Multiple infections by Borrelia burgdorferi and other tick-borne pathogens

Complexity of symptoms, diagnostic tests and consequences for therapy

Conference 8th March 2015
Holiday Inn Regent's Park, London, England

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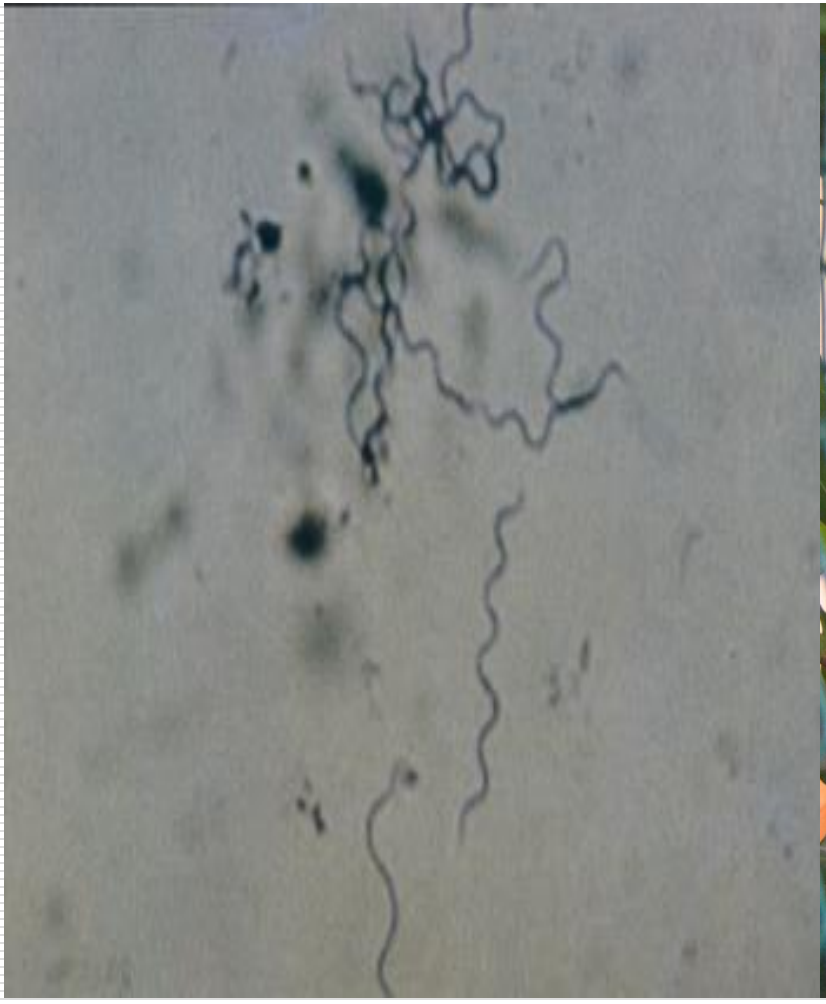
PART 1: LABORATORY TESTS



It remains difficult to culture Lyme Disease except at the margins of an EM rash

Cultures are difficult to obtain due to:

- the small numbers
- slow growth of the organism
- need for special culture media
- can take as long as 10.5 months

Cultures have rarely been positive in knee fluid and CSF.

There are no tests to determine the susceptibility pattern for different antibiotics for an individual LD patient.

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PCR has poor sensitivity

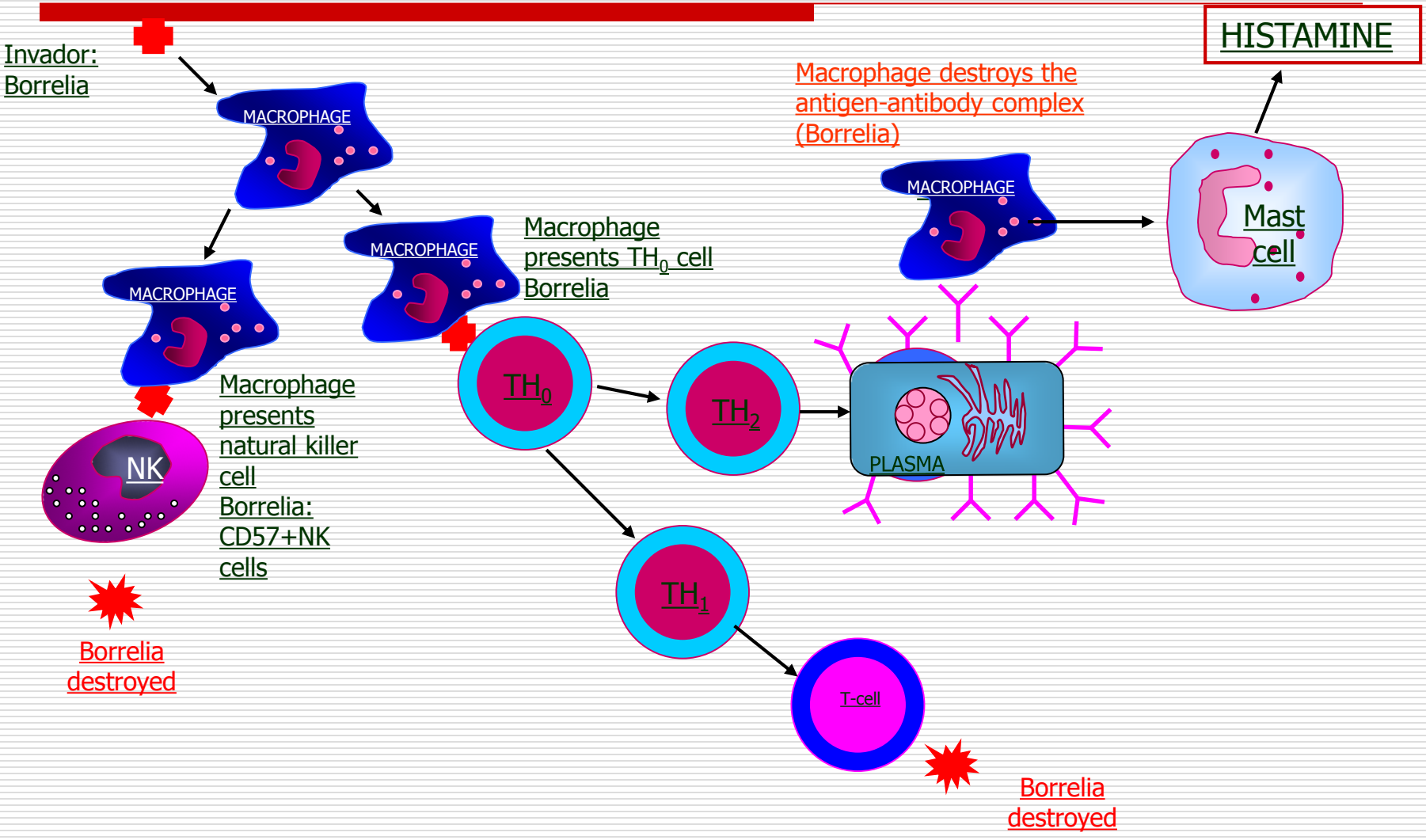
Polymerase chain reaction (PCR) technique amplifies small traces of bacterial DNA

Sensitivity is 5 - 25% in actual practice.

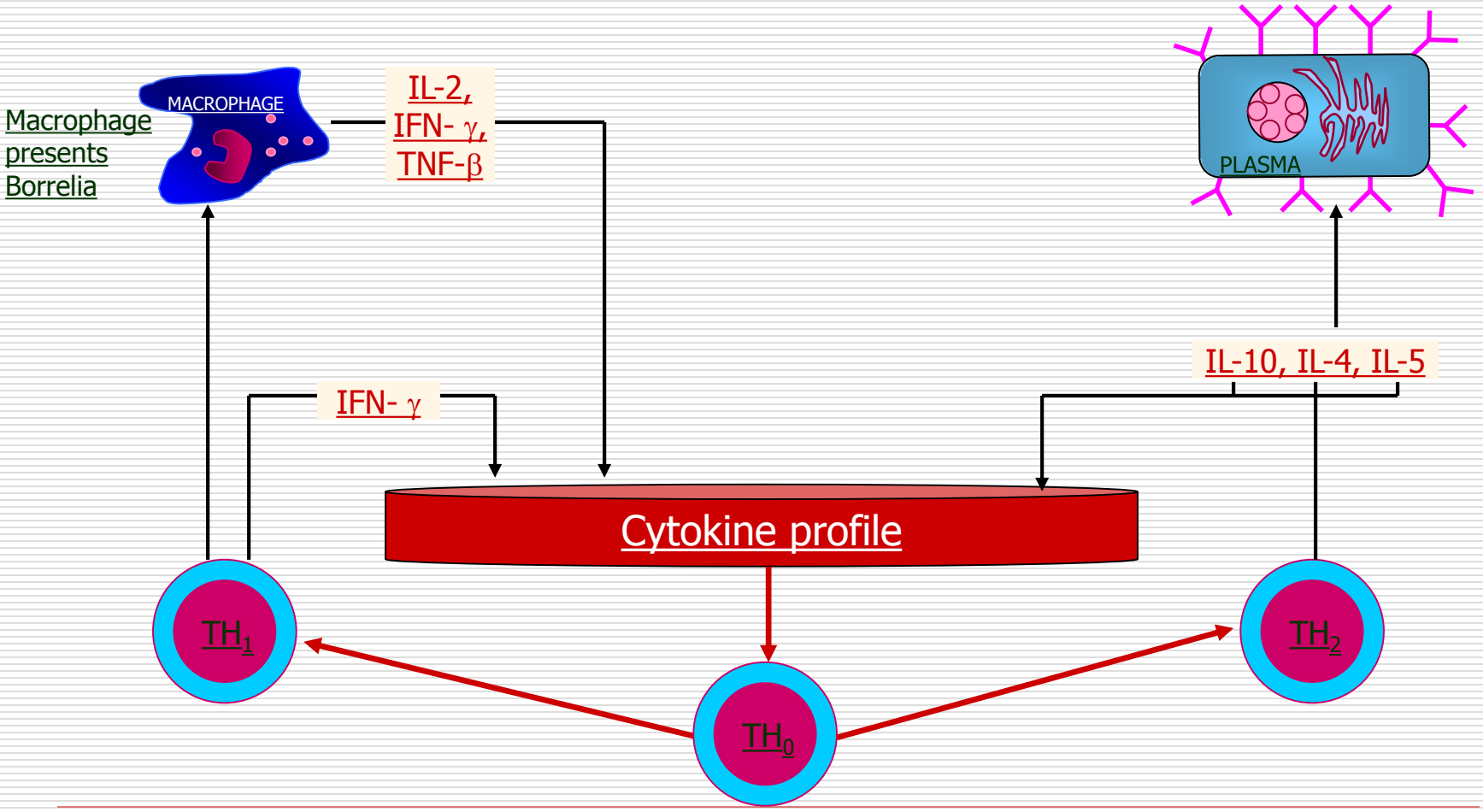
The low sensitivity in blood
- low level of spirochetes in blood
- lack of spirochetemia
- transient spirochetemia
- presence of PCR inhibitors in host blood

Potential for false positives

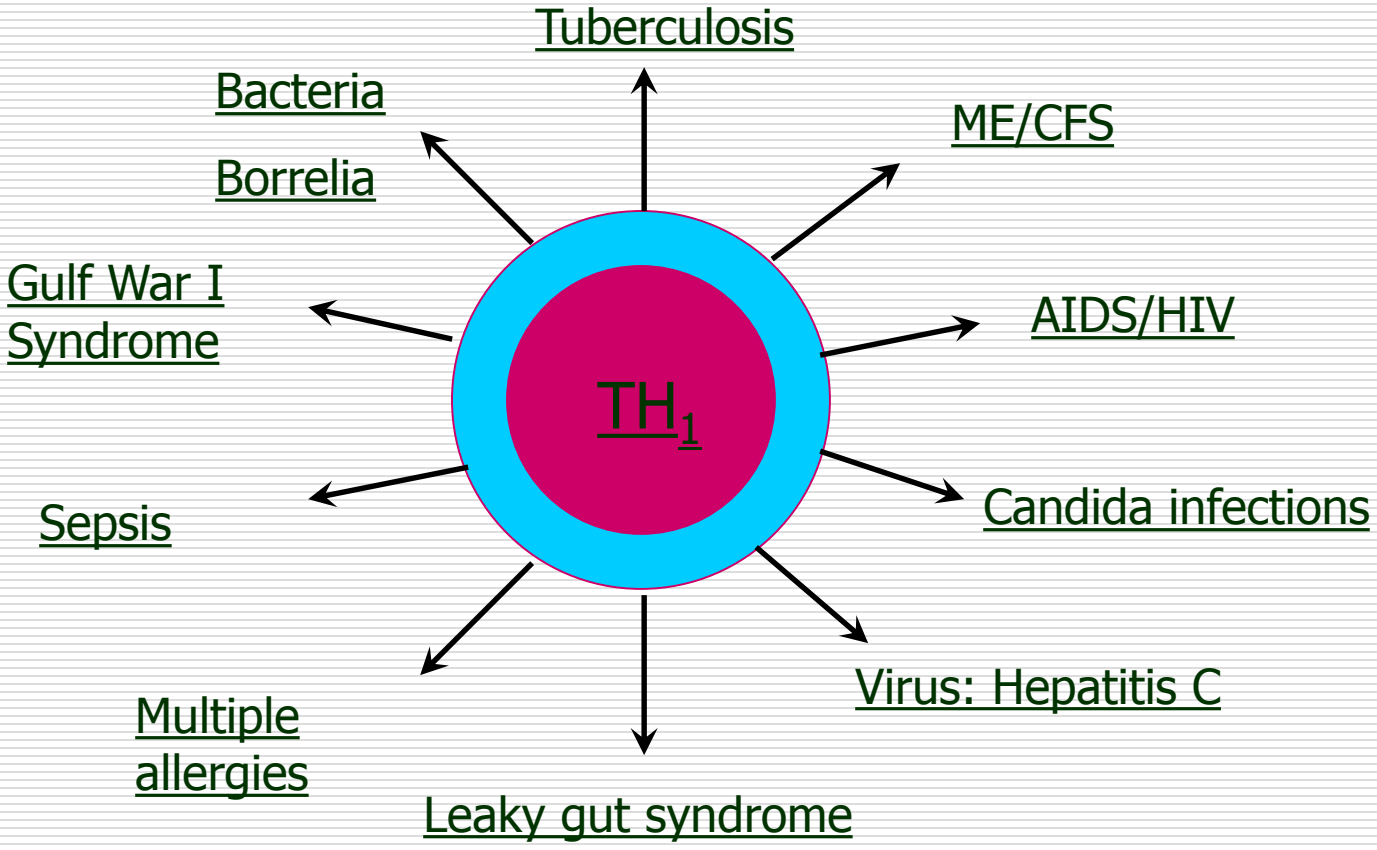
Principles of immune defense



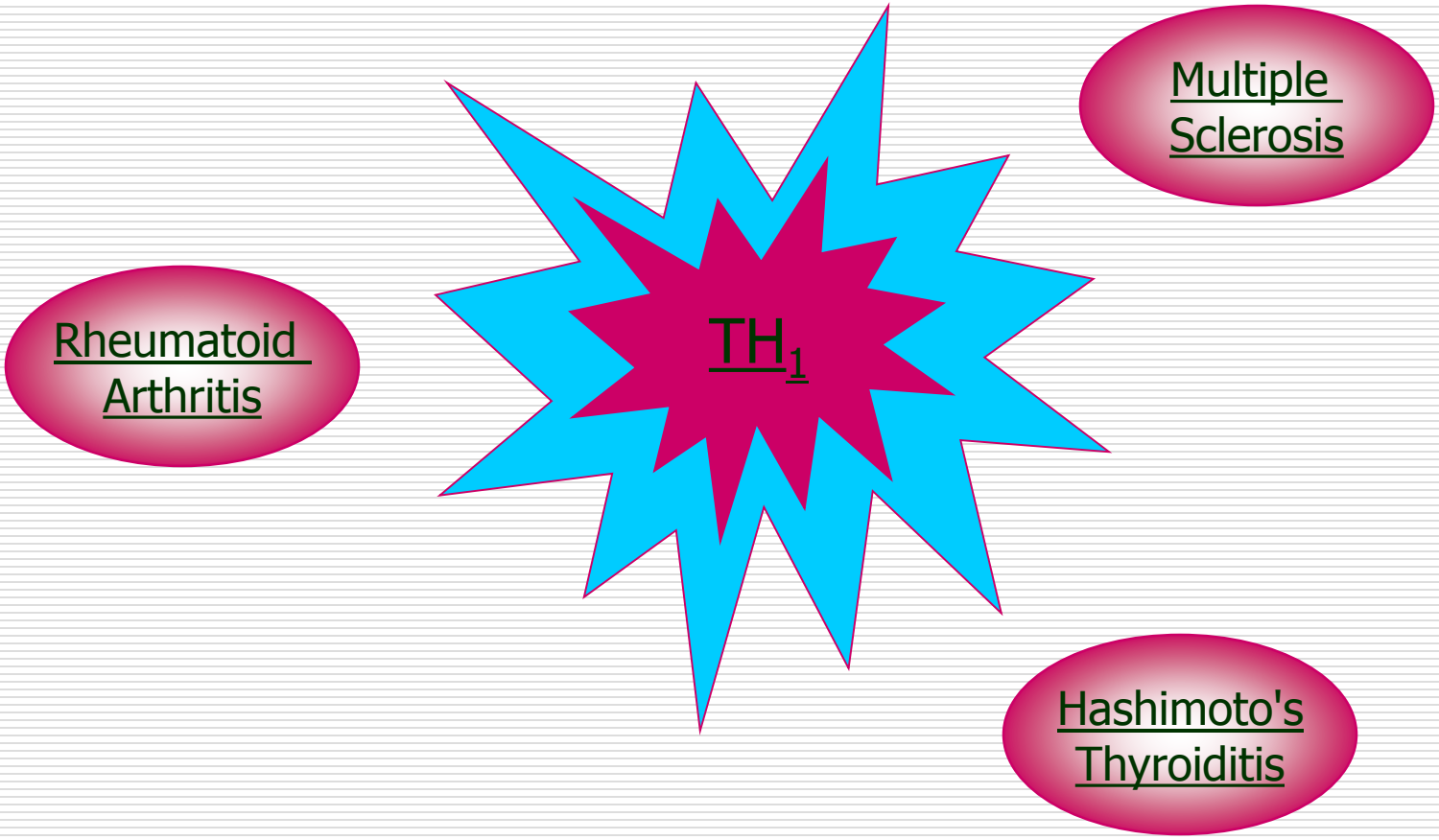
Differentiation of TH1 and TH2 cells by cytokine profiles



