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# Elispot®-LTT: FDA and CDC approved LTT technique in U.S.

## Actual T-cellular activity in the blood against Borrelia burgdorferi, Chlamydia pneumoniae/trachomatis, Ehrlichia/Anaplasma

In May 2011 the U.S. Food and Drug Administration (FDA) and the Centers for Disease Control and Prevention (CDC) have approved the **Elispot®-LTT (T-Spot)** technique beneath the QuantiFERON® TB Gold In-Tube test. Both tests represent Interferon-Gamma Release Assays (IGRAs) in form of Lymphocyte Transformation Tests (LTT).
No other laboratory T-cell tests have been approved (MELISA® or ITT® techniques are not approved!) in the field of all Lymphocyte Transformation Tests (LTT) by the FDA/CDC yet.

In the paper of the CDC regarding Interferon-Gamma Release Assays (IGRAs) from May 2011 the CDC says:
"... A positive result suggests that an infection is likely, a negative result that an infection is unlikely..."
"...Results can be available within 24 hours..."

Center for Disease Control and Prevention. Updated Guidelines for Using Interferon Gamma Release Assays to Detect Mycobacterium tuberculosis Infection, United States. MMWR 2010; 59 (No.RR-5)
http://www.cdc.gov/mmwr/pdf/rr/rr5905.pdf

**New study for the specificity of Borrelia-Elispot-LTT:**
… Borrelia antibody positive **asymptomatic** children (n=20), children with previous clinical LB (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, Clincal and Development Immunology, Vol. 2012, Article ID 294587, 10 pages

**According this study: 100 % Specificity of the Borrelia-Elispot-LTT**

… The sensitivity of ELISPOT is estimated at 84%, and the specificity is 94%...
… ELISPOT assays provide robust, highly reproducible data…
… EIISPOT 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

**According this new studies:**
**82-100 % Specificity of Borrelia-Elispot-LT**
**84 % Sensitivity of Borrelia Elispot-**


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**Elispot®-LTT** is available for:

1. **Borrelia burgdorferi**
2. **Chlamydia pneumoniae**
3. **Chlamydia trachomatis**
4. **Ehrlichia/Anaplasma**
5. **Yersinia species**
6. **Epstein-Barr-Virus (EBV)**
7. **Cytomegalo Virus (CMV)**

Informations about the Elispot®-LTT technique**:**

**www.elispot.com**


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# Borrelia Elispot®-LTT
## Actual cellular activity in the blood against Borrelia burgdorferi

A Borrelia infection leads to a vitalisation of T-lymphocytes apart from the humoral immune answer. The T-cellular immune response vanishes as soon as the Lyme disease is not active anymore.

A therapy success control of a Lyme infection is not possible by the Borrelia antibodies, as the “titer” of antibodies in the blood can be found for years after an infection. Additionally in stage I (e.g. “bull´s eye rash” or “summer flue” after a tick bite) antibodies can be found after several weeks or in stage III they cannot be found in every case (weak immune system).

These diagnostic gaps are closed by the Elispot-LTT for Borrelia, which detects the actual cellular activity against Borrelia burgdorferi in chronic and also acute Lyme disease. The Elispot is so sensitive, that even a single Borrelia can reactivate T-cells in the blood. The Elispot is 20- to 200-fold more sensitive than an ELISA-test on Borrelia and will already find 1 reactive T-cell in 100.000 lymphocytes.

The Elispot for Borrelia is very important for controlling a therapy of a chronic or acute Lyme infection. In general the Elispot-LTT is going to be negative app. 6 to 8 weeks after the end of a successful therapy.

**Advantages of the Elispot-LTT for Borrelia** (- as performed by infectolab -) in contrast to other lymphocyte transformation or proliferation tests from other laboratories are:

- The result is available within 5 days (Other similar tests: 2 – 3 weeks)
- The use of cell stabilising CPDA-tubes means a stability of 3 days for the measured cells after taking the blood (Others: Heparin blood only 24 hours)!
- Better reliability than the different "unspecific" tests using the proliferation of T-cells (e.g. ITT®)

This offers a significant improvement of the stability of the T-cells and a very quick decision possibility for Lyme treating physicians to extend a therapy or to switch to a new treatment option for Lyme disease!

**Elispot-LTT for Borrelia:**

**Material:** 3 x 9 ml CPDA-tubes (yellow cap, kept at room temperature, do not cool or centrifuge)

**Time required for analysis:** 2 days (Results in 5 days)

Indications:

- Diagnosis of chronic Lyme disease
- Diagnosis of acute Lyme disease
- Decision for the length of Lyme disease therapies
- Success control of therapies after Lyme treatment


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# Chlamydia Elispot®-LTT

## Actual cellular activity in the blood against Chlamydia pneumoniae

Chlamydia are intracellular bacteria, which can be the reason for many neurological and/or musculoskeletal symptoms. There are 2 different sub-species of Chlamydia: Chlamydia pneumoniae and Chlymydia trachomatis.

Chlamydia pneumoniae is mainly transmitted by droplet infection from human to human and has a very high degree of penetration. The following diagnosis are described in the literature caused by Chlamydia pneumoniae infections: inflammatory arthritis, tendovaginitis, Multiple Sclerosis, fibromyalgia, Morbus Alzheimer, chronic fatigue syndrome (CFS), asthma, myocarditis, cardiovascular disease, atherosclerosis. An immune suppression is a risk factor for Chlamydia infections and also for other chronic infections like Lyme disease.

A Chlamydia infection leads to a vitalisation of the T-lymphocytes parallel to the humoral immune answer by antibodies (Chlamydia IgG- and Chlamydia IgA-antibodies). The T- cellular immune answer vanishes as soon as the Chlamydia infections show no activity.

