

## Advanced Lyme tests Elispot and Tickplex available in Germany What are they, and how do they work?



I am a member of a non-profit group known as VIRAS (Vector-borne Infections, Research, Analysis and Strategy). We have knowledge and experience of Lyme borreliosis and co-infections, Myalgic Encephalomyelitis (M.E.) and Chronic Fatigue Syndrome (CFS). Our members have a wide range of professional qualifications in science and research, statistics, ethics, biology, psychology, psychotherapy, teaching, nutrition, engineering, business and media.

I was recently very pleased to be able to talk to two scientists by Skype so that I could ask them a few questions about new types of tests for Lyme disease. These tests have not been highlighted before in Britain. After the conversation I looked up references and background information, and then sent my write-up to both scientists to check.

Sitting in her office where she runs her lab in a university in Finland was Docent Leona Gilbert PhD, whom I had never spoken to before; and in my other Skype window there was Dr Armin Schwarzbach PhD at his desk at Arminlabs, of which he is the CEO, in Germany.

Leona I had heard of from friends in Scandinavia, who had alerted me to her new Tickplex advanced tests for Lyme disease. Leona is a tenured academic: an Adjunct Professor in Cell and Molecular Biology at the Department of Biological and Environmental Science at the University of Jyväskylä in Finland, and she is also the CEO of a company that develops tests called Te?ted Oy ([www.teztet.com](http://www.teztet.com)). She is the author of dozens of research papers and has specialised in highly technical methods of investigation and diagnostics in biological systems.

Armin is both a medical doctor and a PhD scientist. I had met him only briefly before, in London, when he addressed a Lyme Disease Parliamentary Symposium in January 2015, and also when he attended a UK Parliamentary drop-in meeting for MPs and expert Lyme doctors in February 2017. He has presented lectures at health department meetings and many scientific conferences in several European and Scandinavian countries and in the USA. He has been working in specialist research and diagnostics laboratories for over 25 years and is a long-time member of the medical and research group ILADS (International Lyme and Associated Diseases Society) that has published guidelines for Lyme disease diagnosis and treatment. This society has been gathering more physicians every year to learn from each other about how best to deal with tick-borne disease patients.

I spoke with Leona first. I had followed a series of presentations by her on YouTube and I'd read her scientific publications about the new test method (1,2). But because the test she developed is so new, and had recently become available at Arminlabs, I asked for an interview to find out more. Also, although I know that I have Lyme disease, I was curious to find out about testing my own blood for the co-infections that are reported by many doctors as being present in their patients. Germs such as **Babesia**, **Bartonella (cat scratch fever)**, **Ehrlichia** and **Rickettsia** can be transmitted by ticks, and then there are also the many opportunistic infections which Lyme patients seem prone to catch because our immune systems have been weakened.

But first of all, the important thing was to ask about the testing for Lyme, or Lyme Borreliosis as it's more properly known. I asked Leona about the fact that her test was so advanced that it could detect the presence of the different forms of the Borrelia germs that can be present in our bodies. She explained that yes, as well as the most well-known form of the Borrelia bacteria, which is a very thin, twisted spirochaete, the Borreliae do transform themselves into other shapes such as the "round bodies". To call them a cyst is a misnomer, but in a way, they behave like spores or cysts, because they

are the stealthy, non-reactive and persistent form of the germ, which have been shown to develop into spirochaetes at certain times.

Under the electron microscope, Leona had observed that the round bodies also have the appendages called flagella, which the spirochaete bacteria use for propelling themselves forwards, and they are visible curled up underneath a cell membrane. Before then, most scientists assumed there was no cell membrane wrapped around the round bodies of *Borrelia*, and that is perhaps why they had not searched to identify what this cell membrane was like.

The cell membrane of the ordinary form, the spirochaete, has been very well characterised over decades of research and lots of proteins have been identified on its surface. They are labelled with names such as Outer Surface Protein A, (OspA) and OspB, OspC etc. One protein on the nose end of the spirochaete is called Decorin Binding Protein, and there are many others along the surface of this long twisted bacterium. But never before has anyone investigated the proteins on the surface of the round bodies.

In fact, each *Borrelia* round body has its own different surface proteins when compared with the spirochaete. These surface proteins on the round bodies will act as antigens which will cause a specific reaction from our immune system that is different from the antigen-evoked reactions of a spirochaete.