Influence of TH1 cells



Development of diseases from TH1 cell overreaction



The current diagnostic strategy of starting with an ELISA test is wrong

Serological tests are performed using an antiquated two-tier concept (according to CDC recommendations):

First step: Sera are screened using an IgG/IgM class-specific **ELISA** (enzyme-linked immunosorbent assay)

Second step: If sera are positive or borderline in the ELISA, this is confirmed using 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 enzyme immunoassay (EIA), but positive Immunoblot

B-C-A GmbH & Co. KG · Morellstraße 33 · 86159 Augsburg

Laboratory results

Patient:
Date of birth: 08/09/1947
Date of testing: 07/08/2009

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-technique (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

Specificity and sensitivity of Borrelia antibodies by ELISA and Immunoblot

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%**

Other recent studies:
Insensitivity of ELISA and antibodies

- "In the case of ELISA, positive or borderline results were observed in only 24 patients (53.3%)." (Wojciechowska-Koszko et al., Feb. 2011)
- "32 patients had specific antiborrelia antibodies confirmed using Western Blot despite a negative ELISA…In patients with persisting difficulties it is necessary to use the Western Blot test…It is probably due to the very low production of specific antibodies caused also by immune deficiency status detected in all our patients." (Durovska et al., 2010)
- "The number of IgM- and/or IgG-positive ELISA results … ranged from 34 to 59%...Comparison of Immunoblots yielded large differences in inter-test agreement…Remarkably, some Immunoblots gave positive results in samples that had been tested negative by all eight ELISAs." (Ang CW et al., Jan. 2011)

Summary: Insensitivity of ELISA vs. Immunoblot

Antibodies in Lyme disease patients the current IDSA/CDC Borrelia ELISA screening model:

Loss of sensitivity: 16 - 28 %

Every 4th - 6th chronic Lyme patient has a positive or borderline Immunoblot, but **not a positive ELISA!**

- Great number of patients will not be identified by the ELISA screening test, and Lyme Disease will consequently be excluded by their diagnosing MDs
- The more specific Immunoblot is the more sensitive test
- The Borrelia ELISA is senseless
- Only a Borrelia Immunoblot should be done for screening Borrelia antibodies

Borrelia antibodies by ELISA and Immunoblot: evidence-based literature on "false seronegativity"

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Borrelia antibodies by ELISA and Immunoblot: evidence-based literature on "false seronegativity"

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Borrelia antibodies by ELISA and Immunoblot: evidence-based literature on "false seronegativity"

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Borrelia antibodies by ELISA and Immunoblot: evidence-based literature on "false seronegativity"

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Evidence that an IgM Western blot response can last longer than 6 months in Lyme disease

"IgM levels rose during exacerbations and fell during remission" for 6 to 18 months after treatment of an EM rash. Steere, 1979

"56% of patients with early Lyme disease had detectable IgM responses to the spirochete 6 months later"

Massarotti 1992

"Serum IgM levels correlated directly with disease activity (p = 0.025) Craft, Yale J Biol Med 1984

"Persistence of specific IgM antibodies may also be associated with more severe disease." Craft, 1984

Case report: Chronic Lyme disease stage III with T-cellular immune response

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 power during his work (high threat of occupation disability)

Diagnosis by neurologists: MS !

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

Borrelia IgM/IgG-ELISA and Immunoblot several different times negative

Therapy: Corticosteroids increased her symptoms + bad side-effects of corticosteroids!

Case report: Initial findings of the Borrelia-LTT and CD57 count on 26th Oct. 2005 before antibiotic treatment

Klinische Angaben: Diagnose unbek.
Material : EDTA, CPD Blut, CPD Blut, Heparinblut, Vollblut

Untersuchung		Ergebnis		Referenzbereich	
Leukozyten	↓	4.2	$x10^3/\mu l$	4.4 - 11.3	IMP
Erythrozyten		4.11	$x10^6/\mu l$	4.1 - 5.1	IMP
Hämoglobin		12.7	g/dl	12.3 - 15.3	PHO
Hämatokrit		37.6	%	36 - 47	RECH
MCV		91.5	fl	80 - 99	RECH
HBE (MCH)		30.9	pg	26 - 34	RECH
MCHC		33.8	g/dl	31 - 36	RECH
Thrombozyten		243	$x10^3/\mu l$	140 - 400	IMP
Differentialblutbild					
Neutrophile		46	%	45 - 75	IMP
Lymphozyten		43	%	20 - 45	IMP
Monozyten		9	%	2 - 13	IMP
Eosinophile	↓	1	%	2 - 4	IMP
Basophile		1	%	0 - 1	IMP
Sonstige Zellen		0	%		MIK
CD3- CD57+ Zellen	↓	3.6	%	5 - 20	[1]
(CD3-,CD57+ absolut)		65	$/\mu l$	60 - 360	RECH[1]
Eine Verminderung der Anzahl CD57+/CD3- Zellen kann für eine chronische Borreliose sprechen.					
Lymphozytentransformationstest					
Spontanaktivität		870	cpm	< 1000	LTT [1]
Ospc	↑	30.1	SI	< 2.0	LTT [1]
P18-Antigen	↑	4.8	SI	< 2.0	LTT [1]
P100-Antigen	↑	8.1	SI	< 2.0	LTT [1]

B. burgdorferi-IgG-Blot	negativ	BLOT
B. burgdorferi-IgM-Blot	negativ	BLOT

Case report: Borrelia-LTT Jan 23rd 2006 after Ceftriaxone IV treatment (8 weeks after the end of therapy)

Ospc	↑ 2.2	SI	< 2.0	LTT 1
P18-Antigen	<1	SI	< 2.0	LTT 1
P100-Antigen	<1	SI	< 2.0	LTT 1

Significant decrease of the LTT (Lymphoycyte Transformation Test)

Lyme-Borreliose

B. burgdorferi-IgG-EIA	< 5	U/ml
B. burgdorferi-IgM-EIA	0.8	Index

Kein serologischer Hinweis auf Infektion mit B. burgdorferi.

On Jan 23rd, 2006, patient is clinically symptom-free and capable of work!

Correct diagnosis: Chronic Neuroborreliosis with Multiple Sclerosis-like symptoms

The spinal tap has poor sensitivity in chronic neurological Lyme Disease

27 subjects with neurological Lyme Disease presenting to Tufts Univ. School of Medicine, Boston

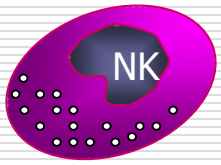
1 out of 27 with antibodies to Lyme disease

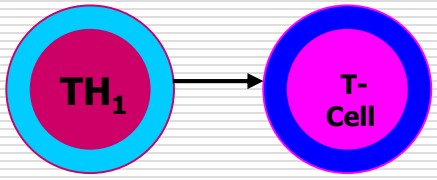
1 out of 27 with an abnormal spinal tap (7 white cells)

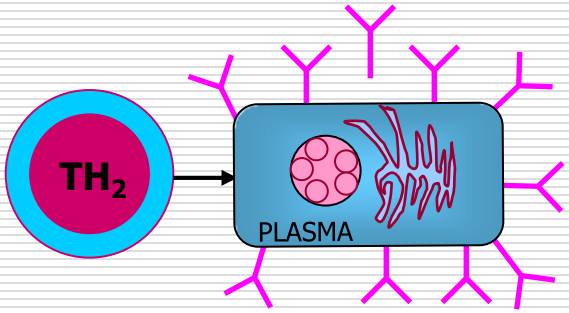
Logigian, Steere, 1990, NEJM

Logigian and Steere 1990 NEJM

Aims of the immune-competent cells







CD57+NK cells

- Lysis antigen-antibody complexes (Borrelia burgdorferi)

Elispot-LTT (T-cells):

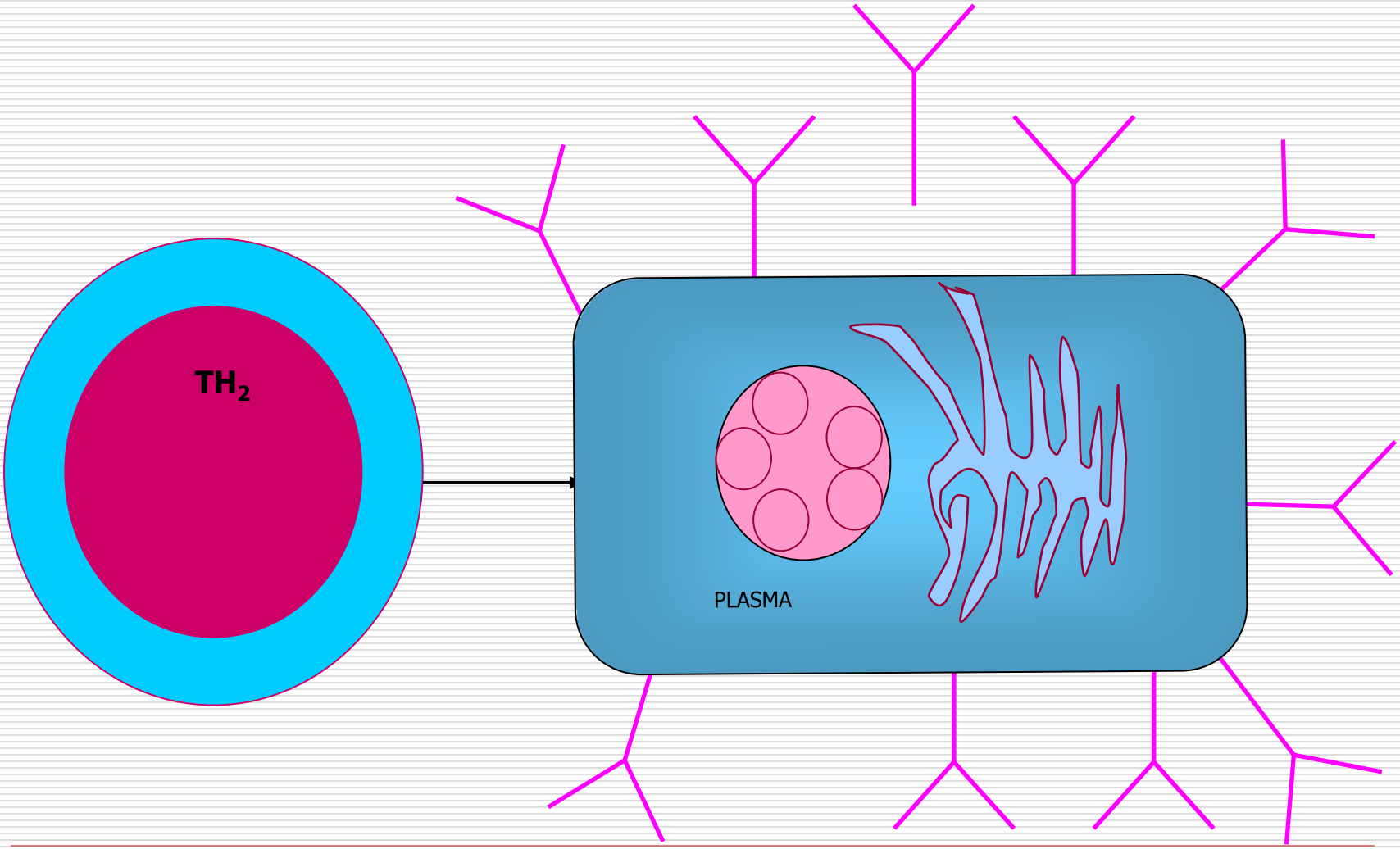
- Borrelia burgdorferi
- Chlamydia pneumoniae
- Anaplasma/Ehrlichia

and others

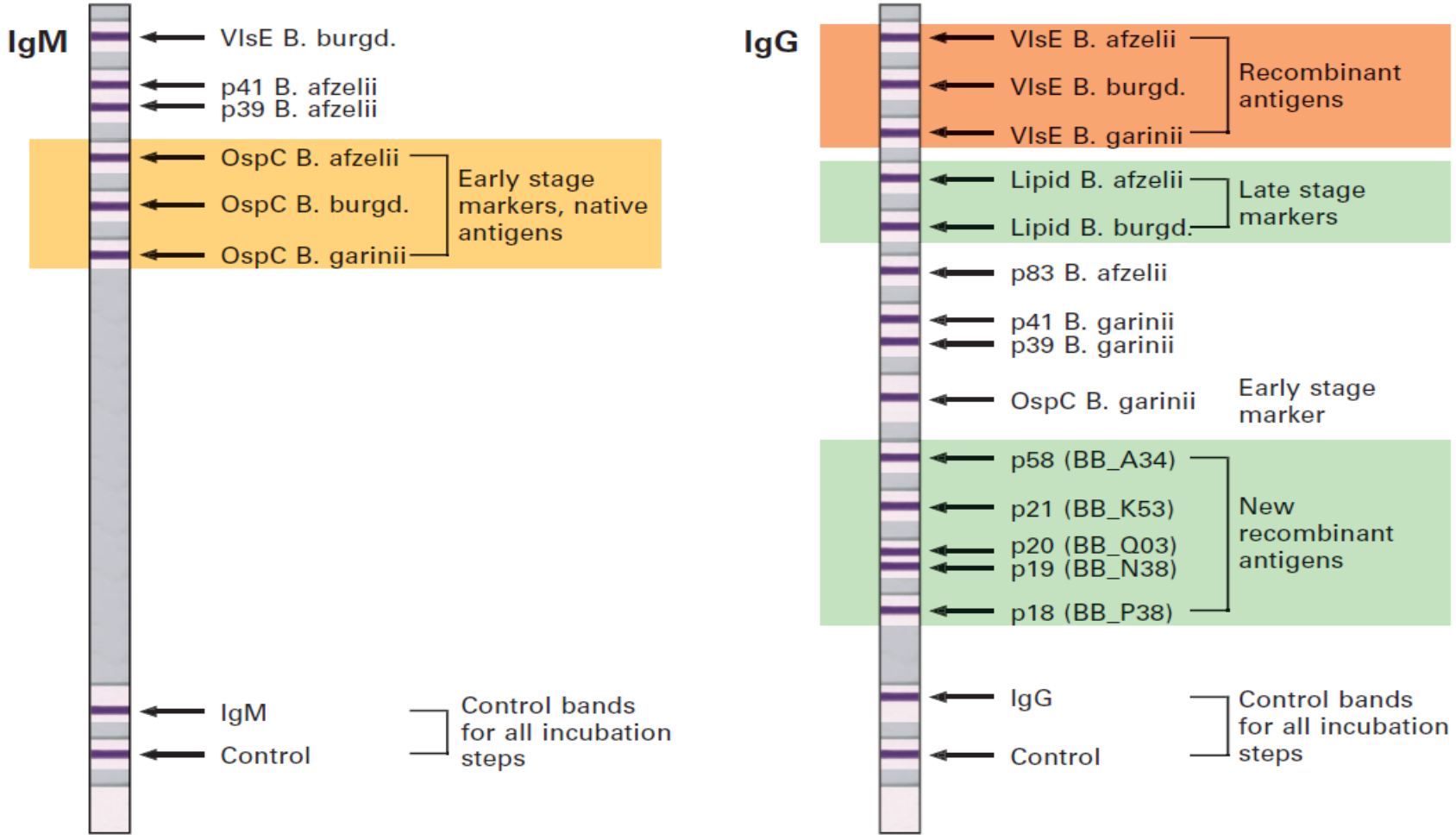
Antibodies (B-cells):

