the Two-Tier protocol. Overall, the C6 ELISA detected 74.9% of the Lyme patients, compared with 55.3% found positive by the Two-Tier protocol. An analysis of reactivity in the subset of IgG and IgM Western Blot-positive Lyme patients showed that 100% of patients with positive IgG Western Blot (n=157) and 95.8% of patients with positive IgM Western Blot (n=192) were also positive by C6 ELISA. Conversely, 47.5% of patients positive on C6 ELISA (n=387) were positive on IgM Western Blot, and 40.7% (n=386) were positive on IgG Western Blot.

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

- EUROIMMUN Anti-Borrelia plus VlsE ELISA (IgG): Data obtained from the test instruction by the manufacturer.
- EUROIMMUN Anti-Borrelia ELISA (IgM): Data obtained from the test instruction by the manufacturer.
- Immunetics C6 Lyme ELISA: Data obtained from the test instruction by the manufacturer.

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

Not applicable.

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?

For serology tests, there is no need for patients to stop their antibiotics treatment. However, to measure the effectiveness of treatment with antibiotics after a Borrelia infection, we advise to wait 3 months after the end of treatment before performing additional Lyme serology tests. Testing after a shorter period of time will not give sufficient reduction in titers to be clinically relevant information.

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.

All immunoblot kits that are used by Innatoss for Lyme serology testing are CE certified tests. We perform IgG and IgM immunoblots for our customers.

EUROIMMUN Anti-Borrelia EUROLINE-RN-AT (IgG)

The EUROLINE test kit provides an in vitro assay for human antibodies of the IgG class against the Borrelia antigens in serum or plasma. The test kit contains test strips coated with purified antigens and recombinant VlsE from *B. burgdorferi*, *B. afzelii* and *B. garinii*.

EUROIMMUN Anti-Borrelia EUROLINE-RN-AT (IgM)

The EUROLINE test kit provides an in vitro assay for human antibodies of the IgM class against the Borrelia antigens in serum or plasma. The test kit contains test strips coated with purified antigens from *B. burgdorferi*, *B. afzelii* and *B. garinii*.

Mikrogen recomLine Borrelia (IgG)

The recomLine Borrelia is an in vitro test for detection of IgG antibodies against *Borrelia burgdorferi sensu strictu*, *B. garinii*, *B. afzelii*, *B. bavariensis*, and *B. spielmanii* in human serum, plasma or cerebrospinal fluid.

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

EUROIMMUN Anti-Borrelia EUROLINE-RN-AT (IgG)

Antigen bands: p18, p19, p20, p21, p58, OspC (p25), p39, p83, p41, LBb, LBa, VlsE Bg, VlsE Bb and VlsE Ba. In addition to the most important serological early phase markers OspC and VlsE of different genospecies, the test contains highly specific p39 (BmpA) and the late phase marker p83. The test also contains immunogenic lipids which were extracted from *Borrelia*. Antibodies against lipid antigens occur primarily in the late stage of infection. Furthermore, five immunoreactive recombinant antigens (p58, p21, p20, p19 and p18) with a high specificity for the detection of anti-*Borrelia* antibodies were produced. These antigens also contribute significantly to the increase in the sensitivity of the Anti-*Borrelia* EUROLINE-RN-AT. Interpretation:

Antibody result		Specific antigen bands: p18, p19, p20, p21, p58, OspC (p25), p39, p83, Lipid Bb, Lipid Ba		
		2 or more bands positive	1 band positive	no band
VlsE Ba or VlsE Bb or VlsE Bg	antigen band positive	positive	positive	positive
	antigen band weak	positive	positive	borderline
	antigen band negative	positive	borderline	negative

EUROIMMUN Anti-*Borrelia* EUROLINE-RN-AT (IgM)

Antigen bands: OspC Bg, OspC Bb, OspC Ba, p39, p41 and VlsE Bb. In the early stage of a *Borrelia* infection, the IgM antibody response is mainly directed against OspC. For the Anti-*Borrelia* EUROLINE-RN-AT the OspC antigens from the *Borrelia* genospecies *B. afzelii*, *B. burgdorferi* and *B. garinii* were purified. The highly specific antigen p39 (BmpA), flagellin p41 and the *Borrelia* main antigen VlsE were produced by recombinant technologies. Interpretation:

Antibody result		Specific antigen bands: p39, VlsE Bb	
		1 band positive	no band
OspC Ba or OspC Bb or OspC Bg	antigen band positive	positive	positive
	OspC Ba or OspC Bg weak positive	positive	borderline
	antigen band negative	positive	negative

