Unravelling Some of the Complexities of Laboratory Testing in Lyme disease and other infections

Testing for viral and bacterial infections in multi-system diseases

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Laboratory example from practice: Negative EIA but positive Western blot

Laboratory results

Antibodies (Humoral immune system)

	Results	Reference
Borrelia burgdorferi-IgG-EIA	2.8 RU/ml	<16
Borrelia burgdorferi-IgM-EIA	7.6 RU/ml	<16
Borrelia burgdorferi-IgG-Blot	positive Bands: OspC +, p41 +, VlsE-Bg +, VlsE-Ba +	
Borrelia burgdorferi-IgM-Blot	positive Bands: OspC-Bg +, OspC-Bb +, OspC-Ba +, p41 (+)	

Interpretation:

The specific Borrelia burgdorferi-IgG/IgM-antibodies by immunoblot (false-negative EIA !) are an indication for a humoral immune-response against Borrelia burgdorferi in blood.

Armin Schwarzbach M.D. Ph.D.
Doctor for laboratory medicine

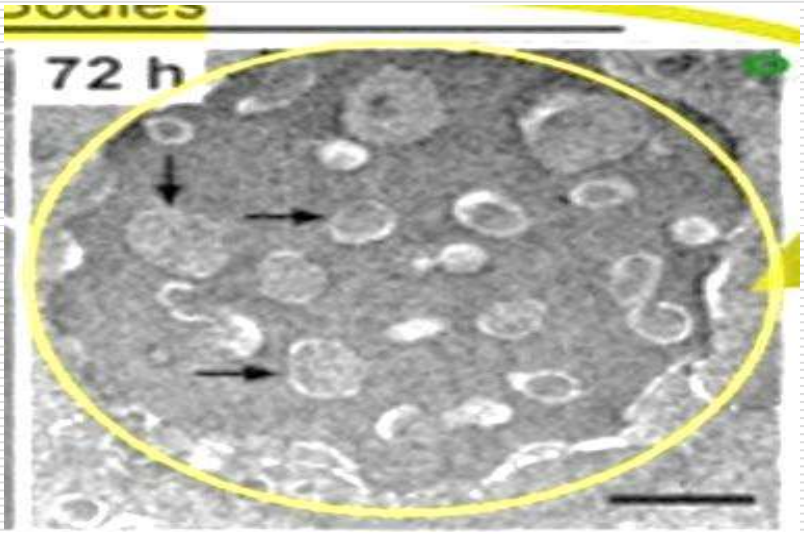
Application of Bayesian decision-making to laboratory testing for Lyme disease and comparison with testing for HIV

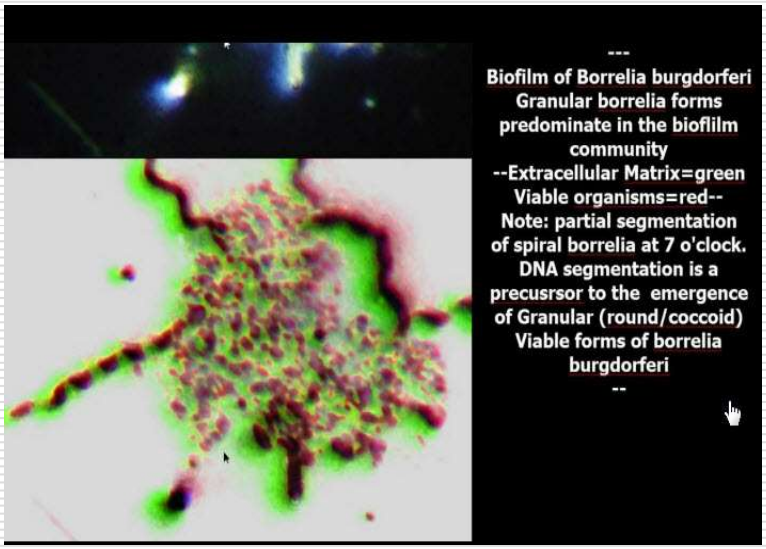
In this study, Bayes' theorem was used to determine the probability of a patient having Lyme disease (LD), given a positive test result obtained using commercial test kits in clinically diagnosed patients. In addition, an algorithm was developed to extend the theorem to the two-tier test methodology. Using a disease prevalence of 5%–75% in samples sent for testing by clinicians, evaluated with a C6 peptide enzyme-linked immunosorbent assay (ELISA), the probability of infection given a positive test ranged from 26.4% when the disease was present in 5% of referrals to 95.3% when disease was present in 75%. When applied in the case of a C6 ELISA followed by a Western blot, the algorithm developed for the two-tier test demonstrated an improvement with the probability of disease given a positive test ranging between 67.2% and 96.6%. Using an algorithm to determine false-positive results, the C6 ELISA generated 73.6% false positives with 5% prevalence and 4.7% false positives with 75% prevalence. Corresponding data for a group of test kits used to diagnose HIV generated false-positive rates from 5.4% down to 0.1% indicating that the LD tests produce up to 46 times more false positives. False-negative test results can also influence patient treatment and outcomes. **The probability of a false-negative test for LD with a single test for early-stage disease was high at 66.8%, increasing to 74.9% for two-tier testing.** With the least sensitive HIV test used in the two-stage test, the false-negative rate was 1.3%, indicating that the LD test generates ~60 times as many false-negative results**.**