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

B-cells (IgG/IgM antibodies): ELISA, Immunoblot

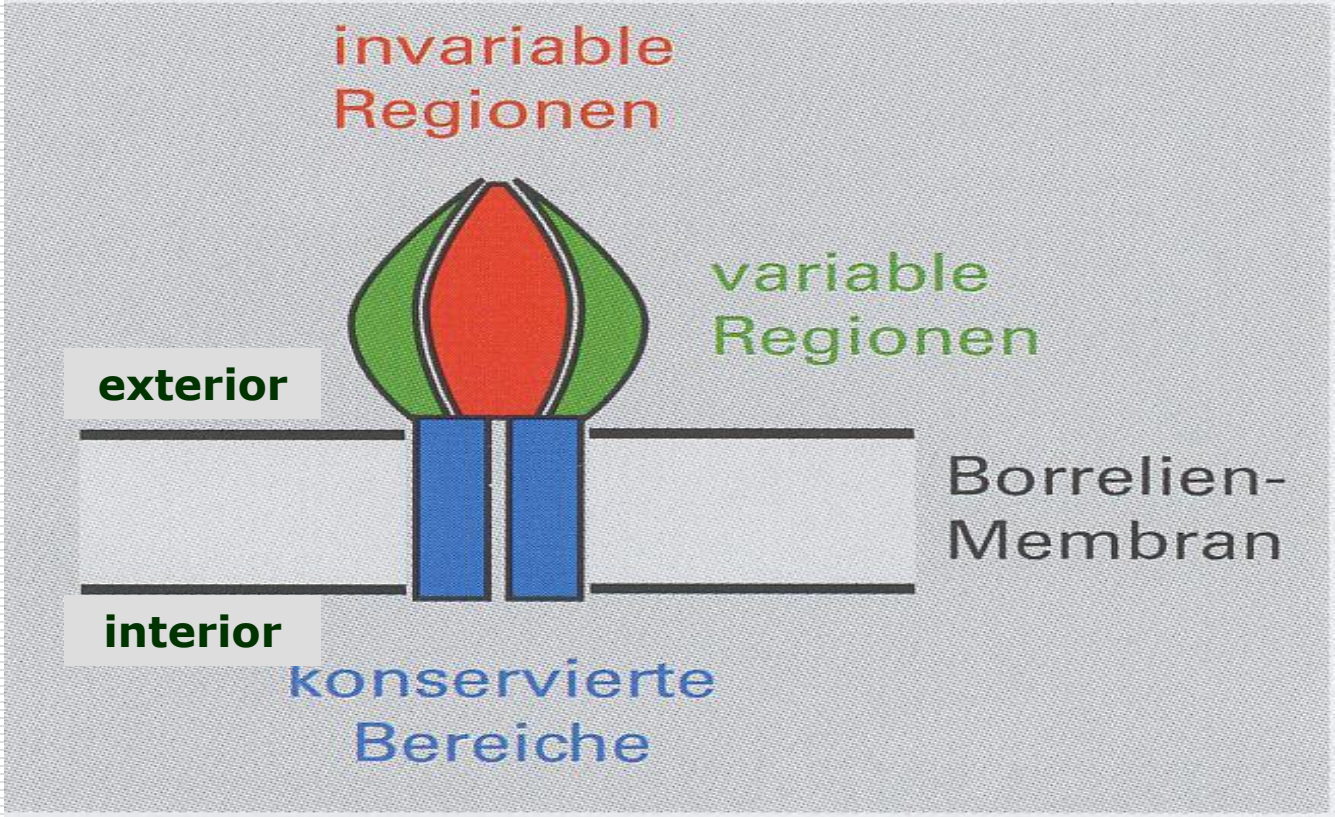


Antibodies by Immunoblot: EUROLINE-RN-AT

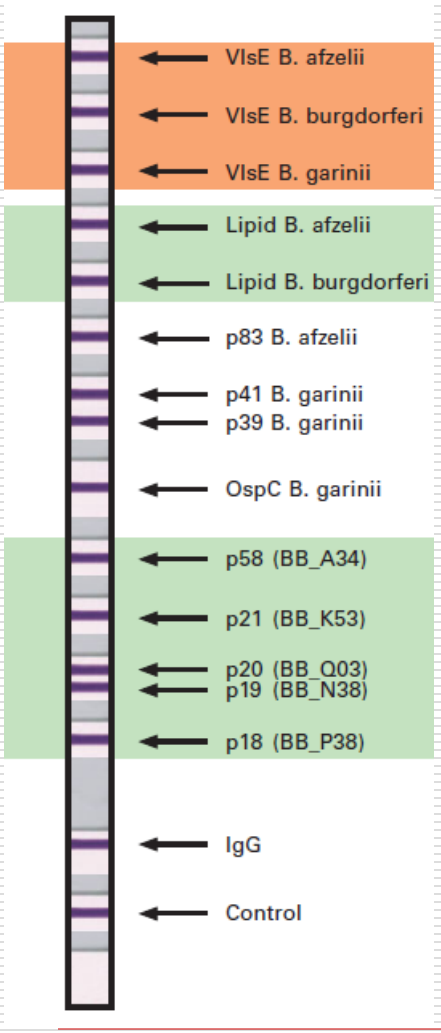


The new surface marker VlsE for B-cellular activity: highly specific, associated with "in vivo" activity

VlsE = Vmp-like sequence Expression site



Antibodies by Immunoblot: EUROLINE-RN-AT



Antigen	Prevalence*	Specificity*
VlsE Ba	66%	99%
VlsE Bb	89%	
VlsE Bg	68%	
Lipid Ba	25%	
Lipid Bb		90%
p83		95%
p39		99%
		96%
	21%	98%
	9%	99%
	7%	100%
(BB_N38)	9%	99%
p18 (BB_P38)	22%	99%

→High specificity: 95-100%
→But: Low sensitivity: 60 %

Anti-Borrelia EUROLINE-RN-AT
Combination of specific Borrelia markers

Recombinant antigens (for example ArminLabs, Germany)
Antigens that are not expressed in bacterial cultures or expressed only in insufficient amounts, e.g. VlsE

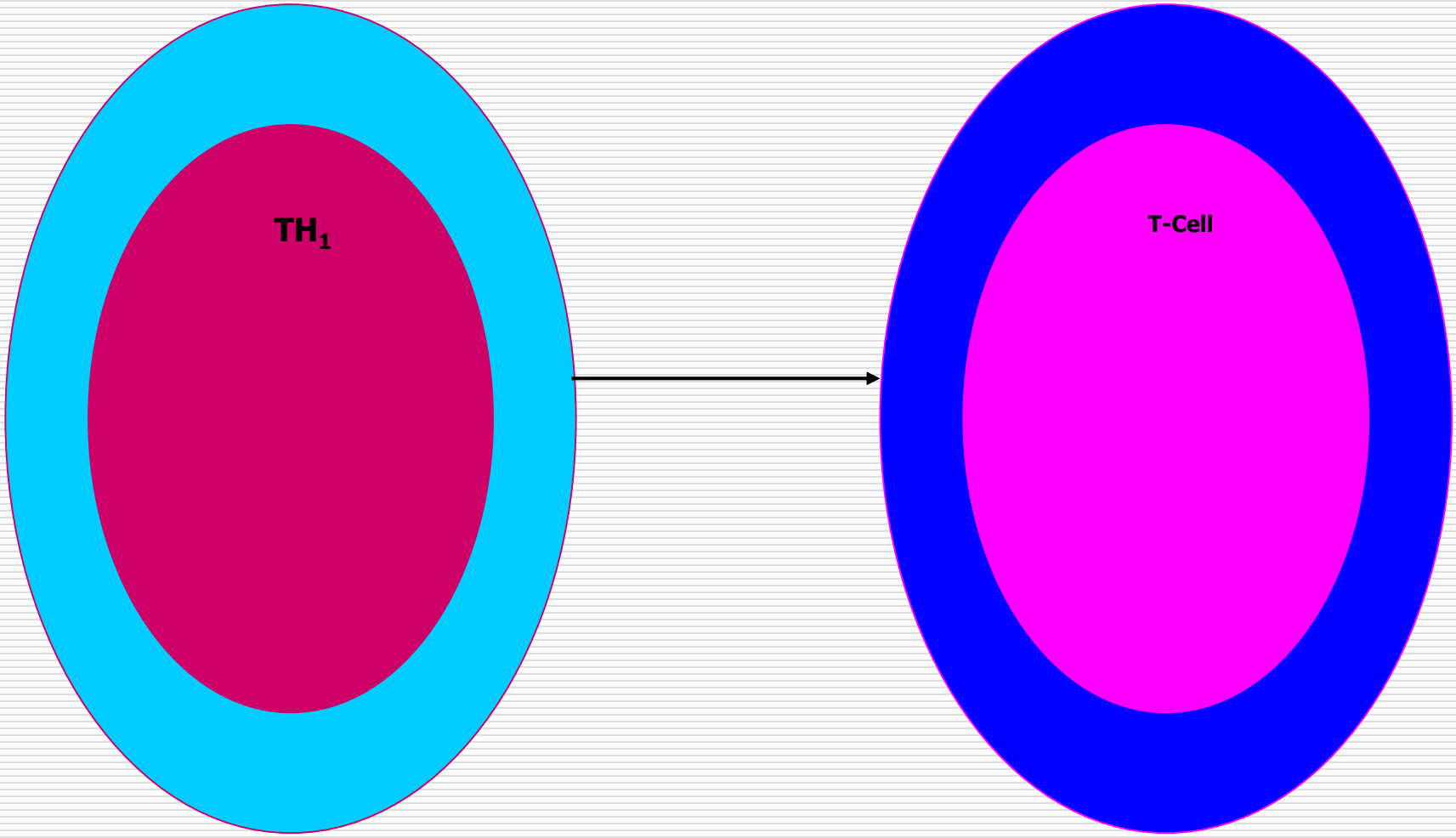
Native antigens (for example Igenex, USA)
High sensitivity and specificity compared to recombinant antigens

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

Specifity: 95-100 %

Sensitivity: ~ 60 % !

T-cells (T-lymphocytes): Elispot Lymphocyte Transformation Test (Elispot-LTT)

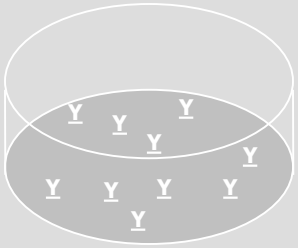


Dr. Leo Joosten, Department of Medicine, Radboud University, Netherlands:

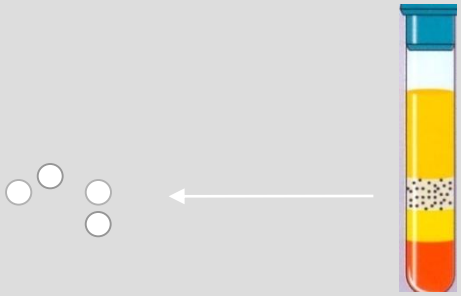
Q: What solutions that are currently being pursued do you believe hold the most promise for diagnosing Lyme disease at a high confidence level? What tests currently available to the general public, other than the Western Blot test, do you believe provide a better degree of certainty?

A: "At the moment, there are cellular-based tests on the market. LTT and **Elispot** are a few of these tests. These tests give us information about the cellular immune response towards *Borrelia* antigens. It seems that these tests will used in the future, apart from serological tests."

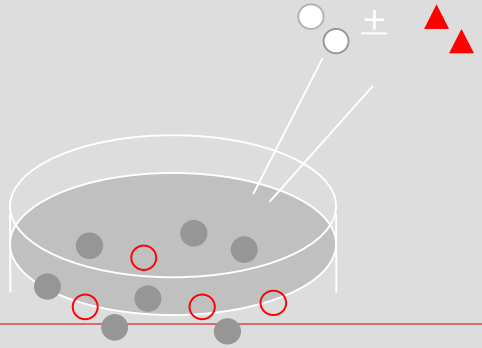
Elispot-LTT: The principle (I)



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

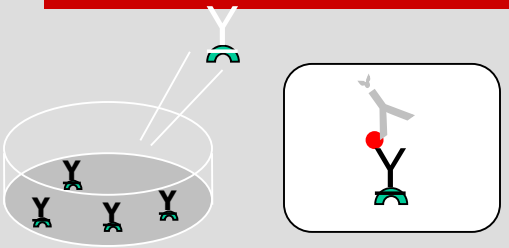


Lymphocytes are isolated

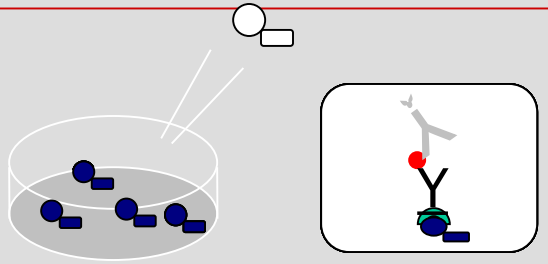


Incubation with cells and antigens, specific cells release cytokines

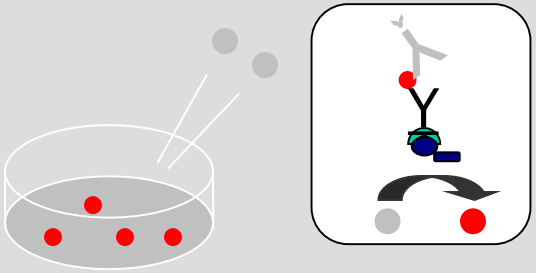
Elispot-LTT: The principle (II)



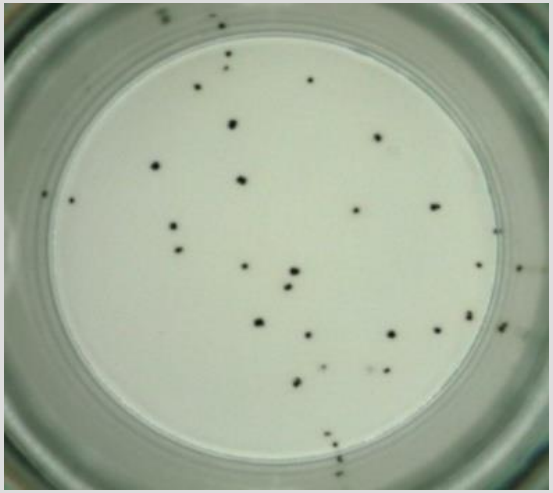
Add biotinylated secondary antibody complex: pr.AB/Cytokine/sec.AB



Add Streptavidin-enzyme conjugate



Add substrate to develop colour



Analysis

Borrelia antigens in the Borrelia EliSpot LTT

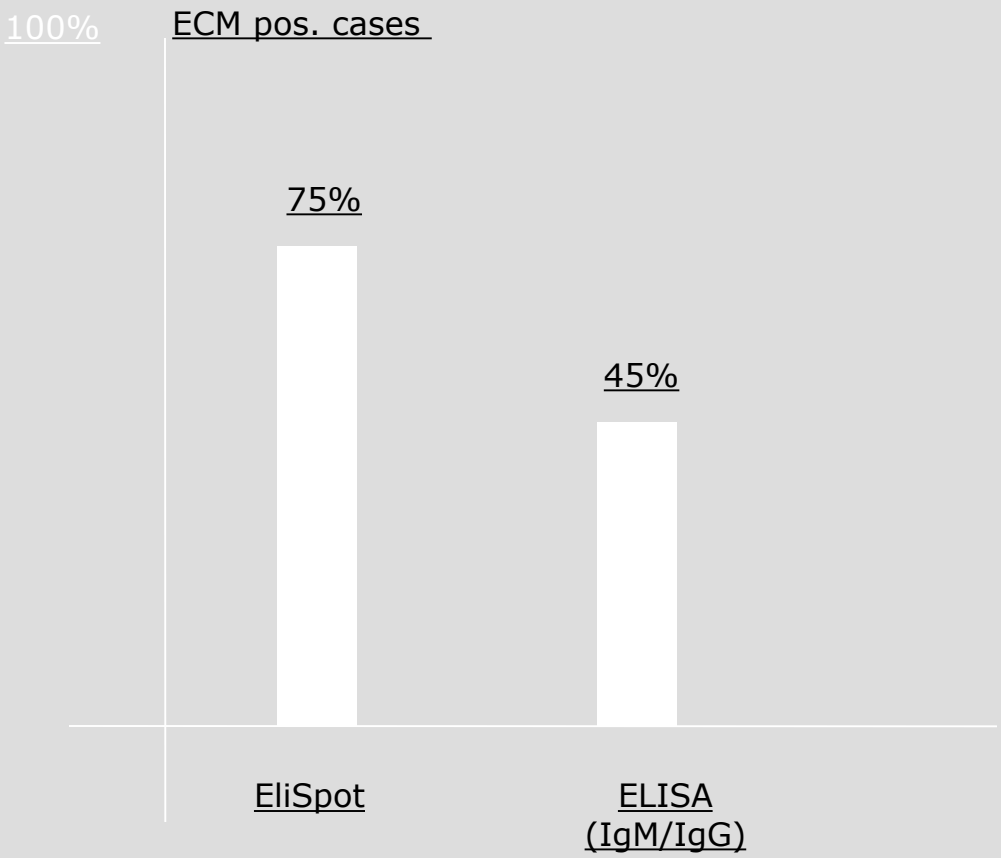
- Borrelia burgdorferi full antigen: Borrelia burgdorferi B31-reference strain (Borrelia burgdorferi sensu stricto)

