Lyme Disease, coinfections and opportunistic infections:

Interactive Workshop for Professionals

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ArminLabs

Germany

arminlabs
DIAGNOSING TICK-BORNE DISEASES

AONM
ACADEMY OF NUTRITIONAL MEDICINE

PART I:
Choosing the best laboratory test combinations

Contamination of ticks in Switzerland: Current data

- **25 % free of pathogens**
- **32 % Borrelia:**
  - **16 % Borrelia afzelii**
  - **11 % Borrelia garinii**
  - **5 % Borrelia sensu stricto**
- **1 % Ehrlichia/Anaplasma**
- **42 % Rickettsia helvetica !**
  (causes myalgia, pericarditis)

17 % of Borrelia contaminated ticks have additional Rickettsia

14 of 113 Lyme disease patients have Rickettsia symptoms (mixed infections)!

Source: Lecture Prof. Sievers, Hochschule Wädenswil, 5th Apr. 2008 Bad Soden-Salmünster

Ticks: Vector for multiple infections

- **Anaplasma phagocytophilum in questing Ixodes ricinus ticks from Romania** *(Matei et al., Ticks and Tick-borne diseases, Vol.6, Issue3, April 2015, Pages 408-413)*

- **The tick-borne pathogen Anaplasma phagocytophilum is an increasing potential public health threat across Europe.** *(Barakova et al. Genetic and Ecologic Variability among Anaplasma phagocytophilum Strain, Northern Italy. Emerging Infectious Diseases, Vol. 20, No. 6, June 2014, www.dcd.gov/eid)*
- **High Percentage of Ixodes ricinus ticks are co-infected with Borrelia, Ehrlichia, and Bartonella in Netherlands**. *(Schoub et al. J. Clin Microbiology 1999 (37:2215-2215)*

- **Candidatus Neoehrlichia mikurensis and Anaplasma phagocytophilum in natural rodent and tick communities in Southern Hungary.** *(Szekeres et al., Ticks and Tick-borne diseases, Vol.6, Issue 2, March 2015, Pages 111-116)*

- **Lyme, Anaplama and B.duncani**
  (Lebech et al. Serologic evidence of granulocytic ehrlichiosis and piroplasma WA 1 in European patients with Lyme neuroborreliosis. Seventh Intl Congress on Lyme Borreliosis 1996:390)

- **PCR evidence of *Bartonella henselae* and *Borrelia burgorferi* was found in both *Ixodes scapularis* ticks and in the CSF of patients presenting with neurological symptoms.** *(Eskow/Mordechai et al. Concurrent infection of the Central Nervous System by Borrelia burgorferi and Bartonella henselae, Archives of Neurology Sept. 2001)*

- **Molecular characterization of Candidatus Rickettsia vini in Ixodes arboricola from Czech Republic and Slovakia.** *(Novakova et al., Ticks and Tick-borne dieseases, Vol.6, Issue3, April 2015, Pages 330-333)*

- **46% positive for Borrelia burgdorferi by culture, 12% positive for Babesia by PCR and 5-10% positive for Bartonella by culture.** *(Hofmeister et al. A Novel Bartonella species in Peromyscus leucopus in conjunction with B. burgdorferi and Babesia microti. J. Infect Disease 1998)*

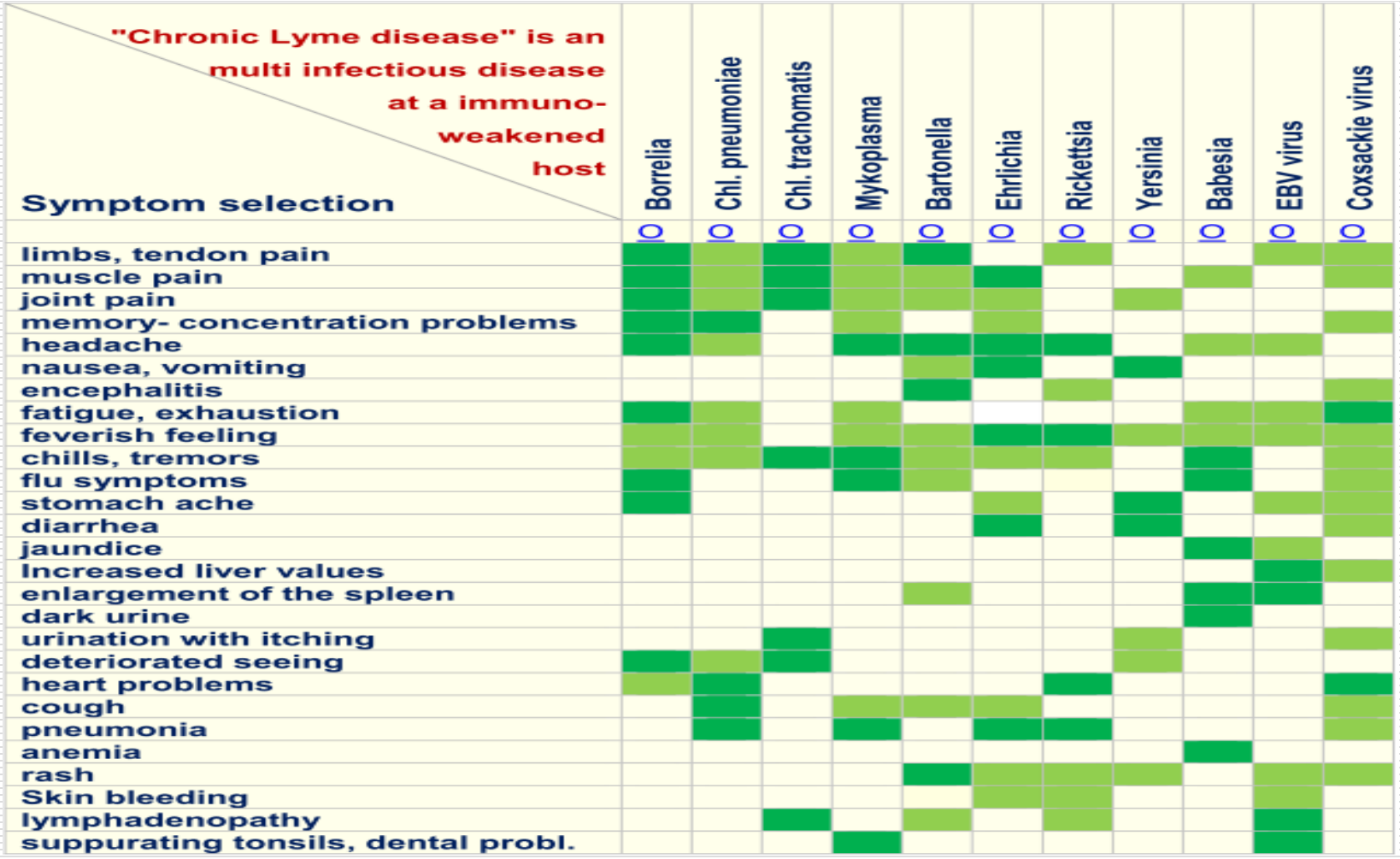
The oldest patient with „Fibromyalgia“ (5300 years ago): "Iceman" Ötzi

Ötzis 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**."

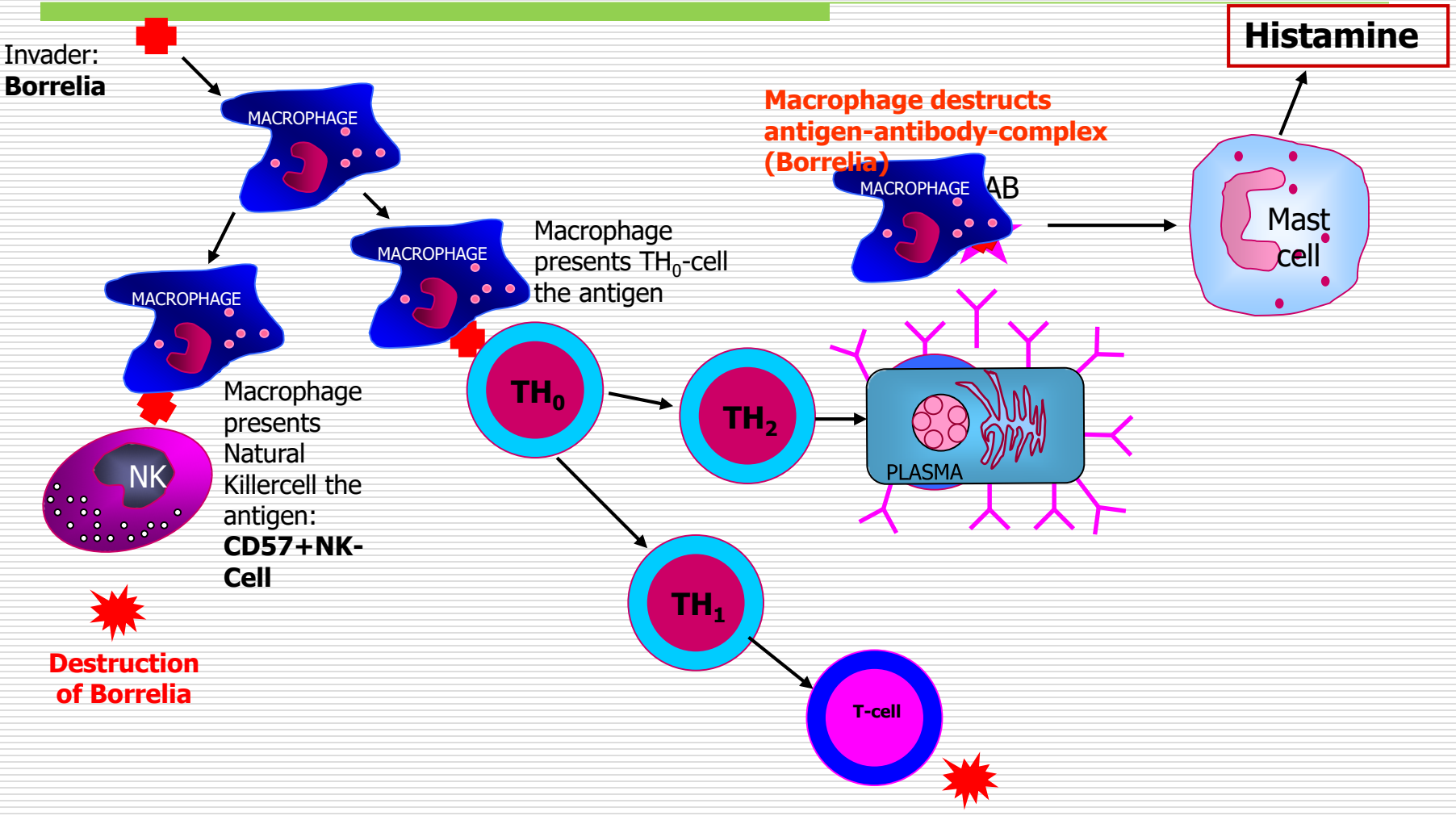
Chronic Lyme disease symptoms

Power loss or reduction (mental/physical) at work, household, sport	>99 %
Fatigue/ Drowsiness/Listlessness	>99 %
Tingling/"Ants running"/Numbness/ „Needle burning" or „burning" skin-sensations	81 %
Neck pain/ neck stiffness	78 %
Shoulder pain	76 %
Headache/Dizziness	76 %
Changing, migrant joint pain (all joints are possible)	68 %
Changing, migrant muscle pain/"Rheumatism"/General weakness of the body	62 %
Feverish infection: in Stage I of Lyme disease as a sign for occurence of borrelia-bacteria in blood	≈20 %
Mental strain/Depressions/Schizophrenia/Psychosis	62 %
Back pain/Sciatic pain syndrome	58 %
Sleeplessness with partly sweating/urge to urinate mostly between 2 and 4 o´clock at night	47 %
Sore throat/Tendence for general infections/HSV or EBV-Infections	39 %
„Burning eyes"/Overproduciton of tears/Blurred vision/Double vision/Lightheadedness	28 %

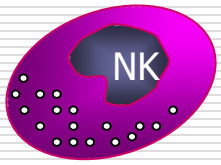
MULTIPLE SYMPTOMS = MULTIPLE INFECTIONS

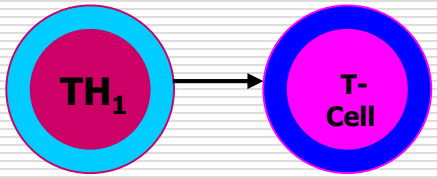


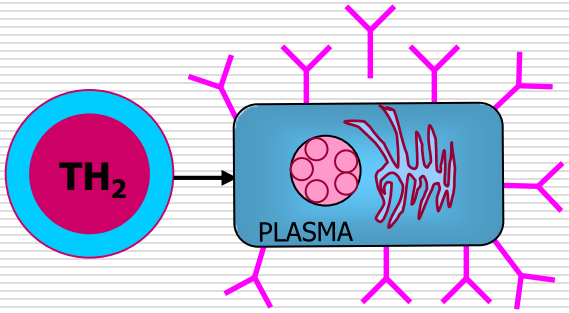
Immune defence principle



Aims of the immune-competent cells







CD57+NK-cells

- Lysis antigen-antibody-complexes (Borrelia burgdorferi)

Elispot (T-cells):

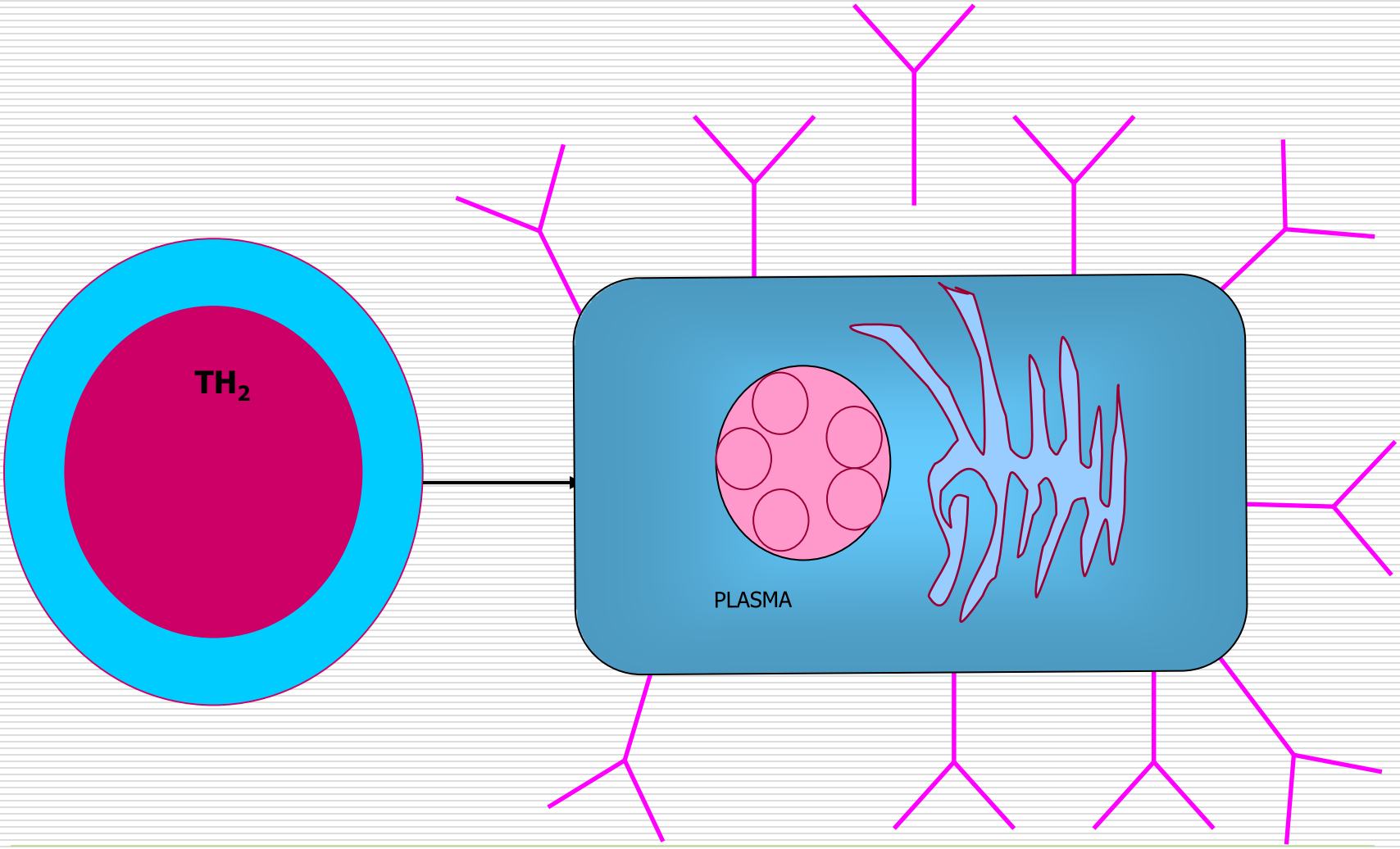
- Borrelia burgdorferi
- Chlamydia pneumoniae
- Anaplasma/Ehrlichia

and others

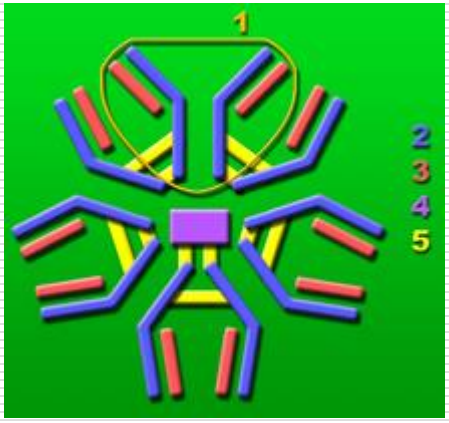
Antibodies (B-cells):

- Borrelia burgdorferi
- Chlamydia, Mycoplasma
- Anaplasma, Ehrlichia, Babesia...