Mikrogen recomLine Borrelia (IgG)

Antigen bands: highly purified recombinant *Borrelia burgdorferi* s.l. antigens (OspA, OspC, p100, VlsE, p39, p58, p18 (= DbpA, decorin-binding protein A), p41 (flagellin)). A reaction with OspC is very characteristic for an early immune response. A strong reaction with the following bands mostly occurs in sera from late stages of the infection: p100, VlsE, p58, p39 and p18. On the contrary, antibodies against OspA are rarely found. The VlsE is a very early marker of the IgG response, but also frequently accompanies the immune response in late manifestation of the infection, and occurs besides p100 and/or p18. Interpretation:

Sum of points	Assessment IgG
≤ 5	negative
6	borderline
≥ 7	positive

Antigen	Points IgG
p100	5
VlsE	5
p58	4
p41	1
p39	5
OspA	5
OspC	5
p18	5

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?

Innatoss always reports all bands that are included on the immunoblot test strips to provide as much information as possible. Bands that are below the cut-off value of the test are also included in results reports, with the notification that they are below the cut-off. Discrepancies between the immunoblots used are often clarified in this way.

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

EUROIMMUN Anti-Borrelia EUROLINE-RN-AT (IgG)

Sensitivity and specificity: The performance characteristics of the Anti-Borrelia EUROLINE-RN-AT were determined by comparing the results with those of a CE-notified Westernblot using Borrelia whole extract and recombinant VlsE (EUROLINE-WB Borrelia). The following panels were investigated: sera from patients with clinically characterized borreliosis (n=274), sera from suspected borreliosis cases (n=198), sera from healthy blood donors and pregnant women (n=117) and sera from patients with other infections (n=28).

In comparison with the reference test, the Anti-Borrelia EUROLINE-RN-AT achieved a sensitivity of 95.6 % at a specificity of 90.3 % and a positive predictive value of 92.3 %. The investigated sera were classified into positive and negative samples using the reference test. In a ROC (receiver operating characteristic) analysis the specificity values obtained for the individual antigens used in the Anti-Borrelia EUROLINE-RN-AT ranged from 95.3 to 100 %. The sensitivity of the individual antigens was between 7.1 and 88.5 %. It can clearly be seen that the VlsE antigens, expressed in vivo, represent the main antigens of Borrelia. Among this group, VlsE from Borrelia burgdorferi stands out through its very high sensitivity. The sensitivity of the new recombinant antigens (p58, p21, p20, p19 and p18) was in a range of 7.1 to 22.4 %.

EUROIMMUN Anti-Borrelia EUROLINE-RN-AT (IgM)

Sensitivity and specificity: The performance characteristics of the Anti-Borrelia EUROLINE-RN-AT were determined by comparing the results with those of a CE-notified Westernblot using Borrelia whole extract and recombinant VlsE (EUROLINE-WB Borrelia). The following panels were investigated: sera from patients with clinically characterized borreliosis (n = 236), sera from suspected borreliosis cases (n = 204), sera from healthy blood donors and pregnant women (n = 159) and sera from patients with other infections (n = 45).

In comparison with the reference test, the Anti-Borrelia EUROLINE-RN-AT achieved a sensitivity of 93.0 % at a specificity of 94.9 % and a positive predictive value of 86.9 %. The investigated sera were classified into positive and negative samples using the reference test. In a ROC analysis, the specificity values obtained for the individual antigens used in the Anti-Borrelia EUROLINE-RN-AT ranged from 96.8 to 99.4 %. The sensitivity values of the antigens vary significantly. The highest sensitivity was achieved

using native OspC antigens from *B. afzelii* and *B. garinii* (88 % and 84 %). IgM antibodies against VlsE have a very low prevalence.

Mikrogen recomLine Borrelia IgG

Diagnostic sensitivity:

	Numbe	IgG positive	IgM positive	IgG/IgM positive
Lyme arthritis	28	27 (96 %)	6 (21 %)	27 (96 %)
ACA	11	11 (100 %)	1 (9 %)	11 (100 %)
Neuroborreliosi	35	29 (83 %)	18 (51 %)	33 (94 %)
Erythema	42	18 (43 %)	30 (71 %)	33 (79 %)

Diagnostic specificity:

<i>recomLine</i> Borrelia	Two comparison tests	
	IgG	IgM
Negative	171	169
Borderline	0	0
Positive	0	0
Specificity	100%	100%

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

- EUROIMMUN Anti-Borrelia EUROLINE-RN-AT (IgG): Data obtained from the test instruction by the manufacturer.
- EUROIMMUN Anti-Borrelia EUROLINE-RN-AT (IgM): Data obtained from the test instruction by the manufacturer.
- Mikrogen recomLine Borrelia IgG: Data obtained from the test instruction by the manufacturer.

