ME, MS, Fibromyalgia, Alzheimer's, Parkinsonism, Autism... Tailored Testing Protocols Holiday Inn Regents Park, 15th November 2015, London, UK

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Aims of the presentation today

- Give an overview of the infections/viruses that it is important to consider testing for in chronic conditions such as ME, MS, RA, Fibromyalgia, Alzheimer's, Parkinsonism and Autism
- Offer tailored testing protocols for these conditions beyond Lyme Disease



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Overview of the different immune components and infections/viruses tested for that may be relevant

- CD3-/CD57+ T-Lymphocytes
- Elispot
- Seraspot
- Chlamydia pneumoniae
- Mycoplasma
- Ehrlichia/Anaplasma
- Bartonella
- Babesia
- Rikettsia
- Epstein Barr Virus (EBV)
- Cytomegalovirus (CMV)
- HSV1/2, HHV6
- Coxsackie virus



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Aims of immune-competent cells



CD57+NK cells

- Destruction of antigen-antibody

complexes

EliSpot (T-cells):

- Borrelia burgdorferi
- Chlamydia pneumoniae
- Anaplasma/Ehrlichia
- Yersinia, EBV, CMV
- Mycoplasma
- Varicella Zoster Virus
- HSV 1&2
- Bartonella
- Babesia

Antibodies (B cells: IgG/IgA/IgM)

- Borrelia burgdorferi
- Chlamydia, Mycoplasma
- Ehrlichia/Anaplasma, Bartonella, Babesia, Rickettsia, Coxsackie Virus...



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T-Cell

CD3-/CD57+ T-Lymphocytes

- 1. Subpopulation of the CD56+ NK cells
- Reduction indicates chronic activity of Lyme disease (symptoms > 1 year)
- 3. Reduction in untreated and inadequately treated Lyme disease
- After the end of therapy for chronic Lyme disease: their normalization represents therapeutic success
- Not highly specific: Also low in other bacterial infections, esp. Chlamydia pneumonia and Mycoplasma pneumoniae

CD3-/CD57+ T-Lymphocytes

Reference range (mean/range)

Lyme patient: Healthy: 46 /ul / 8 - 160 /ul 164 /ul / 60 - 354 /ul

Source: J.J.Burrascano JR., MD, R. Stricker, MD, 2006 ILADS, Crowne Plaza Hotel, Center City Philadelphia



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Reflecting the actual T-cellular activity: The EliSpot Established



The established Borrelia EliSpot (T-Cell-Spot)

 reflects the actual activity of chronic and recent infections
sensitivity is estimated at 84%, and the specificity is 94%
is approved by the FDA in May 2011 for M. tuberculosis
Available for: Borrelia burgdorferi, Ehrlichia, Chlamydia pneumoniae, Chlamydia trachomatis, Yersinia, EBV, CMV
covers the following Antigens for Borrelia subspecies:

Borrelia burgdorferi Fully Antigen: Borrelia b. B31-reference strain (Borrelia b. sensu stricto)

Borrelia b. Peptide-Mix: OspA from Borrelia b. sensu stricto, Borrelia afzelii, Borrelia garinii + OspC native + DbpA recombinant

Borrelia b. LFA-1 (Lymphocyte Function Antigen 1): Own body protein + Borrelia burgdorferi sensu stricto (shared epitope)





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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



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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 tests in 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-LTT

84 % Sensitivity of Borrelia Elispot-LTT



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Currently the EliSpot is available for:

- Borrelia burgdorferi (3 subspecies)
- Chlamydia pneumoniae
- Chlamydia trachomatis
- Ehrlichia
- Yersinia species
- Epstein Barr Virus (EBV)
- Cytomegalovirus (CMV)
- Herpes Simplex Virus ¹/₂
- Mycoplasma
- Bartonella
- Babesia
- Varicella Zoster Virus







Introducing the modern MicroArray: The SeraSpot®



Replaces the Immunoblot at ArminLabs as it

offers a better standardisation and more controls

- combines established ELISA-technique with improved sensitivity of MicroArray analytics
- covers the following Antigens for subspecies:

VlsE(B.b. afzelii), p39(B.b. afzelii), p58(B.b. garinii), p100 (B.b. afzelii), OspC (B.b. afzelii + B.b. garinii + B.b. sensu stricto), DbpA (B.b. afzelii + B.b. garinii + B.b. sensu stricto)



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SeraSpot MicroArray

Microplates are coated with several antigen spots ...processed like an ELISA ...read in a special MicroSpot Reader ...interpreted using multiplex software ...tests for 3 different European Borrelia subspecies: B.b.s.s. + B.b. garinii + B.b. afzelii (i.e., ArminLabs tests for all 3 European subspecies; IGeneX and some other labs do not)





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Borrelia burgdorferi antigens in test systems Combination of specific Borrelia markers

Recombinant antigens

ArminLabs uses these; IGeneX and some other labs do not: Higher sensitivity than native antigens that are not expressed in bacterial cultures or expressed only in insufficient amounts, e.g. VISE has over 99% specificity

Native antigens: ArminLabs uses these, too

High specificity but lower sensitivity than recombinant antigens

- 1. Isolated natively, e.g. OspC
- 2. Cut from a Western blot membrane, e.g. BmpA

Combination of recombinant antigens + native antigens should be used (ArminLabs does; IGeneX and some other labs do not)



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It is possible to quantify the SeraSpot MicroArray, but not the Immunoblot





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Basic diagnostic tests for chronic Lyme Borreliosis