B-cells (IgG/IgA/IgM antibodies): ELISA, Westernblot, SeraSpot Microarray



Immunglobulin M

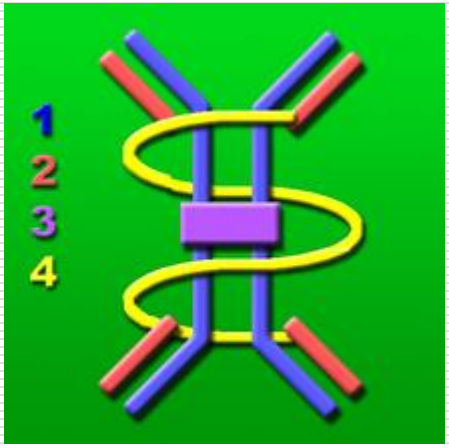


IgM (Immunoglobulin M) antibody molecule consisting of 5 base units.
1: Base unit.
2: Heavy chains.
3: Light chains.
4: J chain.
5: Intermolecular disulfide bonds.

Immunglobulin M

- IgM antibodies appear early in the course of an infection and usually reappear after further exposure. IgM antibodies do not pass across the human placenta (only isotype IgG).
- These two biological properties of IgM make it useful in the diagnosis of infectious diseases. Demonstrating IgM antibodies in a patient's serum indicates recent infection, or in a neonate's serum indicates intrauterine infection (e.g. congenital rubella syndrome).

Immunglobulin A

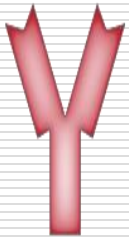


The dimeric IgA molecule
1 H-chain
2 L-chain
3 J-chain
4 secretory component

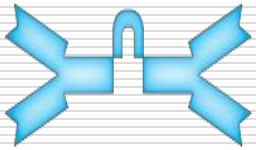
Immunglobulin A

- IgA exists in two isotypes, IgA1 and IgA2. IgA1 predominates in serum (~80%), IgA2 percentages are higher in secretions than in serum (~35% in secretions)
- IgA1 is the predominant IgA subclass found in serum. Most lymphoid tissues have a predominance of IgA1-producing cells.
- In secretory lymphoid tissues (e.g., gut-associated lymphoid tissue), the share of IgA2 production is larger than in the non-secretory lymphoid organs (e.g. spleen, peripheral lymph nodes).
- Both IgA1 and IgA2 have been found in external secretions like colostrum, maternal milk, tears and saliva, where IgA2 is more prominent than in the blood.
- Both IgA1 and IgA2 can be in membrane-bound form.

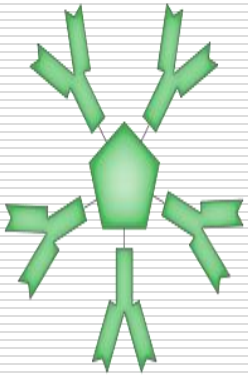
Immunglobulin G



Monomer
IgD, IgE, IgG



Dimer
IgA



Pentamer
IgM

Subclasses Name	Percent	Crosses placenta easily	Complement activator	Binds to Fc receptor on phagocytic cells	Half Life[12]
IgG1	66%	yes (1.47)*	second-highest	high affinity	21 days
IgG2	23%	no (0.8)*	third-highest	extremely low affinity	21 days
IgG3	7%	yes (1.17)*	highest	high affinity	7 days
IgG4	4%	yes (1.15)*	no	intermediate affinity	21 days

Immunglobulin G

- IgG-mediated binding of pathogens causes their immobilization and binding together via agglutination; IgG coating of pathogen surfaces (opsonization) allows their recognition and ingestion by phagocytic immune cells leading to the elimination of the pathogen itself
- IgG activates the classical pathway of the complement system, a cascade of immune protein production that results in pathogen elimination
- IgG also binds and neutralizes toxins
- IgG also plays an important role in antibody-dependent cell-mediated cytotoxicity (ADCC) and intracellular antibody-mediated proteolysis, in which it binds to TRIM21 (the receptor with greatest affinity to IgG in humans) in order to direct marked virions to the proteasome in the cytosol
- IgG is also associated with type II and type III hypersensitivity reactions.

Half life times

A measure of the mean survival time of antibody molecules following their formation, usually
expressed as the time required to eliminate 50 per cent of a known quantity of immunoglobulin from the animal body.

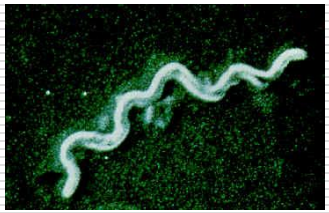
Half-life varies from one immunoglobulin class to another:

Immunglobulin M: 5 days

Immunglobulin A: 14 days

Immunglobulin G: 21 days

Laboratory for Lyme disease diagnosis



Caution major trap!

Lyme disease is not always detectable by antibody tests !

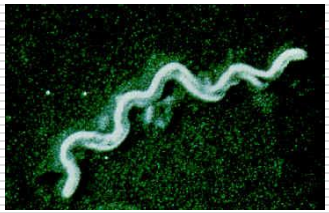
No standardization for antibody tests!

Sensitivity problems of ELISA technique !

Test sensitivity for Borrelia antibodies by ELISA screening: My data with 50 chronic Lyme patients

Test producer		Aeskulap	Virion	Diasorin	Euroimmun
n=	patients	50	50	50	44
ELISA	negative	4%	4%	14%	2%
Immunoblot	positive				
ELISA	negative	14%	12%	14%	16%
Immunoblot	borderline				
Total		18%	16%	28%	18%
Sensitivity	(-40%)	42%	44%	32%	42%

Insensitivity of ELISA vs. Immunoblot: New data



Antibodies in Lyme disease patients stage III by current ELISA screening model:

<u>Loss of sensitivity: 16 – 28 %</u>

Every 4th – 6th chronic Lyme patient has a positive or borderline Immunoblot but <u>no</u> positive ELISA

- A great number of patients will be not identified by the screening ELISA-test and consequently "excluded" for Lyme disease not using the immunoblot as a screening test
- The more specific immunoblot is the more sensitive and the better screening test
- Senselessness of Borrelia ELISA technique in general

The actual diagnostic strategy by ELISA

Serological tests are performed in a two-tier concept (according to the recommendation of CDC):

First step: Screening of sera with the help of an IgG/IgM-class-specific **ELISA**

Second step: Confirmation of the ELISA positive or borderline sera with the help of an IgG/IgM class-specific **immuno-blotting technique**

PROBLEM: The Immunoblot is more sensitive than the ELISA, i.e. the more specific test is more sensitive too:

High risk: Cases of positive Immunoblot but negative ELISA !

Laboratory example from practice: Negative EIA but positive Westernblot

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

Westernblot-results: IgG-/IgM-antibodies in chronic Lyme disease (stage III)

IgG neg/IgM neg	40%
IgG pos/IgM neg	16%
IgG borderline/IgM neg	12%
IgG pos/IgM borderline	10%
IgG borderline/IgM pos	6%
IgG pos/IgM pos	6%
IgG neg/IgM pos	6%
IgG neg/IgM borderline	4%

Westernblot-results: IgG-/IgM-antibodies in chronic Lyme disease (stage III)

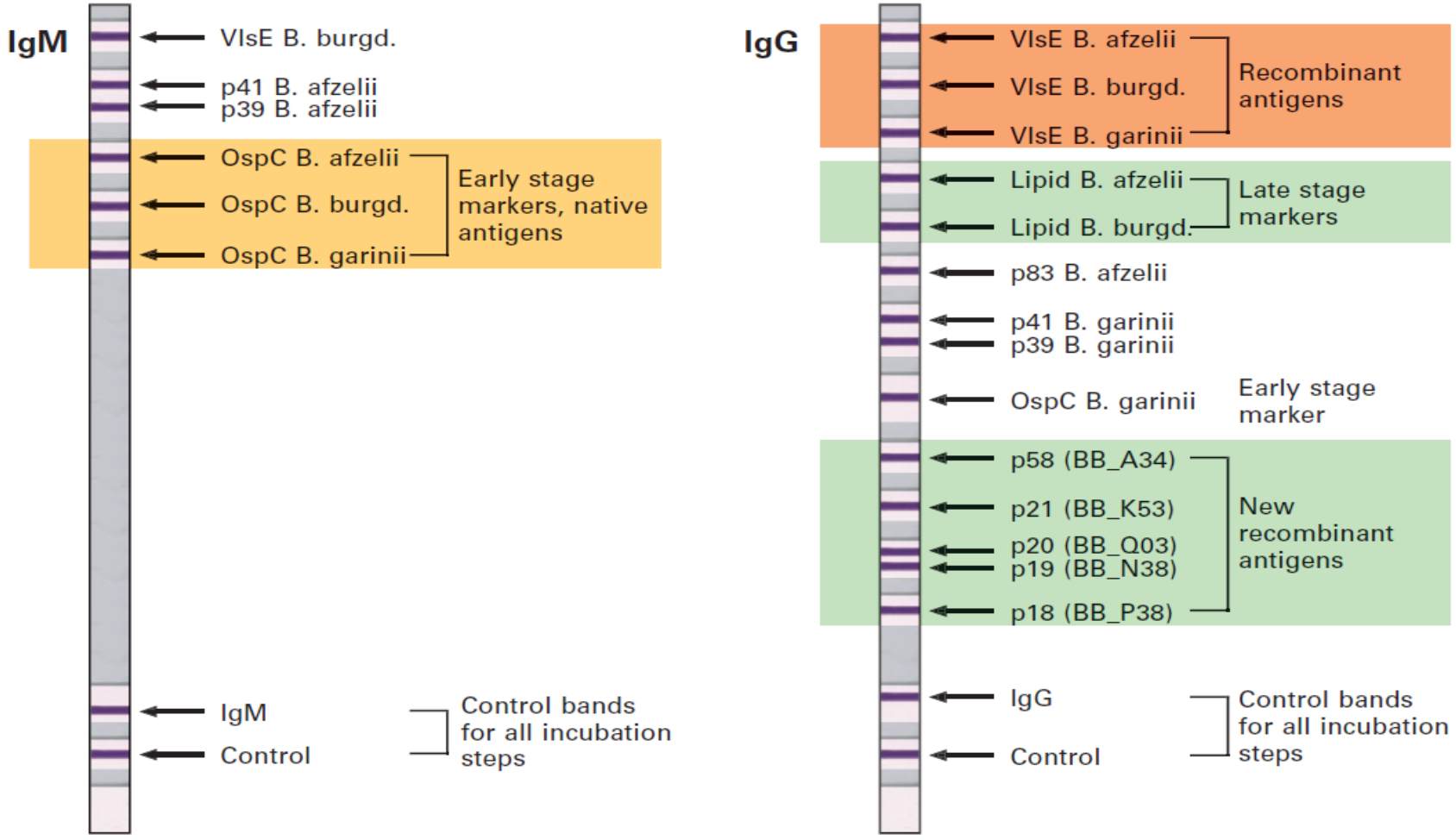
- 40 % Seronegativity
- 28 % IgG-"Rest"-Titer
- 22 % IgM- and IgG-Persistence
- 10 % Isolated IgM-Persistence

Specificity ("false positive") and sensitivity ("false negative") of Borrelia antibody tests

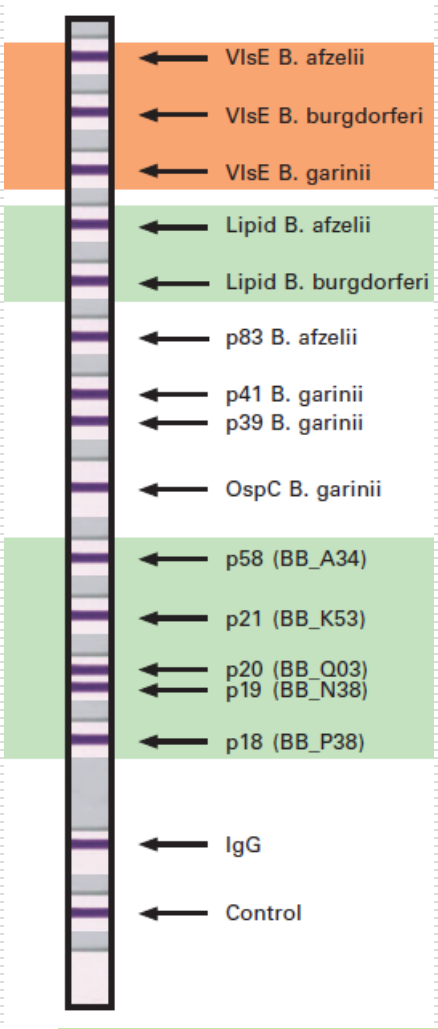
Year	Author/Literature	Specificity/Sensitivity
(1993)	Schmitz et al. Eur J Clin Microbiol Infect Dis 12,419.424	100% / 66%
(1995)	Engstrom SM, Shoop E et al. J Clin Microbiol 33, 419–27.	96% / 55%
(1996)	Ledue TB, Collins MF, Craig WY J Clin Microbiol 34, 2343–50.	100% / 44%
(1999)	Trevejo RT, Krause PJ et al. J Infect Dis 179, 931-8.	100% / 29%
(2001)	Nowakiwski et al. Clin Infect Dis 33, 2023-2027	99% / 66%
(2003)	Bacon RM, Biggerstaff BJ et al. J Infect Dis 187, 1187-99.	99% / 67%
(2005)	Coulter P, Lema C et al. J Clin Microbiol. 43(10), 5080–5084.	- / 25%
(2008)	Steere AC, McHugh G et al. Clin Infect Dis 47,188-95.	99% / 18%
(2008)	Binnicker MJ, Jespersen DJ et al. J Clin Microbiol 46, 2216–21.	100% / 49%
(2009)	Klemann W, Huismans BD. Umwelt-Medizin-Gesellschaft; 22(2) 132-138	- / 60%
(2010)	Schwarzbach A. (unpublished)	92% / 60% Blot - /32-42%ELISA

- **Average** **~99% / ~43%**

Antibodies by immunoblot: EUROLINE-RN-AT



Antibodies by immunoblot: EUROLINE-RN-AT



Antigen	Prevalence*	Specificity*
VlsE Ba	66%	99%
VlsE Bb	89%	9
VlsE Bg	68%	
Lipid Ba	25%	%
Lipid Bb	25	100%
p83		95%
p39		99%
OspC	49%	96%
p58	21%	98%
33)	9%	99%
(BB_Q03)	7%	100%
p19 (BB_N38)	9%	99%
p18 (BB_P38)	22%	99%

→ High specificity: 95-100%

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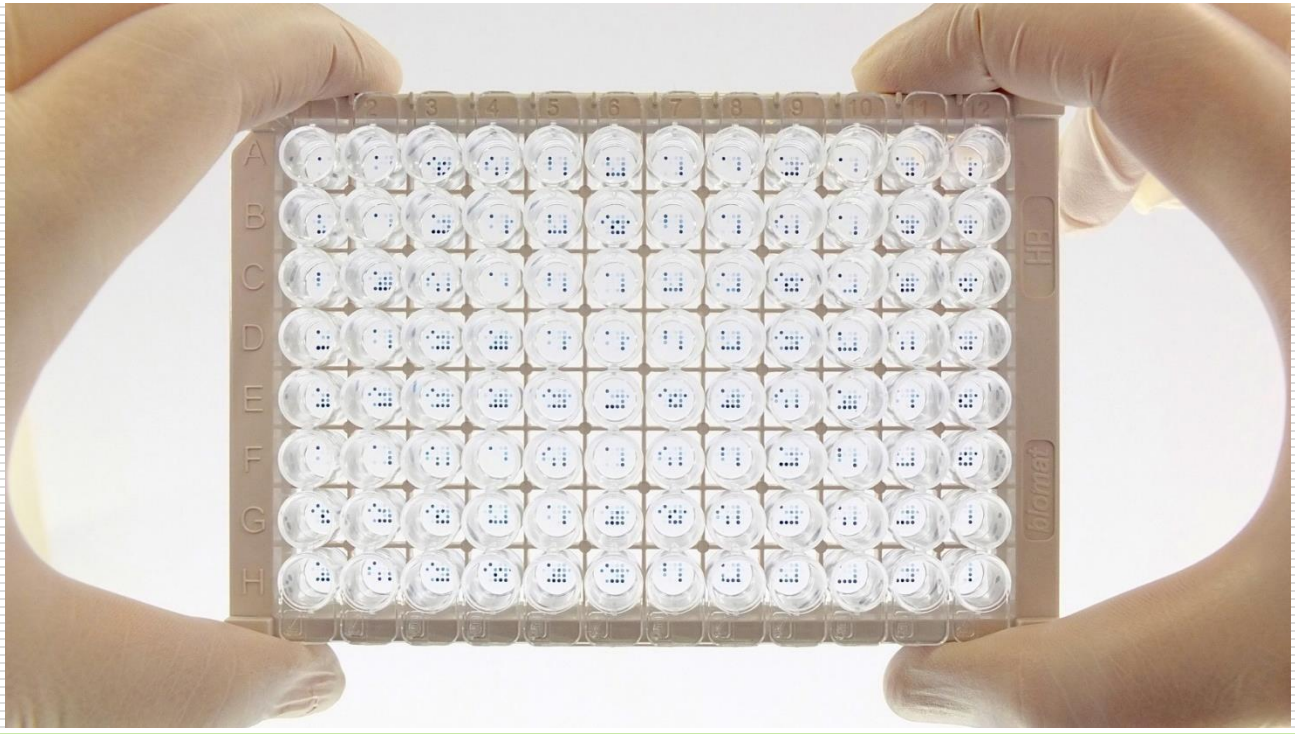
Antibodies by SeraSpot MicroArray