The Elispot-LTT for Chlamydia pneumonia shows the actual T-cellular activity of the infection. The Chlamydia pneumoniae-Elispot-LTT is helpful for the diagnosis of Chlamydia infections and can be used for a therapeutical success control during and after the end of a therapy.

**Advantages of the Elispot-LTT for Chlamydia pneumoniae**:

- The result is available within 5 days!
- The use of cell-stabilising CPDA-tubes means a stability of up to 3 days for the analyzed T-cells!
- The right choice of a targeted antibiotic therapy!

**Elispot on Chlamydia pneumoniae:**

**Material:** 3 x 9 ml CPDA-tubes (yellow cap, kept at room temperature, do not cool or centrifuge)
**Time required for analysis:** 2 days (Results in 5 days)

Indications:

- Diagnosis of Chlamydia pneumoniae infection
- Decision for the length of Chlamydia pneumonia therapies
- Success control of therapies after Chlamydia pneumoniae-specific treatment


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# Borrelia CD 57+ cells

## - Determination of chronic activity in the blood regarding Borrelia burgdorferi

A chronic progression (stage III) of a Lyme infection leads to a weakened immune system. This is reflected by the decrease of the CD3-/CD57+ NK-cells in case of chronic Lyme disease. The CD3-/CD57+ cells are a subpopulation of the Natural-Killer-Cells (NK-cells).

A decrease of the CD57+ cells indicates (an untreated) chronical or not sufficiently treated chronic Lyme disease and does not appear in cases of a acute Lyme infection (e.g. “bull’s eye rash“ or “summer flu“ after a tick bite).

The CD57+ cells reflect the degree of activity for a chronic Lyme disease and increase to a normal level after a successful Lyme disease therapy (during or after the end of treatment).

In contrast there is no decrease of CD57+ cells with clinical similar diseases, such as Multiple Sclerosis (MS), Systemic Lupus Erythematosus (SLE) or an Amyotrophic Lateral Sclerosis (ALS). In addition there is no significant fluctuation of CD57+ cells throughout the day.

CD57+ cells are appropriate laboratory parameters in cases where chronic Lyme disease is suspected and for therapy monitoring. These should be measured parallel to the Borrelia Elispot-LTT, which reflects the actual T-cellular activity.

**Advantages of the CD57+ cells determination of Borrelia**:

1. The result is available within 5 days !
2. The use of cell-stabilising Heparin-tubes assures a stability of the measured NK-cells for 3 days !

Successful Lyme-therapies result in a significant improvement of the number of the CD57-cells. The result helps for a fast decision of Lyme disease therapists to extend the duration of a therapy or start a new treatment approach!

**CD3-/CD57+ NK-cells:**

**Material:** 1 x 6 ml Heparin-tube (green cap) + 1 x EDTA-tube (pink cap, both kept at room temperature, do not refrigerate)
**Time required for analysis:** 2 days (Results in 5 days)

Indications:

- Diagnosis of chronic Lyme disease
- Decision for the length of Lyme disease therapies
- Success control of therapies during and after Lyme treatment


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# "Chlamydia infection with Lyme Borreliosis: Symptoms and laboratory discovery"

**Volker Heisig MD PhD (1), Carsten Nicolaus MD PhD (2), Armin Schwarzbach MD PhD (2)**

Many symptoms of chronic Lyme disease, an infection with the spirochete Borrelia burgdorferi, and Chlamdia infection are "unspecific" for the diagnosis of a Borrelia or Chlamydia infection itself. These "overlapping" symptoms are e.g. a reduction or loss of power, fatigue, drowsiness, sensitivity problems like tingling sensations or numbness, headache, joint pain, muscle pain, general weakness of the body, sleeplessness, forgetfulness, concentration problems, heart problems, cough.
We evaluated 50 patients with chronic neurological and musculoskeletal symptoms, which can be caused either by Borrelia burgdorferi or Chlamydia pneumoniae or by a "mixed infection" of both kinds of bacteria.
The laboratory diagnosis of Lyme disease and Chlamydia is based on direct and indirect laboratory tests in general. In each bacterial infection we find B- and T-cellular immune responses, inducing each other.
Therefore we used commercial available laboratory tests for the antibody detection of Borrelia burgdorferi by IgG/IgM-immmunoblot and for the cellular activity the Borrelia Elispot-Lymphocyte Transformation Test (LTT) and the CD57+ cell-count, for the antibody detection of Chlamydia pneumoniae an ELISA technique for Chlamydia pneumoniae-IgG and –IgA-antibodies and for the cellular activity the Chlamydia pneumoniae Elispot-LTT .
The sensitivity of the Borrelia-Elispot-LTT was 76 %, regarding the Borrelia-IgG/IgM-immunoblot 60%, for the CD57 cell-count 56 % and using all 3 tests together 90%.
The sensitivity of the Chlamydia pneumoniae-IgA-antiobody was 60 %, regarding the Chlamydia pneumoniae-IgG-antibody 60 %, for the Chlamydia pneumoniae Elispot-LTT 67 % and using all 3 tests together 78 %.
The laboratory results showed, that 86 % of our patients with chronic Lyme disease were co-infected with Chlamydia pneumoniae with not high-specific, overlapping symptoms of both illnesses.
This study shows, that there is a need to use modern laboratory tests for the humoral and cellular immune-response in order to reach a higher sensitivity in laboratory and an improved specificity for the diagnosis of an infection.
It is very important to know about the bacterial laboratory results of the humoral and cellular level of patients with chronic neurological and musculoskeletal symptoms to do the right antibiotic decision in the treatment of the infectious situation.
More laboratory studies should be done for Chlamydia trachomatis and other tick-borne diseases like Rickettsia, Bartonella, Ehrlichia, Anaplasma or Babesia infections.

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