- Borrelia burgorferi peptide mix: OspA from Borrelia b. sensu stricto, Borrelia afzelii, Borrelia garinii + OspC native + DbpA recombinant

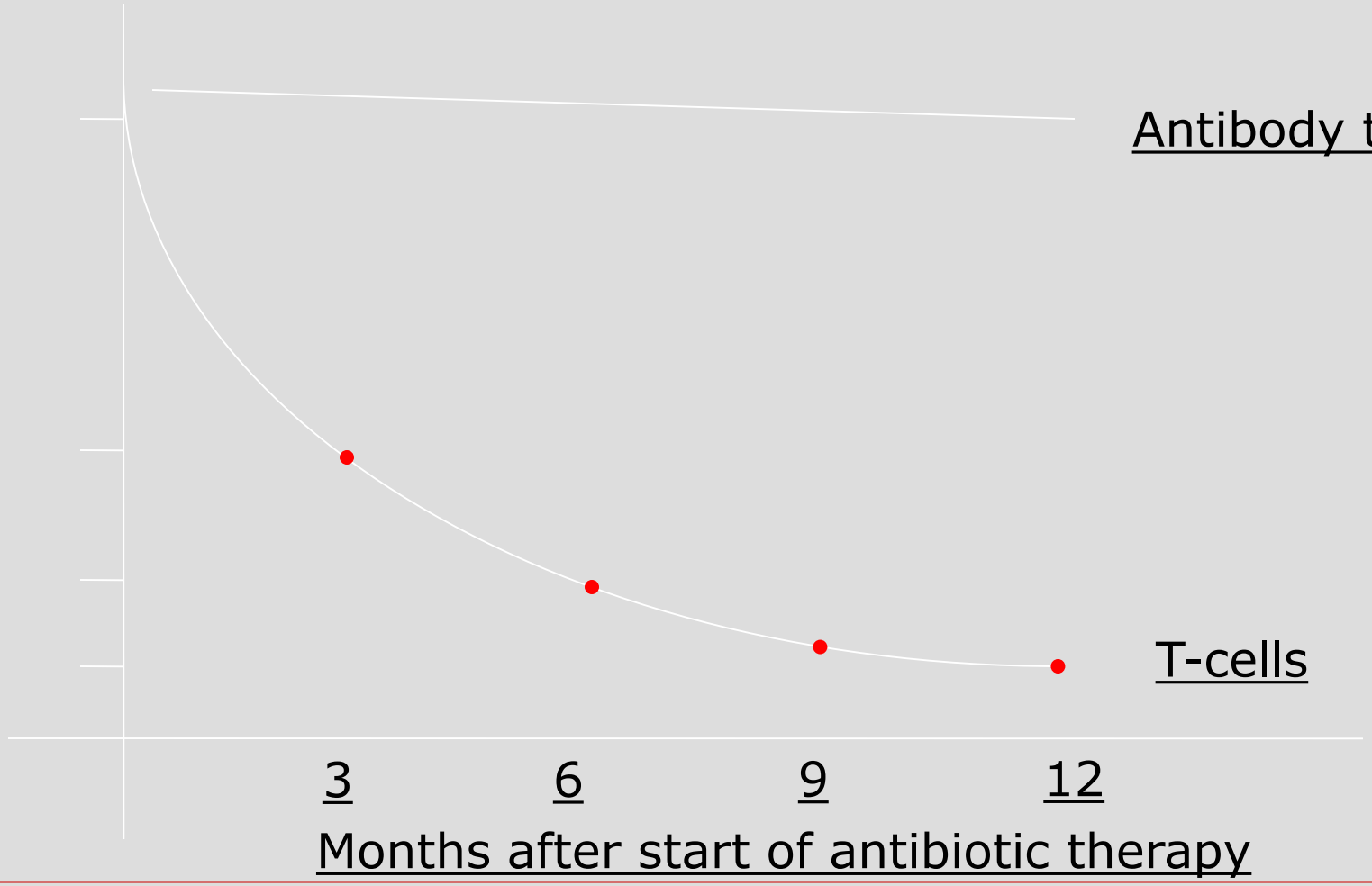
- Borrelia burgdorferi LFA-1 (Lymphocyte Function Antigen 1): Own body protein + Borrelia burgdorferi sensu stricto (shared epitope). Often associated with autoimmune diseases: collagenosis, Rheumatoid Arthritis, vasculitis (ANA, CCP antibodies, ANCA)

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

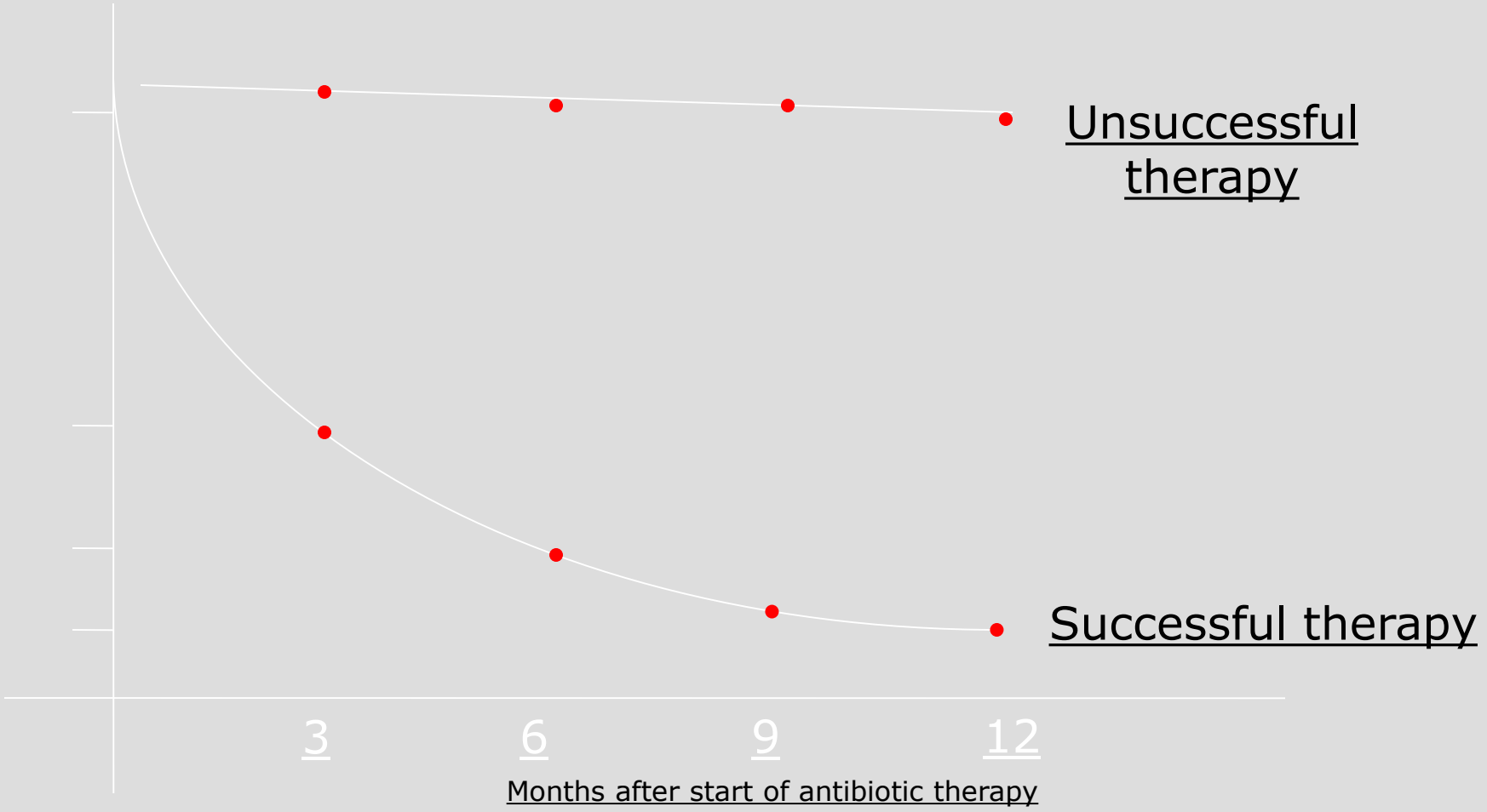
ELISA / EliSpot in Lyme Stage I



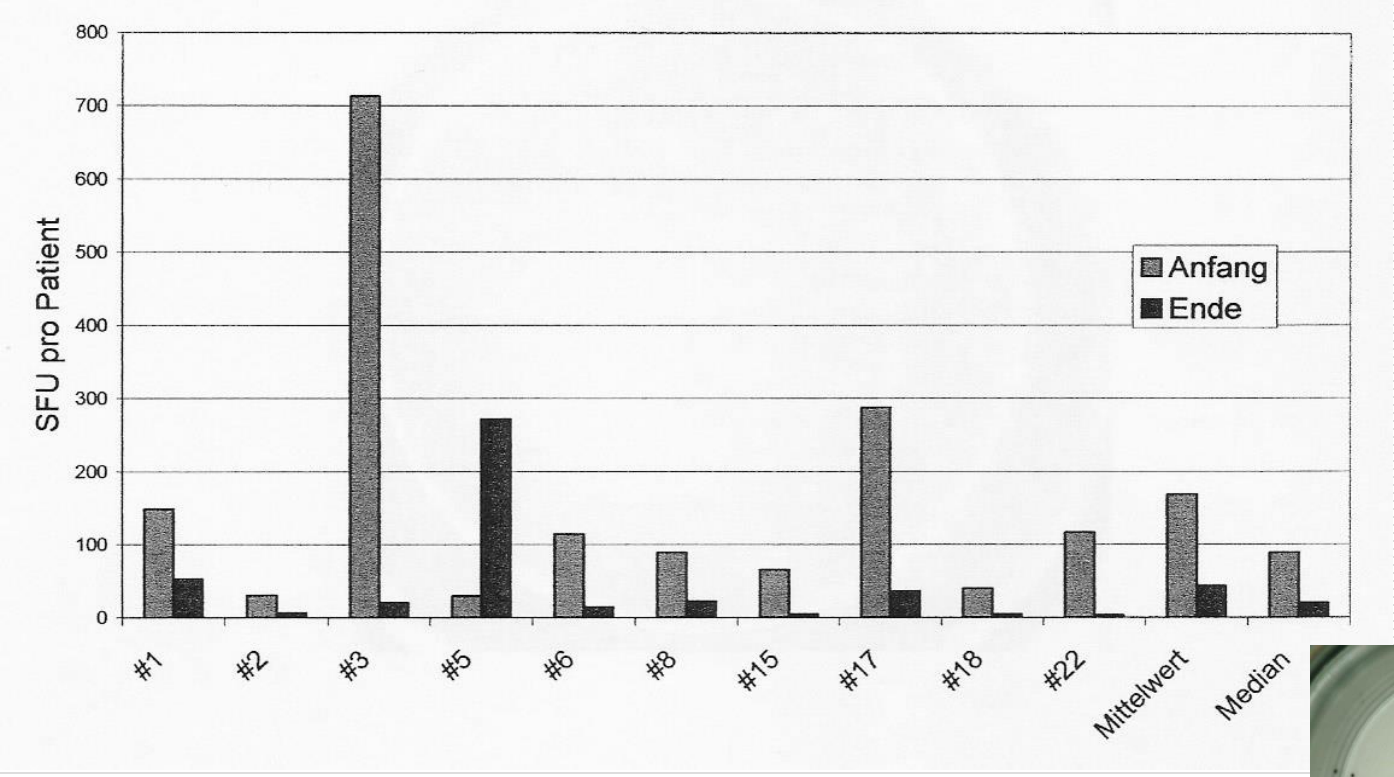
EliSpot-LTT during antibiotics: "Staging" progress

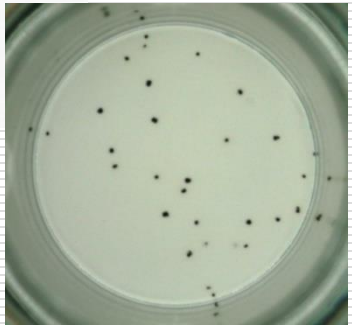


EliSpot-LTT during antibiotics: "Staging" progress



EliSpot-LTT in chronic Lyme Disease





Grey columns: before antibiotic therapy

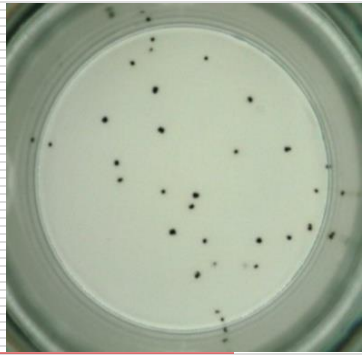
Black columns: after antibiotic therapy

Pfeffer et al. 2003

Borrelia Elispot (LTT / T-Cell-Spot / IGRA: Interferon-Gamma-Release Assay /Lymphocyte Transformation Test)

1. Successful control of antibiotic therapy - **STAGING**:
   - About 2 months after the end of a therapy there should already be a significant reduction
   - Borrelia IgM/IgG titer reduction after 6 - 12 months!

2. Reflects the **current T-cellular activity** of Lyme disease:
   - Indicates active Borrelia infection if a positive Elispot LTT continues after the end of therapy
   - **T-Cell-Spot/IGRA has been 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…"



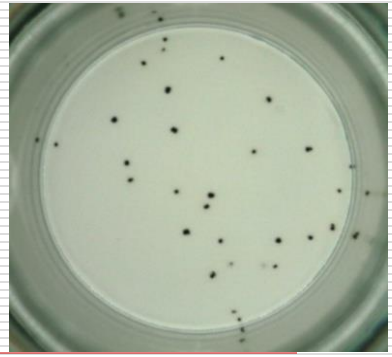
Borrelia Elispot (LTT / T-Cell-Spot / IGRA: Interferon-Gamma-Release Assay /Lymphocyte Transformation Test)

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

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

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

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



ELISPOT-LTT: 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…

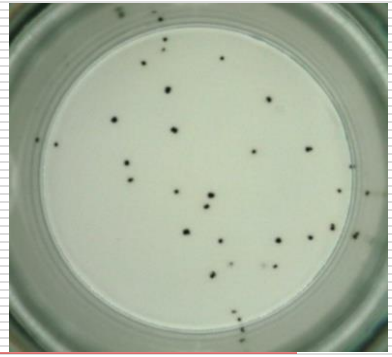
… the two-assays system (ELISPOT + CD57-cell count) complement each other in the quest to understand T cell-mediated immunity in vivo….