Microplates are coated with several antigen spots
…tests for 3 different European Borrelia subspecies:
B.b.s.s. + B.b. garinii + B.b. afzelii



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. VlsE has over 99% specificity

+

Native antigens: ArminLabs uses these, too

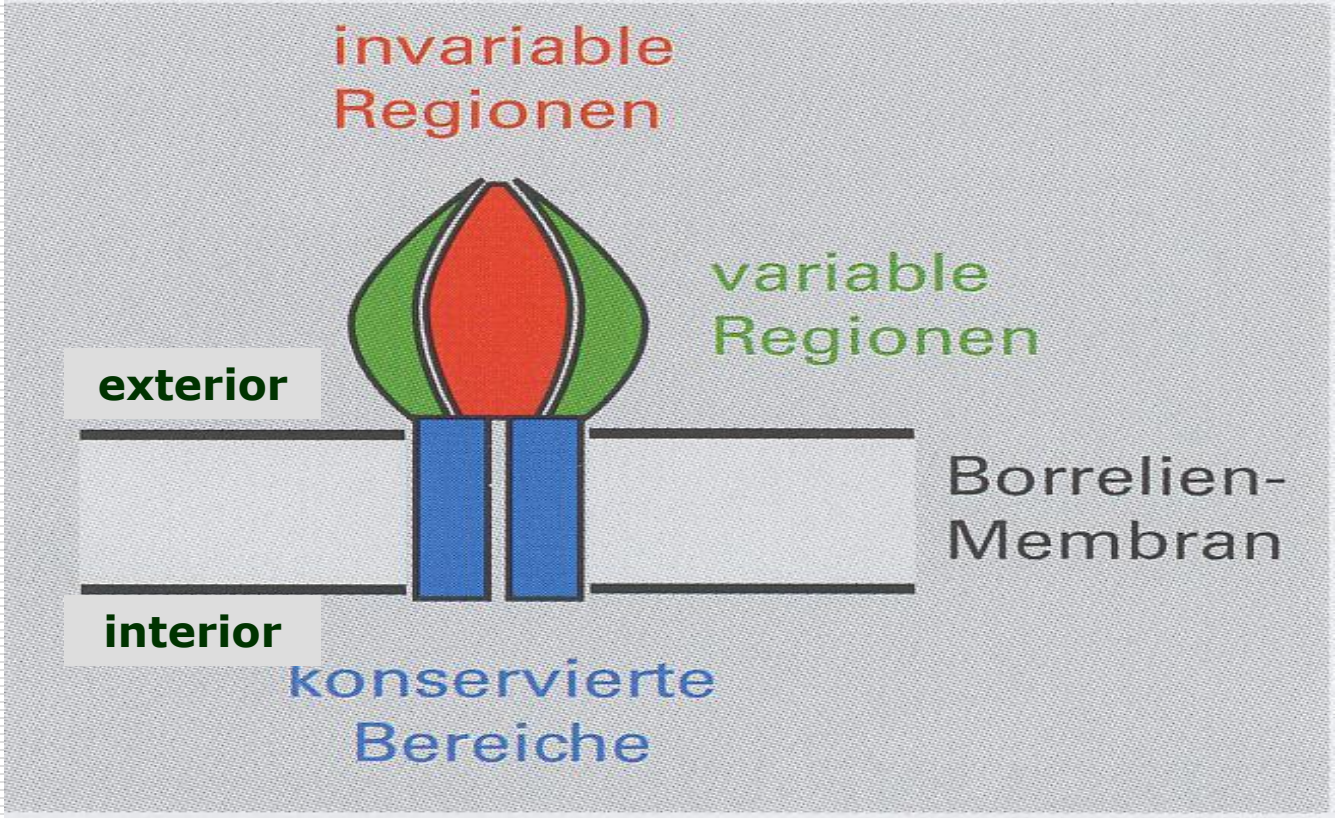
High specificity but lower sensitivity than recombinant antigens

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

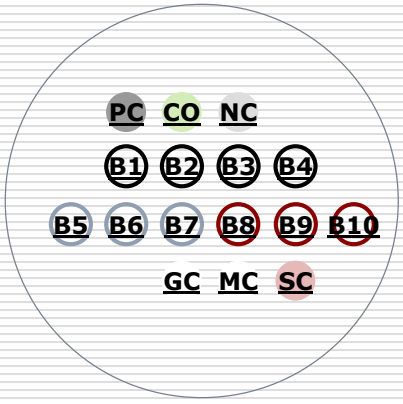
Combination of recombinant antigens + native antigens should be used

The new surface marker VlsE for B-cellular activity: highly specific, "in vivo" activity associated

VlsE = Vmp-like sequence Expression site



SeraSpot: Validation criteria



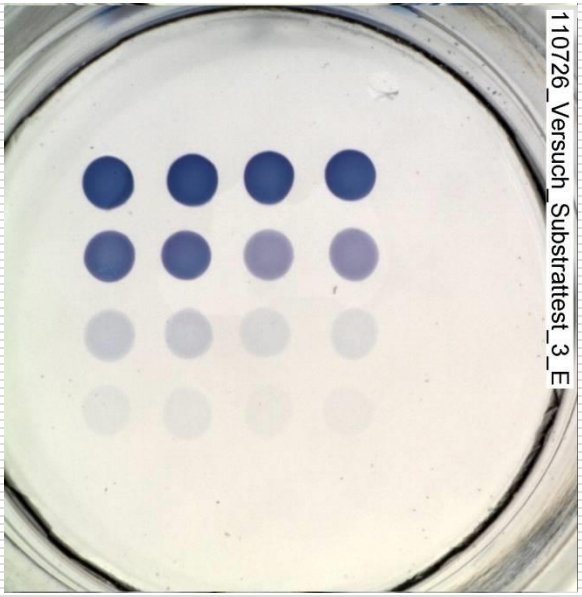
B1 VlsE (AFZ)
B2 p39 (AFZ)
B3 p58 (GAR)
B4 p100 (AFZ)
B5 OspC (AFZ)
B6 OspC (GAR)
B7 OspC (BUR)
B8 DbpA (AFZ)
B9 DbpA (GAR)
B10 DbpA (BUR)

PC Position control
CO Cut-off control
NC Negative control
SC Serum control
GC IgG conjugate control
MC IgM conjugate control

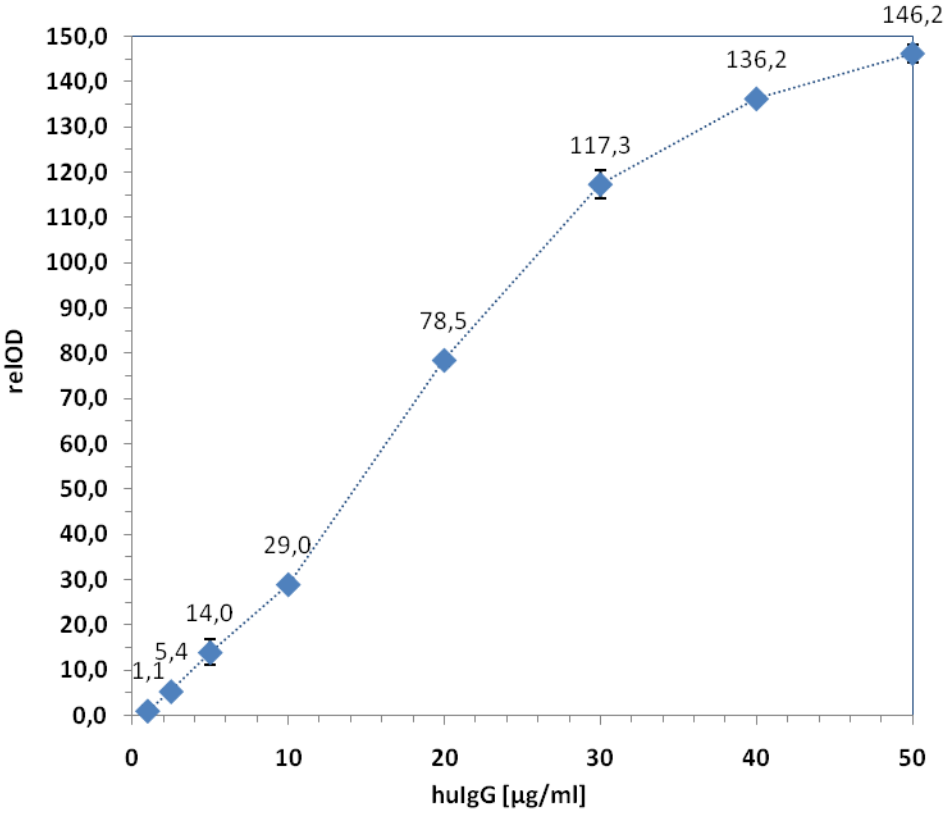
***SeraSpot*® Anti-Borrelia-10 IgG / *SeraSpot*® Anti-Borrelia-10 IgM** arrays include the following control spots:

1. **Position control (PC).** Intensively stained spot, darker than cut-off control. Always stained.
2. **Cut-off control (CO).** Weakly stained spot. Used for evaluating the results of parameter-specific signals.
3. **Negative control (NC).** Pale spot with intensity lower than cut-off control
4. **Function control (IgG-, IgM-conjugate control. GC, MC).** Intensively stained spots with different position for IgG and IgM antibody detection. Serve as an antibody isotype control.
5. **Serum control (SC).** Intensively stained spot always stained in presence of sample. Absence of spot indicates absence of sample.

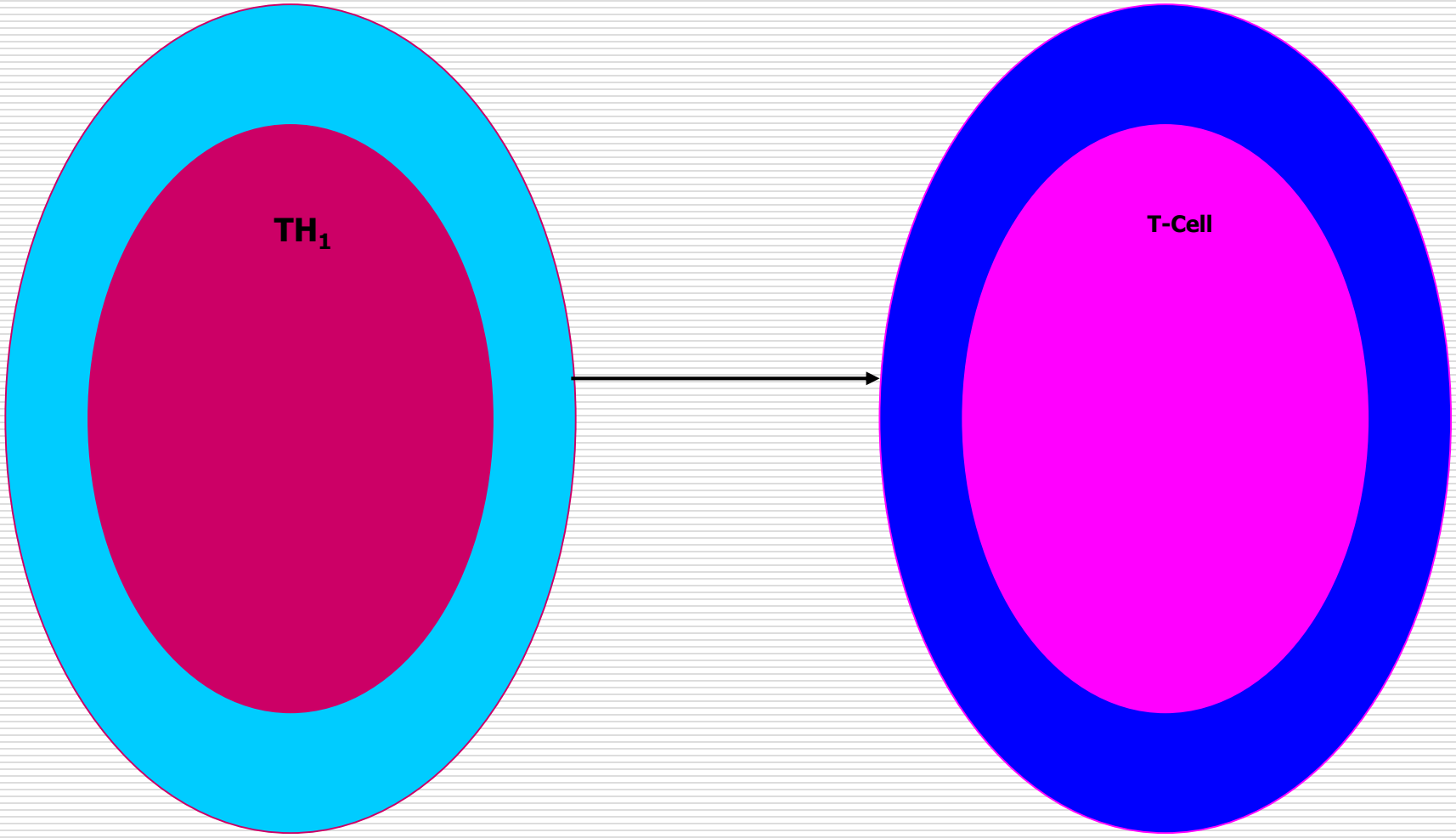
It is possible to quantify the SeraSpot MicroArray, but not the Immunoblot



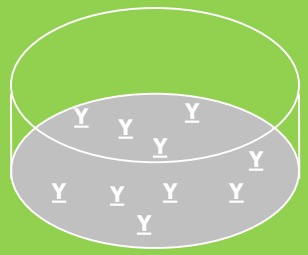
Quantification



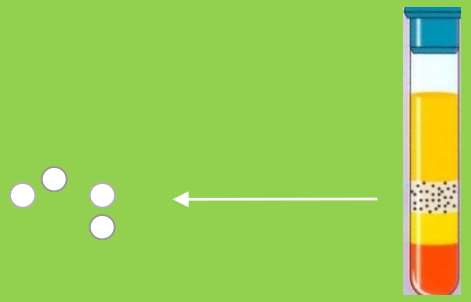
T-cells: EliSpot



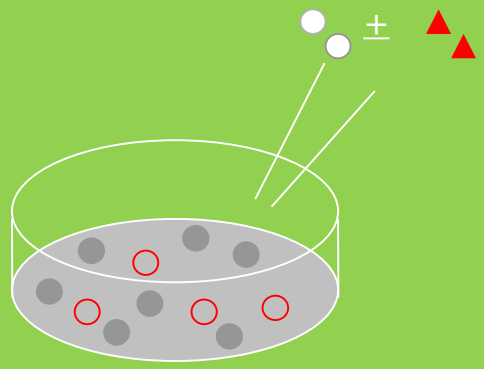
EliSpot: The principle (I)



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

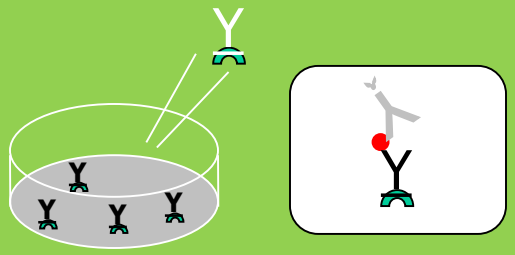


Lymphocyte-isolation

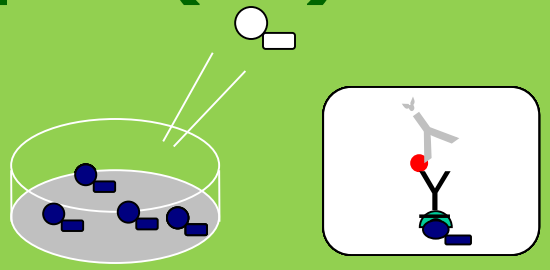


Incubation with cells and antigen, specific cells release cytokine

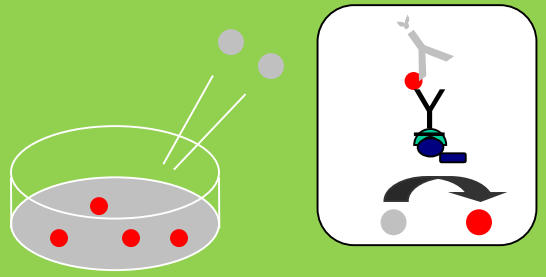
ElisSpot: The principle (II)