For late-stage LD, the two-tier test generated 16.7% false negatives compared with 0.095% false negatives generated by a two-step HIV test, which is over a 170-fold difference. Using clinically representative LD test sensitivities, the two-tier test generated over 500 times more false-negative results than two-stage HIV testing.

Michael J Cook,Basant K PuriInt J Gen Med. 2017; 10: 113–123.
Published online 2017 Apr 10. doi: 10.2147/IJGM.S131909

Round bodies (pleomorphic forms) and biofilm-like colonies of Borrelia burgdorferi in vitro: Antibodies?





...pleomorphic B. burgdorferi should be taken into consideration as being clinically relevant and influence the development of novel diagnostics and treatment protocols...

Merilainen L., Herranen A., *Schwarzbach A.*, Gilbert L. Morphological and biochemical features of B.b. pleomorphic forms, Microbiology, published online ahead of print January 6, 2015, doi: 10/mic.0.000027

Antibodies by Tickplex Basic incl. round bodies www.tezted.com

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Patient: [redacted] F

Date of birth: Date of Reception: Date of Report: Barcode-ID: Physician:

Material: CPDA, Heparin, EDTA, Serum

FINAL REPORT

Analysis		Result	Units	Reference Range
Tickplex Plus				
B.burg. +afz. +gar. IgG		negative 0.620		negative
B.burg. +afz. +gar. IgM		negative 0.470		negative
B.burg. +afz. +gar. + round bod. IgG	+	positive 2.700		negative
B.burg. +afz. +gar. + round bod. IgM		negative 0.790		negative

Testing the other arm of the immune system: T-cells

Using T-cells to show a cellular response against antigens is much more sensitive, and **indicates active infection (in contrast to antibodies, which can remain for months or years long after an infection is gone)**. EliSpot (enzyme-linked immunosorbent spot) technology has long been used in Germany to do exactly this: it quantifies T-cells that secrete signature proteins (such as a given cytokine) against a specific antigen. The Borrelia EliSpot evaluates the number of spot-forming units using a stimulation index (SI) based on IGRA (Interferon Gamma Release Assay).

Humana Press; 3rd ed. 2018 edition (14 July 2018)