- 1. Borrelia IgM and IgG antibodies by the Microarry (SeraSpot) technique, incl. VIsE: Sensitivity around 60%, specificity around 99%
- 2. Borrelia Elispot (LTT): <u>current</u> Borrelia activity: Sensitivity around 84%, specificity 82-100%
- 3. CD3-/CD57+ cells: <u>chronic</u> Borrelia activity: Sensitivity around 70%, specificity ? (i.e. also low in Chlamydia or Mycoplasma infections)

All 3 tests together: >90% sensitivity+99% specificity

Monitoring 1 month after the end of therapies to verify whether the therapy has been successful or not:

Laboratory STAGING process



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Lyme Borreliosis: The great imitator

20-30% of autistic disorders can be caused by Borrelia and 58% by Mycoplasma (Bransfield et al.: Med Hypotheses.2008; 70(5):967-74)

Multiple Sclerosis, myelopathies, polyneuropathies, brain tumor, encephalopathy. (Neurosurgery.1992May;30(5): 769-73)

Can cause meningitis, encephalitis, neuritis, mania, depression, schizophrenia, anorexia, dementia. (Am J Psychiatry. 1994 Nov;151(11):1571-83) "90% of chronic fatigue patients are Lyme positive." (Informal study by American Lyme Disease Alliance at www.lymealliance.org)

"Most fibromyalgia patients are Lyme positive." (Rheum Dis Clin North Am. 1998 May;24 (2):323-51 & report of Lida Mattman,M.D.)

"Borrelia can cause Parkinsonism" (Arch.of Path.& Lab.Med.127(9):1204-6)

Pure Lyme dementia exists and has a good outcome after antibiotics. It is advisible to do Lyme serology in demented patients. (Blank et al.: Journal of Alzheimer's disease, Volume 4/2014, 1087-1093)



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Chlamydia pneumoniae

<u>Bacteria</u>: Chlamydophila pneumoniae (gram-negative, intracellular); cystic and aberrant forms, biofilms

<u>Vector/transmission</u>: airborne infection, human to human, ticks? Or reactivated in Lyme disease (horses, koalas, frogs are infected), aerogen transmission (cough) from horses to horse-riders?

<u>Symptoms</u>: cough, slight throat pain, hoarseness, sinusitis, atypical pneumonia, meningoencephalitis, bronchiolitis obliterans, myocarditis, Guillain-Barre Syndrome; arthritis, tendovaginitis (4-6 weeks)

<u>Associations</u>: Alzheimer's, Multiple Sclerosis, depression, Fibromyalgia, ME/CFS, heart attacks, acute ischemic stroke (AIS), arteriosclerosis, autism, Parkinsonism, Rheumatoid Arthritis, etc.



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Mycoplasma infection



Bacteria: Mycoplasma pneumoniae/fermentans (gram-positive, intracellular)

Transmission: airborne infection, human to human, ticks?

<u>Symptoms</u>: Fatigue (100%), fever, joint pain, joint swelling, muscle pain, headache, insomnia, anxiety, emotional volatility, lack of concentration, memory loss, autism

ME, "Gulf War I syndrome"



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Source: CDC

Bacteria: Ehrlichia chaffeensis, Anaplasma phagocytophilum (gramnegative, obligatory intracellular in granulocytes or monocytes)

Human Granulocytic Ehrlichiosis (HGE) or

Human Monocytic Ehrlichiosis (HME)

Vector: Ixodes ricinus

Spectrum of hosts: game (e.g. deer), domestic animals, humans

<u>Symptoms</u> (incubation time: days up to 4 weeks): rapid onset of beginning illness with fever, headache and prostration, headaches are "sharp, knife-like and often located behind the eyes", muscle pain, not joint pain, neurological symptoms, psychiatric symptoms, rarely: diffuse vasculitic rash, including palms and soles (<10%)



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Bartonella

 <u>Bacteria</u>: B. henselae (cat scratch disease), B. quintana (Trench fever, bacillary angiomatosis), B. bacilliformis (Carrion's disease/Oroya fever), 5 other subspecies known to be pathogens for humans (gram-negative, facultative intracellular bacterium in endothelial cells/erythrocytes)

<u>Vector/transmission</u>: cat-scratch surface wounds, Ixodes ricinus (Germany/Europe: up to 40% of ticks are contaminated), fleas, mosquitoes, sand flies

<u>Symptoms (incubation time 3 - 38 days): tiredness (100%)</u>, headache (80%), muscle twitches, tremors, seizures, fever in the mornings (30%, in spates of up to 6 weeks, otherwise 1 - 3 weeks), swollen lymph nodes, arthralgia (often), myalgia, insomnia, depression, agitation, severe mood swings, lack of concentration and alertness, dizziness, anxiety, outbursts, antisocial behaviour, restlessness, gastritis, intestinal symptoms, sore soles (especially in the morning), tender subcutaneous nodules along the extremities, occasional lymphadenopathy and light sweats, striae; <u>Complications</u>: endocarditis, retinitis, epilepsy, aseptic meningitis, hepatosplenomegaly



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Bartonella striae





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Babesia

Bacteria: Babesia microti, Babesia divergens, Babesia duncani

<u>Vector/transmission</u>: Ixodes ricinus, Dermacentor reticulatus, blood transfusions