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

Result reports that are send to patients and/or doctors include a conclusion field in which information is provided regarding the interpretation of the test results. We explain that increased IgM antibodies may reflect a recent infection, and that IgG antibodies generally represent an infection that has occurred a longer time ago. IgG antibodies often require more time to be formed than IgM.

We also inform patients and/or doctors that IgM antibodies may be measurable for a year or more. After the infection is cleared (with or without the help of antibiotics), antibody levels slowly decrease. In some cases, only IgM antibodies remain. This makes interpretation complex since a ELISA values just above the cut-off may just as well indicate a very recent infection as an older one that has been resolved/treated. For

proper interpretation, the clinical context, exposure risk, season, information on antibiotics treatment, and how long symptoms have been present are crucial information.

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?

For serology tests, patients do not need to stop their antibiotics treatment. However, if the goal is to measure the effectiveness of treatment with antibiotics for a Borrelia infection, we advise to wait 3 months after the end of treatment before performing Lyme serology tests. Testing after a shorter period of time will not give sufficient reduction of antibody levels to be relevant information on treatment effect.

Indirect tests looking for other immune reactions to infection:

c. LTT/Elispot/other T-cell test (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?

We have implemented the ELISpot Interferon- γ LymeSpot assay in which the production of Interferon- γ (IFN- γ) is measured after stimulation of T-cells with Borrelia-specific antigens. We are assessing whether it would provide added value for our customers in addition to the current Lyme diagnostic tests. The tests will only be offered to patients when we can start cell isolation on the day of blood collection.

In addition, we are working on the development of our own cellular, point-of-care test for early detection of Lyme disease within the European research project ID-LYME (for more information, please see <https://www.id-lyme.eu/>). As the laboratory and clinical development of this test are ongoing, technical details and sensitivity or specificity data cannot be provided yet.

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- γ LymeSpot assay

This kit is manufactured by Autoimmun Diagnostika (Strassberg, Germany) and is the only CE-marked ELISpot kit for the determination of IFN- γ in human T-cells after antigen-specific stimulation.

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.

Not applicable.

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

ELISpot Interferon- γ LymeSpot assay: Borrelia B31 Lysate and Borrelia OSP-mix.

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

ELISpot Interferon- γ LymeSpot assay: Data on studies done is available upon request from the manufacturer AID Diagnostika but is not included in the test kit instructions.

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.

See previous answer.

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.

ELISpot Interferon- γ LymeSpot assay

For interpretation of results, a stimulation index is used. This index is calculated by dividing the mean number of spots in the patient sample by the mean number of spots in the control sample. The interpretation is different when the unstimulated value is 0 or 1 or higher.

Cut-off thresholds reported by the manufacturer are:

- Interpret the result as reactive when SI is >4
- Interpret the result as borderline when SI is between 2 and 4
- Interpret the result as negative when SI is < 2
- If the number of spots in the sample control is 0 or 1 the number of spots in the OSP Mix must be >5 or in the Borrelia lysate >10 to interpret as reactive.

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?

At the time of the blood draw for T-cell mediated assays, patients should not be taking antibiotics or immunosuppressive medication. As antibiotic treatment interferes with the test, no reliable results can be provided. The time needed between the end of treatment and the test depends on the type of antibiotics used (i.e. on the half-life of the drug; how fast is it cleared from the body).

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?

Not applicable; CD57/NK-cell tests are not performed by Innatoss.

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

Not applicable.

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

Not applicable.

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?

Not applicable.

Direct testing methods

e. PCR

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

Not applicable; PCR tests are currently not performed by Innatoss.

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

Innatoss does not perform PCR testing on ticks.

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

The general limitation of PCR testing in screening tests for Lyme borreliosis is the low or unknown sensitivity of this method when applied to blood or urine. However, it can be considered to use PCR testing in case of potential Lyme arthritis (in synovial fluid) or in case of suspected acrodermatitis chronica atrophicans, Borrelia lymphocytoma (in biopsy of skin lesion), or neuroborreliosis (in liquor cerebrospinalis). In these cases, PCR testing will indicate whether Borrelia is still present. This is the main strength of direct detection and clinically relevant.

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

The available research data on the specificity and sensitivity of PCR testing on different human biological tissues/body fluids are summarized in the Dutch CBO guideline for Lyme disease. Please see:

https://www.nvvg.nl/files/39/Lymeborreliose%20Richtlijn_lymeziekte_definitief_18_juli_2013.pdf).