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

According to this new study:

82 - 100 % Specificity of Borrelia-Elispot-LTT

84 % Sensitivity of Borrelia Elispot-LTT



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
- Dressler F et al, The T-cell proliferative assay in the diagnosis of Lyme disease. Ann Intern Med 1991; 115: 533-9
- Krause et al, T cellproliferation induced by Borrelia burgdorferi in patients with Lyme borreliosis. Autologous serum required for optimum stimulation. Arthritis Rheum 1991; 34: 393-402
- 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

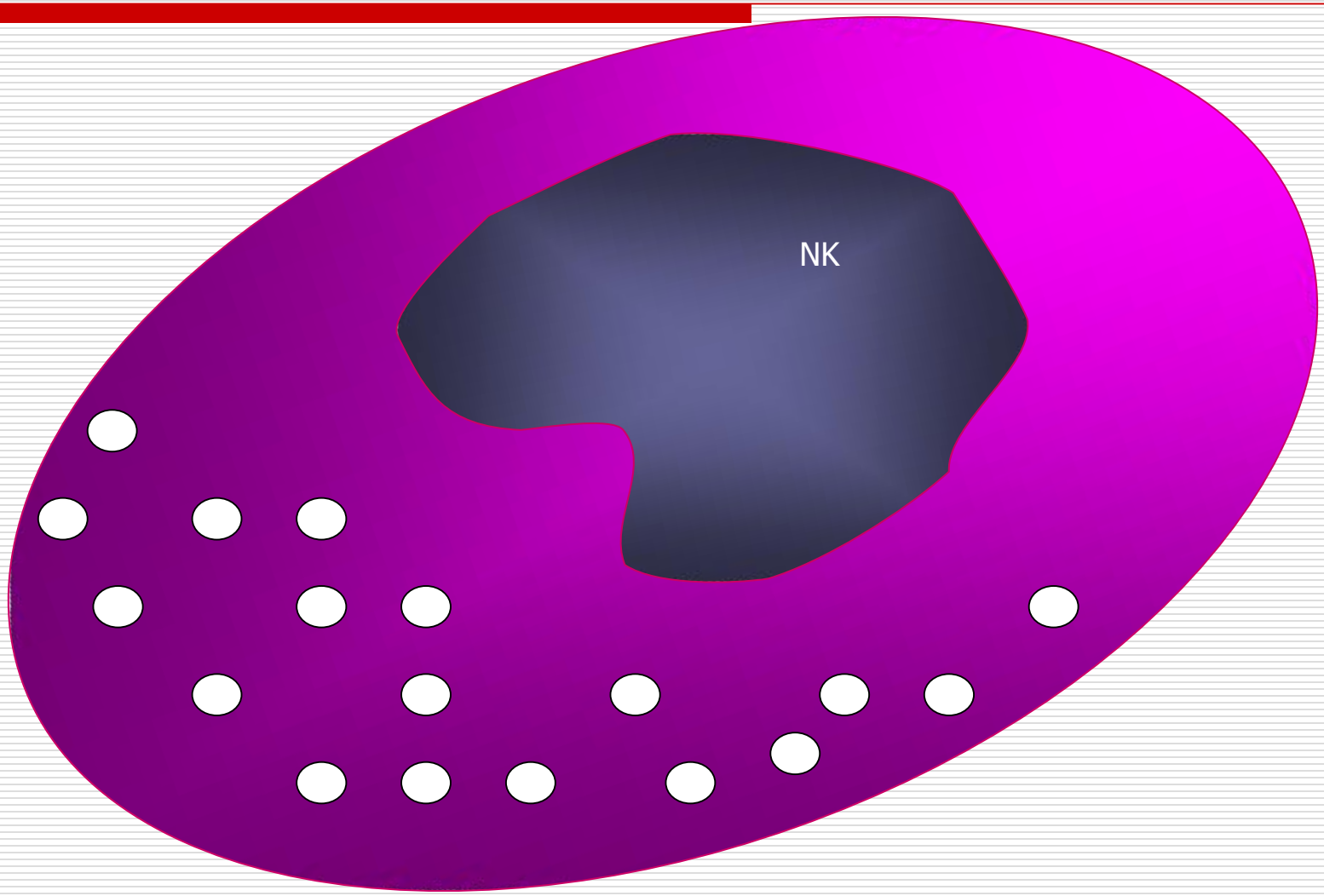
LTT: Evidence-based literature

- "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

Currently the Elispot is available for:

- Borrelia burgdorferi (3 subspecies)
- Chlamydia pneumoniae
- Chlamydia trachomatis
- Ehrlichia
- Yersinia species
- Epstein Barr Virus (EBV)
- Cytomegalovirus (CMV)

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

CD3-/CD57+ T-Lymphocytes

Reference range (mean/range)

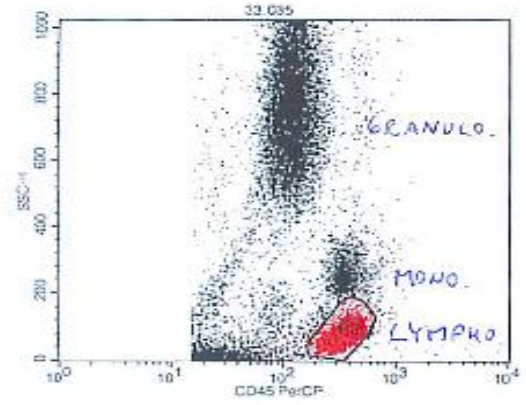
Lyme patient:	46 /ul / 8 – 160 /ul
Healthy:	164 /ul / 60 – 354 /ul

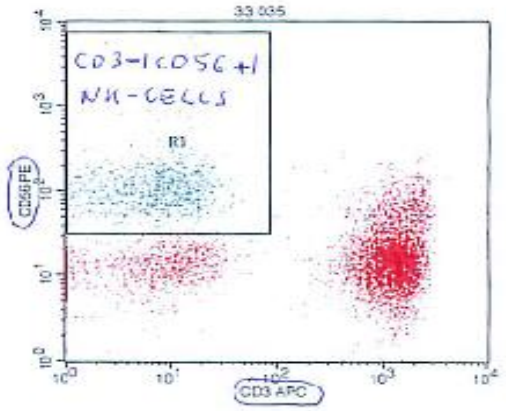
Source: J.J.Burrascano JR., MD, R. Stricker, MD, 2006 ILADS, Crowne Plaza Hotel, Center City Philadelphia

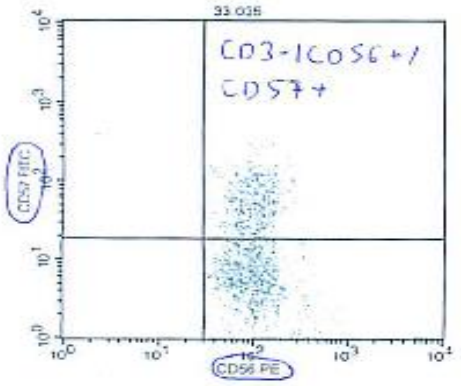
Low CD57 count: Flow Cytometry

Messung der CD57+ NK Zellen

Sample ID: 33 Patient ID: 12171428
Acquisition Date: 09-Feb-11







Gate: Lymphos

Gate	Events	% Gated
Lymphos	6720	100.00
NK Zellen	1208	17.98

NK-Zellen % = 17.98 %LY

CD57+ NK-Zellen %NK = 33.69 %NK Zellen

CD57+ NK Zellen%LY = 6.06 %LY

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: Evidence-based 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

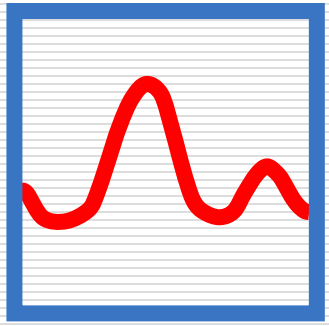
Basic diagnostic tests for chronic Lyme Borreliosis

1. **Borrelia IgM and IgG antibodies by the Immunoblot technique, including VlsE**
2. **Borrelia Elispot (LTT): actual Borrelia activity**
3. **CD3-/CD57+ T-Lymphocytes: chronic Borrelia activity**

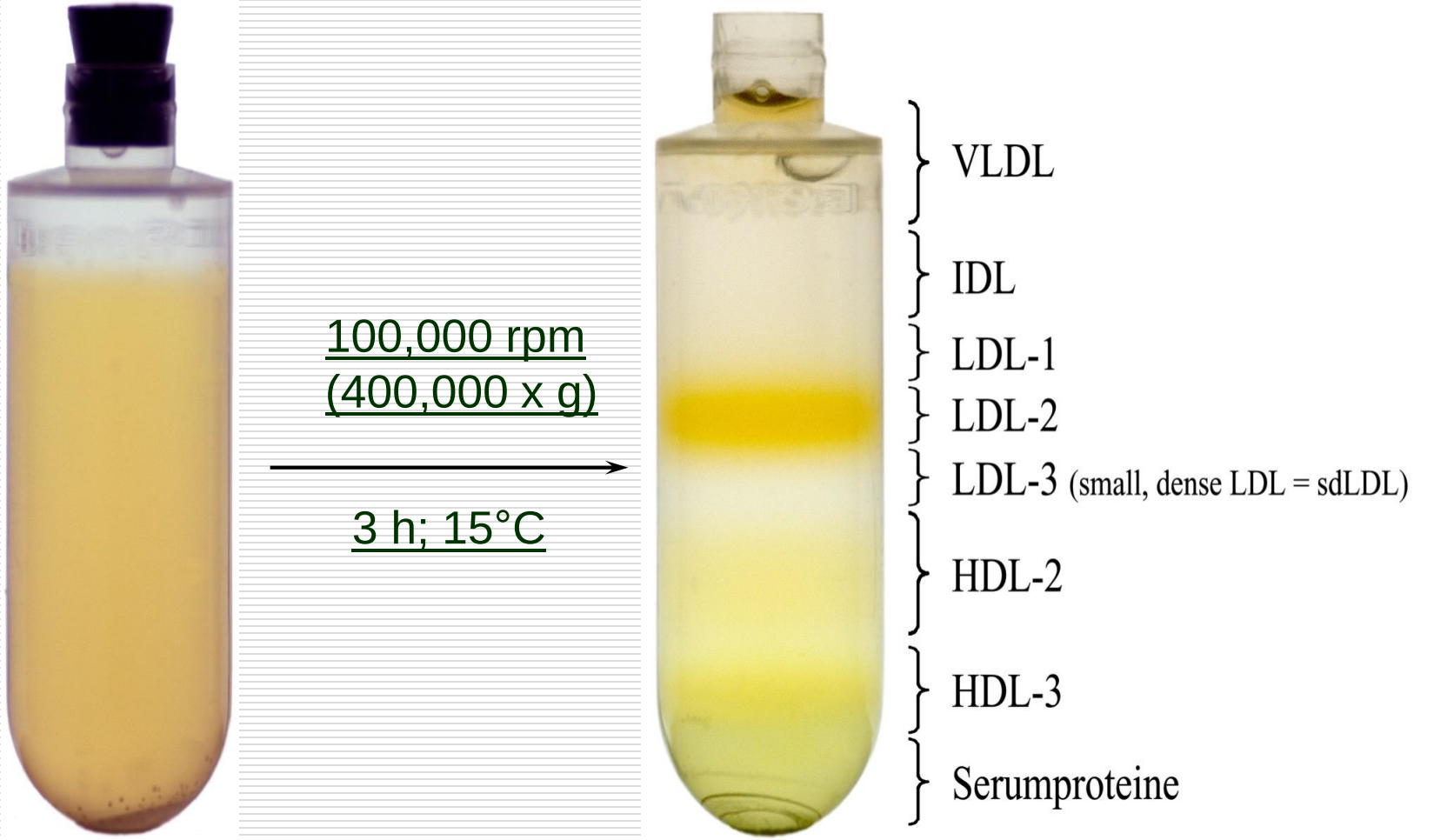
Monitoring 6 - 8 weeks after the end of therapies to verify whether the therapy has been successful or not

Laboratory STAGING process

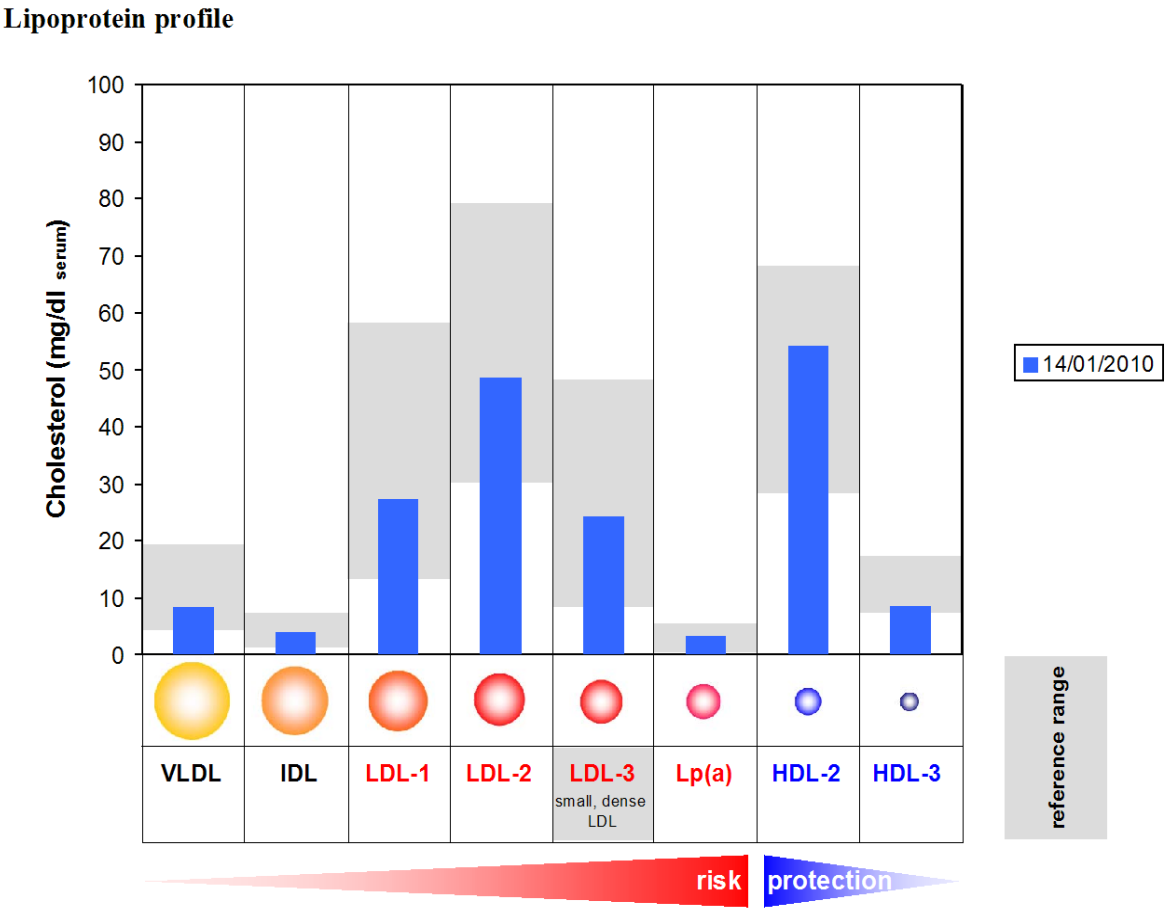
LipoDens
a Rapid Single-step Ultracentrifugation Method for Determination of Lipoprotein Subfractions