Add biotinylated secondary antibody Complex: pr.AB/Cytokine/sec.AB



Add Streptavidin-enzyme conjugate



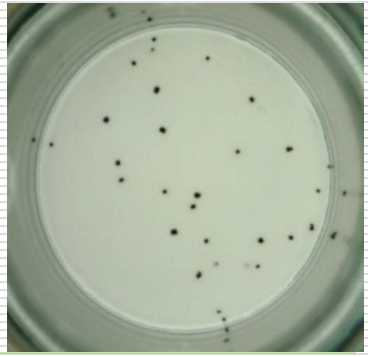
Add substrate color development

analysis

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…"



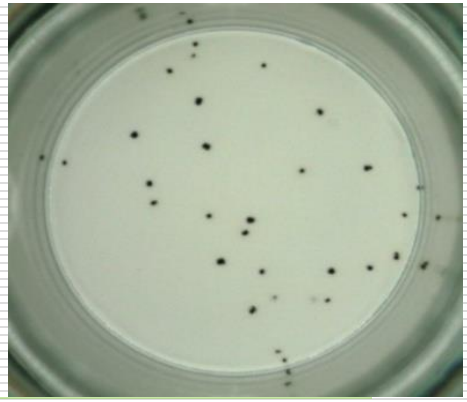
Borrelia Elispot
(T-Cell-Spot / IGRA: Interferon-Gamma-Release Assay)

Study for the specificity of Borrelia-Elispot:

… 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 Borrelia-Elispot

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…

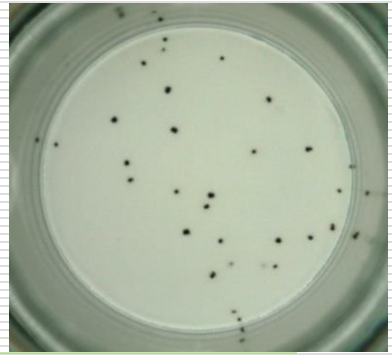
… ELISPOT can be retested to gain additional information in follow-up assays…

… 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**



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

Example Borrelia EliSpot

arminlabs
DIAGNOSING TICK-BORNE DISEASES

Armin Schwarzbach MD PhD
Specialist for laboratory medicine

ArminLabs GmbH - Zirbelstr.58 3rd floor, 86154 Augsburg, Germany

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)

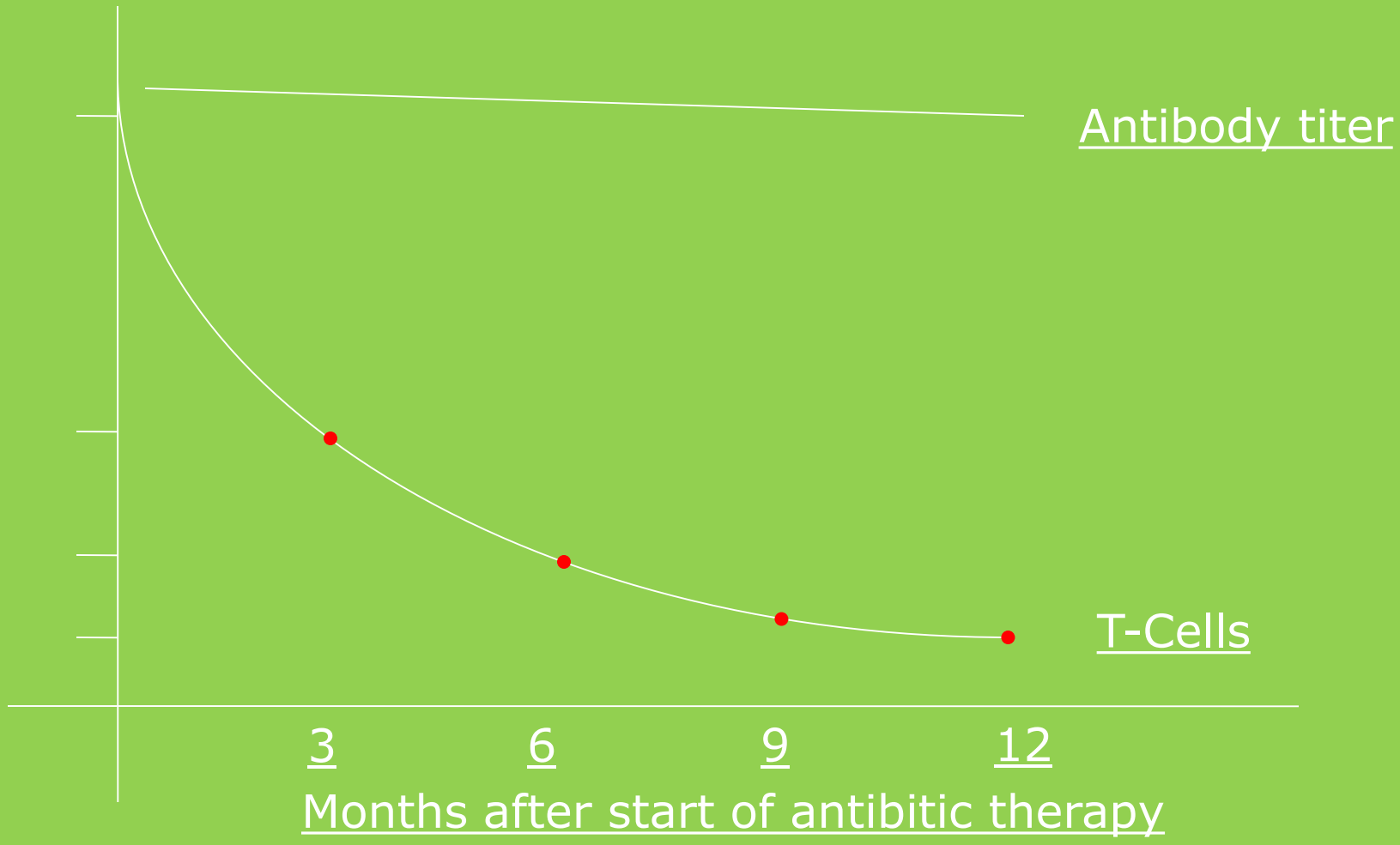
Report validated by

Armin Schwarzbach MD PhD

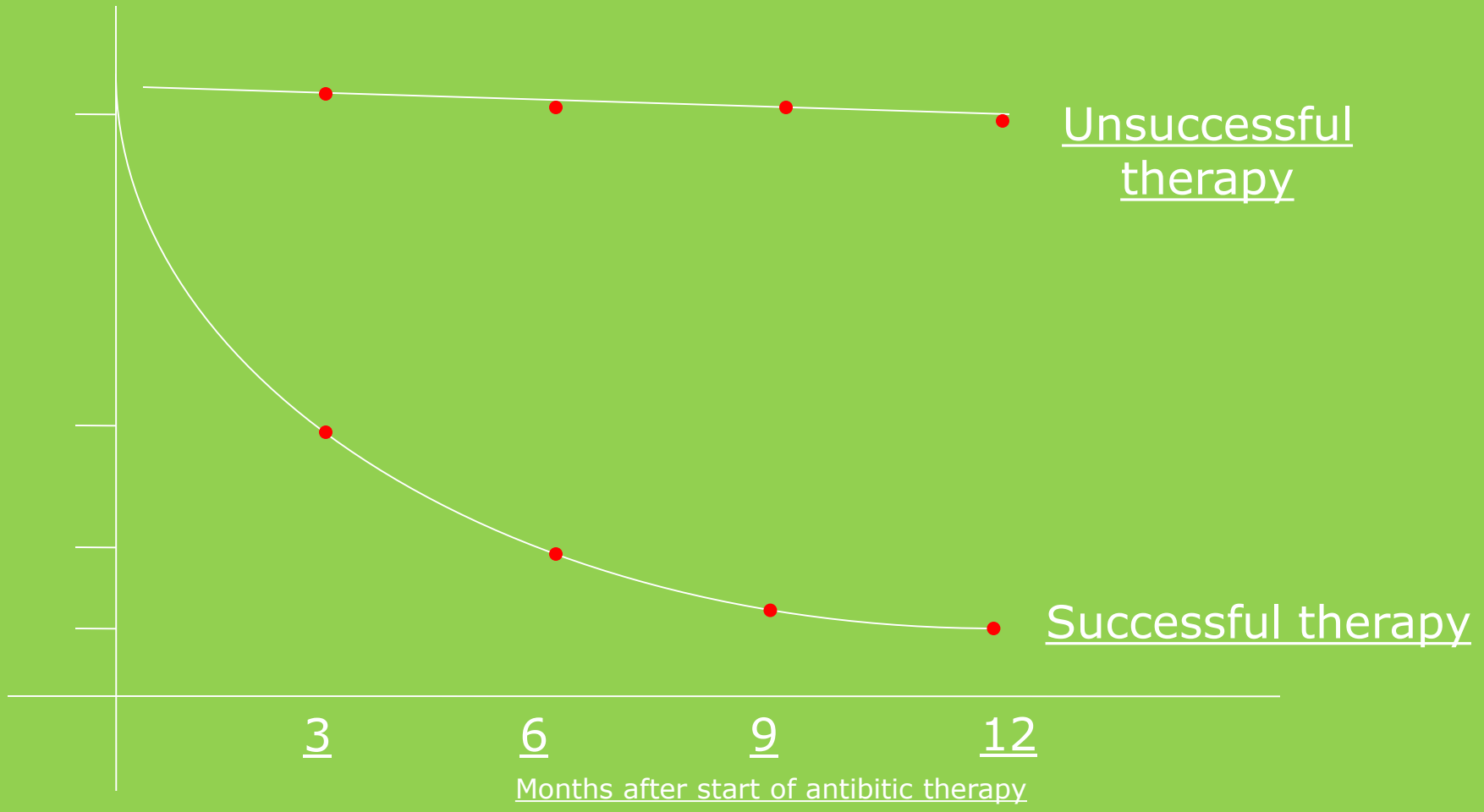
Specialist for laboratory medicine

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@arminlabs.com Amtsgericht Augsburg HRB 29350

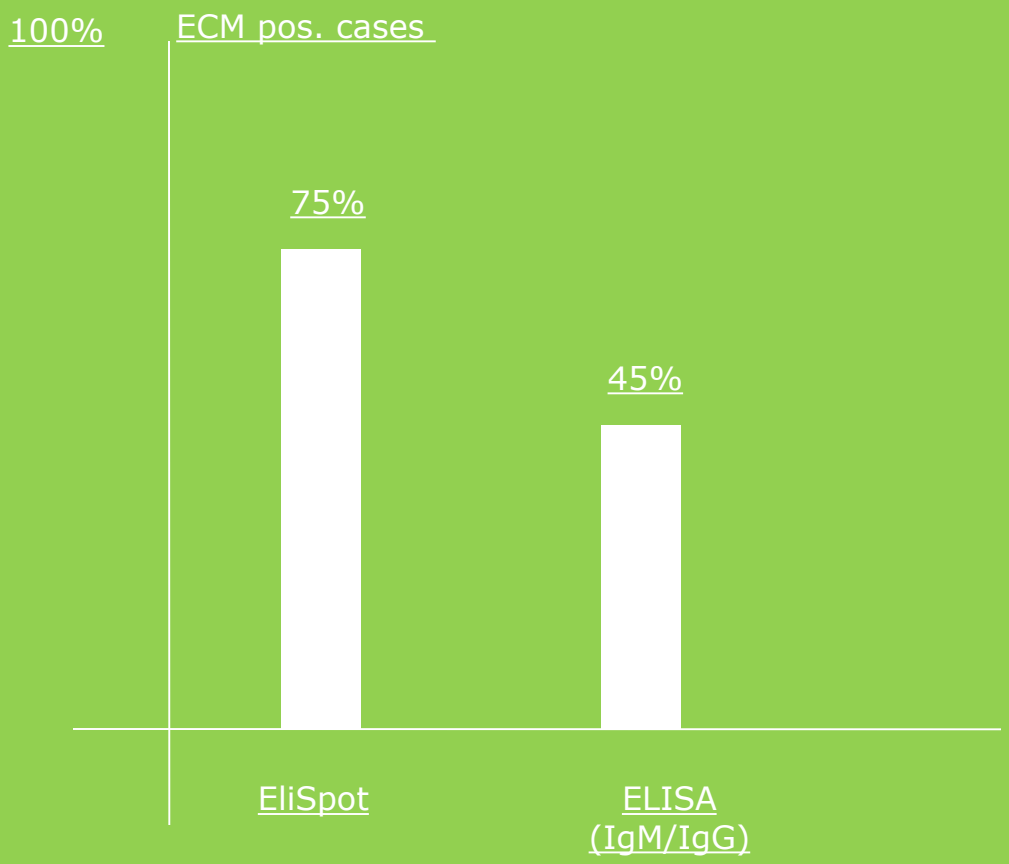
EliSpot during antibotics: "Staging" process of activity



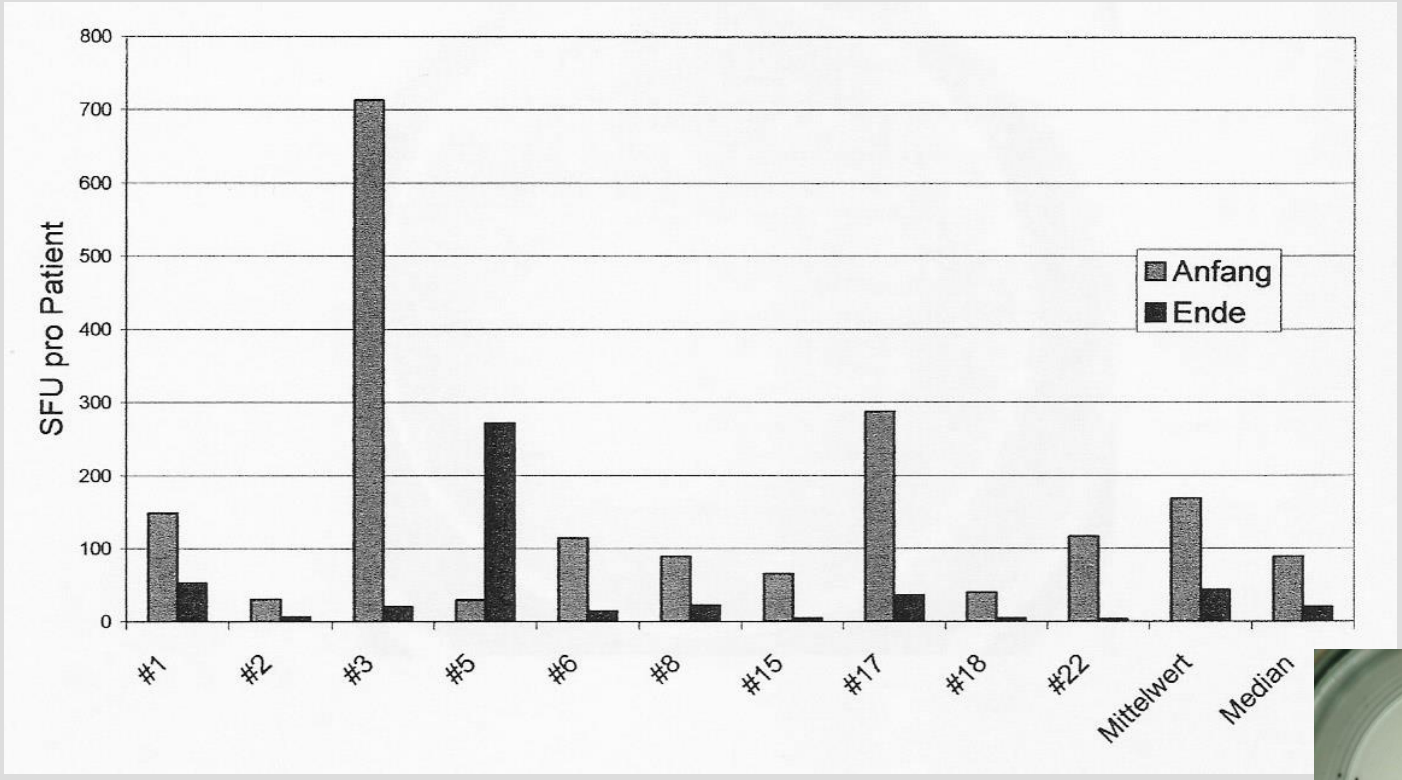
EliSpot during antibiotics: "Staging" process of activity



ELISA vs. EliSpot in Lyme stage I



EliSpot-LTT in chronic Lyme disease



Grey columns: before antibiotic therapy

Black columns: after antibiotic therapy

Pfeiffer et al., 2003

EliSpots can be done for

- Borrelia burgdorferi (3 subspecies: B.b. sensu stricto + B.b. garinii + B.b. afzelii)
- **Borrelia miyamotoi !**
- Chlamydia pneumoniae
- Chlamydia trachomatis
- Ehrlichia
- Yersinia species
- Epstein Barr Virus (EBV)
- Cytomegalovirus (CMV)
- Herpes Simplex Virus 1/2

Currently the EliSpot is available for:

- Borrelia burgdorferi (3 subspecies: B.b. sensu stricto + B.b. garinii + B.b. afzelii)
- Borrelia myamotoi
- Chlamydia pneumoniae
- Chlamydia trachomatis
- Ehrlichia
- Yersinia species
- Epstein Barr Virus (EBV)
- Cytomegalovirus (CMV)
- Herpes Simplex Virus 1/2

New: Borrelia miyamotoi EliSpot

arminlabs
DIAGNOSING TICK-BORNE DISEASES

ArminLabs GmbH
Zirbelstr. 58, 2nd floor
86154 Augsburg
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