Hosts: game (e.g. deer), domestic animals, humans

Symptoms (incubation time 5 days – 9 weeks): Rapid onset of beginning illness with severe fever, headache (can be severe/dull, global, involves the whole head, described like the head is in a vice), sweats (usually at night, but can be day-sweats as well), fatigue (worse with exercise), "air-hunger", need to sigh and take a deep breath, dry cough without apparent reason, stiffness of neck, nausea, diminished appetite, tiredness, feeling of weakness, permanent exhaustion even worse during stress, dizziness, haemolytic anaemia, hemoglobinuria, haemangiomata, (seldom) hepatosplenomegaly, muscle pain, dizziness, mental dullness and slowing of reactions and responses, hypercoagualability, stomach pain, emotional lability, "mental dullness", kidney problems, dyspnoea, influenza-like symptoms (could be lethal)

<u>Risk factors</u>: Splenectomy, HIV, organ transplantation, blood transfusions



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Rickettsia

Bacteria: Rickettsia conorii (Boutonneuse Fever), R. rickettsia (RMSF), R. helvetica, R. slovaca, R. prowazekii (gramnegative, obligate intracellular in endothelial cells)

<u>Vector/hosts:</u> rodent, dogs, humans, Ixodes ricinus, Dermacentor reticulatus

<u>Symptoms (incubation period 5 - 7 days): fever, nausea,</u> vomiting, severe headache, lymphadenitis, exanthema

<u>Complications</u> (app. 13%): peri-/myocarditis, kidney insufficiency, pneumonia, encephalitis, gastrointestinal bleedings, anaemia, hepatitis, myalgia, meningitis



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Epstein Barr Virus (EBV)

<u>Virus:</u> Epstein Barr Virus (obligate intracellular), double stranded DNA virus, one of the Herpesviruses, "Mononucleosis"

<u>Transmission</u>: "kissing disease", saliva, drinking from the same glass, toothbrush, blood, sex, blood-transfusion, organ transplantation

<u>Symptoms (incubation period several weeks)</u>: fatique, fever, flulike symptoms, nausea, loss of appetite, lymphadenitis (swollen lymph nodes in the neck), rash, sore throat, weakness, sore muscles

<u>Complications</u>: enlarged spleen, swollen liver, association with Non-Hodgkin Lymphoma



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Cytomegalovirus (CMV)

<u>Virus:</u> Cytomegalovirus (obligate intracellular), double-stranded DNA virus, one of the Herpes viruses

<u>Transmission</u>: body fluids (urine, saliva, breast milk, sexual transmission), organ transplantation, blood transfusion

<u>Symptoms</u> (incubation period several weeks): fatique, fever, flulike symptoms, lymphadenitis (swollen cervical lymph nodes), sore throat, splenomegaly

<u>Complications</u>: congenital infection with hearing loss, vision loss, seizures, mental disabilities, lack of coordination; immune suppressed patients: hepatitis, colitis, retinitis, pneumonitis, esophagitis, polyradiculopathy, transverse myelitis, subacute encephalitis; arterial hypertension, artheroscleroris, aortic aneurysms; association with Non-Hodgkin Lymphoma



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Herpes Simplex Virus 1 / 2 (HSV 1 / 2)

<u>Virus:</u> Herpes Simplex Virus (Human Herpes Virus HHV 1 / 2) (obligate intracellular), double-stranded DNA virus, one of the Herpes viruses

Transmission: Saliva, sharing drinks, sexually transmitted

<u>Symptoms</u> (incubation time 2-20 days): Watery blisters on the skin or mucous membranes of the mouth, lips, genitals, anus, flu-like symptoms (fever, muscle aches, swollen lymph nodes, problems urinating, herpes keratitis (pain, light sensitivity, discharge))

<u>Complications</u>: Multiple Sclerosis (neurovirulent), loss of vision, encephalitis, latent infection; reactivation by organ transplantation or HIV: encephalitis, pneumonitis, bone marrow suppression



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Human Herpes Virus 6 (HHV6)

<u>Virus:</u> Human Herpes Virus 6 (obligate intracellular), doublestranded DNA virus, one of the Herpes viruses

<u>Transmission</u>: Saliva, latency in salivary glands, haematopoetic (blood-building) system

<u>Symptoms</u>: Exanthema subitum (roseola infantum, sixth disease) with high temperature followed by a rash

<u>Complications</u>: Multiple Sclerosis (neurovirulent), cofactor in CFS, fibromyalgia, AIDS, optic neuritis, cancer, temporal lobe epilepsy, Hashimoto thyroiditis, liver dysfunction, liver failure; reactivation by organ transplantation: encephalitis, pneumonitis, bone marrow suppression,



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Coxsackie Virus

<u>Virus:</u> Coxsackie Virus (obligate intracellular), belongs to Picornaviridae/ enterovirus family, is a single-stranded RNA virus divided into group A and group B

<u>Transmission</u>: fecal-oral contamination, droplets, body fluids, utensils, toys, diaper-changing table

Symptoms: Group A: Herpangina, AHC (acute hemorrhagic conjunctivitis, HFM (hand-foot-and-mouth disease), Group B: myocarditis, pericarditis, pleurodynia, hepatitis; Group A and B: fever, rashes, sore throat, diahorrea, cough, fatigue, conjunctivitis, loss of appetite, headache, night sweats, aseptic meningitis

<u>Complications</u>: CNS disease similar to poliomyelitis, systemic neonatal disease, IDDM (insulin-dependent diabetes mellitus), Group A: generalized myositis with flaccid paralysis, Group B: focal muscle injury, degeneration of neuronal tissue with spastic paralysis



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Tailored testing protocols

- Fibromyalgia/Rheumatoid Arthritis
- Multiple Schlerosis
- 🗆 ME
- Dementia
- Yersinia species
- Epstein Barr Virus (EBV)
- Cytomegalovirus (CMV)





Fibromyalgia

"Most fibromyalgia patients are Lyme positive." (Rheum Dis Clin North Am. 1998 May;24 (2):323-51 & report of Lida Mattman,M.D.)