The Elispot technique

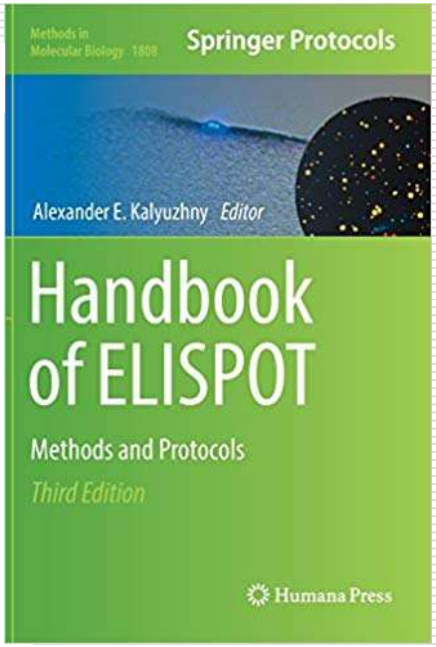
Chapter 1

Unique Strengths of ELISPOT for T Cell Diagnostics

Paul V. Lehmann and Wenji Zhang

Abstract

The T cell system plays an essential role in infections, allergic reactions, tumor and transplant rejection, as well as autoimmune diseases. It does so by the selective engagement of different antigen-specific effector cell lineages that differentially secrete cytokines and other effector molecules. These T cell subsets may or may not have cytolytic activity, can preferentially migrate to different tissues, and display variable capabilities to expand clonally. The quest of T cell immune diagnostics is to understand which specific effector function and T cell lineage is associated with a given clinical outcome, be it positive or adverse. No single assay can measure all of the relevant parameters. In this chapter, we review the unique contributions that ELISPOT assays can make toward understanding T cell-mediated immunity. ELISPOT assays have an unsurpassed sensitivity in detecting low frequency antigen-specific T cells that secrete effector molecules, including granzyme and perforin. They provide robust, highly reproducible data – even by first time users. Because
cytometry, ELISPOT is ideally su
ditions. These include defining (
establishing the fine–specificity
concentrations of the antigen in se
secretory products released by T
because T cells survive ELISPOT



"The quantification of single cell interferon-gamma (IFN-γ) release for assessing cellular immune responses using the Enzyme-linked immunospot (ELISPOT) assay is an invaluable technique in immunology."[1]

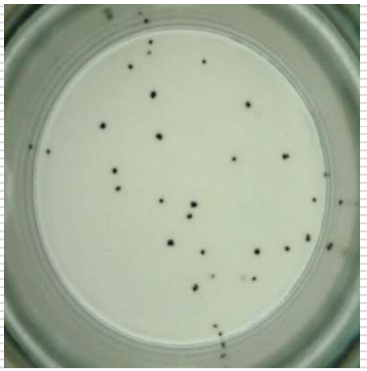
Source: 1 Sedegah M. The Ex Vivo IFN-γ Enzyme-Linked Immunospot (ELISpot) Assay Methods Mol Biol. 2015;1325:197-205; Humana Press; 3rd ed. 2018 edition (14 July 2018)

EliSpot (Interferon-Gamma Release Assay)

Reflects the **current T-cellular activity** of bacteria and viruses

- **T-Cell-Spot/IGRA was approved by the FDA in May 2011 for M. tuberculosis**

- **"… 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…"



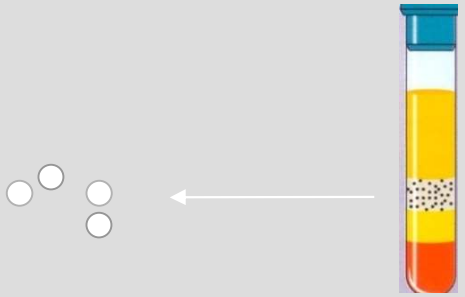
… ELISPOT assays provide robust, highly reproducible data, and can be retested to gain additional information in follow-up assays…

… the tests in the two-assay system (ELISPOT + CD57 cell count) complement each other in the quest to understand T cell-mediated immunity in vivo….

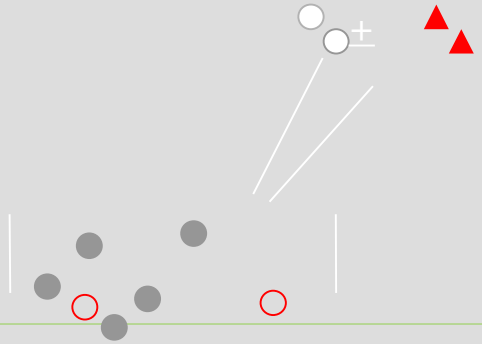
Source: Lehman PV et al.: Unique Strengths of ELISPOT for T Cell Diagnostics in: Kalyuzhny AE. Handbook of ELISPOT: Methods and Protocols, Methods in Molecular Biology, Vol. 792. 2nd Ed: Springer; 2012: 3-23.

Elispot LTT: Methodology (I)

Elispot well coated with monoclonal, cytokine-specific antibodies (IFNy, IL10, etc.)