ArminLabs GmbH – CEO: Armin Schwarzbach MD PhD
Zirbelstraße 58, 2nd floor - 86154 Augsburg - Germany - Phone: 0049 821 780 931 50 www.arminlabs.com
Email: info@arminlabs.com - VATReg-No.: DE815543871 - Amtsgericht Augsburg HRB 29350

LTT: Evidence-based literature

- Sigal LH et al, Cellular immune findings Lyme disease. Yale J Biol Med 1984, 57 : 595-8
- Sigal LH et al, Proliferative responses of mononuclear cells in Lyme disease. Reactivity to Borrelia burgdorferi antigens is greater in joint fluid than in blood. Arthritis Rheum 1986; 29: 761-9
- Dattwyler RJ et al, Seronegative Lyme disease. Dissociation of specific T- and B-lymphocyte responses to Borrelia burgdorfer. N Engl J Med 1988; 319: 1441-6
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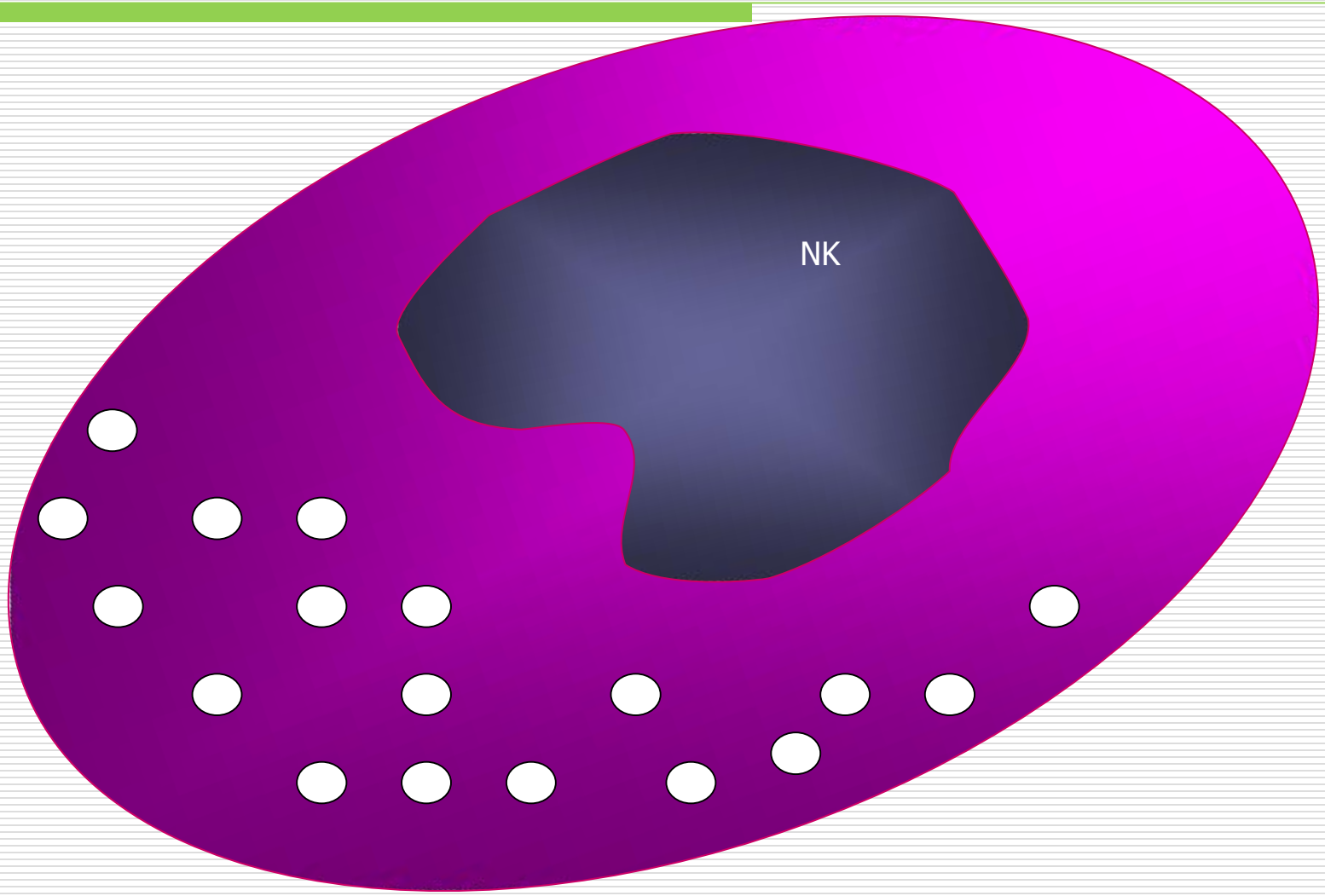
- Buechner SA et al, Lymphoproliferative responses to Borrelia burgdorferi in patients with erythema migrans, acrodermatitis chronica atrophicans, lymphadenosis benigna cutis and morphea. Arch Dermatol 1995; 131_ 673-7
- Breier F et al, Lymphoproliferative responses to Borrelia burgdorferi in circumscribed scleroderma. Brit J Dermatol 1996; 134: 285-91
- Huppertz et al, Lymphoproliferative responses to Borrelia burgdorferi in the diagnosis of Lyme arthritis in children and adolescents. Eur J Pediatr 1996; 155: 297-302
- Valentine-Thon E et al, A novel lymphocyte transformation test for Lyme borreliosis. Diagn Microbiol Infect Dis 2007; 57: 27-34
- Von Baehr V et al, Untersuchungen zur diagnostischen Wertigkeit des Lymphozytentransformationstestes bei Patienten mit Borreliose. J Lab Med 2007; 31(3): 149-158

LTT: Evidence-based literature

- "TB Elimination: Interferon-Gamma-Release Assays, www.cdc.gov/tb, May 2011
- Von Baehr, V.: The lymphocyte transformation test for the diagnosis of Lyme borreliosis, Clin Microbiol Infect. 2014 Oct 29
- 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
- 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
- Chenggang Jin et al.: An enhanced ELISPOT assay for sensitive detection of antigen specific T cells responses to Borrelia burgdorferi, Cells 2013, 2, 607-620; doi 10.3390/cells2030607
- Von Baehr, V. et al: The Lymphocyte Transformation Test for Borrelia detects active Lyme Borreliosis and verifies effective antibiotic treatment, Open Neurol. J. 2012, 6: 104-112

CD57+ Natural Killer cells (NK cells): CD57 flow cytometry



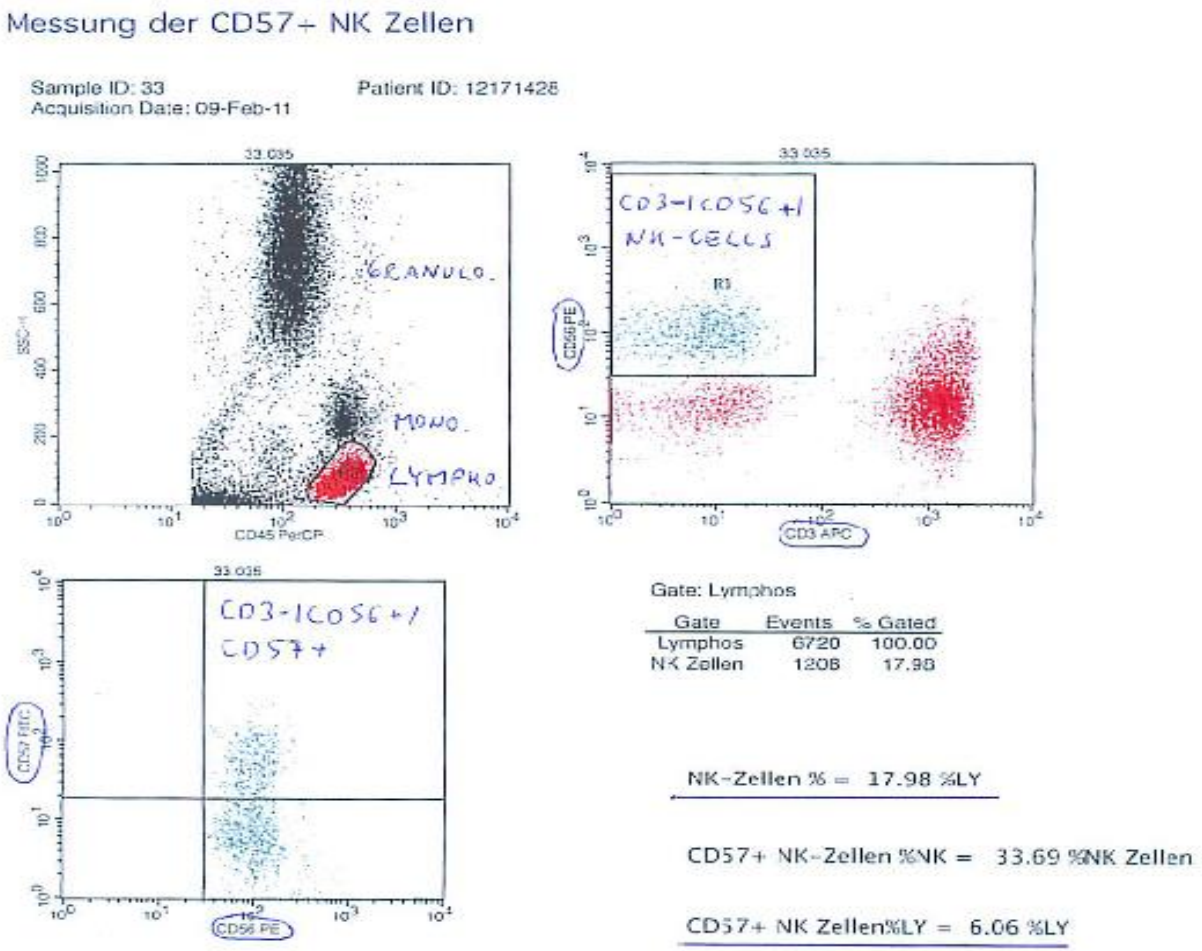
CD3-/CD57+ T-Lymphocytes

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

Reference range

Lyme patient:	< 130 /ul
Healthy:	> 130 /ul

Low CD57-count: Flow Cytometry



Low CD57-count: Laboratory report

No serological evidence for an infection with Anaplasma.

CD 57 Flow Cytometry				
Leucocytes		3.31	/ul	2.6-10.0
Peripheral Lymphocytes		34.10	%	18.0-51.0
Lymphocytes		11.29	/µl	468-5100
Natural killer cells		17.98	%	6-29
Natural killer cells		203	/µl	60-700
CD 57 positive NK-cells		6.06	%	2-77
CD 57 positive NK-cells	-	68	/µl	100-360

The CD57-cell-count is an indication for a chronic immune-suppressive situation caused by Borrelia burgdorferi.

Blood Count				
Hemoglobin		14.8	g/dl	14-18
Erythrocytes		4.94	mill./ul	4.5-5.9
Hematocrit		44.0	%	40-54
MCH		30.0	pg	28-32
MCHC		33.6	g/dl	32-36
MCV		89.1	fl	80-98
Thrombocytes		222	tsd/ul	150-350
Leucocytes	-	3.31	tsd/ul	4-10

Differential Blood Count			
Basoph. Granulocytes	0.60	%	0-2
Eosin. Granulocytes	3.30	%	0-4
Neutroph. Granulocytes	49.6	%	40-70
Lymphocytes	34.1	%	25-40
Monocytes	12.4	%	2-14

CD 57+: Literature

- Stricker RB, Winger EE. Normalization of the CD57 natural killer cell subset associated with prolonged antibiotictherapy in patients with chronic Lyme disease. Clin Immunol (2002) 103, 117–8.
- Stricker RB, Winder EE. Decreased CD57 lymphocyte subset in patients with chronic Lyme disease. Immunology Letters 76 (2001) 43-48
- Stricker RB, Burrascano JJ, Winger EE. Longterm decrease in the CD57 lymphocyte subset in a patient with chronic Lyme disease. Ann Agric Environ Med 2002, 9, 111-113
- Sincovics JG, Horvarth JC., Human natural killer cells: A comprehensive review. International Journal of Oncology (2005) 27, 5-47
- Focosi D, Petrini M. CD57 Expression on Lymphoma Microenvironment As a New Prognostic Marker Related to Immune Dysfunction Journal of Clinical Oncology, (2007) 25, 10, 1289-1291 American Society of Clinical Oncology. DOI: 10.1200/JCO.2006.10.2251
- Focosi D., Bestagno M., Burrone O., Petrini M . (2010) CD57 T lymphocytes and functional immune deficiency . Journal of Leukocyte Biology Volume 87
- Nielsen et al. Functional significance of CD57 expression on human NK cells and relevance to disease; Front Immunol. 2013 Dec 9;4:422
- Alco et al Decreased Numbers of CD57+CD3- Cells Identify Potential Innate Immune Differences in Patients with Autism Spectrum Disorder; in vivo 30: 83-90 (2016)

Low CD57+ cells in Autism Spectrum Disorder

In vivo *30*: 83-90 (2016)

Decreased Numbers of CD57+CD3− Cells Identify Potential Innate Immune Differences in Patients with Autism Spectrum Disorder

DARIO SINISCALCO[1,2,3], TATIANA MIJATOVIC[4], EUGENE BOSMANS[4], ALESSANDRA CIRILLO[5], PETER KRUZLIAK[6,7], VINCENT C. LOMBARDI[8], KENNY DE MEIRLEIR[9] and NICOLA ANTONUCCI[5]

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[2]*Centre for Autism – La Forza del Silenzio, Caserta, Italy;*
[3]*Cancellautismo – Non-profit Association for Autism Care, Florence, Italy;*
[4]*R.E.D. Laboratories, Zellik, Belgium;*
[5]*Biomedical Centre for Autism Research and Treatment, Bari, Italy;*
[6]*2nd Department of Internal Medicine, Faculty of Medicine, Masaryk University, Brno, Czech Republic;*
[7]*Laboratory of Structural Biology and Proteomics, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic;*
[8]*Nevada Center for Biomedical Research, Reno, NV, U.S.A.;*
[9]*Himmunitas vzw, Brussels, Belgium*

Abstract. *Background/Aim: Autism spectrum disorders (ASD) are complex, and severe heterogeneous neurodevelopmental pathologies with accepted but complex immune system abnormalities. Additional knowledge regarding potential immune dysfunctions may provide a greater understanding of this malady. The aim of this study was to evaluate the CD57+CD3− mature lymphocyte subpopulation of natural killer cells as a marker of immune dysfunction in ASD. Materials and Methods: Three-color flow cytometry-based analysis of fresh peripheral blood samples from children with autism was utilized to measure CD57+CD3− lymphocytes. Results. A reduction of CD57+CD3− lymphocyte count was recorded in a significant number of patients with autism. Discussion and conclusion: We demonstrated that the number of peripheral CD57+CD3− cells in children with autism often falls below the clinically accepted normal range. This implies that a defect in the counter-regulatory functions necessary for balancing pro-inflammatory cytokines exists, thus opening the way to chronic inflammatory conditions associated with ASD.*

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Key Words: Autism, CD57+CD3− lymphocytes, HNK-1, immune dysfunction.

Autism and autism spectrum disorders (ASD) are complex and severe, heterogeneous neurodevelopmental pathologies. Their multifactorial nature suggests they originate from the interactions of several genes with environmental, lifestyle and immunological factors (1). ASD diagnostic criteria substantially changed in the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) (2). The revised diagnosis represents a new and hopefully more accurate depiction of these disorders.

Despite significant progress in our understanding of the associated immunobiology of autism, the pathogenesis, as well as defined molecular mechanisms, remain unclear (3). The dramatic increase in the prevalence of autism (one in 42 boys and one in 189 girls) (4) underscores the urgent need for a broader understanding of the immunological underpinnings of this disease (3, 5). The DSM-5 criteria of autism diagnosis continue to limit this disorder to the evaluation of social, communication skills, and behavioral criteria. However, others are proposing to sub-type of ASD based on a combination of socio-behavioral and biomedical criteria (6-8). Our long-lasting clinical experience points towards a multifactorial disorder combining disorders from four major sub-groups, namely immune disorders, infection, intestinal dysfunction and environmental or maternal toxicant exposure (*i.e.* valproic acid, endocrine disrupting plasticizers, ethanol, air pollution, organophosphates and heavy metals). Sufficient immunological evidence presently exists to encourage the identification of more specific biological criteria (9), thus enabling better diagnostic categorization, and in turn, leading to better management of the complex clinical picture associated with ASDs.