Ultracentrifugation of a serum sample



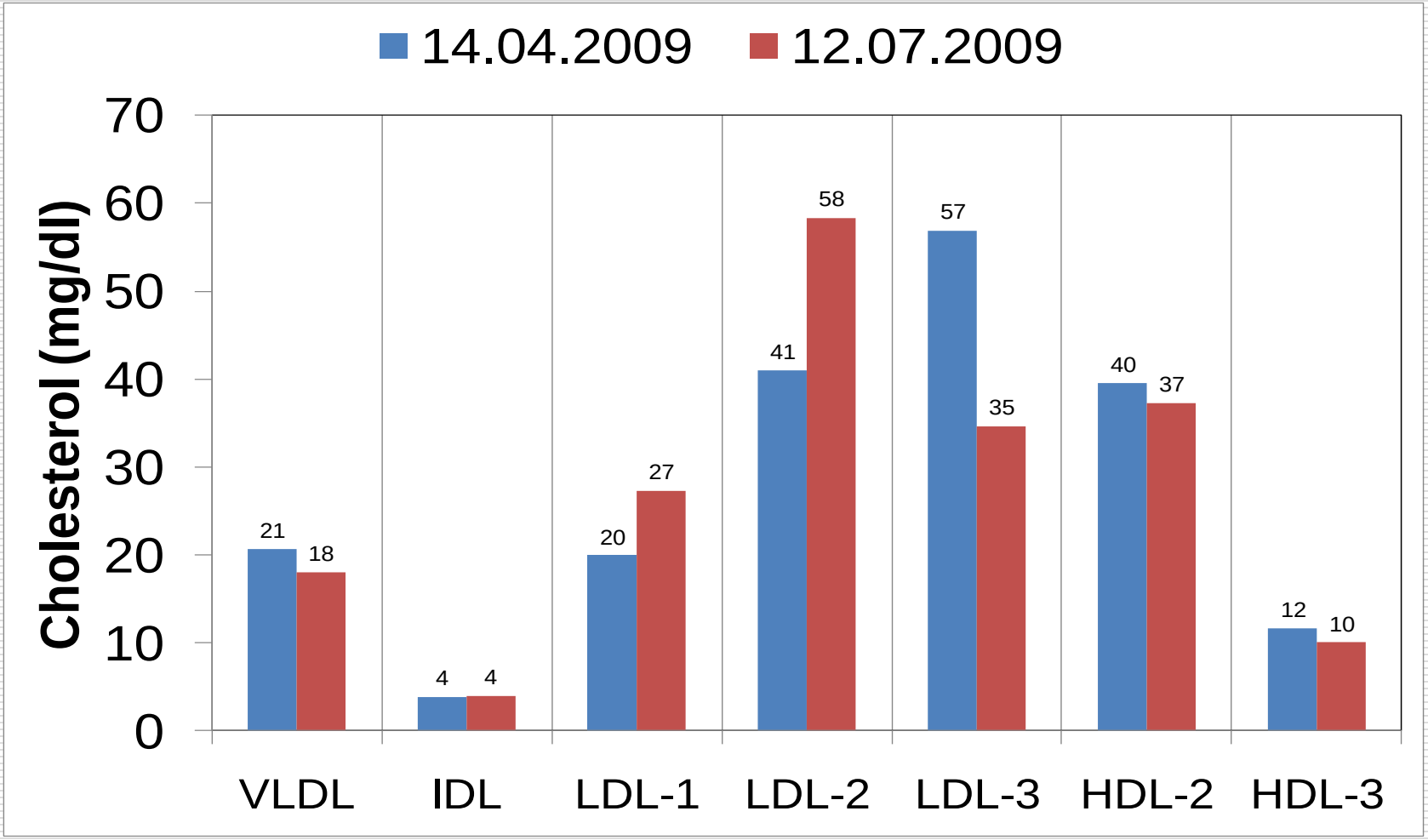
LipoDens laboratory report



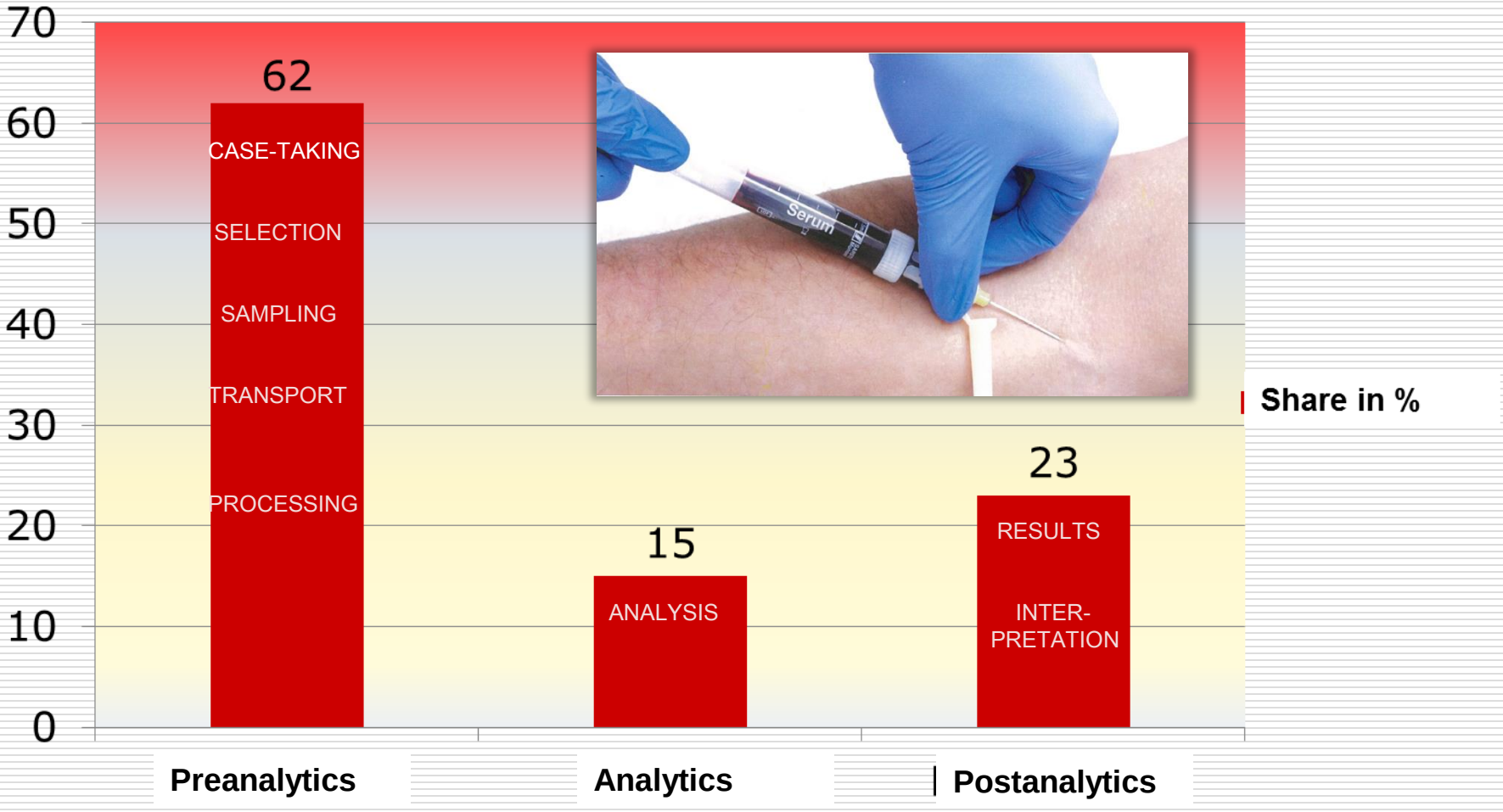
Parameters

- Serum-Chol
- Serum-Trig
- LDL-Chol
- HDL-Chol
- LDL-Chol / HDL-Chol
- Trig / HDL-Chol
- Non-HDL-Chol
- **VLDL-Chol**
- **VLDL-Chol / VLDL-Trig**
- **IDL-Chol**
- **sdLDL-Chol (LDL-3)**

Effect of lifestyle changes on LP subfractions

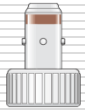


Sources of error in laboratory work

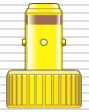


Preanalytics: Use the right tubes in the right order when taking blood

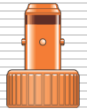
- **Serum tube (antibodies)**
  Stability of antibodies: weeks



- **CPDA tube (Elispot-LTT)**
  Vitality of T-cells for LTT: Up to 3 days !
  DON`T USE HEPARIN FOR LTT!

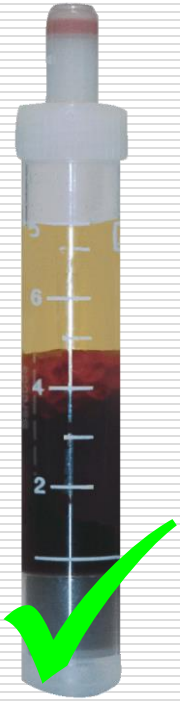


- **Heparin tube (CD57+cells)**
  Stability of CD57-cells: Up to 3 days

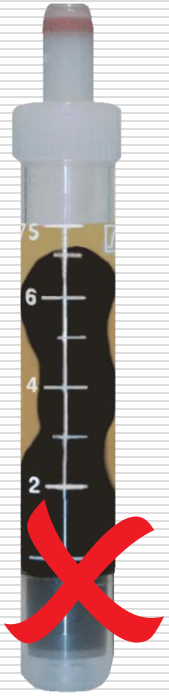


- **EDTA tube (Blood count for CD57+cells)**
  Stability of leucocytes up to 3 days

Preanalytics: Use the right tubes in the right way after taking blood



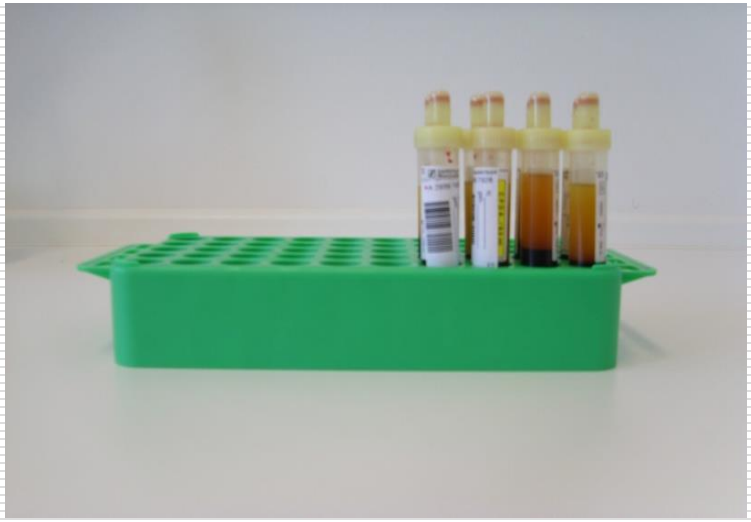
Serum tube:
Keep upright!



Serum tube after a lying position!

Preanalytics: Best workflow for best qualitiy

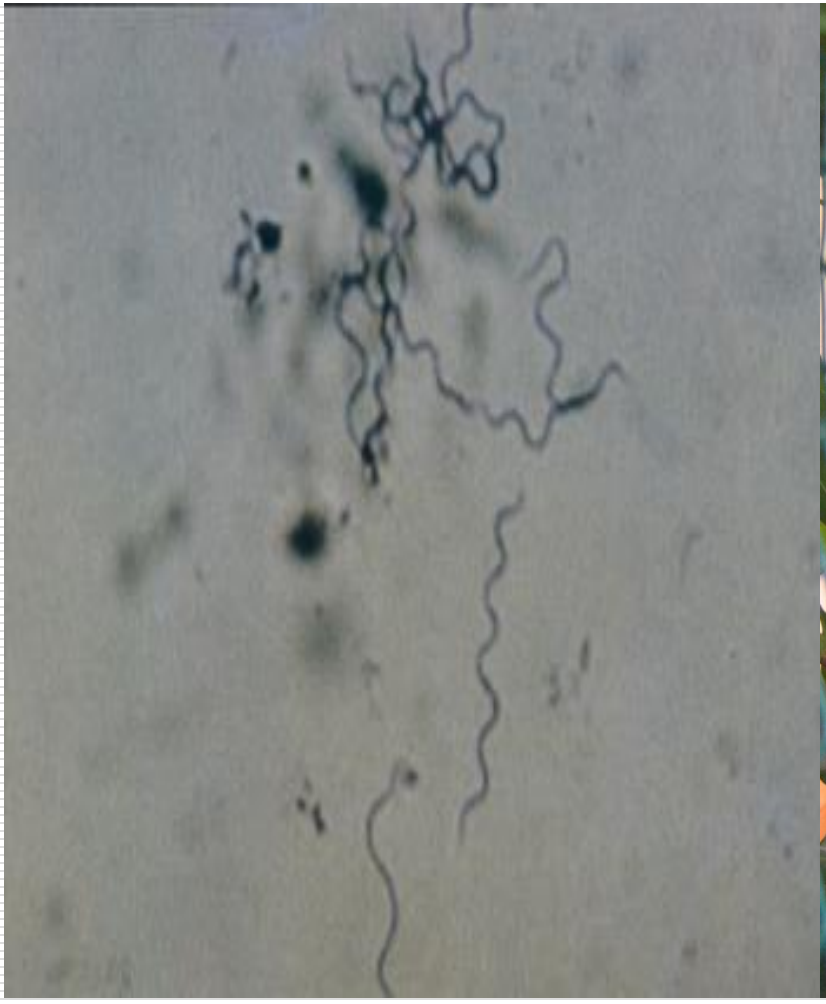
- **Shake all tubes carefully 5-10 times after taking blood**
- **Put all blood samples in an upright position for 30 minutes, centrifuge only serum (NOT other tubes)!**
- **Store at room temperature, not in the sun, no refrigerator or cooling of all tubes**
- **Protect all tubes in a special test kit for transportation/logistics**



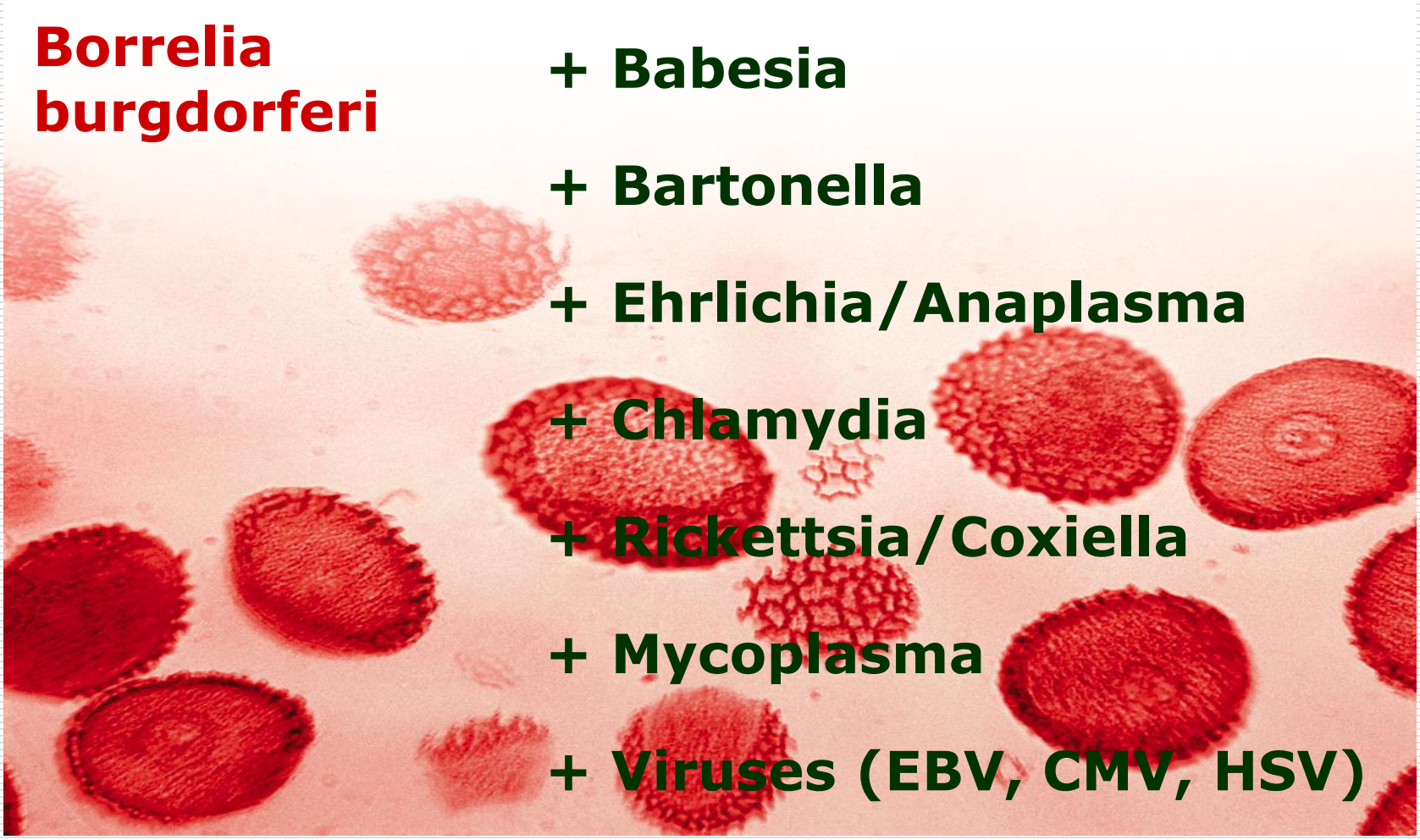
Responsibility ?! End of Part I …

"Who entered the settings for the radiation lamp?"

PART 2: CO-INFECTIONS



LYME BORRELIOSIS and CO-INFECTIONS



Ehrlichia / Anaplasma

Bacteria: Ehrlichia chaffeensis, Anaplasma phagocytophilum (gram-negative, obligatory intracellular in granulocytes)

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 (length: 1 up to 60 days) up to lethal ending, rarely: diffuse vasculitic rash, including palms and soles (<10%)

Risk factors: the elderly, severe underlying illness, immune suppression (children)

Diagnosis of Ehrlichia/ Anaplasma

Ehrlichia/Anaplasma Elispot-LTT

Ehrlichia/Anaplasma-DNS-PCR in blood (EDTA blood): direct detection

Bacteria detection in Giemsa blood smear

Ehrlichia-IgM and Ehrlichia-IgG antibodies

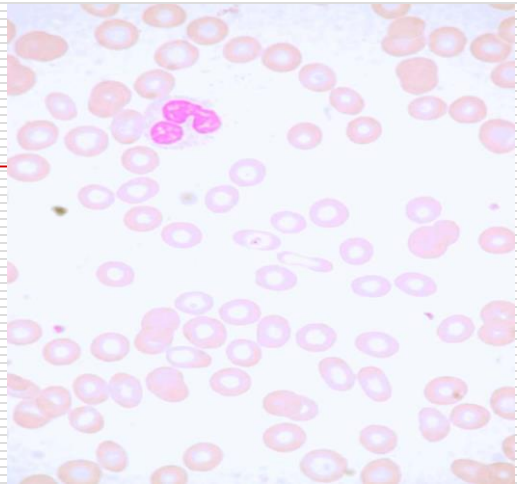
Leucopenia / Thrombocytopenia / Anaemia

Elevated liver enzymes

Ehrlichia/Anaplasma: Therapy

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

Babesia



Bacteria: Babesia microti, Babesia divergens, B. WA1

Vector/transmission: Ixodes ricinus, blood transfusion

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 anemia, 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, immune suppression (children), organ transplantation, the elderly

Babesia haemangiomata

Human Babesiosis World map With Vector map

Alan MacDonald MD: Editorial Comment: Geographies with Endemic Babesiosis do not exist in a vacuum. Endemic Lyme borreliosis travels with Endemic human Babesiosis!!!!!.