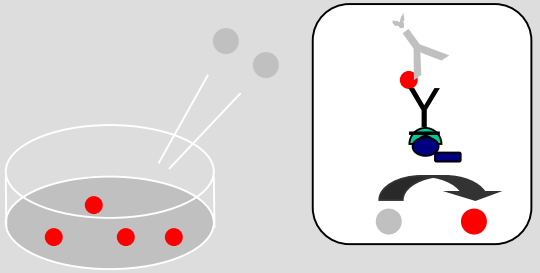


Lymphocytes are isolated



Elispot LTT: Methodology (II)

Add Streptavidin-enzyme conjugate



Add substrate to develop colour

Analysis

Example Borrelia EliSpot laboratory test report

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Page: 1 of 1

Patient:

Date of birth: Date of Reception: Date of Report: Barcode-ID: Physician:

Material: CPDA, Heparin, EDTA, Serum

FINAL REPORT

Analysis		Result	Units	Reference Range
Borrelia burgdorferi Elispot				
Borrelia burgdorferi Fully Antigen	+	15	SI	< 2
Borrelia b. OSP-Mix (OSPA/OSPC/DbpA)	+	16	SI	< 2
Borrelia burgdorferi LFA-1	+	10	SI	< 2

The results of the EliSpot-Tests are an indication for an actual cellular activity against Borrelia burgdorferi.

Explanation of antigens:

- Borrelia burgdorferi Fully Antigen: Borrelia b. B31-reference strain (Borrelia b sensu stricto)
- Borrelia burgorferi Peptide-Mix: OspA from Borrelia b. sensu stricto, Borrelia afzelii, Borrelia garinii + OspC native + DbpA recombinant
- 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. If positive or borderline positive look at: ANA, CCP-antibodies, ANCA.

(Native : cultured antigens/ Recombinant: genetic technology produced)

Report validated by

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page 1

Borrelia antigens in the Borrelia EliSpot

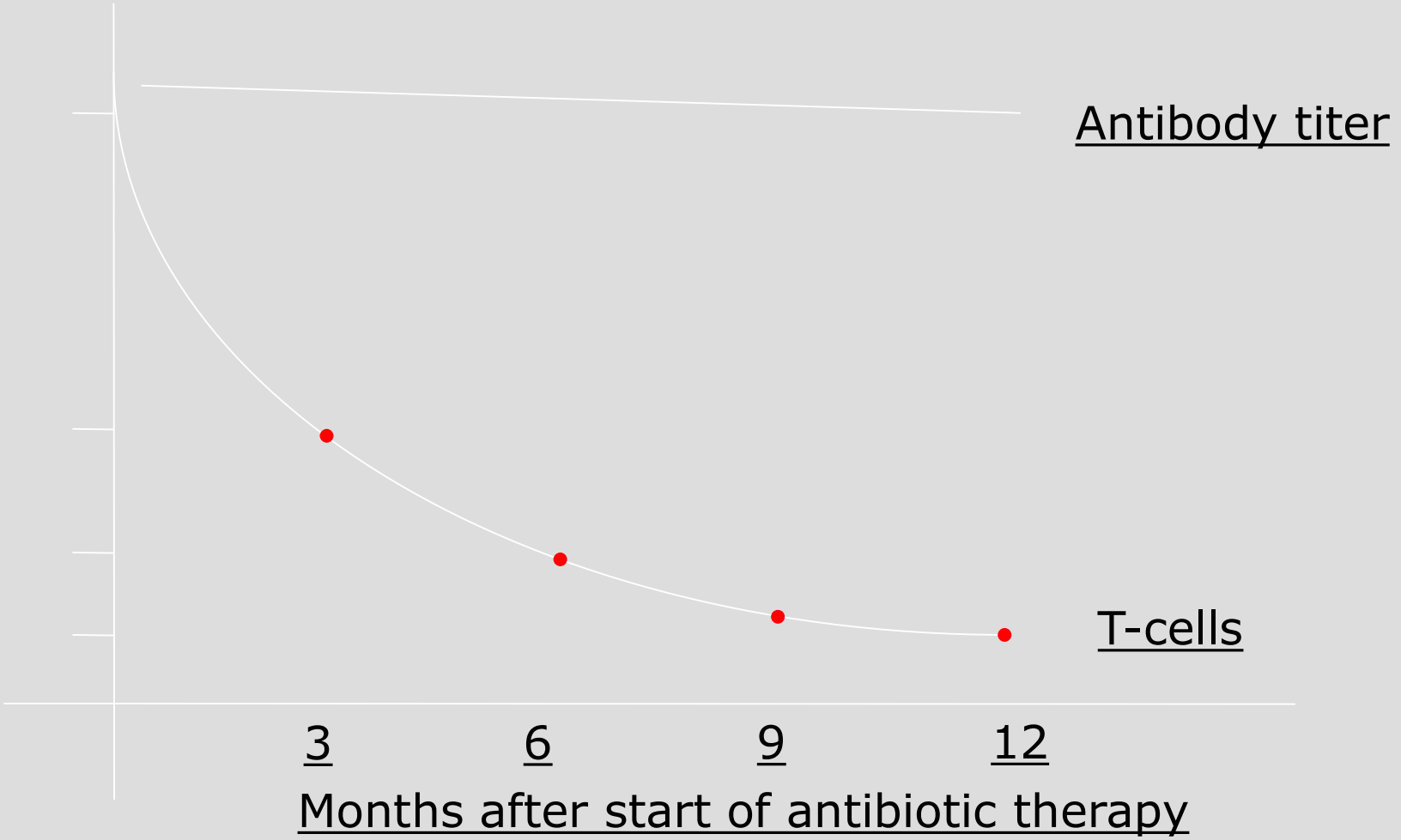
- Borrelia burgdorferi full antigen: Borrelia burgdorferi B31-reference strain (Borrelia burgdorferi sensu stricto)
- Borrelia burgorferi peptide mix: OspA from Borrelia b. sensu stricto, Borrelia afzelii, Borrelia garinii + OspC native + DbpA recombinant
- Borrelia burgdorferi LFA-1 (Lymphocyte Function Antigen 1): Own body protein + Borrelia burgdorferi sensu stricto (shared epitope). Often associated with autoimmune diseases: collagenosis, Rheumatoid Arthritis, vasculitis (ANA, CCP antibodies, ANCA)