0258-851X/2016 $2.00+.40

Basic diagnostic tests for chronic Lyme disease

1. **Borrelia IgM and IgG antibodies by Microarry (SeraSpot): Sensitivity 60%, specificity 99%**

2. **Borrelia Elispot (LTT) = current Borrelia activity: Sensitivity 84%, specificity 82-100%**

3. **CD3-/CD57+ cells = chronic Borrelia activity: Sensitivity 70%, specificity ? (low in Chlamydia and other bacterial infections)**

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

Monitoring 4-6 weeks after end of therapies to verify whether the therapy has been successful or not:

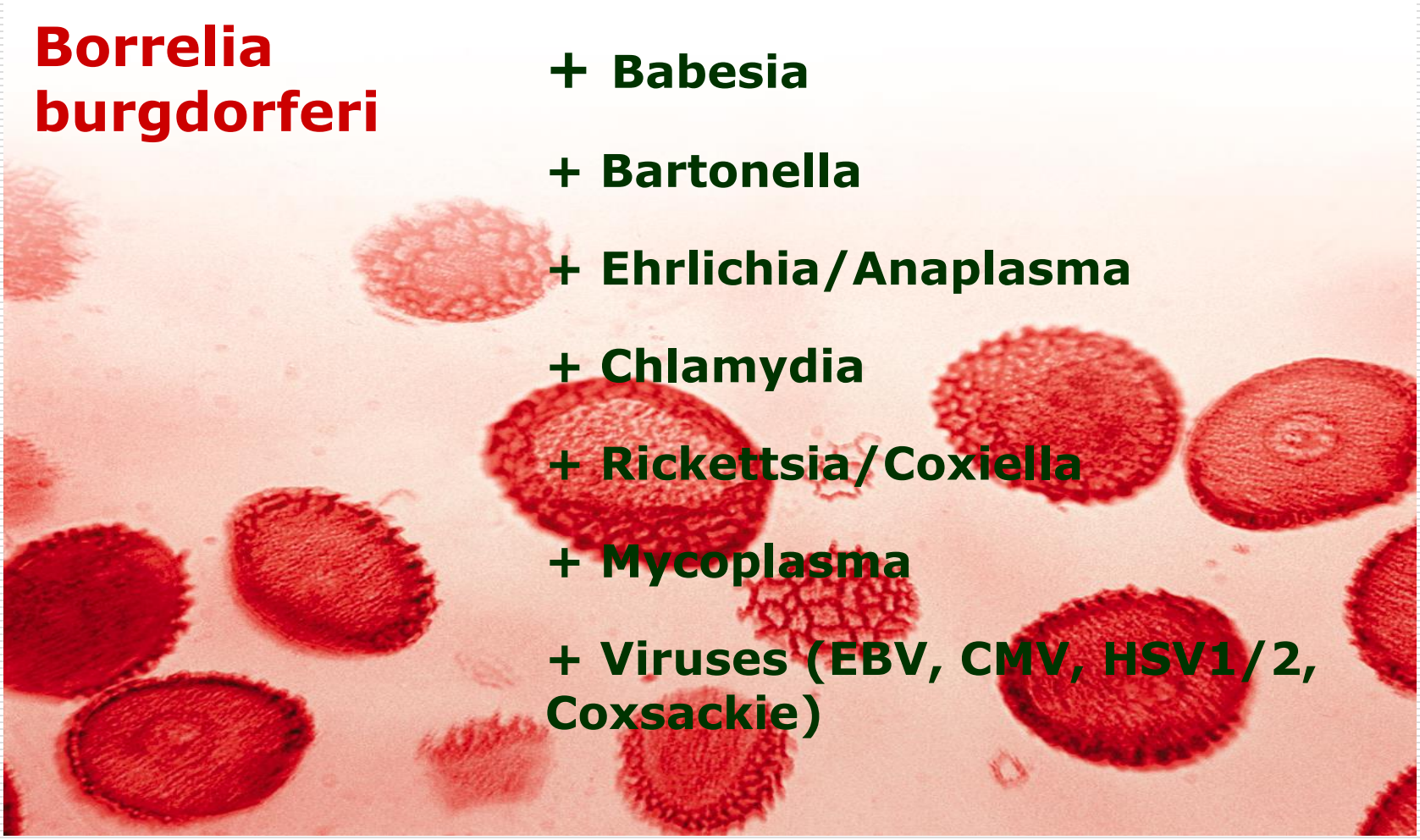
Laboratory STAGING process

Laboratory "Staging" for chronic Lyme disease

Screening	**Immunoblots: IgG, IgM with VlsE** **Borrelia Elispot LTT** **CD3-/CD57+ NK cells** **ANA titer ("para"- infectious)**
???	**Enzymimmunoassays** (ELISA): IgG,IgM, **VlsE** Direct detection of Borrelia by **PCR** technique
"STAGING" process before, during and after therapy	**Borrelia Elispot/LTT (actual activity)** **CD3-/CD57+ NK cells (chronic activity)** **ANA , IL10, IFN-gamma, TFN-alpha**

PART II:
Interpreting the laboratory results

LYME BORRELIOSIS and CO-INFECTIONS



Borrelia burgdorferi

+ Babesia

+ Bartonella

+ Ehrlichia/Anaplasma

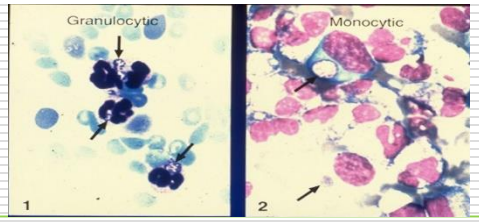
+ Chlamydia

+ Rickettsia/Coxiella

+ Mycoplasma

+ Viruses (EBV, CMV, HSV1/2, Coxsackie)

Ehrlichia / Anaplasma



Source: CDC

Bacteria: Ehrlichia chaffeensis, Anaplasma phagocytophilum (gram-negative, 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

Symptoms (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%)

Laboratory tests Ehrlichia/ Anaplasma

Ehrlichia-chaffeensis-IgG/IgM-antibodies

Anaplasma phagocytophilum-IgG/IgM-antibodies

Ehrlichia/Anaplasma Elispot (T-cell test)

Ehrlichia/Anaplasma-DNS-PCR in blood (EDTA-blood)

Leucopenia / Thrombocytopenia / Anemia

Elevated liver enzymes

Ehrlichia/Anaplasma: Therapy

- Macrolides (**Azithromycin, Clarythromycin**)
- Tetracycline (**Doxycyclin, Minocyclin**)
- Quinolones (Ciprofloxacin, Levofloxacin)
- Rifampicin (During pregnancy!)

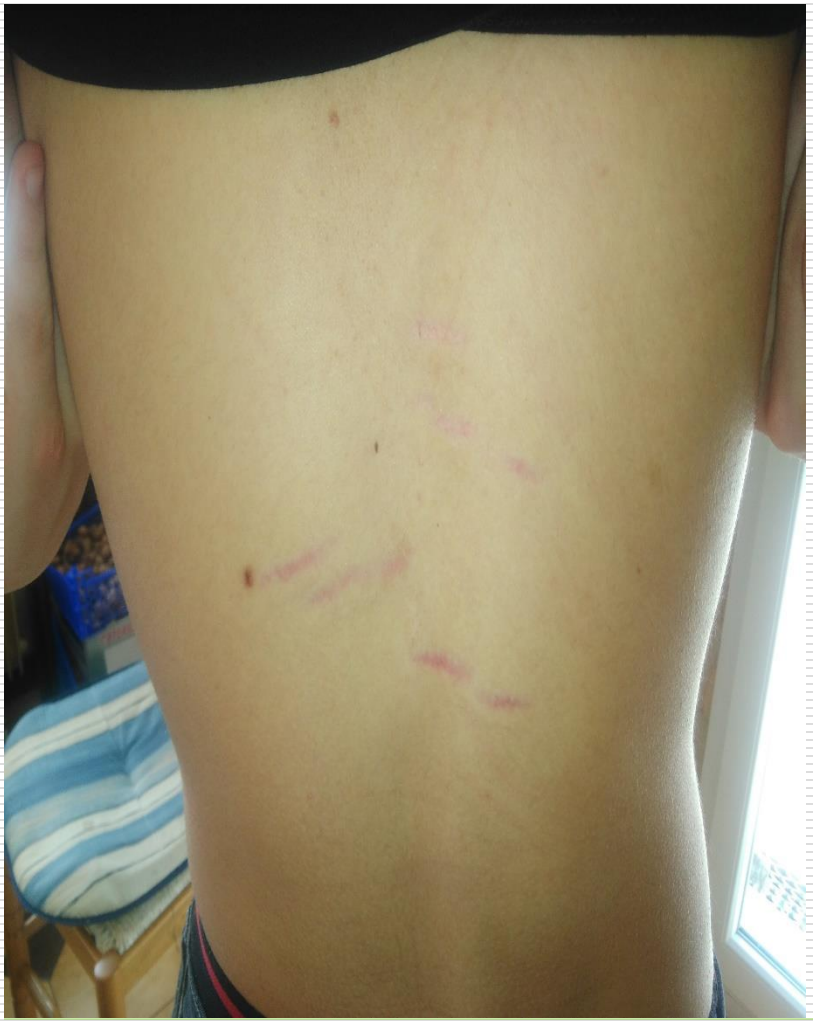
Bartonella

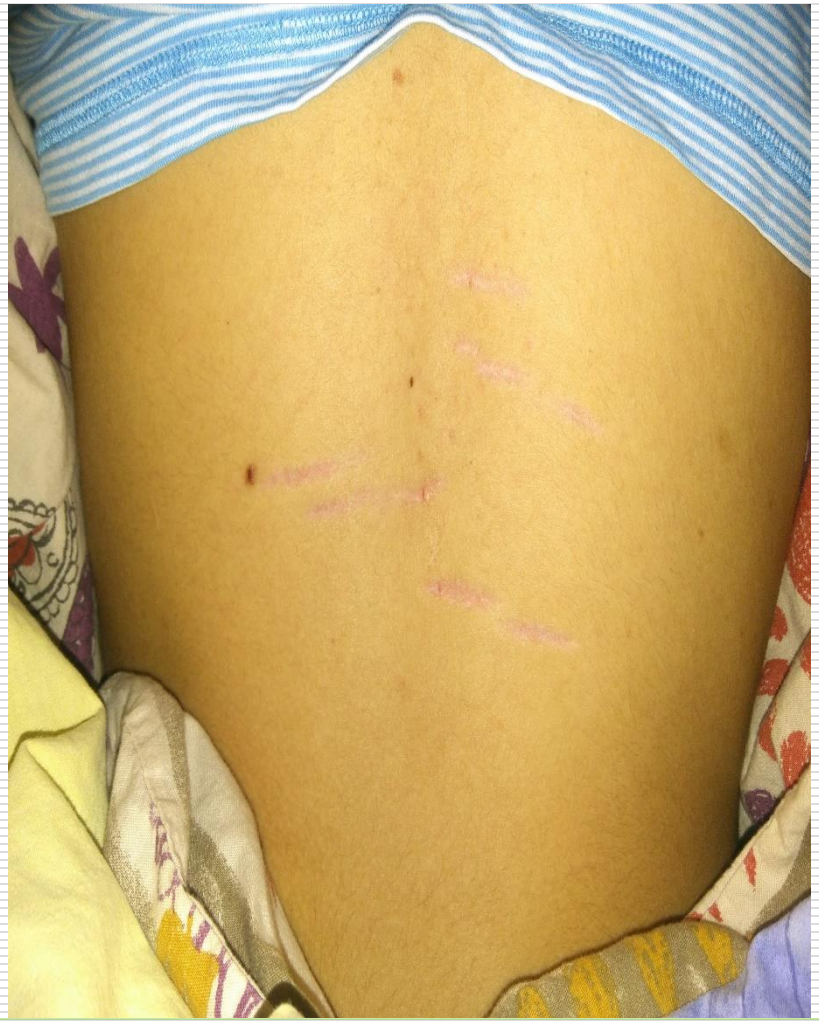
Bacteria: B. henselae (cat scratch disease), B. quintana (Trench fever, bacillary angiomatosis), B. bacilliformis (Carrion´s disease/Oroya fever), 5 other subspecies known to be pathogens for humans (gram-negative, facultative intracellular bacterium in endothelial cells/erythrocytes)

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

Symptoms (incubation time 3 - 38 days): tiredness (100%), 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; Complications: endocarditis, retinitis, epilepsy, aseptic meningitis, hepatosplenomegaly

Bartonella striae





Laboratory tests Bartonella

Bartonella henselae-IgG/IgM-antibodies

Bartonella quintana-IgG/IgM-antibodies

Bartonella-PCR in blood (EDTA)

Histology: PCR on biopsies (striae/hemangioma/lymphadenitis)

Elevated vascular endothelial growth factor (VEGF): seldom increased, but in such cases activity marker for monitoring

Bartonella: Therapy

- Macrolides (**Azithromycin, Clarythromycin**)
- Tetracyclin/**Doxycyclin**
- Quinolones (Ciprofloxacin, Levofloxacin)
- Rifampicin
- Ceftriaxone/Cefotaxime

Babesia

Bacteria: Babesia microti, Babesia divergens, Babesia duncani

Vector/transmission: 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)

Risk factors: Splenectomy, HIV, organ transplantation, blood transfusions

Laboratory tests Babesia

Babesia microti-IgG/IgM-antibodies

Babesia-DNS-PCR in blood (EDTA blood)

Babesia-FISH in blood (EDTA blood)

Blood smear

Rarely:

- Hamolytic anemia (erythrocytes, haptoglobin)
- Thrombocytopenia
- Leucocytopenia
- Increase of liver enzymes (sGOT, sGPT, sGGT)
- Increase of Creatinine, Urea
- Hemoglobinuria

Babesia: Therapy

- Clindamycin
- Malarone
- Atovaquon
- Lariam
- **Plaquenil (Hydroxychloroquin)** 2x200 mg/day
- **Artemisin** 2x400 mg/day

Rickettsia

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

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

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

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

Laboratory tests Rickettsia

Rickettsia-IgG/IgM-antibodies

Rickettsia PCR in blood (EDTA blood)

Rickettsia: Therapy

- **Doxycyclin**/Tetracyclin
- Ciprofloxacin
- Chloramphenicol
- Erythromycin (Children)

Chlamydia pneumoniae

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

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

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

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

Laboratory tests Chlamydia pneumoniae

Chlamydia pneumoniae

Chlamydia pneumoniae-IgA and Chlamydia pneumoniae-IgG:

half-life time of local-standing IgA-antibodies 2 weeks

New study Chlamydia pneumoniae-IgA in AIS: 60.8 %

"Chlamydia pneumoniae seropositivity in adults with acute ischemic stroke: A case-control study", NK Rai et al., Official Journal of Indian Academy of Neurology, 14, 2011 p. 93-97)

Chlamydia pneumoniae PCR in blood/sputum/pharyngeal secretion

Chlamydia pneumoniae: Therapy

- Macrolides (**Azithromycin, Clarythromycin**)
- **Doxycyclin/Minocyclin**
- Levofloxacin
- **Metronidazol**

Mycoplasma pneumoniae

Bacteria: Mycoplasma pneumoniae (gram-positive, intracellular)

Transmission: airborne infection, human to human, ticks?

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

Myalgic Encephalitis (ME), "Gulf War I syndrome", Guillain-Barre Syndrome, Amyatrophic Lateral Sclerosis (ALS)

Laboratory tests Mycoplasma pneumoniae

Mycoplasma pneumoniae-IgA and Mycoplasma pneumoniae-IgG-antibodies (half-life time of local-standing IgA-antibodies: 2 weeks)

Mycoplasma pneumoniae PCR or bacterial culture in blood/sputum/secretion

Mycoplasma: Therapy

- Macrolides (**Azithromycin, Clarythromycin**)
- **Doxycyclin/Minocyclin**
- **Metronidazol**
- Levofloxacin, Ciprofloxacin

Epstein Barr Virus (EBV)

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

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

Symptoms (incubation period several weeks): fatique, fever, flu-like symptoms, nausea, loss of appetite, lymphadenitis (swollen lymph nodes in the neck), rash, sore throat, weakness, sore muscles

Complications: enlarged spleen, swollen liver, association with Non-Hodgkin Lymphoma

Laboratory tests Epstein Barr Virus (EBV)

Epstein Barr Virus-IgG/IgM-antibodies

Epstein Barr Virus-Anti-EBNA-antibodies (former infection)

Epstein Barr Virus Early Antigen-antibodies (reactivated or chronic)

Epstein Barr Virus Elispot (T-cell test)

- EBV lytic antigen: sign for replication
- EBV latent antigen: sign for latency

Cytomegalovirus (CMV)

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

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

Symptoms (incubation period several weeks): fatique, fever, flu-like symptoms, lymphadenitis (swollen cervical lymph nodes), sore throat, splenomegaly

Complications: 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

Laboratory tests CMV

CMV-IgG/IgM-antibodies

CMV Elispot (T-cell test)

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

Virus: 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

Symptoms (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))

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

Laboratory tests HSV 1 / 2

Herpes Simplex Virus 1 / 2 – IgG/**IgA**/IgM – antibodies
(half-life time of local-standing IgA-antibodies: 2 weeks)

Herpes Simplex Virus 1 / 2 - Elispot (T-cell test)

Human Herpes Virus 6 (HHV6)

Virus: Human Herpes Virus 6 (obligate intracellular), double-stranded DNA virus, one of the Herpes viruses

Transmission: Saliva, latency in salivary glands, haematopoetic (blood-building) system

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

Complications: 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,

Laboratory tests HHV6

HHV6-IgG/IgM-antibodies

HHV6-DNS by PCR in blood (EDTA-blood)