The NEW ENGLAND JOURNAL of MEDICINE

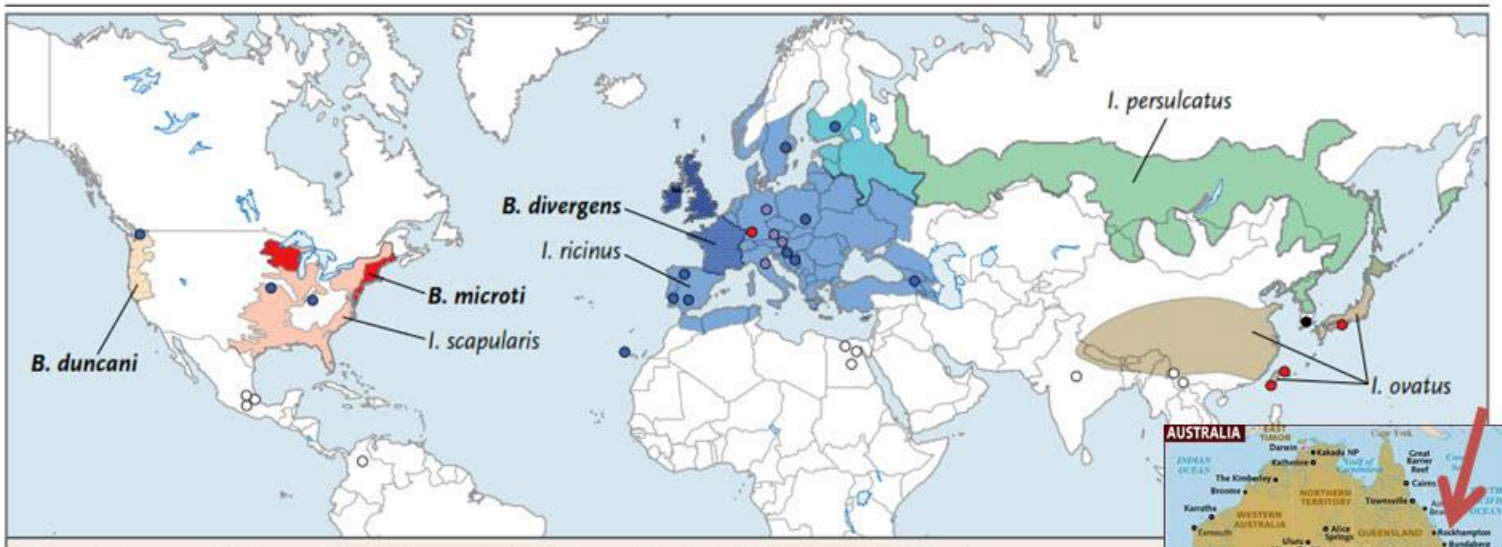


Figure 1. Geographic Areas Where Human Babesiosis and Ixodes Tick Vectors Are Endemic.

IMAGE Credit: New England Journal of Medicine: Vannier,E., and Krause, P., 2012, 366:2397-2401 (Modified to Include Australian endemic Human babesiosis)

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Babesia: Diagnosis

Babesia-DNS-PCR in blood (EDTA blood): direct detection

Babesia FISH (EDTA blood): direct detection

Blood smear: direct detection

Babesia-IgM and Babesia-IgG antibodies

Rarely:

- Haemolytic anaemia (erythrocytes, haptoglobin)
- Thrombocytopenia
- Leucocytopenia
- Increase of liver enzymes (sGOT, sGPT, sGGT)
- Increase of creatinine, urea
- Haemoglobinuria

Babesia: Therapy

- Clindamycin
- Malarone 250/200 mg 1x/day
- Malarone junior 65/25 mg 1x/day
- Atovaquon 750 mg 2x/day
- Lariam 250 mg
- **Plaquenil (Hydroxychloroquine)** 2 x 200 mg/day
- **Artemisia annua** 2 x 400 mg/day

Bartonella (cat scratch fever)

Bacteria: Bartonella henselae, Bartonella quintana (gram-negative, optional intracellular in endothelial cells / erythrocytes)

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

Symptoms (incubation time 3 - 38 days): headache (80%), tiredness (100%), amyostasia, muscle twitches, tremors, seizures, fever in the mornings (30%, in thrusts up to 6 weeks, otherwise 1 – 3 weeks), swollen lymph nodes, arthralgia (often), myalgia, insomnia, depression, agitation, severe mood swings, amentia, 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; Complications: endocarditis, retinitis, epilepsy, aseptic meningitis, hepatosplenomegaly

BLO: No or only minimal musculoskeletal symptoms (according to JJ. Burrascano)!

Risk factors: immune suppression (children)

Bartonella striae

Bartonella: Diagnosis

PCR on Bartonella in blood (EDTA): direct detection

Histology (haemangioma/lymphadenitis)

Bartonella henselae/quintana-IgM and Bartonella henselae/quintana-IgG

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

Bartonella: Therapy

- Macrolides (**Azithromycin, Clarythromycin**)
- Tetracycline/**Doxycycline**
- Quinolones (Ciprofloxacin, Levofloxacin)
- Rifampicin
- Ceftriaxone/Cefotaxime

Rickettsia

Bacteria: Rickettsia conorii, R. rickettsii, R. helvetica, R. slovaca, R. prowazekii (not gram-stainable, obligatory intracellular in endothelial cells)

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

Symptoms (incubation period 5 - 7 days): fever, lymphadenitis, exanthema

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

Rickettsia: Diagnosis

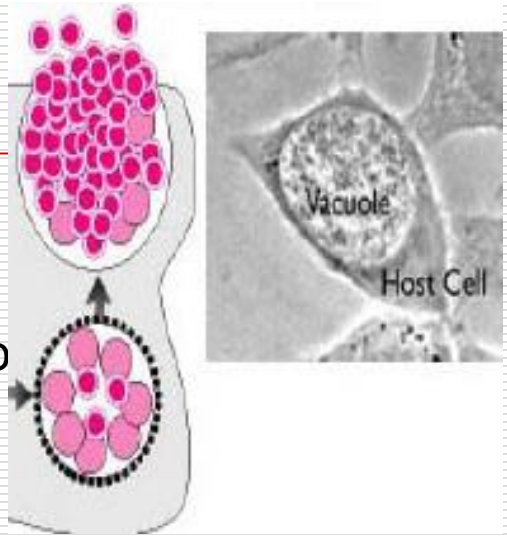
PCR on Rickettsia in blood (EDTA blood): direct detection

Rickettsia rickettsia/conorii IgM and IgG antibodies

Rickettsia: Therapy

- **Doxycycline**/Tetracycline
- Ciprofloxacin
- Chloramphenicol
- Erythromycin (children)

Chlamydia pneumoniae infection



Bacteria: Chlamydophila pneumoniae (gram-negative, intracellular)

Vector/Transmission: airborne infection, human to ticks ? Or reactivated in Lyme disease (horses, koalas, frogs are infected)

Symptoms: **cough, slight throat pain, hoarseness, sinusitis,** atypical pneumonia, meningoencephalitis, bronchiolitis obliterans, myocarditis, Guillain-Barre Syndrome

After infection (4 - 6 weeks): arthritis, tendovaginitis

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

Risk factors: immune suppression (children/the elderly)

Chlamydia pneumoniae: Laboratory tests

Chlamydia pneumoniae EliSpot-LTT

Antibodies for Chlamydia pneumoniae-IgA and Chlamydia pneumoniae-IgG: Half-life of local-standing IgA-antibodies 2 weeks

New study IgA in acute ischemic stroke: 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)

PCR of Chlamydia pneumoniae in blood/sputum/pharyngeal secretion: direct detection

Chlamydia pneumoniae: Therapy

- Macrolides (**Azithromycin, Clarythromycin**)
- **Doxycycline/Minocycline**
- Levofloxacin
- **Metronidazole**

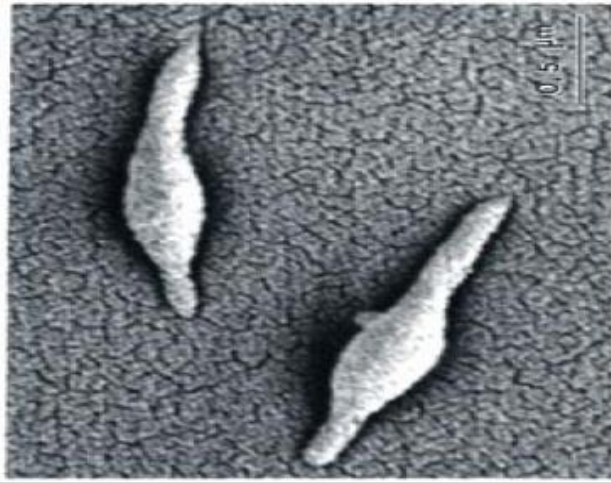
Mycoplasma infection

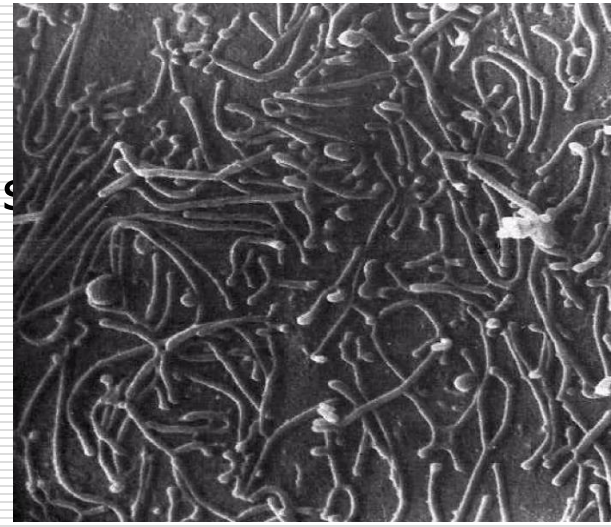
Bacteria: Mycoplasma pneumoniae/fermentans (gram-positive, intracellular)

Transmission: airborne infection, human to human, ticks ?

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

Risk factors: immune suppression (children/the elderly), ME/CFS, Gulf War I syndrome





Mycoplasma: Laboratory tests

Mycoplasma pneumoniae IgM- and -IgG antibodies

Bacterial culture

PCR of Mycoplasma pneumoniae in blood/sputum/secretion: direct detection

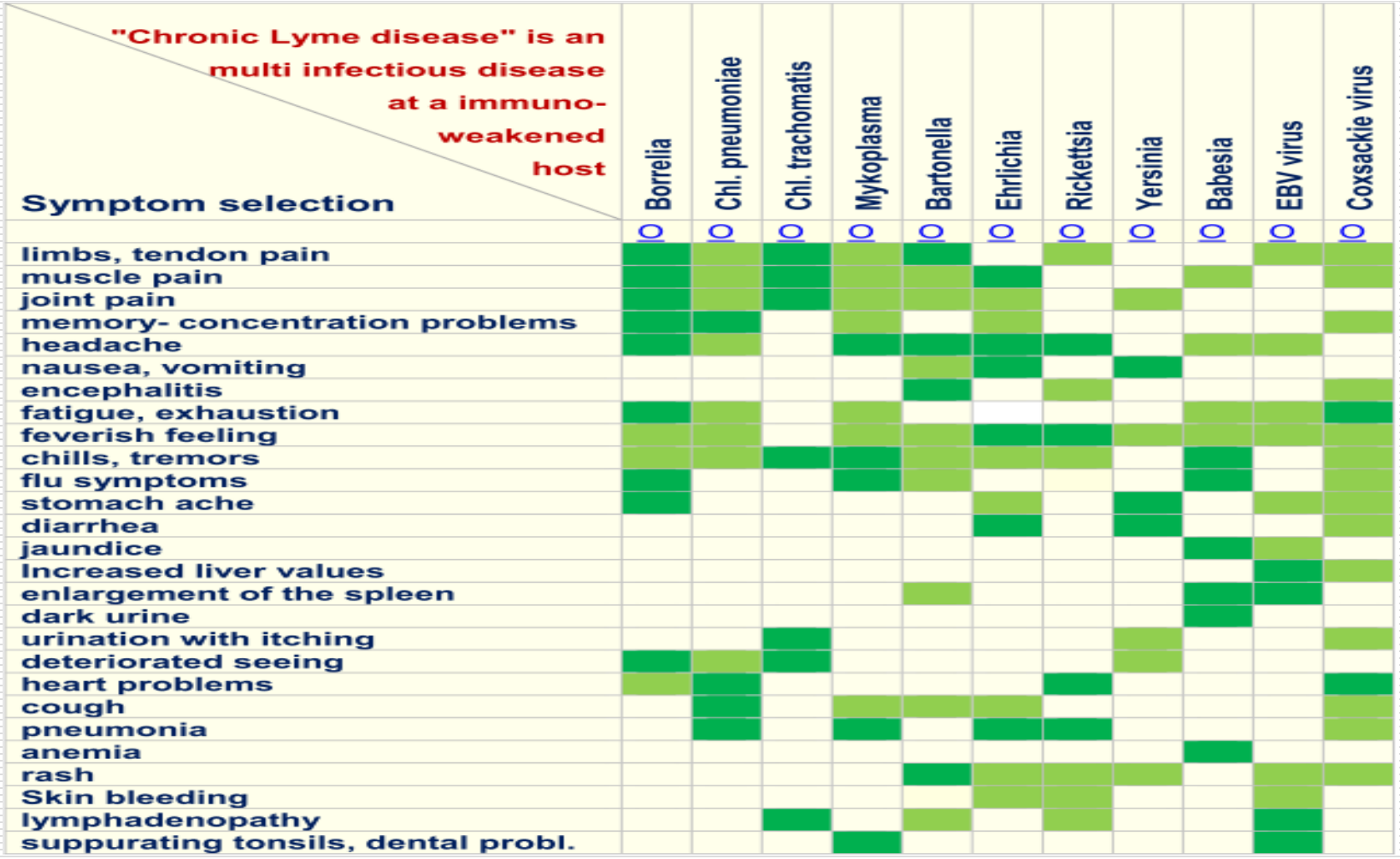
Mycoplasma: Therapy

- Macrolides (**Azithromycin, Clarythromycin**)
- **Doxycycline/Minocycline**
- **Metronidazole**
- Levofloxacin, Ciprofloxacin

Other complicating / reactivated viruses or bacteria

- ☑ Yersinia enterocolitica
- ☑ Herpes Simplex Virus Typ I/II
- ☑ Cytomegalovirus (CMV)
- ☑ Toxoplasma gondii
- ☑ Epstein-Barr-Virus (EBV)
- ☑ HHV 6
- ☑ HHV 8
- ☑ Coxsackie-Virus

MULTIPLE SYMPTOMS = MULTIPLE INFECTIONS ?