Explanation: Native = cultured antigens; Recombinant: produced using genetic technology

ELISA vs. EliSpot in Lyme stage I

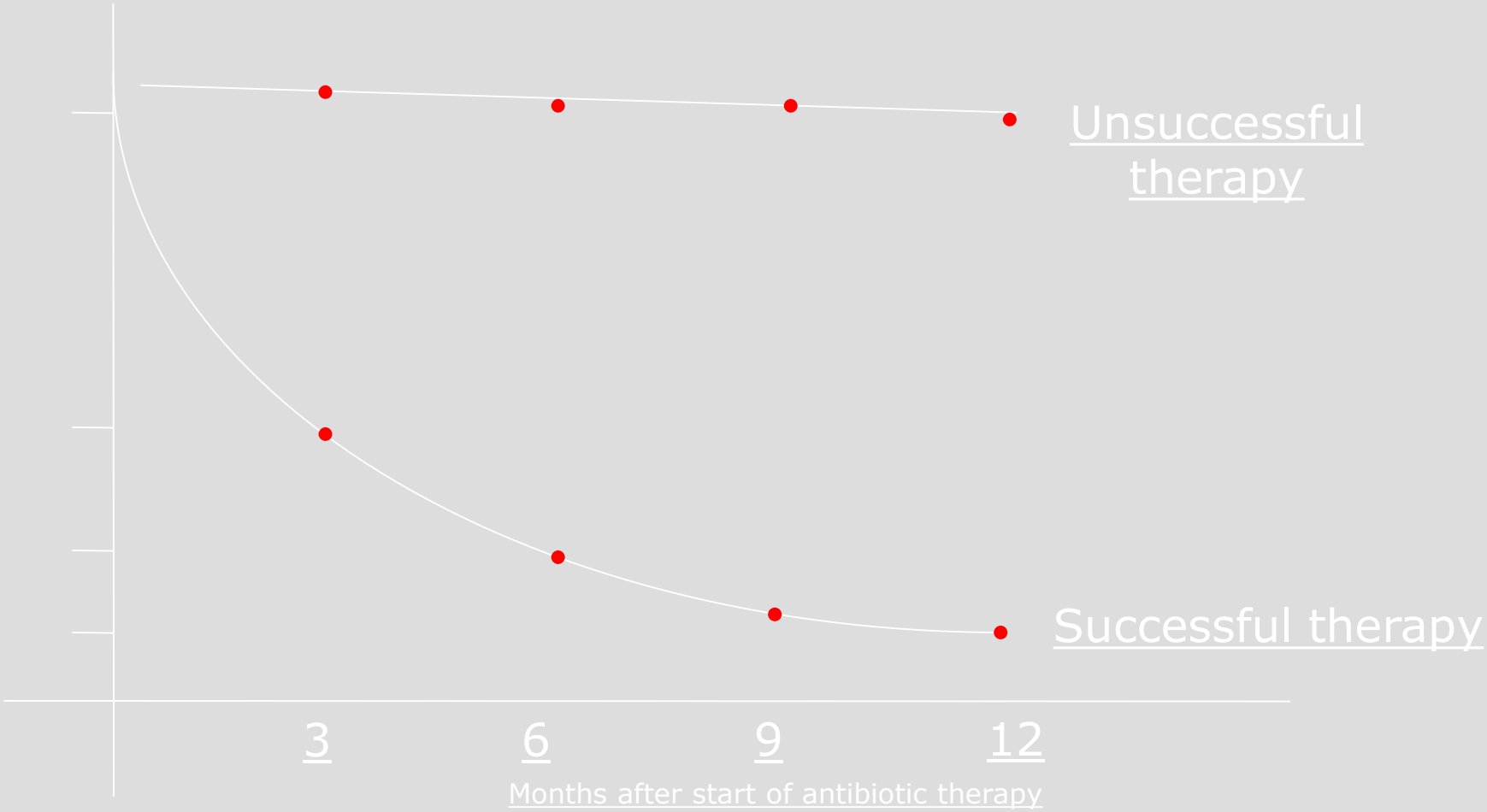
VP13

EliSpot during antibiotics: "Staging" process



VP13 dont you want to align format of legends between 98 and 99?
Vanessa Priss; 03.03.2017

EliSpot during antibiotics: "Staging" process of activity



Borrelia Elispot (IGRA: Interferon-Gamma-Release Assay)

… 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, I, 153-167

… 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

ELISPOT: New T-Cell Test a "Game Changer" for Lyme Disease

… The sensitivity of the ELISPOT is estimated at 84%, and the specificity is 94%...

… ELISPOT assays provide robust, highly reproducible data…

… ELISPOT can be retested to gain additional information in follow-up assays…

… the two-assay system (ELISPOT + CD57-cell count) complement 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

94 % **Specificity of Borrelia-Elispot**

84 % **Sensitivity of Borrelia Elispot**

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EliSpot Test Results Compared to "Standard" Laboratory

Samples (n=31)	Standard Lab C6		
EliSpot	Positive	Negative	Total
Positive	9	17	26
Negative	1	4	5
Total	10	21	31

EliSpot versus C6	
EliSpot Positive	84%
Standard Lab Positive	32%
Ratio	38%
"Standard" Lab Missed Cases	62%

Samples (n=13)	Standard Lab Western Blot		
EliSpot	Positive	Negative	Total
Positive	2	9	11
Negative	0	2	2
Total	2	11	13

EliSpot versus WB	
EliSpot Positive	85%
Standard Lab Positive	15%
Ratio	18%
"Standard" Lab Missed Cases	82%

Samples (n=14)	Standard Lab Two-Tier test		
EliSpot	Positive	Negative	Total
Positive	2	10	12
Negative	0	2	2
Total	2	12	14

EliSpot versus Two-Tier	
EliSpot Positive	86%
Standard Lab Positive	14%
Ratio	17%
"Standard" Lab Missed Cases	83%

Comparing Lyme Testing

Key terms:
ELISA – Enzyme Linked Immuno Sorbent Assay
Specificity - True negative rate
Sensitivity - True positive rate

Borrelia Testing Method	**Summary**	**Testing accuracy**	**Clinical application**	
ELISA IgG / IgM	Tests B-cell imunmune response against Borrelia	Poor sensitivity Poor specificity	Screening for Borrelia antibodies	
ELISA C6	Tests part of B-cell immune response against Borrelia	Poor sensitivity Poor specificity	Alternative partly screening for Borrelia antibodies	
IgG/IgM Seraspot	Tests B-cell immune response (modern Westernblot)	Poor sensitivity High specificity	Confirmation test for Borrelia antibodies (modern Westernblot)	
Tickplex Basic	**Tests B-cell immune response including "roundbodies"**	**High sensitivity High specificity**	**Screening for Borrelia antibodies including "roundbodies"**	**Recommended**
Western blot	Tests B-cell immune response	Poor sensitivity High specificity	Confirmation test for Borrelia antibodies	
PCR	Assesses presence of DNA of Borrelia in blood	Poor sensitivity High specificity	Reflects current presence of Borrelia	
Elispot	**Tests T-cell activity against Borrelia**	**High sensitivity High specificity**	**Reflects current activity last 6-8 weeks**	**Recommended**
Borrelia culture	Asseses presence of Borrelia in blood	Poor sensitivity High specifictiy	Reflects current presence of Borrelia	

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GERMANY

Augsburg, 12 September 2016

September 2016

New at ArminLabs: The Borrelia miyamotoi EliSpot

Dear Sir or Madam,

A special form of an infection with Borrelia is the infection with the spirochete Borrelia miyamotoi, which was detected in Japan in 1995. However, the infection occurs increasingly worldwide. In the past years, more and more Borrelia miyamotoi have been found in ticks (England, Germany, USA, amongst others) and related diseases have been documented at the same time.