Coxsackie Virus

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

Transmission: 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

Complications: 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

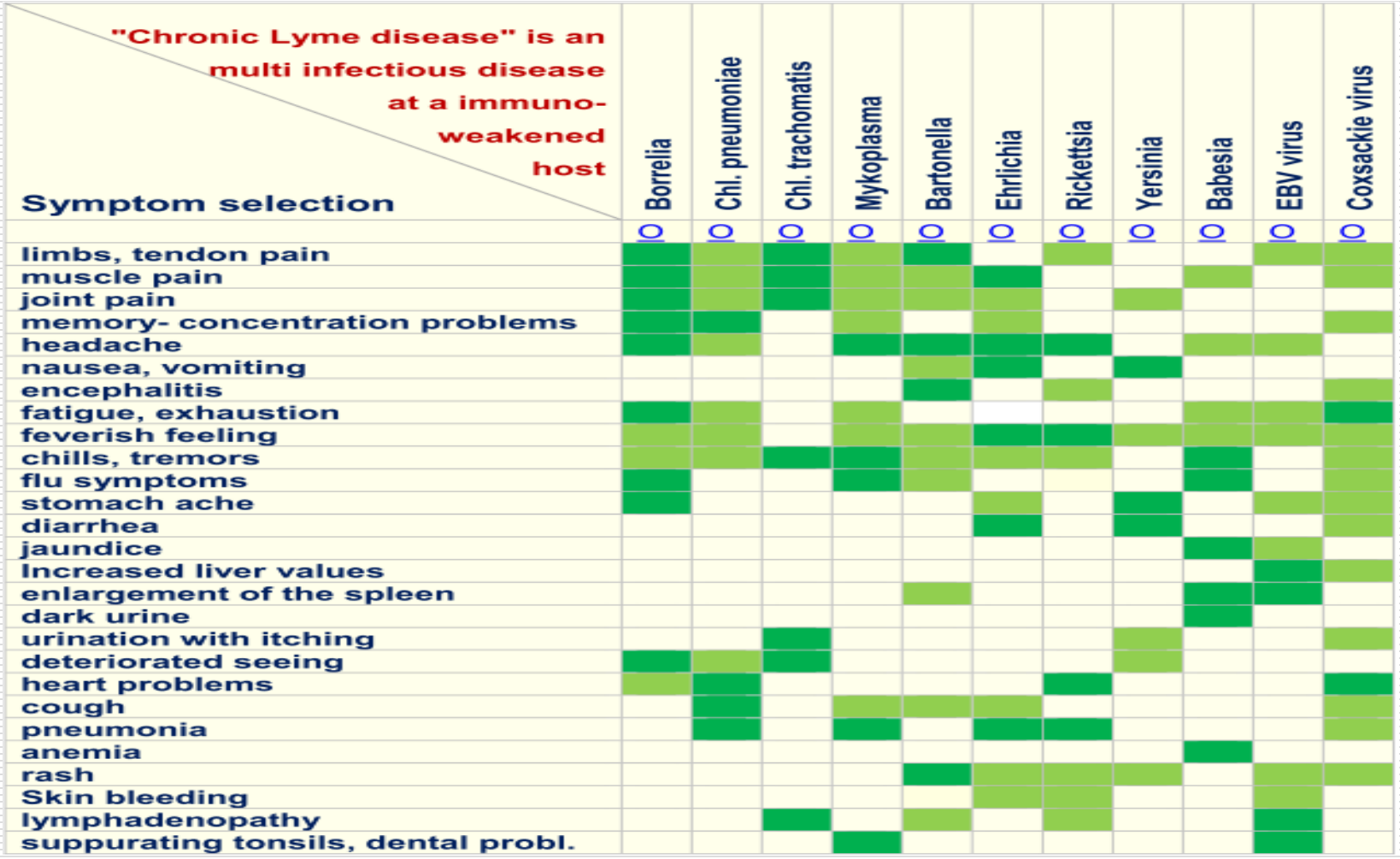
Laboratory tests Coxsackie Virus

Coxsackie Virus Type A7/B1 – IgG/**IgA**/IgM-antibodies
(half-life time of local-standing IgA-antibodies: 2 weeks)

Responsibility ?!

Who is responsible for this technique ?

MULTIPLE SYMPTOMS = MULTIPLE INFECTIONS



The coinfections checklist for patients, developed by Dr. Schwarzbach

arminlabs
DIAGNOSING TICK-BORNE DISEASES

ArminLabs GmbH
Zirbelstr. 58, 2nd floor
86154 Augsburg
GERMANY

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			
13	Heart problems, disturbance of cardiac rhythm			
14	Cough, expectoration			
15	Headache			
16	Impaired liver function/ liver laboratory values			
17	Pneumonia, bronchitis			
18	Swollen lymph nodes			
19	Tonsilitis			
20	Enlargement of the spleen			
21	Fatigue / exhaustion, intermittent or chronic CFS			
22	Muscle pain, muscle weakness			
23	Shivering, chill			
24	Blurred, foggy, cloudy, flickering, double vision			
25	Nausea, vomiting			
26	Dark urine			
27	Itching or pain when urinating			

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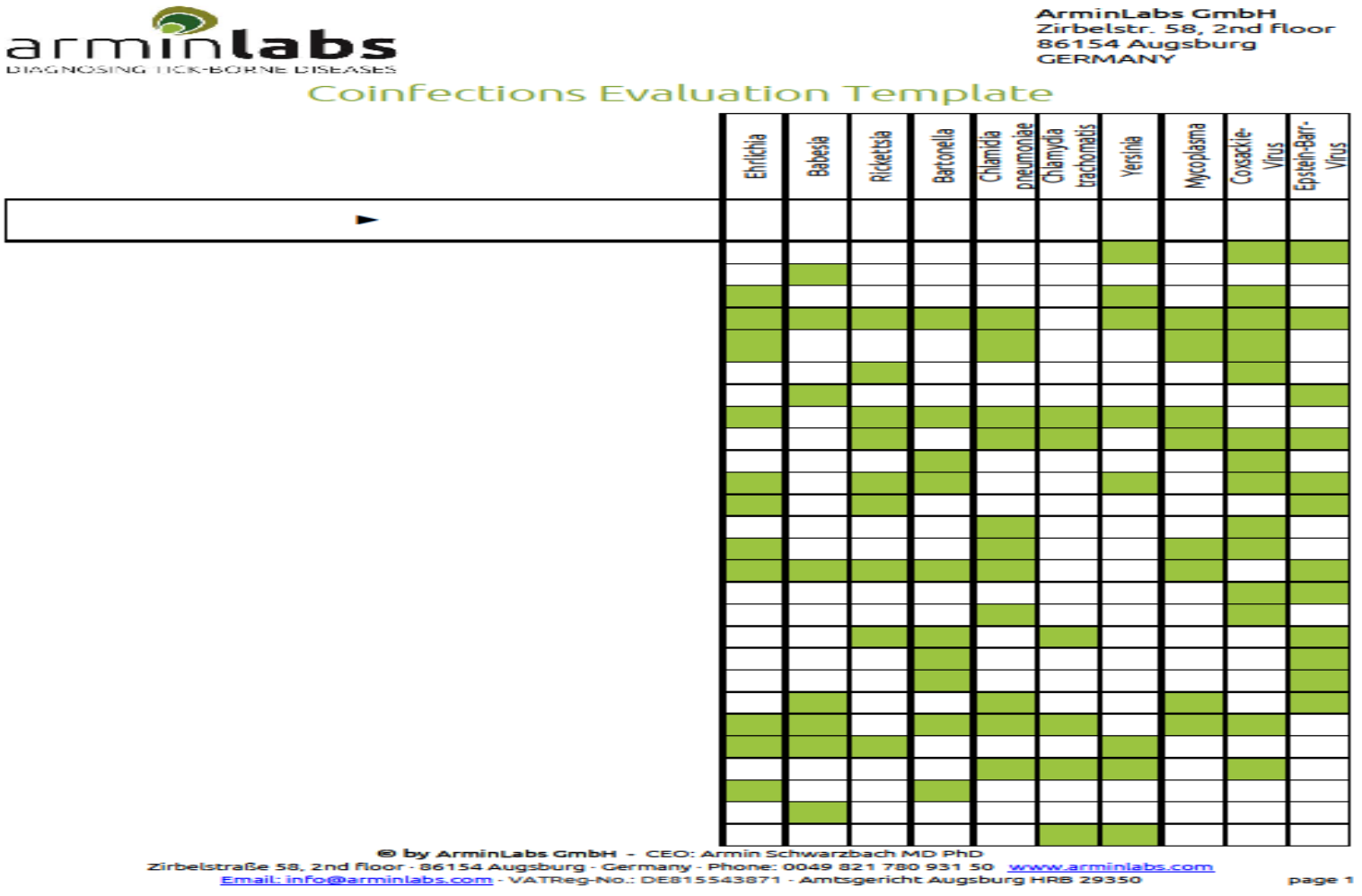
Email: info@arminlabs.com · VATReg-No.: DE815543871 · Amtsgericht Augsburg HRB 29350

Evaluation template for doctors/naturopaths, developed by Dr. Schwarzbach



Coinfections checklist: Patient 1

B.C.
Name, First name

15th Oct. 2010
Date

►	**Symptoms - Please tick the appropriate symptoms** (to be filled in by the patient)	X	**Score-Points** (to be filled in by the physician)	**Ranking**
01	Stomach-ache	X	Ehrlichia: **5**	**4**
02	Anaemia		Babesia: **5**	**4**
03	Diarhoea		Rickettsia: **5**	**4**
04	Fever or feverish feeling	X	Bartonella: **6**	**3**
05	Lack of concentration, memory disturbance, forgetfulness	X	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	X	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	X		
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	X		
22	Muscle pain	X		
23	Shivering	X		
24	Blurred vision			
25	Nausea, vomiting	X		
26	Dark urine	X		
27	Painful or ichty urinating			

Laboratory test results: Patient 1

		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 (immunoblot)				
Borrelia Blot IgG antibodies	+	**positive**		negative
		Bands: OspC (+),p41 +, 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.

Laboratory test results: Patient 1

		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 EliSpot				
CMV EliSpot	+	**4**	SI	<2

Laboratory test results: Patient 1

		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

Summary Patient 1

Coinfections checklist (symptoms):

Multiple infection with

Borrelia burgdorferi + Chlamydia pneumoniae + Mycoplasma pneumoniae + Coxsackie virus +

Epstein Barr Virus + Rickettsia + Yersinia

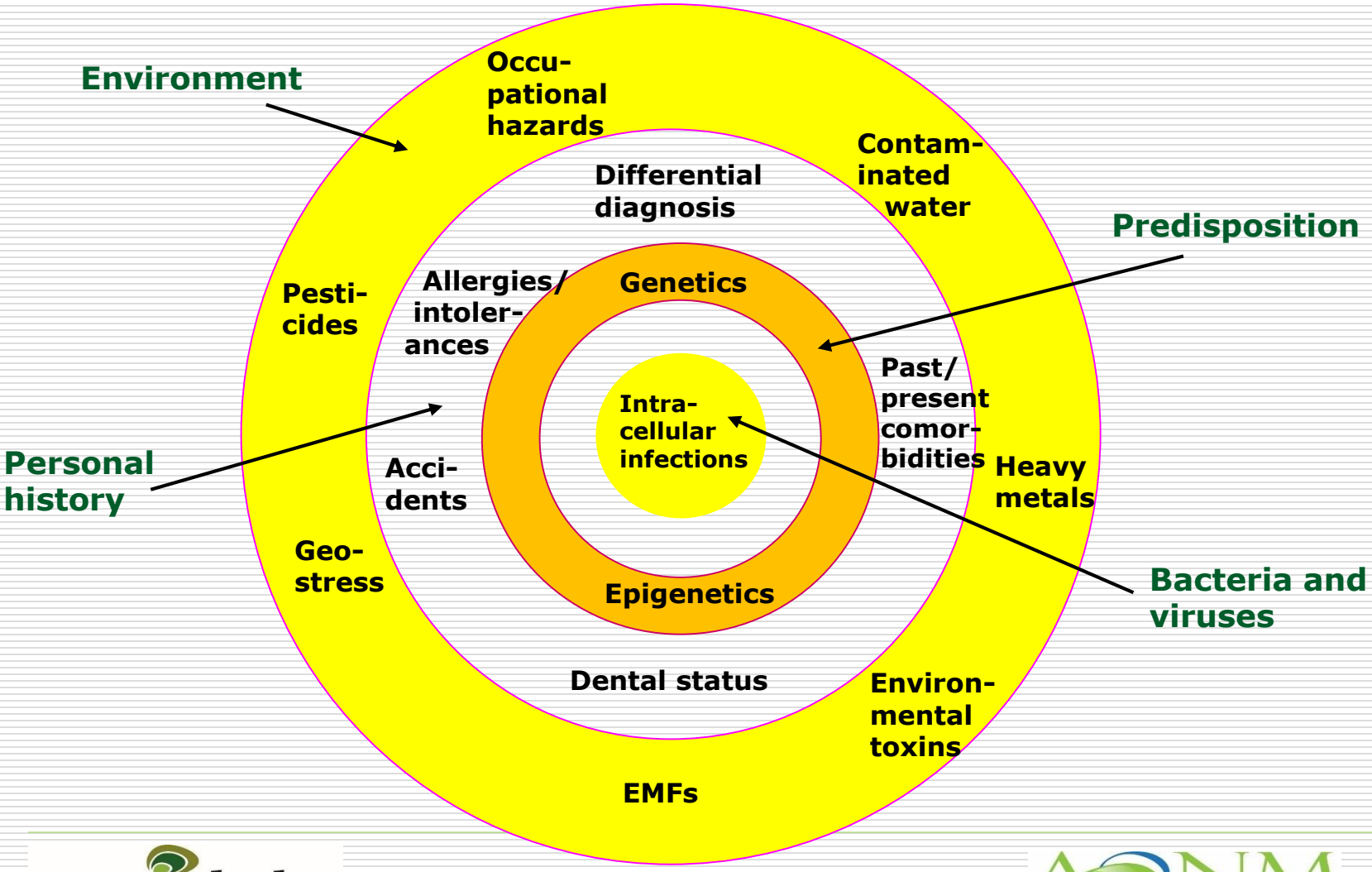
Laboratory test results:

Multiple infections with

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

5 bacteria + 3 viruses

Vital to begin by "peeling the onion“



Numerous criteria need considering to decide on therapies

How long has the infection lasted? Severity?
How old is the patient? Organ function?
What is the specific presentation? (arthritic? vascular? Neurological? neuropsychiatric?, etc.)
What other comorbidities?
Medications the PX is on?
Is there cardiac involvement?
Which have the higher load: bacterial infections or viruses? (CD3/CD57 cells)
Very important:
What coinfections?

If considering antibiotics …

In certain cases antibiotics may be the best approach, but need to check:
How well can the patient tolerate them? (e.g. beware of clarithromycin if patient has a CYP3A4 deletion: what SNPs in Phase 1 and Phase 2 of detoxification? Methylation defects?)

Oral or IV ?

Dysbiosis ?

Studies to back the usage?
Donta? Fallon? Burrascano? Cameron?

Evidence based antibiotic options

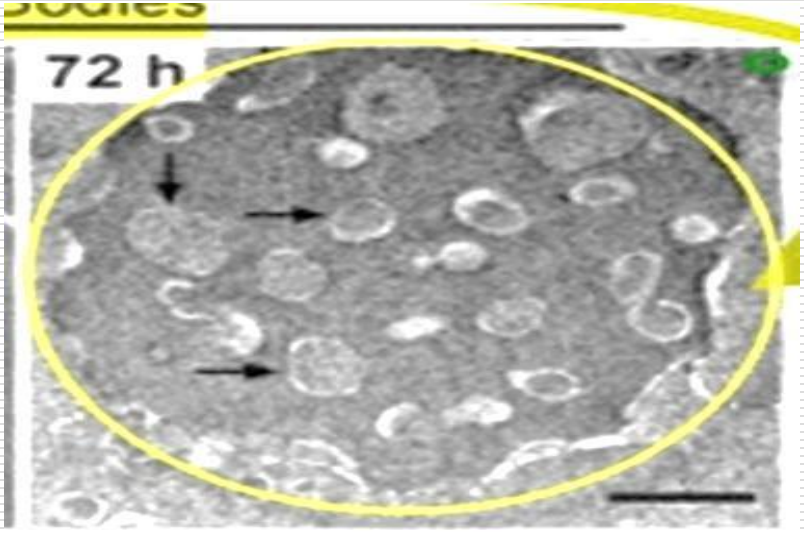
Table 5: Effective antibiotics in Lyme borreliosis

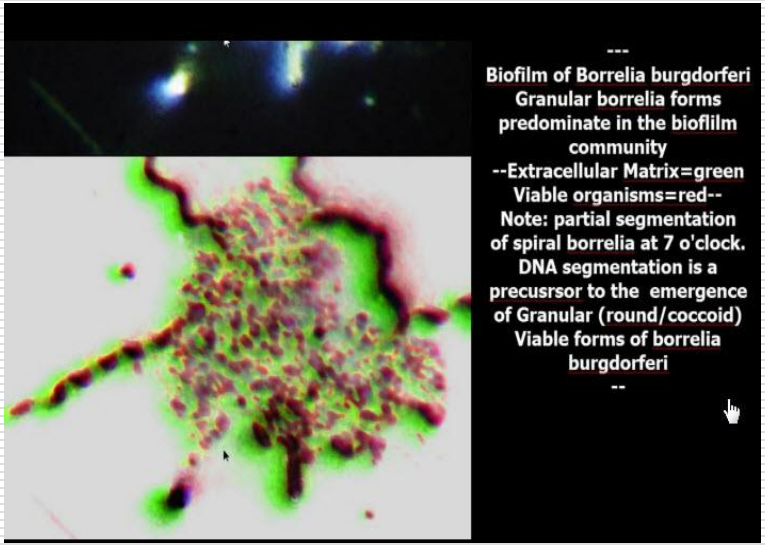
Antibiotic	Effective intra-cellularly	Enters the CSF	Effective against encysted forms	Plasma half-life
Betalactams				
Ceftriaxone	—	(+)*	—	8 hrs
Cefotaxime	—	(+)*	—	1 hr
Cefuroxime axetil	—	—	—	1 hr
Benzathine benzylpenicillin	—	+	—	3 days
Phenoxymethyl penicillin	—	—	—	30 min
Amoxicillin	—	—	—	1 hr
Tetracyclines and glycylcyclines				
Doxycycline	+	14%	—	15 hrs
Minocycline	+	40%	—	15 hrs
Macrolides**				
Clarithromycin	+	5%	—	4 hrs
Azithromycin	+	—	—	68 hrs tissue half-life
Nitroimidazoles				
Metronidazole	+	+	+	7 hrs
Co-drugs				
Hydroxychloroquine	+	+	+	30-60 days tissue half-life

* The betalactams have a poor ability to enter the CSF but, on account of their wide therapeutic spectrum, attain concentrations in the CSF which are clearly above the minimum inhibitory concentration (MIC).[74]

** Macrolides are not used in cases of QTc intervals (frequency-corrected QT intervals) of more than 440 milliseconds with heart rates between 60 and 100 bpm.[67,68]

Biofilms and pleomorphic forms





…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

Antibiotics: Influences on micro-biome and mitochondria

Randomized Trial of Longer-Term Therapy for Symptoms Attributed to Lyme Disease

Anneleen Berende, M.D., Hadewych J.M. ter Hofstede, M.D., Ph.D., Fidel J. Vos, M.D., Ph.D., Henriët van Middendorp, Ph.D., Michiel L. Vogelaar, M.Sc., Mirjam Tromp, Ph.D., Frank H. van den Hoogen, M.D., Ph.D., A. Rogier T. Donders, Ph.D., Andrea W.M. Evers, Ph.D., and Bart Jan Kullberg, M.D., Ph.D.