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The oldest patient with "Fibromyalgia" (5,300 years ago): "Iceman" Ötzi





Ötzi's enemies: Ticks! "Zink's team found almost two-thirds of the genome of Borrelia burgdorferi, a bacterium that causes Lyme disease. Zink speculates that tattoos on the iceman's spine and ankles and behind his right knee could have been an attempt to treat the joint pain that occurs when the condition goes untreated."



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Fibromyalgia/Rheumatoid Arthritis: Laboratory tests suggested

- 1. Borrelia SeraSpot + Borrelia EliSpot + CD57-cells
- Chlamydia pneumoniae IgG/IgA antibodies + Chlamydia pneumoniae EliSpot
- Chlamydia trachomatis IgG/IgA-antibodies + Chlamydia trachomatis EliSpot
- 4. Mycoplasma pneumoniae IgG/IgA antibodies + EliSpot
- Ehrlichia/Anaplasma IgG/IgM antibodies + Ehrlichia/Anaplasma EliSpot
- 6. Rickettsia IgG/IgM antibodies
- 7. Yersinia IgG/IgA antibodies + Yersinia EliSpot
- 8. Coxsackie Virus IgG/IgA antibodies
- 9. HHV6 IgG/IgM antibodies
- 10.ANA (antinuclear antibodies) + CCP (cyclic citrullinated peptide) antibodies





Multiple Sclerosis, myelopathies, polyneuropathies, brain tumor, encephalopathy. (Neurosurgery.1992May;30(5): 769-73)

1986 (USA): Relapsing fever/Lyme disease – Multiple sclerosis. Medical Hypotheses, volume 21, issue 3, pages 335-343

2000 (Poland): Lyme borreliosis and Multiple sclerosis: Any Connection? A Seroepidemic study. Ann Agric Environ Med. issue 7, 141-143

2001 (Norway): Association between Multiple sclerosis and Cystic Structures in Cerebrospinal Fluid. Infect 29:315

2004 (Switzerland): Chronic Lyme borreliosis at the root of Multiple sclerosis – is a cure with antibiotics attainable?



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Case report: Initial diagnosis of Multiple Sclerosis

43-year-old patient, suffering since May 2005 from

- Persistent paraesthesia of the left leg
- 80% blindness of the left eye
- Progressive myalgia
- Recurrent dizziness
- Substantial loss of stamina (high risk of occupational disability)

Diagnosis by neurologists: MS

Spinal fluid and laboratory tests were negative (no Borrelia antibody AI, no chronic IgG synthesis in the form of oligoclonal bands in the spinal fluid)

Borrelia IgM/IgGELISA and Immunoblot were tested several times and found to be negative

Therapy: Corticosteroids increased her symptoms + she suffered bad side-effects from the corticosteroids



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Case report: Initial findings on 26th Oct. 2005 before antibiotic treatment

			Armin Speciali	Schwarzbach MD PhD ist for laboratory medicine	
ArminLabs GmbH - Zirbelstr.58 3rd floor, 86154	Augsbu	urg, Germany		Page: 1 or 1	
Patient:					
Date of birth: Date of Reception:	Date	e of Report:	Barcode-ID:	Physician:	
Material: CPDA, Heparin, EDTA, Sei	um				
A h t		Den la		D-6	
Analysis		Result	Units	Reference Range	
Borrelia burgdorferi antibodies (Westernb	lot)				
Borrelia-Blot-IgG-antibodies		negative		negative	
Borrelia-Blot-IgM-antibodies		negative		negative	
The specific Borrelia burgdorferi-Ig Borrelia burgdorferi. Please look at	G/IgM-a the resu	ntibodies are no in ults of the Borrelia-f	dication for a hum EliSpot and the CDS	oral immune response against 7+cell-count.	
CD 57 Flow Cytometry					
CD 57 + NK-cells (%)		28	%	2-77	
CD 57 + NK-cells (absolute)	-	40	/ul	100-360	
The CD57-cell-count indicates a o burgdorferi.	hronic	immune-suppressiv	ve situation, which	n can be caused by Borrelia	
Borrelia burgdorferi Elispot					
Borrelia burgdorferi Fully Antigen	+	30	SI	< 2	
Borrelia b. OSP-Mix (OSPA/OSPC/DbpA)	+	5	SI	< 2	
Borrelia burgdorferi LFA-1	+	8	SI	< 2	
The results of the EliSpot-Tes	ts are ar	n indication for an a ArminLabs GmbH	octual cellular activit	y against Borrelia burgdorferi.	
	CEO: A	Armin Schwarzbach M	D PhD		

Zirbelstraße 58, 3rd floor 86154 Augsburg Germany Phone: 0049 821 218 2879 www.arminlabs.com e-mail: service@arminlabs.com Amtsgericht Augsburg HRB 29350

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Case report: Borrelia EliSpot 8 weeks after Ceftriaxone IV treatment