An **antigen** is just a name for a molecule, almost always a protein, which the immune system recognises as something that should not really be there, something that will cause harm or inflammation. So our amazing immune systems start to make very specific defence proteins that will stop these antigens, and they are called the **antibodies**. Like an antidote to a poison, we have antibodies responding to an antigen. The cells that make antibodies are in the blood and lymphatic fluids, and they are called **B cells**. [I use this phrase to remember what they do B cells Beat the Bugs by producing anti-Bodies.]

Another part of the immune white cells in our blood are called the **T cells**. They don't make antibodies – but they are crucially important because they tell the B cells what to do. We will hear more about these T cells while looking at the **Elispot** tests when talking with Armin.

I learned from Leona that there are antibodies in the blood of Lyme patients which no one has looked for until now, because they did not know the properties of the antigens on the cell membrane of the round bodies which evoke those antibodies.. It makes sense to look for the antibodies to these antigens, because some patients will have more round bodies in their blood than spirochaetes.

Leona told me that she has had enquiries about her tests from the CDC (Centres for Disease Control and Prevention) and the NIH (National Institutes of Health) in America, as well as the Department of Health in Finland. I asked her how she had made her proteins for the kits but she said they are a bit of a trade secret, and that's when she mentioned that there are **4 patents pending** associated with the methodology. Two of her peer-reviewed research publications (1, 2) describe how she discovered this exceptionally useful information about the round bodies, and how she devised the tests.

Her basic **Tickplex** tests for ***Borrelia burgdorferi*, *Borrelia afzelii*, and *Borrelia garinii***, including the **persistent forms** of all three. Also, it can identify the acute antibody types called the IgM and the older antibodies which stay in the blood for longer (the IgG antibodies).

The **Tickplex Plus** test is ideal for people who suspect other pathogens are causing their illness; perhaps because they have failed courses of treatment for Lyme disease or their symptoms point to a complex infection. The additional pathogens tested for include ***Babesia*, *Bartonella*, *Ehrlichia* and**

**Rickettsia.** Also, the opportunist infections such as **Coxsackie virus, EBV, Human Parvovirus B19, Mycoplasma fermentans and Mycoplasma pneumoniae** are covered. These two tests, Tickplex and Tickplex Plus, are both validated with CE and are IVD marked and accredited for medical devices standard ISO 13485:2016.

Leona explained the validation of third test, the **Tickplex Premium** test, is ongoing and should be available next spring. It is well on its way to validation, but she explained that because it covers so many more pathogens, and its ability to detect each pathogen has to be assessed separately, the validation process is taking longer than the Basic and Plus tests. The Tickplex Premium will include many more pathogens in addition to the ones on the Tickplex Plus and these will include **Tick-borne Encephalitis Virus (TBE), Brucella, Chlamydia pneumoniae, Cytomegalovirus and Chlamydia trachomatis** and others.

In the validation of Tickplex technology a double-blinded study, supported by the Finnish government, and involving 1,100 patients and 200 healthy controls, was carried out to see how well the Tickplex Plus test performed. The sensitivity was 95% and the specificity 98%. It was by far the best study result the FSI has seen regarding infectious diseases caused by ticks (3.)

The tests will be good value for money, because even the basic test will be looking for far more antibodies than the ones used by government or National Health Service labs. The cost of the Tickplex Plus for instance is about £450 – but imagine how much it would cost to pay for separate tests for 10 different species of bacteria, plus the 3 species of Borrelia. Given that ticks are carrying multiple infections, it makes sense to test for several pathogens in each patient at one time. She explained that the assessments of positive and negative results are done by a machine, which measures the optical density of each antibody that shows up as a circular spot. If a spot on the test plate has a high optical density, it will indicate a high level of infection – so all the Tickplex tests are not only qualitative, but also quantitative.

Armin Schwarzbach is already carrying out the Tickplex series of tests at his Arminlabs, so I then turned to him, to ask if the costs to the patient could ever be reduced? He said that in Germany, there are strict rules about not going below a threshold in the pricing of medical tests. The costs of paying well-trained scientists and technicians, and maintaining the highest levels of laboratory standards, are very high, and the German authorities are keen to keep up these standards. For example, Arminlabs were accredited in the summer of 2017, to the highest level possible internationally under the Dakks German standards board – and this cost Arminlabs EUR 50,000 to pay the fees of the many different experts who had to visit the labs to inspect them. The full accreditation is called **DIN EN ISO 15189**. It means that every aspect of the labs and every procedure done there, are recognised as being of the highest possible quality.

I asked him why he also offers another type of test, called the **Elispot**, for Lyme borreliosis and for some of the co-infections, especially as Leona's tests were so good! His reply is very important for Lyme disease patients to learn about.

