

Augsburg, 26th Jan. 2016

To whom it may concern

Statement about Borrelia-Elispot

Lyme Borreliosis does not only show humoral immune responses by antibodies, but can activate T-lymphocytes at the same time. Once *Borrelia burgdorferi* is not active anymore, the T-cellular immune response should cease.

It is not possible to test the treatment success by *Borrelia* antibodies, because the "titer" of antibodies can persist in the blood over years. Recent *Borrelia burgdorferi* infections (e.g. 'bull's-eye rash' or 'summer flu') can develop antibodies after weeks and in around 40% do not show them at all.

The *Borrelia* EliSpot can eliminate some problems. The test reflects the actual *Borrelia burgdorferi* activity of chronic and recent *Borrelia burgdorferi* infections. It is highly sensitive and can detect even one single *Borrelia burgdorferi*-reactive T-cell in the sample. With detection levels that can be as low as one cell in 100,000, this test is one of the most sensitive cellular assays available. The EliSpot is between 20 and 200 times more sensitive than a conventional ELISA. It displays a similar sensitivity as a RT-PCR (Real Time PCR) analysis but detects the secreted protein instead of the mRNA (messenger RNA).

The EliSpot is a CE-certified, externally controllable and standardized test, which can be also helpful when monitoring therapies. Results should usually be negative about 4 to 8 weeks after completion of an effective therapy.

The Enzyme Linked ImmunoSpot (EliSpot) belongs to the IGRA (Interferon-Gamma-Release Assay) test systems.

The *Borrelia* EliSpot includes the following antigens, which have the same specificities used for *Borrelia*-antibody detection in CE-certified Westernblot systems with over 90% specificity:

- *Borrelia burgdorferi* complete antigen: an antigen which stimulates the immune response and reacts with products, e.g. antibody of that response, cf. hapten.
Borrelia burgdorferi B31-reference strain (*Borrelia burgdorferi sensu stricto*)
- *Borrelia burgdorferi* Peptide-Mix:
OspA from *Borrelia b. sensu stricto*, *Borrelia afzelii*, *Borrelia garinii* + OspC native + DbpA recombinant
Explanation: Native = cultured antigens / Recombinant: produced with genetic technology
- *Borrelia burgdorferi* LFA-1 (Lymphocyte Function Antigen 1):
Own body protein + *Borrelia burgdorferi sensu stricto* (shared epitope). Often associated with autoimmune diseases: collagenosis, rheumatoid arthritis, vasculitis (ANA, CCP-antibodies, ANCA)

The EliSpot reflects the actual T-cellular activity of Lyme disease:

Indication of an actual active *Borrelia burgdorferi* infection in cases of a positive EliSpot post-treatment.

The T-Cell-Spot/IGRA has been approved for *M. tuberculosis* by the FDA in May 2011:

"... A positive result suggests that an infection is likely, a negative result suggests that an infection is unlikely..." "...Results can be available within 24 hours..."

ELISPOT is named the new T-Cell Test is a "Game Changer" for Lyme disease:

"... The sensitivity of ELISPOT is estimated at 84%, and the specificity is 94%..." "... ELISPOT assays provide robust, highly reproducible data..." "... ELISPOT can be retested for the acquisition of additional information in follow-up assays..." "... the two assays systems (ELISPOT + CD57-cell count) compliment each other in the quest to understand T cell-mediated immunity in vivo...."

(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: 3-23)

... The ELISPOT assay showed ... a specificity of 82% in Neuroborreliosis...

Nordberg et al.: Can ELISPOT be applied to a clinical setting as a diagnostic utility for Neuroborreliosis?, Cells 2012, 1, 153-167

... Borrelia antibody positive asymptomatic children (n=20), children with previous clinical Lyme Borreliosis (n=24), and controls (n=20). Blood samples were analyzed for Borrelia-specific interferon-gamma...by ELISPOT...We found no significant differences in cytokine secretion between groups...

Skogman et al.: Adaptive and Innate Immune Responsiveness to Borrelia burgdorferi sensu lato in Exposed Asymptomatic Children and Children with Previous Clinical Lyme Borreliosis, Clinical and Development Immunology, Vol. 2012, Article ID 294587, 10 pages

Best regards

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