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ArminLabs GmbH - Zirbelstr.58 3rd floor, 86154	Augsburg, Germany		r oget i of i
Patient:			
Date of birth: Date of Pereption:	Date of Peport:	Barcode-ID:	Physician
Date of birth. Date of Reception.	Date of Report.	Barcode-ID.	Physician.
Material: CPDA, Heparin, EDTA, Seru	m		
Analysis	Result	Units	Reference Range
Borrelia burgdorferi antibodies (Westernblo	>t)		
Borrelia-Blot-IgG-antibodies	negative		negative
Borrelia-Blot-IgM-antibodies	negative		negative
The specific Borrelia burgdorferi-IgG, Borrelia burgdorferi. Please look at th	/lgM-antibodies are no ne results of the Borrelia	indication for a hun a-EliSpot and the CD	noral immune response against 57+cell-count.
CD 57 Flow Cytometry			
CD 57 + NK-cells (%)	28	%	2-77
CD 57 + NK-cells (absolute)	180	/ul	100-360
The CD57-cell-count indicates no chro	onic immune-suppressiv	e situation.	
Borrelia burodorferi Elispot			
Borrelia burgdorferi Fully Antigen	0	SI	< 2
Borrelia b. OSP-Mix (OSPA/OSPC/DbpA)	0	SI	< 2
Borrelia burgdorferi LFA-1	0	SI	< 2
The results of the EliSpot-Tests	are no indication for ar	a actual cellular activ	ity against Borrelia burgdorferi.

By Jan 23rd, 2006, the patient was clinically symptom-free and able to work

ArminLabs GmbH CEO: Armin Schwarzbach MD PhD Zirbelstraße 58, 3rd floor 86154 Augsburg Germany Phone: 0049 821 218 2879 www.arminlabs.com e-mail: service@varminlabs.com

Correct diagnosis: Chronic Neuroborreliosis with Multiple Sclerosis-like symptoms



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Multiple Sclerosis: Laboratory tests suggested

- 1. Borrelia SeraSpot + Borrelia EliSpot + CD57-cells
- Chlamydia pneumonia IgG/IgA antibodies + Chlamydia pneumoniae EliSpot
- 3. Mycoplasma pneumoniae IgG/IgA antibodies + EliSpot
- 4. Bartonella IgG/IgM antibodies + EliSpot
- 5. Coxsackie Virus IgG/IgA antibodies
- 6. EBV EliSpot
- 7. CMV EliSpot
- 8. HHV6 IgG/IgM antibodies





"90% of chronic fatigue patients are Lyme positive." (Informal study by American Lyme Disease Alliance at www.lymealliance.org)



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- 1. Borrelia-SeraSpot + Borrelia-EliSpot + CD57-cells
- Chlamydia pneumoniae IgG/IgA antibodies + Chlamydia pneumoniae EliSpot
- 3. Mycoplasma pneumoniae IgG/IgA antibodies + EliSpot
- 4. Bartonella IgG/IgM antibodies + EliSpot
- 5. Parvovirus B19 IgG/IgM antibodies
- 6. Coxsackie Virus IgG/IgA antibodies
- 7. EBV EliSpot
- 8. CMV EliSpot
- 9. Herpes Simplex Virus 1 / 2 IgG/IgA/IgM antibodies + Herpes simplex Virus EliSpot

10.HHV 6-IgG/IgM antibodies





Alzheimer's Disease

"Pure Lyme dementia exists and has a good outcome after antibiotics. It is advisible to do Lyme serology in demented patients.2 (Blank et al.: Journal of Alzheimer's disease, Volume 4/2014, 1087-1093)



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Alzheimers / Dementia

- 1. Borrelia SeraSpot + Borrelia-EliSpot + CD57 cells
- 2. Chlamydia pneumoniae IgG/IgA antibodies + Chlamydia pneumoniae EliSpot
- 3. Mycoplasma pneumoniae IgG/IgA antibodies + EliSpot
- 4. Coxsackie Virus IgG/IgA antibodies
- Herpes simplex virus 1 / 2 IgG/IgA/IgM antibodies + Herpes simplex virus EliSpot
- 6. EBV EliSpot
- 7. CMV EliSpot





Parkinsonism

"Borrelia can cause Parkinsonism" (Arch.of Path.& Lab.Med.127(9):1204-6)



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Parkinsonism

- 1. Borrelia SeraSpot + Borrelia EliSpot + CD57 cells
- Chlamydia pneumoniae IgG/IgA antibodies + Chlamydia pneumoniae EliSpot
- 3. Mycoplasma pneumoniae IgG/IgA antibodies + EliSpot
- 4. Bartonella IgG/IgM antibodies + EliSpot
- 5. Coxsackie Virus IgG/IgA antibodies
- 6. EBV EliSpot
- 7. CMV EliSpot





Autism

"20 - 30% of autistic disorders can be caused by Borrelia and 58% by Mycoplasma" (Bransfield et al.: Med Hypotheses 2008; 70(5):967-74)



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Autism

- 1. Borrelia SeraSpot + Borrelia-EliSpot + CD57-cells
- Chlamydia pneumoniae IgG/IgA antibodies + Chlamydia pneumoniae EliSpot
- 3. Mycoplasma pneumoniae IgG/IgA antibodies + EliSpot
- 4. Bartonella IgG/IgM antibodies + EliSpot
- 5. Coxsackie Virus IgG/IgA antibodies
- 6. EBV EliSpot
- 7. CMV EliSpot
- 8. ASL titer





Multiple symptoms = Multiple infections





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MULTIPLE SYMPTOMS = MULTIPLE INFECTIONS

"Chronic Lyme disease" is an multi infectious disease at a immuno- weakened host Symptom selection	Borrelia	Chl. pneumoniae	Chl. trachomatis	Mykoplasma	Bartonella	Ehrlichia	Rickettsia	Yersinia	Babesia	EBV virus	Coxsackie virus	
	0	0	0	0	0	0	0	0	0	0	0	
limbs, tendon pain												
ioint pain												
memory- concentration problems												
headache												1=
nausea, vomiting												
encephalitis												
fatigue, exhaustion												
feverish feeling												
chills, tremors												
flu symptoms												
stomach ache												
diarrhea												
jaundice												
Increased liver values												
enlargement of the spleen												
dark urine												
urination with itching												
beart problems	_											
couch											_	
pneumonia												
anemia												
rash											_	
Skin bleeding												1=
lymphadenopathy												1=
suppurating tonsils, dental probl.												1三