N Engl J Med 2016; 374:1209-1220 | March 31, 2016 | DOI: 10.1056/NEJMoa1505425

Comments open through April 6, 2016

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Patients with Lyme disease, which is caused by the *Borrelia burgdorferi* sensu lato complex (including *B. afzelii* and *B. garinii* in Europe), often report persistent symptoms.[1] These symptoms are also referred to as the post–Lyme disease syndrome or chronic Lyme disease and may occur after resolution of an erythema migrans rash or after other — possibly unnoticed — manifestations of early Lyme disease, regardless of whether a patient re… mainly with…

Previous ra… prolonged … persistent … debate abo… recommen… prolonged … Lyme Emp… shorter-ter… (ceftriaxon… of clarithro…

QUICK TAKE VIDEO SUMMARY

Although we did not find a significant benefit of longer-term antibiotic therapy, we did find that there were side effects from the use of antibiotics; however, these side effects were similar among the study groups. The majority of patients (68.6%) reported a drug-related adverse event. During the open-label ceftriaxone phase, the incidence of serious adverse events was low; no patient had a serious adverse event related to the use of catheters, and 4 of 280 patients (1.4%) had allergic reactions. During the randomized phase, photosensitivity related to doxycycline use and rash related to clarithromycin–hydroxychloroquine use were the most common adverse events, and no serious adverse event was thought to be related to the randomized study drugs or placebo.

The PLEASE study (Persistent Lyme Empiric Antibiotic Study Europe)

Natural remedies can be an option

A structured natural programme tailored to the patient's bacterial and viral infections that also strengthens their innate immunity and mitochondria

E.g. tinctures containing formulas in a liposomal base that can enter cells more easily and cross the blood-brain barrier, such as Andrographis, Astragalus, Artemisinin, Japanese Knotweed, Coriander, Stevia, Propolis f.e. Klinghardt's "Lyme Cocktail"

Or tinctures in a water/alcohol base: f.e. Nutramedix "Lee Cowden" remedies

Natural infusions for immune support

Myer's Cocktail IV

Vitamin C	5000 mg
Vitamin B1	100 mg
Vitamin B6	25 mg
Vitamin B12	1000 µg
Dexpanthenol	250 mg
Magnesium	3,125 mmol

in 500 ml isotonic saline solution

Infusion time around 60 minutes / 1 infusion per week for 4 weeks

John Myers MD, John Hopkins University, Baltimore, Maryland, USA

IV Glutathione

IV Alpha Lipoic Acid

Naturopathy: For example herbal support for TH1 cells

- Stevia (Stevia rebaudiana)
- Samento (*Pentacyclic Alkaloid Type Uncaria tomentosa*)
- Cumanda (*Cmpsiandra angustifolia*)
- Quina (*Cinchona calisaya)*
- Takuna (*Cecropia strigosa*)
- Noni *(Morinda citirfolia)*
- Banderol *(Otaba species)*
- Barberry *(Mahonia aquifolium)*
- Glucane (*Saccheromyces cerivisiae*)
- Procyanidin (*Vitis vinifera*)
- Melatonin
- DHEA
- Selen
- Zinc
- Magnesium

Naturopathy: For example herbal support for TH2 cells

- Myrrh (*Commiphora molmol*)
- Statins (block cholesterol synthesis) – red wine
- Progesterone

Multiple infections with bacteria + viruses: Complimentary therapy options for example…

A) Lee Cowden Protocol against viruses

Takuna + Burbur-Pinella + Samento + Serrapeptase + Stevia:

"For Coxsackie, EBV and CMV Dr. Cowden recommends the Cowden Support Program (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."

Additionally: **Stevia** (against Borrelia burgdorferi)

B) Nutrined Program against bacteria and viruses

1. Multimessenger (improves NK cells)
2. Artemisinin SOD (with Curcumin)
3. ATP Fuel (mitochondrial support)
4. Transfer Factor Lym Plus (working against Lyme, bacteria and viruses)
5. Messenger N1 (working against Mycoplasma/Chlamydia and viruses)
6. Lumbrokinase (working against Biofilms)

Complimentary therapy option: Stevia

Original article

European Journal of Microbiology and Immunology (2015)
DOI: 10.1556/1886.2015.00031

EFFECTIVENESS OF *STEVIA REBAUDIANA* WHOLE LEAF EXTRACT AGAINST THE VARIOUS MORPHOLOGICAL FORMS OF *BORRELIA BURGDORFERI IN VITRO*

P. A. S. Theophilus, M. J. Victoria, K. M. Socarras, K. R. Filush, K. Gupta, D. F. Luecke, E. Sapi*

Department of Biology and Environmental Science, University of New Haven, West Haven, CT, USA

Received: September 7, 2015; Accepted: October 26, 2015

Lyme disease is a tick-borne multisystemic disease caused by *Borrelia burgdorferi*. Administering antibiotics is the primary treatment for this disease; however, relapse often occurs when antibiotic treatment is discontinued. The reason for relapse remains unknown, but recent studies suggested the possibilities of the presence of antibiotic resistant *Borrelia* persister cells and biofilms.

In this study, we evaluated the effectiveness of whole leaf Stevia extract against *B. burgdorferi* spirochetes, persisters, and biofilm forms *in vitro*. The susceptibility of the different forms was evaluated by various quantitative techniques in addition to different microscopy methods. The effectiveness of Stevia was compared to doxycycline, cefoperazone, daptomycin, and their combinations. Our results demonstrated that Stevia had significant effect in eliminating *B. burgdorferi* spirochetes and persisters. Subculture experiments with Stevia and antibiotics treated cells were established for 7 and 14 days yielding, no and 10% viable cells, respectively compared to the above-mentioned antibiotics and antibiotic combination. When Stevia and the three antibiotics were tested against attached biofilms, Stevia significantly reduced *B. burgdorferi* forms. Results from this study suggest that a natural product such as Stevia leaf extract could be considered as an effective agent against *B. burgdorferi*.

Keywords: *Borrelia burgdorferi*, biofilms, persister cells, *Stevia rebaudiana*, antibiotic resistance

Abbreviations: ATCC – American type culture collection; BSK-H – Barbour–Stoner–Kelly H; CefP – cefoperazone; DapM – daptomycin; DoxC – doxycycline; EPS – extracellular polymeric substances; Log phase – logarithmic phase; PBS – phosphate buffered saline; PI – propidium iodide; PTLDS – post-treatment Lyme disease syndrome

Introduction

Lyme disease is a leading tick-borne multisystemic disease caused by the spirochete *Borrelia burgdorferi*. The bacterium is transmitted by Ixodes ticks, which could feed on white-footed mice, rodents, deer, and birds [1, 2]. In the United States, there are approximately 300,000 people diagnosed with Lyme disease each year [3]. The frontline treatment for Lyme disease is antibiotics such as doxycycline for adults and amoxicillin for children [4–8]. These antibiotics are found effective in most cases of patients diagnosed with Lyme disease [5–8]. However, according to the Centers for Disease Control (CDC), approximately 10–20% of the Lyme disease patients treated with antibiotics for a recommended 2 to 4 weeks experienced symptoms of fatigue, pain or joint and muscle aches [9]. In some patients, the symptoms even lasted for more than 6 months [9]. This condition was termed as "post-treatment Lyme disease syndrome (PTLDS)" or "chronic Lyme disease" [9].

The mechanism associated with this condition in patients remains unclear. Though not proven, there are a couple of suggested explanations, such as the inability of the immune system to completely clear *B. burgdorferi* persisters [10], or due to the presence of antigenic debris, which might cause immunological responses [11]. Another possibility of *Borrelia* evading the host immune clearance after antibiotic treatment is not well understood [12, 13].

Previous *in vivo* studies on mice, dogs, and nonhuman primates have shown that *B. burgdorferi* could not be fully eliminated by various antibiotics such as doxycycline, ceftriaxone, and tigecycline. Also, a recent study had demonstrated the presence of *Borrelia* DNA in mice following 12 months of antibiotic treatment [14]. However, the culturing of viable organisms in *Borrelia* growth media could not be achieved in these studies [14–17]. A recent study reported the presence of *Borrelia* DNA from a patient with PTLDS after antibiotic treatment [18]. Prospective clinical studies demonstrated no significant effective antibiotic therapy and failed to show evidence of the continued presence of

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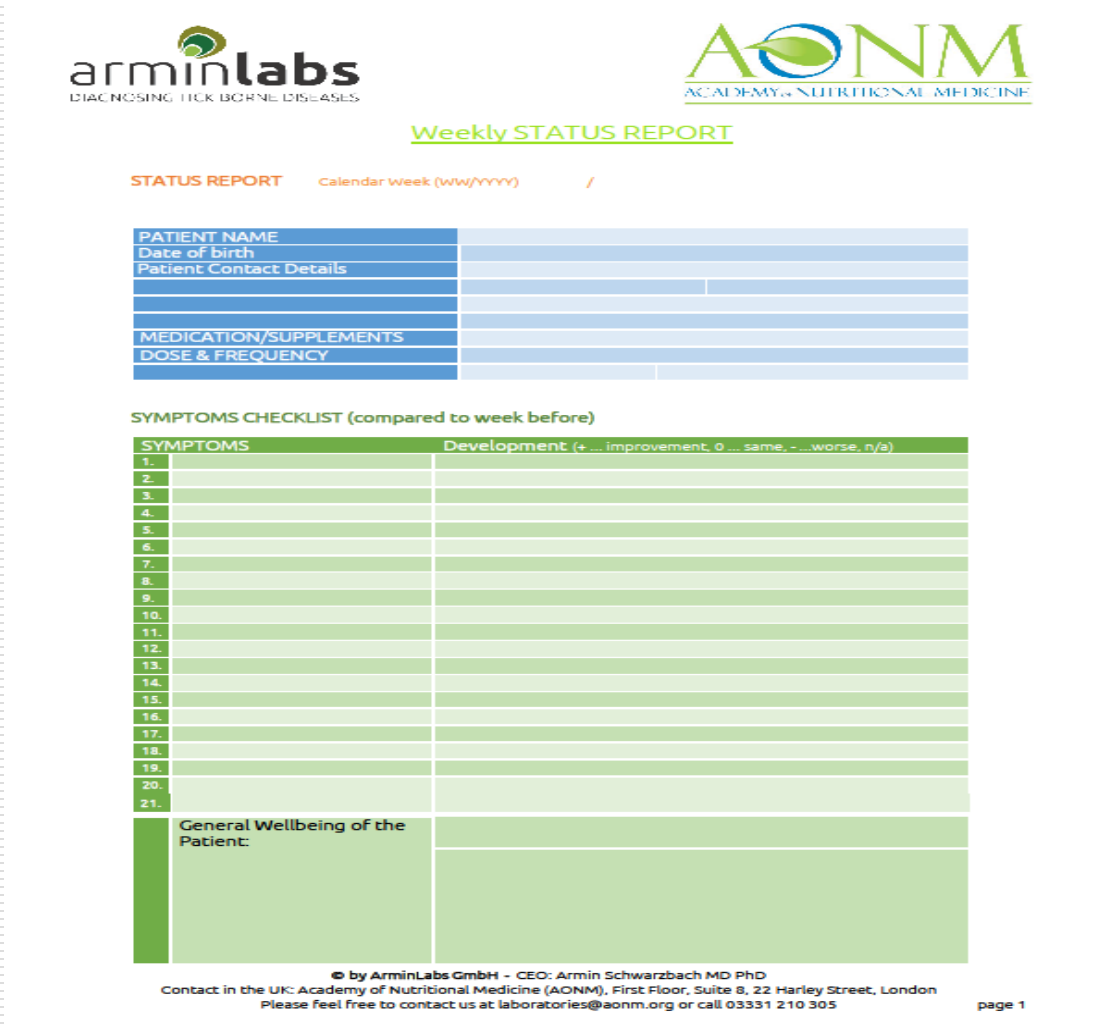
New development:
Monitoring of symptoms by Weekly status report



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DIAGNOSING TICK BORNE DISEASES

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Weekly STATUS REPORT

STATUS REPORT Calendar week (WW/YYYY) /

PATIENT NAME		
Date of birth		
Patient Contact Details		
MEDICATION/SUPPLEMENTS		
DOSE & FREQUENCY		

SYMPTOMS CHECKLIST (compared to week before)

	SYMPTOMS	Development (+ ... improvement, 0 ... same, - ...worse, n/a)
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.		
12.		
13.		
14.		
15.		
16.		
17.		
18.		
19.		
20.		
21.		
	General Wellbeing of the Patient:	

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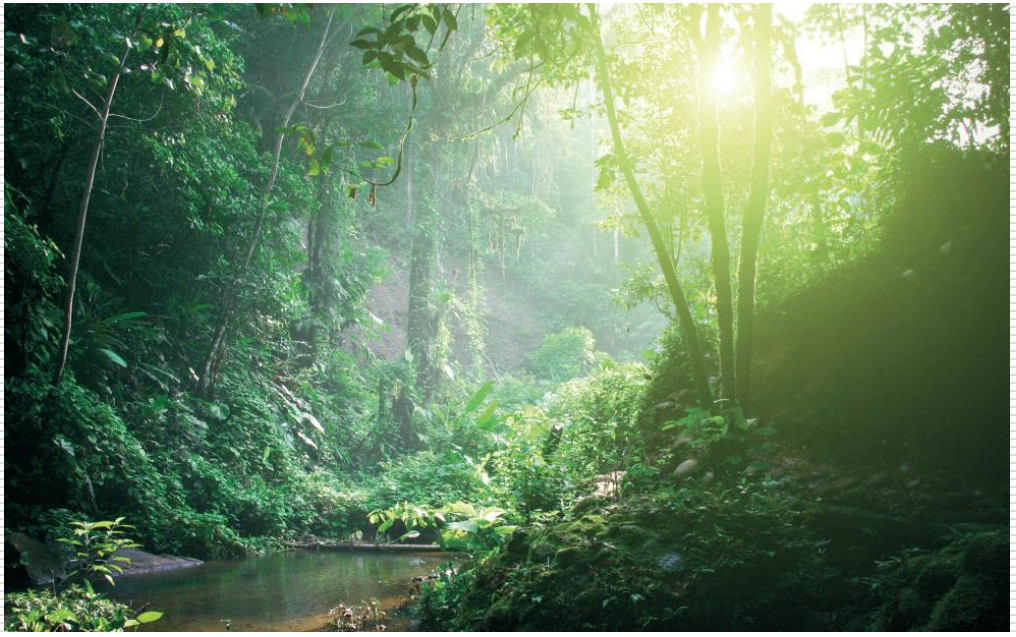
Contact in the UK: Academy of Nutritional Medicine (AONM), First Floor, Suite 8, 22 Harley Street, London
Please feel free to contact us at laboratories@aonm.org or call 03331 210 305 page 1

Natural Therapies for Chronic Illness with Judy Rocher and Professor Eva Sapi

Saturday 26 November 2016, 10:00 - 16:00

Holiday Inn London – Regent's Park, Carburton Street



An interactive workshop on the most up-to-date nutritional and herbal protocols for Lyme Disease and its co-infections.
£50.00
https://www.eventbrite.co.uk/e/natural-therapies-for-chronic-illness-with-judy-rocher-tickets-26581022554

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Thank you very much for your attention!

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