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?

For PCR testing, patients do not need to stop their antibiotics treatment.

Other tests

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

Innatoss has implemented a new direct testing method for the detection of Borrelia. The test is called Nanotrap[®] Lyme Antigen and directly detects Borrelia-antigen in urine after a concentration step using nanoparticles. With this innovation, Borrelia infections can be detected without the need for blood draws. This test has been developed by Ceres Nanosciences (see <https://www.lymedx.com/>) and can detect an active infection in all stages of disease (from early infection to late stage Lyme disease). Innatoss will be the only laboratory in Europe that will perform. Ceres has published (Magni et al, 2015) that in fully characterized EM patients in the US the sensitivity of the test is 100% and specificity is also 100%. Innatoss is still verifying these numbers for Europe. The Nanotrap Lyme Antigen test is a so-called lab developed test which means that you cannot buy the kit in a box. This is common practice for less common diseases. Lab developed tests do not have to be FDA approved or CE marked. They do not need to be validated.

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?

Acute Lyme disease: C6, IgG, and IgM ELISA; if positive: confirmation by IgG or IgM immunoblot(s).

Chronic Lyme disease: C6, IgG, and IgM ELISA in parallel with IgG or IgM immunoblot(s). In addition Nanotrap Lyme is useful for direct detection of Borrelia-antigen in urine.

Very early disease: Serological tests will be less useful in very early stages of disease, unless a previous serological test result is available. Small increases can be detected more easily when a prior sample is present to compare the results with. In addition Nanotrap Lyme is useful for direct detection of Borrelia-antigen in urine.

Patients with limited financial resources: screening with C6, IgG, and IgM ELISA. These tests can give a good first impression on whether there is/has been an infection and have the lowest costs.

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.

Innatoss does not carry out tests for other tick-borne co-infections in its own lab. We do test for Q fever, which is sometimes listed as a co-infection. Innatoss also sends patient samples to other diagnostic labs such as Stein und Kollegen to have them tested for specific co-infections (for example Babesia, Bartonella, Anaplasma, Chlamydia and Rickettsial disease). We also implemented CE-marked immunoblots that cover different

viral infections, STDs and autoimmune diseases that may cross react in Lyme tests and that may have symptoms similar to Lyme disease.

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

Innatoss has a medical advisor who is a GP. We offer consultations with our medical advisor in case more information or an advice is needed by either patients or doctors based on test results.

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

Serological Lyme tests are not affected by antibiotic or steroid use. T-cell tests (at Innatoss still under development or validation) are affected by antibiotic treatment so need to be performed when patients are not being treated. Please be aware that antibiotics and steroids will affect the immune response of the patient. Even though the tests may not be affected as such, the formation of e.g. antibodies will be different when someone is taking steroids.

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

No, this is not needed. We do recommend to consult a doctor in advance since Innatoss does not provide treatment and we find it important that the results are used for treatment decisions.

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

UK patients can order Lyme tests using our website. The link to our webshop (EN) [ihttps://www.innatoss.com/en/shop/](https://www.innatoss.com/en/shop/)

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 contact us using the email address info@innatoss.com. Most of our staff is well versed or fluent in English. If medical terms pose a problem patients will be connected to someone who is fluent in English and understand the topic.

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

Patients can find the tests that Innatoss performs and the prices on our website.

In Dutch: <https://www.innatoss.com/nl/winkel/>

In English: <https://www.innatoss.com/en/shop/>

Questions specific for Innatoss

1. You offer a bundle of 5 tests. Please can you explain why this combination offers an improvement which is worth the extra cost?

In the Innatoss 5-in-1 test, we perform a combination of 3 ELISA's (C6, IgG, and IgM). The ELISA results are directly confirmed in immunoblots. This testing scheme leads to an improved sensitivity and specificity; the chance of detecting a true Borrelia infection is increased and the risk of false positive results is decreased. The 5-in-1 test provides a complete overview of any Borrelia antibodies that are present, by providing more extensive testing than regularly performed in hospitals.

By testing in parallel, we are able to detect more often exposure to Borrelia. Using the combination of 3 ELISAs increase chance of detection without increasing the costs too much. In the immunoblots, traces of antibodies that remain after many years are more easily visible than in ELISAs, most likely due to a higher avidity of these antibodies.

By testing immunoblots and ELISAs in parallel, rare *B. spielmanii* infections are e.g. identified in the recomLine blot that would not have resulted in a positive ELISA. The recomLine blot also point towards the Borrelia strain that was responsible for the infection.

