Modern Diagnostic Tests for Chronic Lyme Disease and Co-infections

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Germany

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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(2010) Schwarzbach A. (unpublished)	92% / 60% Blot - /32-42% ELISA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%

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

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

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

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

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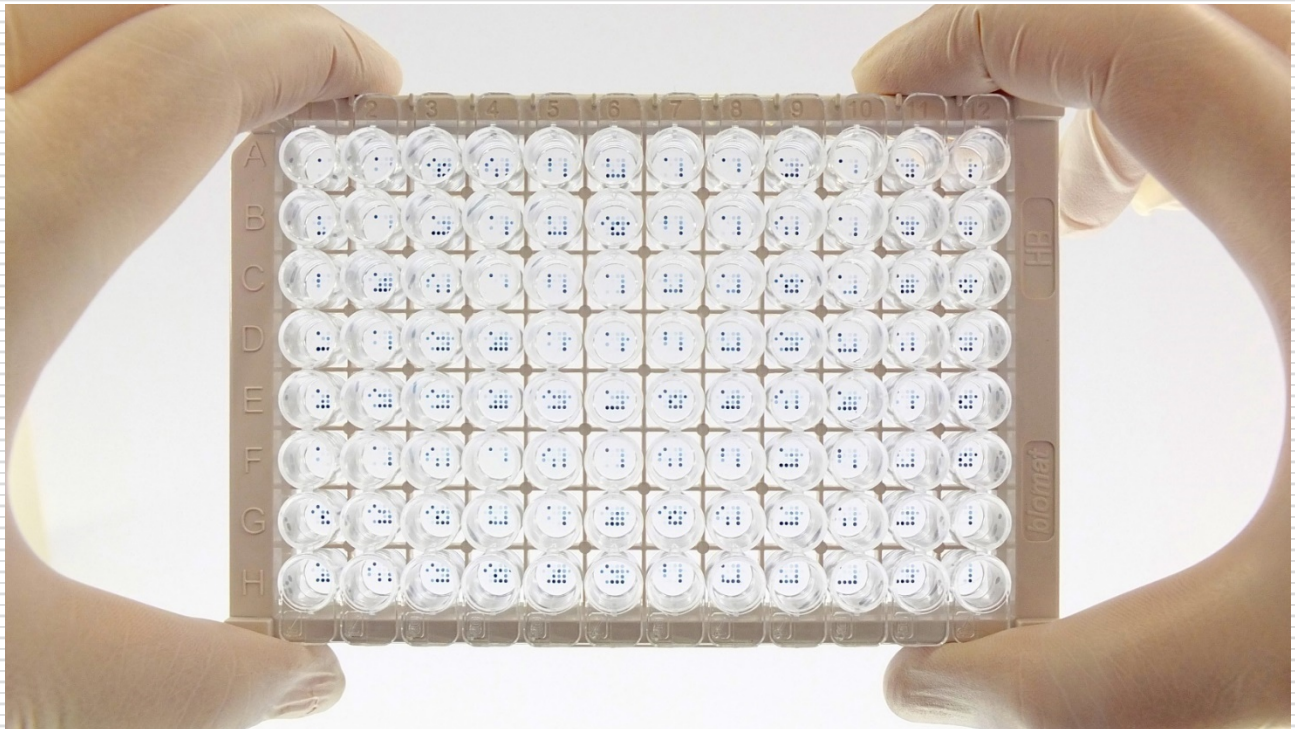
Antibodies by SeraSpot MicroArray

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



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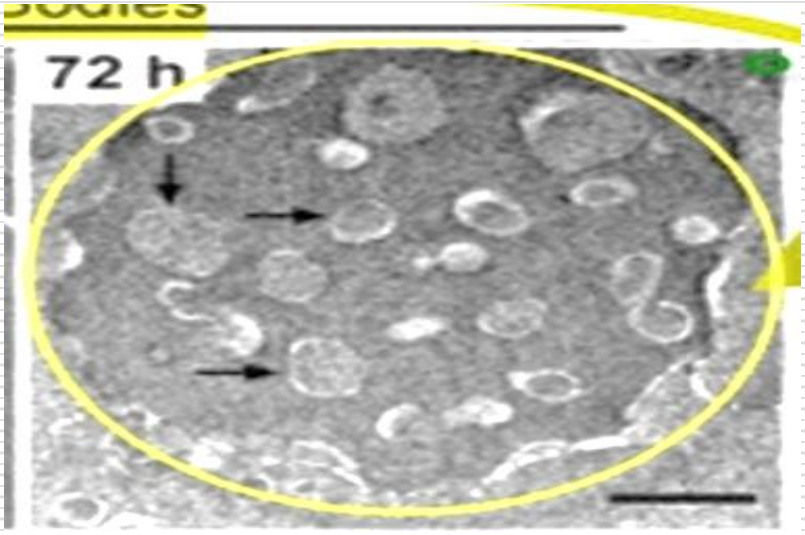
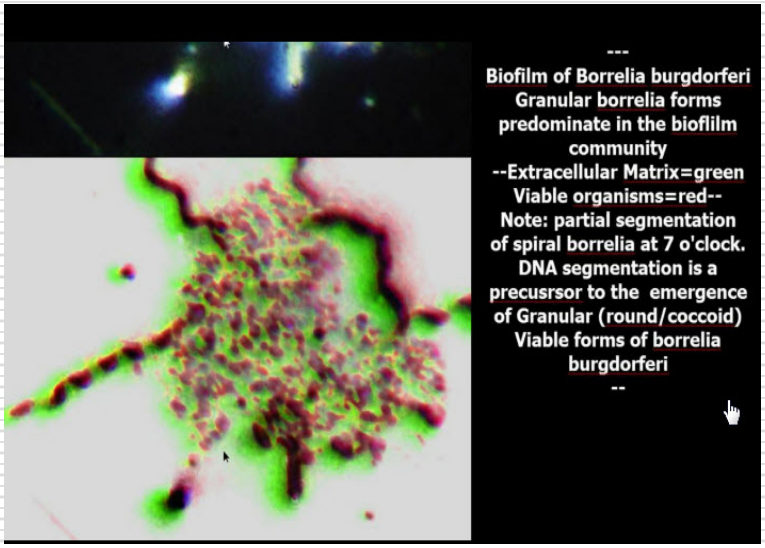
Round bodies (pleomorphic forms) and biofilm-like colonies of Borrelia burgdorferi in vitro: Antibodies?