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The coinfections checklist for patients, developed by Dr. Schwarzbach





Coinfections-Checklist

Name, first name Date (DD/MM/YYYY)

►	Actual and former symptoms Please mark with a cross	x	Score-Points (filled in by physician/naturopath)	Ranking
1	Stomach ache, gut problems		Ehrlichia:	
2	Anaemia		Babesia:	
3	Diarhoea intermittent		Rickettsia:	
4	Fever or feverish feeling		Bartonella:	
5	Lack of concentration, memory disturbance, forgetfulness		Chl.pneumoniae:	
6	Encephalitis/Inflammation of the brain (NMR)		Chl.trachomatis:	
7	Yellowish colour of the skin/eyes		Yersinia:	
8	Painful joints, swollen joints		Mykoplasma:	
9	General aches and pains, tendon problems		Coxsackie-Virus:	
10	Flu-like symptoms intermittent		EBV/CMV:	
11	Rash(es)			
12	Small red/purple spots of the skin			



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Evaluation template for therapists, developed by Dr. Schwarzbach





Coinfections Evaluation Template



Coinfections checklist: Patient 1

<u>B.C.</u> Name, First name

15th Oct. 2010 Date

	Symptoms - Please tick the appropriate		Score-Points	Ran-
•	symptoms (to be filled in by the patient)	×	(to be filled in by the	king
			pnysician)	
01	Stomach-ache	×	Ehrlichia: 5	4
02	Anaemia		Babesia: 5	4
03	Diarhoea		Rickettsia: 5	4
04	Fever or feverish feeling	Х	Bartonella: 6	3
05	Lack of concentration, memory disturbance, forgetfulness	Х	Chl.pneumoniae: 8	1
06	Encephalitis (Inflammation of the brain)		Chl.trachomatis: 3	6
07	Yellowish colour of the skin (Jaundice)	X	Yersinia: 4	5
08	Painful joints	Х	Mykoplasma: 7	2
09	General aches and pains	X	Coxsackie-Virus: 7	2
10	Flu-like symptoms	X	EBV: 6	3
11	Rash			
12	Petechiae			
13	Heart-problems	Х		
14	Cough			
15	Headache	X		
16	Impaired liver function/ liver parameters			
17	Pneumonia			
18	Swollen or inflamed lymph nodes			
19	Tonsilitis			
20	Enlargement of the spleen (Splenomegaly)			
21	Fatigue / exhaustion	Х		
22	Muscle pain	Х		
23	Shivering	X		
24	Blurred vision			
25	Nausea, vomiting	X		
26	Dark urine	X		
27	Painful or ichty urinating			



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Laboratory results: Patient 1

	Results	Reference range
Borrelia burgdorferi antiboo	dies	
Borrelia IgG Immunoblot	positive Bands: p39+,p41+,p83+, VIsE-Bq+, VIsE-Bl	negative p58+,p83+,LBb+, b+,VlsE-Ba+
Borrelia IgM Immunoblot	negative	negative
Elispot-LTT Borrelia		
Borrelia Full Antigen	+ SI >3	< 2
Borrelia Peptide Mix	+ SI 4	< 2
Borrelia LFA-1	SI 2	< 2
CD3/CD57 NK cells	- 49 /ul	>100 /ul
Chlamydia pneumoniae ant	ibodies	
Chl. pneumoniae IgG EIA*	+ 71.3 RE/ml	< 16.0 = neg.
Chl. pneumoniae IgA EIA*	+ 2.29 ratio	< 0.8 = neg.
Elispot LTT Chlamydia pneu	moniae	
Chlamydia pneumoniae	+ SI 46	< 2
* Enzyme immunoassay		



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Coinfections checklist: Patient 2

A.K. Name, First name

27th March 2011 Date

	Symptoms - Please tick the appropriate symptoms (to be filled in by the patient)	×	Score-Points (to be filled in by the physician)	Ran- king
01	Stomach-ache		Ehrlichia: 3	3
02	Anaemia		Babesia: 3	3
03	Diarhoea		Rickettsia: 3	3
04	Fever or feverish feeling	×	Bartonella: 4	2
05	Lack of concentration, memory disturbance, forgetfulness		Chl.pneumoniae: 5	1
06	Encephalitis (Inflammation of the brain)		Chl.trachomatis: 3	3
07	Yellowish colour of the skin (Jaundice)		Yersinia: 2	4
08	Painful joints	X	Mykoplasma: 5	1
09	General aches and pains	X	Coxsackie-Virus: 4	2
10	Flu-like symptoms	×	EBV: 3	3
11	Rash			
12	Petechiae			
13	Heart-problems			
14	Cough			
15	Headache			
16	Impaired liver function/ liver parameters			
17	Pneumonia			
18	Swollen or inflamed lymph nodes			
19	Tonsilitis			
20	Enlargement of the spleen (Splenomegaly)			
21	Fatigue / exhaustion	X		
22	Muscle pain	×		
23	Shivering			
24	Blurred vision			
25	Nausea, vomiting			
26	Dark urine			
27	Painful or ichty urinating			



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Laboratory results: Patient 2