He explained that some patients who are infected with tick-borne pathogens have B cells that are so damaged by the infections that they can no longer produce antibodies, or they produce them intermittently at a very low level. This means that they will be “seronegative”, and this is a major problem for patients with tick-borne disease because they will be misdiagnosed as not having the infection. Even the best antibody test in the world is not going to find antibodies if the blood doesn't have any antibodies there!

I also asked him if he would be using the new tests that take patients' urine samples using “nanotrap” technology – and he replied that he would not want to use them because they only look for one antigen, the OspA, and this has been found to be a very variable protein, so patients will have

different versions of it in their blood and they might test falsely negative..

Seronegativity, with no antibodies in the blood, is a situation where the T-cells become important – because they are the cells that tell the B cells to make antibodies and there is a better chance that they have not yet been totally damaged by the infection. **Elispot** tests are designed to look at how T cells are operating in a patient. The manufacturer that Armin uses supplies Elispot kits, detecting different infections, to dozens of laboratories and hospitals all over Europe, including 21 health and research institutes in the UK (including Porton Down). The company with this excellent international reputation is Autoimmun Diagnostika GmbH (elispot.com).

The T cells command B cells to make antibodies by sending out chemicals called **Gamma Interferons**, and the Elispot tests have been designed to be sensitive enough to detect the activity of a single T cell's output of Gamma Interferon in each blood sample. In some patients, there is still a possibility that their T cells are quiescent, but the beauty of a T cell test is that when the Gamma Interferons are detected, there can be no dispute that a patient has an ongoing infection. Armin explained that the chance of a result being a false positive is very small. The Elispot tests for Lyme disease have been assessed for their sensitivity and in reality they are over twice as sensitive as the Lyme tests used by the UK's NHS labs: a combined ELISA and Western Blot (2 tier test) as used in the UK can have sensitivity down to ~22% and that is the method used by the NHS for most patients. Whereas the Elispot Lyme test has a sensitivity of at least 84%. and in practice this reaches higher levels. In Germany there are around 20 different laboratories offering the **Elispot**, both for *Borrelia burgdorferi* as well as every other type of infection and virus for which the Elispot has been developed.

Armin emphasised that it is crucial to remember that **Lyme Disease should be a clinical diagnosis**. Lyme Disease is not a laboratory diagnosis. Laboratory tests are there to support the clinical suspicion of Lyme disease developed by appropriate specialists, using symptoms and differential diagnosis in a face-to-face consultation, together with assessment of a patient's full medical history.

I asked Armin whether his Elispot tests are covered by insurance under the German health system and he assured me that the biggest provider, which has 8 million Germans insured with them, definitely does pay for the Elispots.

I also asked him about how patients can get their blood samples to him in time – i.e. do the samples deteriorate when they are sent, say, from Scotland to Germany? He explained that the blood collection tubes contain chemicals that preserve the integrity of the blood cells for up to 5 days. Even so, it is best to send a sample if possible at the beginning of the working week. Results are usually ready within 7 to 14 days. “Do patients have to make sure they are not taking antibiotics when they have their blood samples taken?” I asked him – and he said that it is better to stop taking them for a week before the blood is drawn. One week should be long enough for most types of antibiotics to have been cleared from our systems.

I thanked Leona and Armin, and so concluded my interview with two eminent hard-working scientists, who were kind enough to answer my questions with great patience, and to give up their time in their busy schedules. Patients in the UK should be aware that, however scientifically accurate both of these tests are, only a few doctors in the UK will have the knowledge to realise their value, nor in general will any doctors accept foreign test results. Many people though, have chosen to have these tests for their own information, and some have found doctors willing to include the test results in their evaluations. Things have to change, and they will do so, as soon as doctors and consultants have taken the time to learn about the Lyme Borreliosis Complex.

Denise Longman MSc  
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## Further Reading

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## Disclaimer:

This enquiry about blood tests was carried out in the knowledge that looking at the antibody or immune cell responses in Lyme Borreliosis patients can never be as definitive as using direct testing methods, which look for the bacteria themselves. Tests which culture the bacteria from a patient's blood or tissues are the gold standard for proving that an infection is present, but they are not readily available for European patients. Tests which probe for the DNA of the bacteria in a sample of blood or tissues can also be definitive, but have not yet proved to be sensitive enough in practice.

Arminlabs is not the only laboratory testing for Lyme borreliosis in Germany, and across Europe there are many others, including public health laboratories and independent organisations. The author would be happy to interview any scientists who are available to describe and explain their testing and diagnosis for Lyme Disease Complex.