Borrelia miyamotoi is the human pathogen of relapsing fever. An infection with Borrelia miyamotoi can cause the following symptoms: relapsing fever, chills, headaches, joint and muscle pain, fatigue, nausea/vomiting, sometimes conjunctivitis, and cough at an incubation period of 5-15 days. Typically, the symptoms appear for 2-9 days. They can recur in periods of different lengths or even persist. Contrary to an infection with Borrelia burgdoferi, an erythema migrans does typically not appear.

Atypical symptoms of an infection with Borrelia miyamotoi are as follows: abdominal pain, diarrhoea, hepatitis, myocarditis, arrhythmia, pulmonary symptoms (like ARDS), disseminated intravascular coagulation (DIC), facial nerve paralysis, hearing loss, iritis, polyneuropathies or neuropsychiatric symptoms.

Laboratory diagnostics via detection of antibodies is not available in routine laboratories at the moment. As of now, the analysis of the cellular activity against Borrelia miyamotoi is performed at ArminLabs by means of the certified EliSpot method.

The EliSpot (Enzyme-Linked ImmunoSpot) belongs to the group of the interferon gamma release assays (IGRA). The following EliSpot tests have been available at ArminLabs so far: Borrelia burgdorferi, Ehrlichia/Anaplasma, Chlamydia pneumoniae/trachomatis, Yersinia, EBV, CMV, Herpes Simplex Virus ½. As of now, ArminLabs has extended its EliSpot analytics and is able to offer the Borrelia miyamotoi EliSpot.

Please write on the order form by hand if the Borrelia miyamotoi EliSpot is not listed on your Order Form.

Borrelia miyamotoi EliSpot

Material: 1x CPDA blood tube

The costs for the Borrelia miyamotoi EliSpot are the same as for the Chlamydia pneumoniae EliSpot and can be found on your Order Form.

Yours sincerely,

The ArminLabsTeam

EliSpot is available for:

- Borrelia burgdorferi (3 subspecies: B.b. sensu stricto + B.b. garinii + B.b. afzelii)
- Borrelia myamotoi
- Bartonella
- Babesia
- Chlamydia pneumoniae
- Chlamydia trachomatis
- Mycoplasma pneumoniae
- Ehrlichia/Anaplasma
- Yersinia enterocolitica
- Epstein Barr Virus (EBV)
- Cytomegalovirus (CMV)
- Herpes Simplex Virus 1 / 2
- Varicella Zoster Virus (VZV)
- HHV-6, HHV-7

Also:
Candida
Aspergillus niger

Brand-New available:

Corona Virus EliSpot
current infection?
cellular immunity?

References on the Elispot: examples

Navarrete MA ELISpot and DC-ELISpot Assay to Measure Frequency of Antigen-Specific IFNγ-Secreting Cells, in Hnasko R (Editor), Elisa Methods and Protocols 2015.

Navarrete MA, Bertinetti-Lapatki C, Michelfelder I et al (2013) Usage of standardized antigen-presenting cells improves ELISpot performance for complex protein antigens. J Immunol Methods 391:146–153

Czerkinsky CC, Nilsson LA, Nygren H et al (1983) A solid-phase enzyme-linked immunospot (ELISPOT) assay for enumeration of specifi c antibody-secreting cells. J Immunol Methods 65:109–121

Keilholz U, Weber J, Finke JH et al (2002) Immunologic monitoring of cancer vaccine therapy: results of a workshop sponsored by the Society for Biological Therapy. J Immunother 25:97–138
Scheibenbogen C, Lee KH, Mayer S et al (1997) A sensitive ELISPOT assay for detection of CD8+ T lymphocytes specifi c for HLA class I-binding peptide epitopes derived from infl uenza proteins in the blood of healthy donors and melanoma patients. Clin Cancer Res 3:221–226

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