	Results	Reference range
Borrelia burgdorferi antibo	dies	
Borrelia IgG Immunoblot	negative	negative
Borrelia IgM Immunoblot	positive	negative
	Bands: OspC-Bg/Bb/I	3a+, p41+
Borrelia burgdoferi Elispot-	LTT	
Borrelia full antigen	+ SI 27	< 2
Borrelia peptide mix	SI 1	< 2
Borrelia LFA-1	SI 3	< 2
CD3/CD57 NK cells	- 41 /ul	>100 /ul
Chlamydia pneumoniae ant	ibodies	
Chl. pneumoniae IgG EIA	+ 119.0 RE/ml	< 16.0 = neg.
Chl. pneumoniae IgA EIA	+ 1.62 ratio	< 0.8 = neg.
Chlamydia pneumoniae Elis	spot-LTT	
Chlamydia pneumoniae	+ SI 10	< 2
Mycoplasma pneumoniae a	ntibodies	
Mycoplasma pn. IgG EIA	+ 71.1 RE/ml	< 22
Mycop. pneumoniae IgA EIA	+ 1.2 ratio	< 1.1
Ehrlichia Elispot-LTT		
Ehrlichia	+ SI 3	< 2



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Coinfections checklist: Patient 3

F.H. Name, First name

12th Apr. 2011 Date

	Symptoms - Please tick the appropriate symptoms (to be filled in by the patient)	×	Score-Points (to be filled in by the physician)	Ran- king
01	Stomach-ache		Ehrlichia: 6	3
02	Anaemia		Babesia: 4	5
03	Diarhoea		Rickettsia: 4	5
04	Fever or feverish feeling		Bartonella: 3	6
05	Lack of concentration, memory disturbance, forgetfulness	×	Chl.pneumoniae: 8	1
06	Encephalitis (Inflammation of the brain)		Chl.trachomatis: 3	6
07	Yellowish colour of the skin (Jaundice)		Yersinia: 2	7
08	Painful joints	×	Mykoplasma: 7	2
09	General aches and pains	X	Coxsackie-Virus: 3	4
10	Flu-like symptoms		EBV: 3	6
11	Rash			
12	Petechiae			
13	Heart-problems	×		
14	Cough	X		
15	Headache	X		
16	Impaired liver function/ liver parameters			
17	Pneumonia			
18	Swollen or inflamed lymph nodes			
19	Tonsilitis			
20	Enlargement of the spleen (Splenomegaly)			
21	Fatigue / exhaustion	×		
22	Muscle pain	×		
23	Shivering	X		
24	Blurred vision			
25	Nausea, vomiting			
26	Dark urine			
27	Painful or ichty urinating			



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Laboratory results: Patient 3

ACADEMY or NUTRITIONAL MEDICINE.

	Results	Reference range	
Dowelio huwadowiowi owtih	a di a a		
Borrella Durgdorferi antibe	odies		
Borrella IgG Immunobiot	negative	negative	
Borrelia IgM Immunoblot	negative	negative	
Borrelia burgdoferi Elispo	t-LTT		
Borrelia full antigen	+ SI 27	< 2	
Borrelia peptide mix	+ SI 7	< 2	
Borrelia LFA-1	SI 1	< 2	
CD3/CD57 NK cells	- 96 /ul	>100 /ul	
Chlamydia pneumoniae ar	ntibodies		
Chl. pneumoniae IgG EIA	+ 46.8 RE/ml	< 16.0 = neg.	
Chl. pneumoniae IgA EIA	0.35 ratio	< 0.8 = neg.	
Chlamydia pneumoniae El	ispot-l TT		
Chlamydia preumoniae		<)	
chiamydia pheumoniae			
Ehrlichia Elispot-LTT			
Ehrlichia	+ SI 24	< 2	
ANA (EIA)	+ 43.9 units	< 20	
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DIAGNOSING TICK-BORNE DISEASES

Coinfections checklist: Patient 4

<u>B.C.</u> Name, First name

15th Oct. 2010 Date

	Symptoms - Please tick the appropriate symptoms (to be filled in by the patient)	×	Score-Points (to be filled in by the physician)	Ran- king	1
01	Stomach-ache	×	Ehrlichia: 5	4	1
02	Anaemia		Babesia: 5	4	1
03	Diarhoea		Rickettsia: 5	4	Ī
04	Fever or feverish feeling	X	Bartonella: 6	3	Ī
05	Lack of concentration, memory disturbance, forgetfulness	X	Chl.pneumoniae: 8	1	Ì
06	Encephalitis (Inflammation of the brain)		Chl.trachomatis: 3	6	Ì
07	Yellowish colour of the skin (Jaundice)	×	Yersinia: 4	5	1
08	Painful joints	×	Mykoplasma: 7	2	1
09	General aches and pains	×	Coxsackie-Virus: 7	2	1
10	Flu-like symptoms	×	EBV: 6	3	1
11	Rash				Ī
12	Petechiae				1
13	Heart-problems	X			-
14	Cough				ł
15	Headache	×			1
16	Impaired liver function/ liver parameters				1
17	Pneumonia				1
18	Swollen or inflamed lymph nodes				1
19	Tonsilitis				1
20	Enlargement of the spleen (Splenomegaly)				Ī
21	Fatigue / exhaustion	X			1
22	Muscle pain	×			I
23	Shivering	×			ł
24	Blurred vision				Ì
25	Nausea, vomiting	X			
26	Dark urine	X			1
27	Painful or ichty urinating				1



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Laboratory test results: Patient 4