The new co-infections checklist (to be filled out by the patient)

arminlabs
DIAGNOSING TICK-BORNE DISEASES

ArminLabs GmbH
Zirbelstr. 58, 3rd 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	Stomach ache, gut problems			
27	Anaemia			

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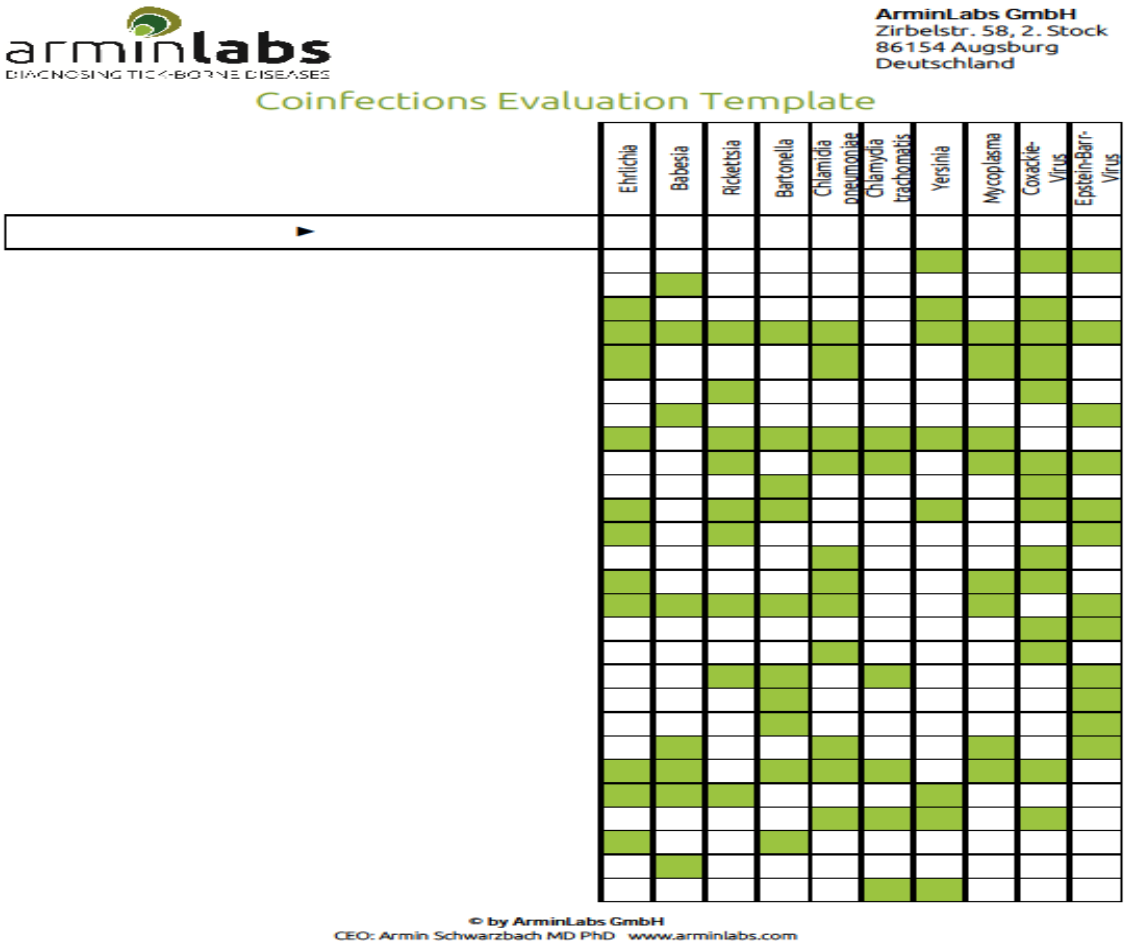
Evaluation template for doctors/naturopaths



Co-infections 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 LTT				
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 LTT				
Yersinia Elispot LTT	+	**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 LTT				
Chlamydia pneumoniae Elispot LTT	+	**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 LTT				
CMV Elispot LTT	+	**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 LTT				
EBV-Elispot-LTT (lytic)	+	**17**	SI	< 2
EBV-Elispot-LTT (latent)	+	**8**	SI	< 2
CD 57 Flow Cytometry				
CD 57 positive NK cells	-	**37**	/µl	100-360

Summary Patient 1

Co-infections checklist (symptoms):

Multiple infection with

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

Epstein Barr Virus + Rickettsia + Yersinia

Laboratory test results:

Multiple infection with

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

5 bacteria + 3 viruses !

Correlation of modern laboratory tests for Borrelia burgdorferi and Chlamydia pneumoniae

	Borrelia burgdorferi				Chlamydia pneumoniae		
	IgG / IgM	LTT	CD57		ChlP IgG	ChlP IgA	LTT
positive	22 = 44%	21 = 42%	27 = 56%		27 = 54%	26 = 52%	15 = 50%
negative	20 = 40%	12 = 24%	21 = 44%		20 = 40%	20 = 40%	10 = 33%
borderline	8 = 16%	17 = 34%			3 = 6%	4 = 8%	5 = 17%
			no value = 2				no value = 20

Summary of my study: Borrelia burgdorferi and Chlamydia pneumoniae

- Sensitivities in my study (n=50):

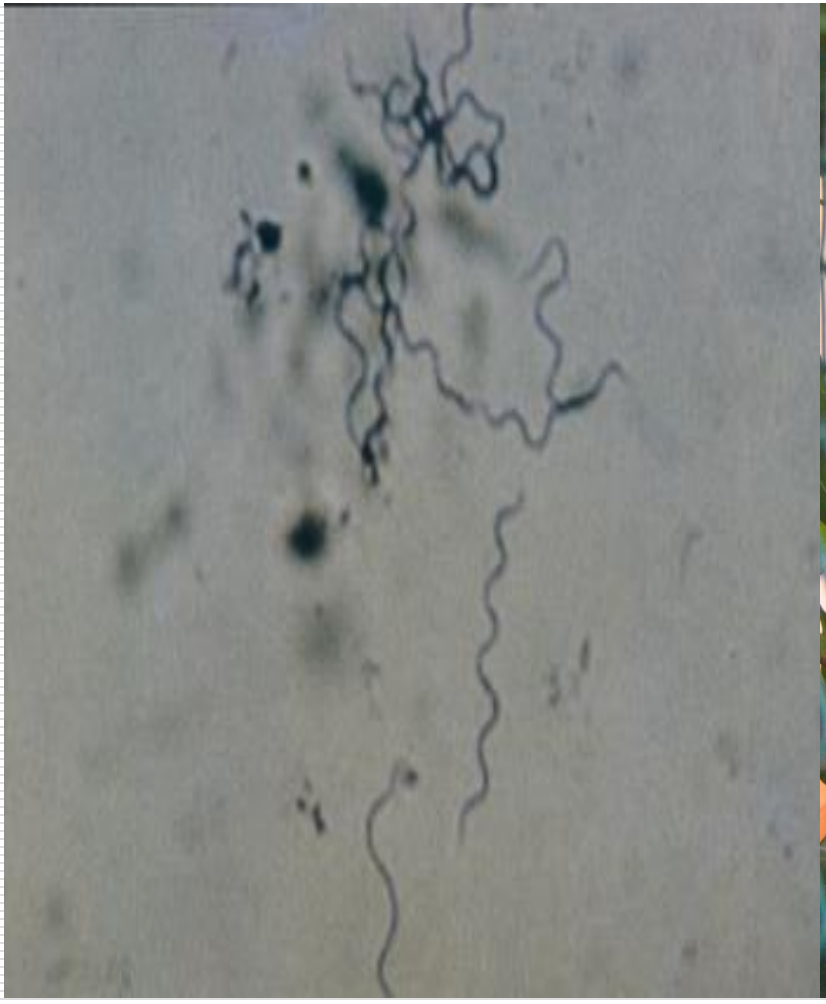
Chlamydia pneumoniae

- - Chl. Pneumoniae-IgA: 60 %
- - Chl. Pneumoniae-IgG: 60 %
- - Chl. Pneumoniae-Elispot-LTT: 67 %
- **All 3 tests together: 78 %**

Borrelia burgdorferi

- - Borrelia-Elispot-LTT 76 %
- - Borrelia-IgG/IgM-immunoblot: 60 %
- - CD57-count: 56 %
- **All 3 tests together: 90 %**

PART 3: THERAPEUTIC OPTIONS



Modular therapeutic concept

1. **Antibiotics**
2. **Nutrition** (anti-inflammatory diet, basic nutrition)
3. **Nutritional supplements** (vitamins, minerals, essential fatty acids, probiotics)
4. **Pain therapy**
5. **Complementary therapies** (Naturopathy, Homeopathy, Biological Medicine): e.g. Samento, Cumanda, Noni, Banderol, Tacuna, Teasel, Cat's claw, Artemisia annua, Resveratrol, Andrographis, garlic….
6. **Rehabilitation and exercise therapy**
7. **Stress management and relaxation therapy**
8. **Mental Coaching, change of "Lifestyle"**

Aims and goals of therapies

- Destruction of bacteria or viruses (for example antibiotics: bacteriostatic and bactericide antibiotics)
- Consider the different structures of bacteria or viruses (pleomorphic forms, biofilms, intracellular), and diagnose co-infections for the right selection of therapies
- Sufficiently long treatments (consider the life cycles of Borrelia burgdorferi and co-infections)
- Low risk-benefit ratio: Side effects

Oral antibiotics:

0,0001 – 0,04 % (1: 1 000 000 – 4: 10 000)

Infusions with antibiotics:

0,001 – 0,03 % (1: 100 000 – 3: 10 000)

Therapeutic options: antibiotics

Antibiotics for Borrelia, Chlamydia and Mycoplasma:

- Macrolides (Azithromycin, Clarythromycin)
- Doxycycline/Minocycline
- Metronidazole
- Cefalosporines (Ceftriaxone, Cefuroxim, Cefotaxim)

Remedies that have an intracellular action:

- Hydroxychloroquin (Plaquenil)
- Artemisia annua intensae

Therapeutic options: viruses and biofilms

Viruses (EBV, CMV, Coxsackie, etc.)

- Dimepranolacedoben/Inosin 50 mg/kg of body weight daily (Delimmun)
- Immunmodulation (vitamins, etc.)
- Herbal products / alternative pathways (e.g. Takuna)

Biofilms

- Serrapeptase
- Lumbrokinase

Lyme disease: Antibiotics Stage I (recent infection)

Stage I (recent infection)

Oral therapy (Duration: minimum until the "bull's eye rash" or lymphocytic infiltration have disappeared): up to 4 - 6 weeks.

Doxycycline (from 8 years old upwards)

(works against Ehrlichia as a co-infection)

Cefuroxim *

Amoxicillin *+ (Probenicid)

(* for children under 8 years and pregnant women)

Chronic Borrelia infections: Example for antibiotic strategy

Azithromycin 500/600 mg oral 1 x 1 per day/ 3 days per week
Contra-indications (Heart rhythm problems: QT-time, AV block, pregnancy)

+

Doxycycline 200/400 mg 1 x 1 per day
or
Minocycline 50/100 mg 1 x 1 per day

+

Hydroxychloroquine 200 mg 2 x 1 per day
or
Artemisia annua 200 mg 2 x 2 per day

(+)

After that 1-2 weeks single therapy with:

Metronidazole 400 mg 2 x 1 per day

Whole Body Concept – WBC

1. Improve compatibility and effectiveness of the antibiotics
2. Strengthen the immune system
3. Relieve pain
4. Produce “bad” conditions so as to inhibit the reproduction of bacteria
5. Mobilize “hidden“ Borrelia bacteria in the connective tissue
6. Reduce the inflammation/cytokine storm and balance the TH 1 and TH 2 systems
7. Treat anxiety, depression, concentration problems, etc.
8. Support the organ system (liver, kidney, intestines, heart, brain)
9. Detoxify
10. Improve overall capacity/muscle power

Myer's Cocktail Immune System Support (ME/CFS…)

Vitamin C (Ascorbic acid)	5000 mg
Vitamin B1 (Thiamin)	100 mg
Vitamin B6 (Pyridoxine)	25 mg
Vitamin B12 (Cyanocobalamin)	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

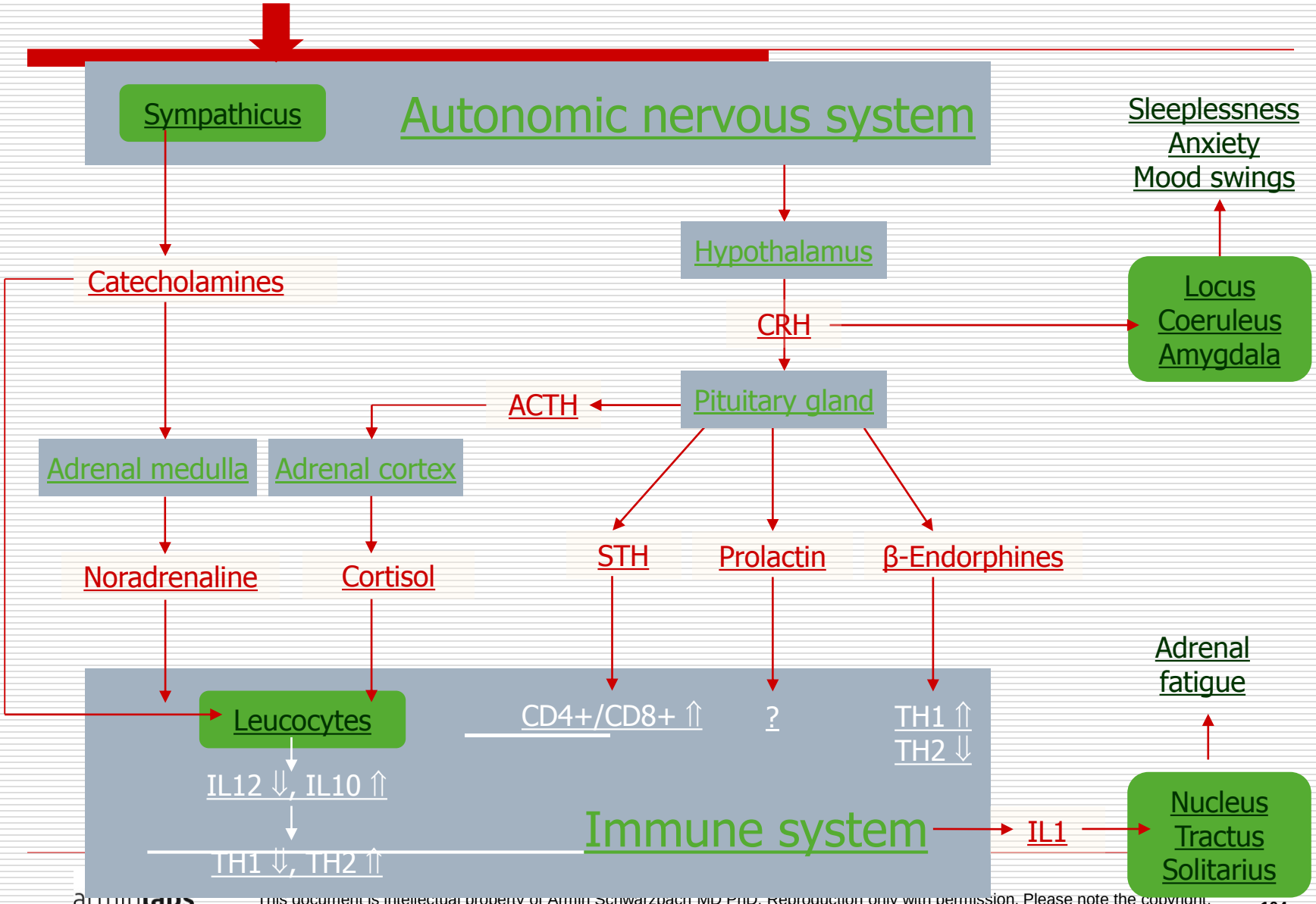
Naturopathy: For example, Cowden Support Protocol - Herbal support for TH1 cells

- 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, Cowden Support Protocol - Herbal support for TH2 cells

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

Stress from infections



Infections: Herbal support

Adrenal fatigue

- Schizandra
- Astragalus
- Rhododendron caucasicum
- Ginseng
- Rhodiola

Stress/Anxiety/Sleeplessnes

- Amantilla (Valeriana officinalis)

Dr. Leo Joosten, Department of Medicine , Radboud University, Netherlands:

If you believe in chronic Lyme disease, what do you believe are the most effective ways to treat it?

"If chronic or persistent Lyme disease exists, we will need more than antibiotics for treatment. Anti-inflammatory components or anti-cytokine treatment will be options."

Summary and conclusions

- Sensitivity of ELISA in chronic Lyme disease: 32 - 42%
- Sensitivity of Immunoblot in chronic Lyme disease: 60%
- ELISA tests are too insensitive and useless
- Negative antibodies in the Immunoblot cannot exclude Lyme disease
- IgM antibody persistence is a sign of chronic Lyme disease
- There is a possibility of false negative CSF results
- 86% of patients with chronic Lyme disease are co-infected with Chlamydia pneumoniae (**multiple infections**)
- Borrelia or Chlamydia/Mycoplasma pneumoniae symptoms are not highly specific (overlapping symptoms)
- Patients can be co-infected by other bacteria in the tick (Babesia, Bartonella, Rickettsia, Ehrlichia/Anaplasma)
- **Ranking** of the co-infections can be performed by a modern co-infections checklist

Summary and conclusions

- **Staging** of Lyme disease and co-infections should be performed by modern laboratory tests: recombinant Borrelia Immunoblots, incl. VlsE, Elispot-LTT, CD57
- Multiple infections Borrelia+Chlamydia+Mycoplasma:

  Very often they are the reason for ME/CFS, Multiple Sclerosis, ALS, Parkinsonism, Dementia, Fibromyalgia, Rheumatoid Arthritis, Autism, Apoplectic Strokes, Heart attacks
- Give preference to antibiotic that have intracellular action
- Use combinations of antibiotics: Azithromycin/Clarythromycin + Doxycycline/Minocycline + Artemisia annua intensae
- Use additional therapies against biofilms
- No antibiotics without probiotics!

Thank you very much for your attention !

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