Using 2 immunoblot types with varying antigens increases the chance that less common antibodies are detected. The RN-AT blot is more VlsE based and will score samples with only VlsE reactivity as positive (as such this is an ideal follow-up for a positive C6 ELISA) whereas the recomLine blot will score samples with only VlsE reactivity as negative. A patient with a positive C6 and negative recomLine blot will thus be given a "not infected" result, while the same C6 ELISA combined with an RN-AT blot would give a "infected" result.

Having all data at the same time will allow for a better overall interpretation of the results in a shorter time. In case of limited resources all tests can be done sequentially. If for example all ELISAs are positive, confirmation by immunoblot is formally required, but the chance of a positive confirmation is > 95% and most doctors would treat their patient.

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**

Innatoss Laboratories is a young research-intensive organization, established in 2012. Innatoss' mission is to significantly reduce health problems associated with infectious diseases such as Q fever and Lyme disease. We do so by providing diagnostic tests that allow for adequate and timely treatment. Our tests are reliable and predictive. They come to market after sound validation and with easy access to the population at risk. The Innatoss team consists of professionals with extensive experience in the pharmaceutical and diagnostic industry, as well as starters. We work closely with patient organizations and university medical centers contributing to meaningful and scientifically sound innovations. Innatoss is located at the Pivot Park, an industry park in Oss (The Netherlands), on which many life science companies are located and that provides facilities to the companies for common use. The combination of knowledge, experience, energy and drive is what makes Innatoss a successful, ambitious and involved organization.

- **Your plans and hopes for the future**

In 10 years' time, Innatoss will be a global reference laboratory for cellular diagnostic tests for infectious diseases. We intend to be a major manufacturer of diagnostic tests in this field. Our area of attention will remain Q fever and Lyme disease. We expect to expand operations to the most relevant countries in the EU, to Australia, the USA and Africa. Our focus on intracellular infections will provide us with new insights in the immune response against those bacterial infections. This will pave the way to new solutions for other diseases as well. Solutions for primary physicians to support their diagnoses, for regular hospitals, their patients and people at risk. Our innovations will be supported by new ideas from academic medical centers. We thrive on open innovation with partners such as patient organizations, public health institutes, diagnostic laboratories and companies worldwide. Academia will provide the knowledge, Innatoss will create marketable solutions based on requirements from all parties. As such, we will bridge the gap between a great concept and a great product.

- **Your perspectives on the situation of Lyme patients in the UK, such as trends or differences from other countries' experience**

In general, the situation of Lyme patients in the UK is not that different from those in other countries. All stakeholders, patients, labs and doctors alike, struggle with the currently available test methods. All would prefer to have direct detection methods that will provide clear evidence that antibiotic therapy is required.

The UK is not unique in having a large number of patients who truly suffer and who contribute their symptoms to a *Borrelia* infection. In the Netherlands, we have

experienced however that Q-fever may give very similar symptoms. It is therefore important that doctors and patients remain aware that symptoms may be due to other diseases, whether they are infectious or not. Treatment may be very different!

The UK is special in that the infection rate of ticks appears much more unevenly distributed over the country. Data on distribution are missing. In some parts of the country, the infection rate is very low, which means that doctors have little experience in recognizing an erythema migrans and diagnostic labs have limited experience with performing and interpreting the tests. For that reason, it made sense to concentrate Lyme testing in 2 labs, one in England and one in Scotland. This however gives little room for professional discussions and development of novel ideas. It is therefore crucial that NHS consultants and diagnostic specialists interact with colleagues abroad.

- **Your views on the current difficulties and possible future developments in Lyme testing**

Serological tests and T cell tests indicate that an infection took place and are not proof of an active infection. However, using these tests in a more intelligent manner, looking at specific antigen bands in immunoblots and at changing titers in ELISAs, will definitely help in identification of active infections. As long as no true improvements have been made, it is important to use these methods as well as possible, instead of providing yes/no answers, or not testing at all.

An important difficulty is that there are no direct detection methods readily available that indicate that an active infection is ongoing that requires antibiotic treatment. This is an important field that will require active research and development with significant funding. The Nanotrap Lyme Antigen test is a major step forward, provided it works as well with the European strains as with the US strains.

Once the direct tests are available, it becomes crucial to understand how patients without an active infection but with ongoing symptoms can be distinguished and subsequently treated. Any treatment that affects the immune system can be either helpful in these patients or detrimental. Therefore, precision diagnostics and therapy are essential for this group.

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