		Results	Unit	Reference range
Borrelia burgdorferi antibodies (ELISA)				
Borrelia IgG antibodies (ELISA)	+	71.9	RU/ml	< 16=neg. >22.0=pos.
Borrelia IgM antibodies (ELISA)		4.72	RU/ml	< 16=neg. >22.0=pos.
Borrelia burgdorferi antibodies (immunol	plot)			
Borrelia Blot IgG antibodies	+	positive		negative
		Bands: O	spC (+),p4	1 +, VlsE-Bb +
Borrelia Blot IgM antibodies		negative		negative
Borrelia burgdorferi EliSpot				
Borrelia burgd. full antigen	+	4	SI	< 2
Borrelia OSP mix (OSPA/OSPC/DbpA)	+	3	SI	< 2
Borrelia LFA-1		1	SI	< 2
Yersinia antibodies				
Yersinia IgG antibodies (EIA)	+	1.9	ratio	< 0.8=neg.; >1.1=pos.
Yersinia IgA antibodies (EIA)	+	8.6	ratio	< 0.8=neg.; >1.1=pos.



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Laboratory test results: Patient 4

		Results	Unit	Reference range
Yersinia EliSpot				
Yersinia EliSpot	+	20	SI	< 2
Chlamydia pneumoniae antibodies				
Chlam.pneum. IgG antibodies (ELISA)	+	1.2	ratio	< 0.8=neg.; >1.1=pos.
Chlam.pneum. IgA antibodies (ELISA)	÷	3.5	ratio	< 0.8=neg.; >1.1=pos.
Chlamydia pneumoniae EliSpot				
Chlamydia pneumoniae EliSpot	+	18	SI	< 2
Mycoplasma pneumoniae antibodies				
Mycoplasma pneumoniae IgG (EIA)	+	1.1	ratio	< 0.8=neg.; >1.1=pos.
Mycoplasma pneumoniae IgM (EIA)		0.3	ratio	< 0.8=neg.; >1.1=pos.
Mycoplasma pneumoniae IgA (EIA)	+	2.0	ratio	< 0.8=neg.; >1.1=pos.
Cytomegalovirus				
Cytomegalovirus IgG antibodies (EIA)	+	3.7	ratio	< 0.8=neg.; >1.1=pos.
Cytomegalovirus IgM antibodies (EIA)		0.3	ratio	< 0.8=neg.; >1.1=pos.
Cytomegalovirus Cytomegalovirus IgG antibodies (EIA) Cytomegalovirus IgM antibodies (EIA)	+	3.7 0.3	ratio ratio ratio	< 0.8=neg.; >1.1=pos. < 0.8=neg.; >1.1=pos. < 0.8=neg.; >1.1=pos.

Cytomegalovirus EliSpot



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+



57

<2

SI

Laboratory test results: Patient 4

		Results	Unit	Reference range
Coxsackie-Virus antibodies				
Coxsackie Virus IgG Type B1 (IFT)	+	1:400	titer	< 1:100
Coxsackie Virus IgA Type B1 (IFT)	+	1:100	titer	< 1:10
Rickettsia antibodies				
Rickettsia rickettsii IgG antibodies	+	1:256	titer	< 1:64
Rickettsia typhi IgG antibodies		< 1:64	titer	< 1:64
Epstein-Barr-Virus antibodies				
EBV-CA IgG antibodies (EIA)	+	7.1	ratio	< 0.8=neg; >1.1=pos
EBV-EBNA antibodies (EIA)	+	4.2	ratio	< 0.8=neg; >1.1=pos
EBV-CA IgM antibodies (EIA)		0.4	ratio	< 0.8=neg; >1.1=pos
Epstein-Barr Virus EliSpot				
EBV-EliSpot (lytic)	+	17	SI	< 2
EBV-EliSpot (latent)	+	8	SI	< 2
CD 57 flow cytometry				
CD 57 positive NK cells		37	/µl	100-360



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Summary Patient 4

Coinfections checklist (symptoms):

Multiple infections with Borrelia burgdorferi + Chlamydia pneumoniae + Mycoplasma pneumoniae + Coxsackie virus + Epstein Barr Virus + Rickettsia + Yersinia

Laboratory test results:

Multiple infections with

Borrelia burgdorferi + Chlamydia pneumoniae + Mycoplasma pneumoniae + Coxsackie-Virus + Epstein Barr Virus + Rickettsia rickettsii + Yersinia + Cytomegalovirus



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Complementary therapy options: for example...

A) Lee Cowden Protocol against viruses

Takuna + Burbur-Pinella + Samento + Serrapeptase:

"For Coxsackie, EBV and CMV Dr. Cowden recommends the Cowden Support Programme (CSP) plus Takuna. For patients with high viral loads he recommends 30 drops of Takuna 4 times daily or 30 drops 2 times per day for patients with lower viral loads."

B) Nutrined Program against bacteria and viruses

- 1. Multimessenger
- 2. Artemisinin SOD
- 3. ATP Fuel
- 4. Transfer Factor Lym Plus (against Lyme, bacteria and viruses)
- 5. Messenger N1 (against Mycoplasma/Chlamydia and viruses)
- 6. Lumbrokinase (against biofilms)



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How to obtain ArminLabs tests in the UK

- The Academy of Nutritional Medicine is the hub for ArminLabs tests in the UK. Please visit the ArminLabs tab on their webpage <u>www.aonm.org</u>
- The tab is:<u>http://www.aonm.org/Armin%20Labs</u>
- Their number is 03331 210 305
- They will advise you, arrange for a blood draw if requested, arrange a courier of the bloods to Germany, etc.



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Thank you very much for your attention!





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