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

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

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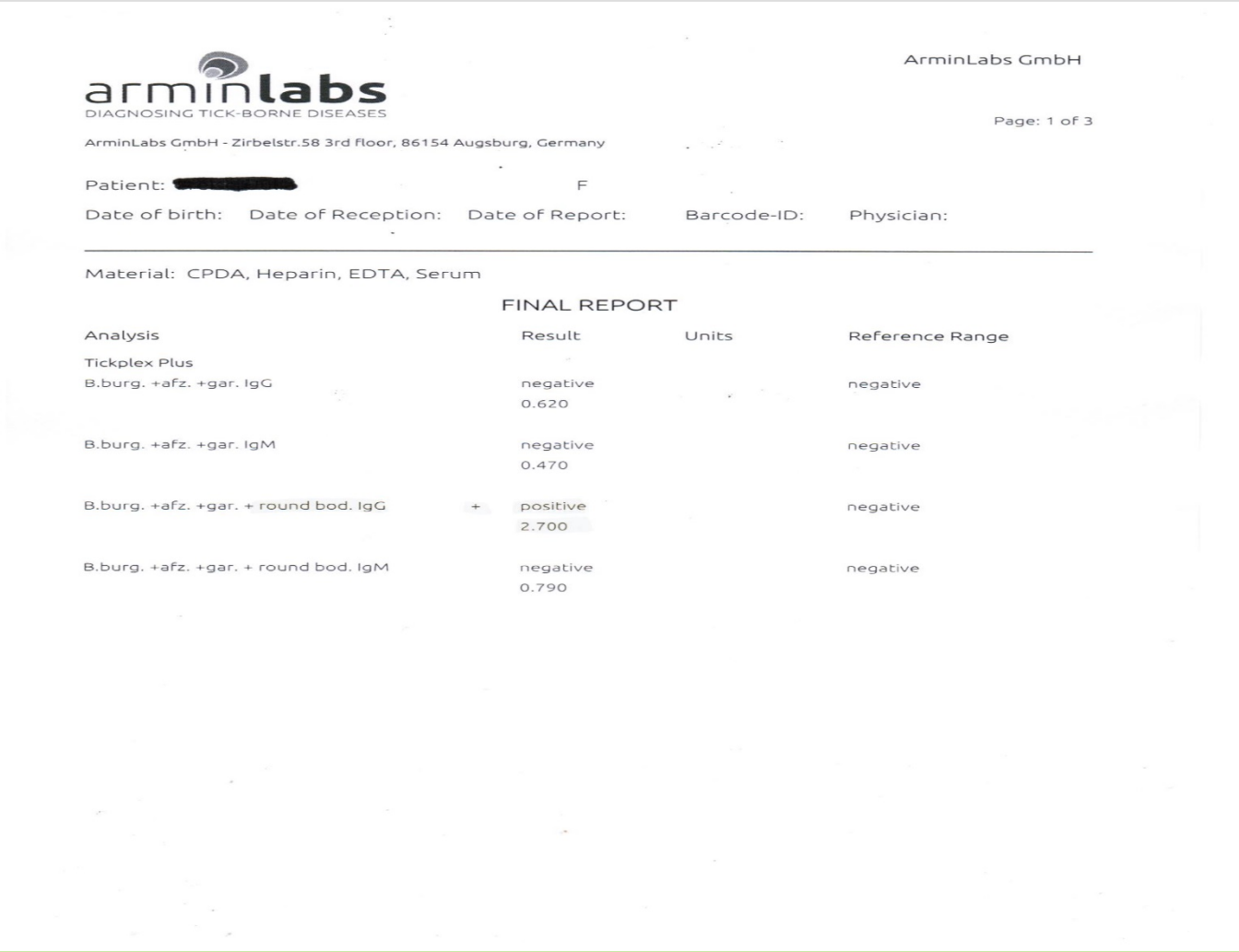
Antibodies by Tickplex Basic incl. round bodies www.tezted.com



arminlabs
DIAGNOSING TICK-BORNE DISEASES

ArminLabs GmbH

Page: 1 of 3

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

Patient: [redacted] F

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

Material: CPDA, Heparin, EDTA, Serum

FINAL REPORT

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

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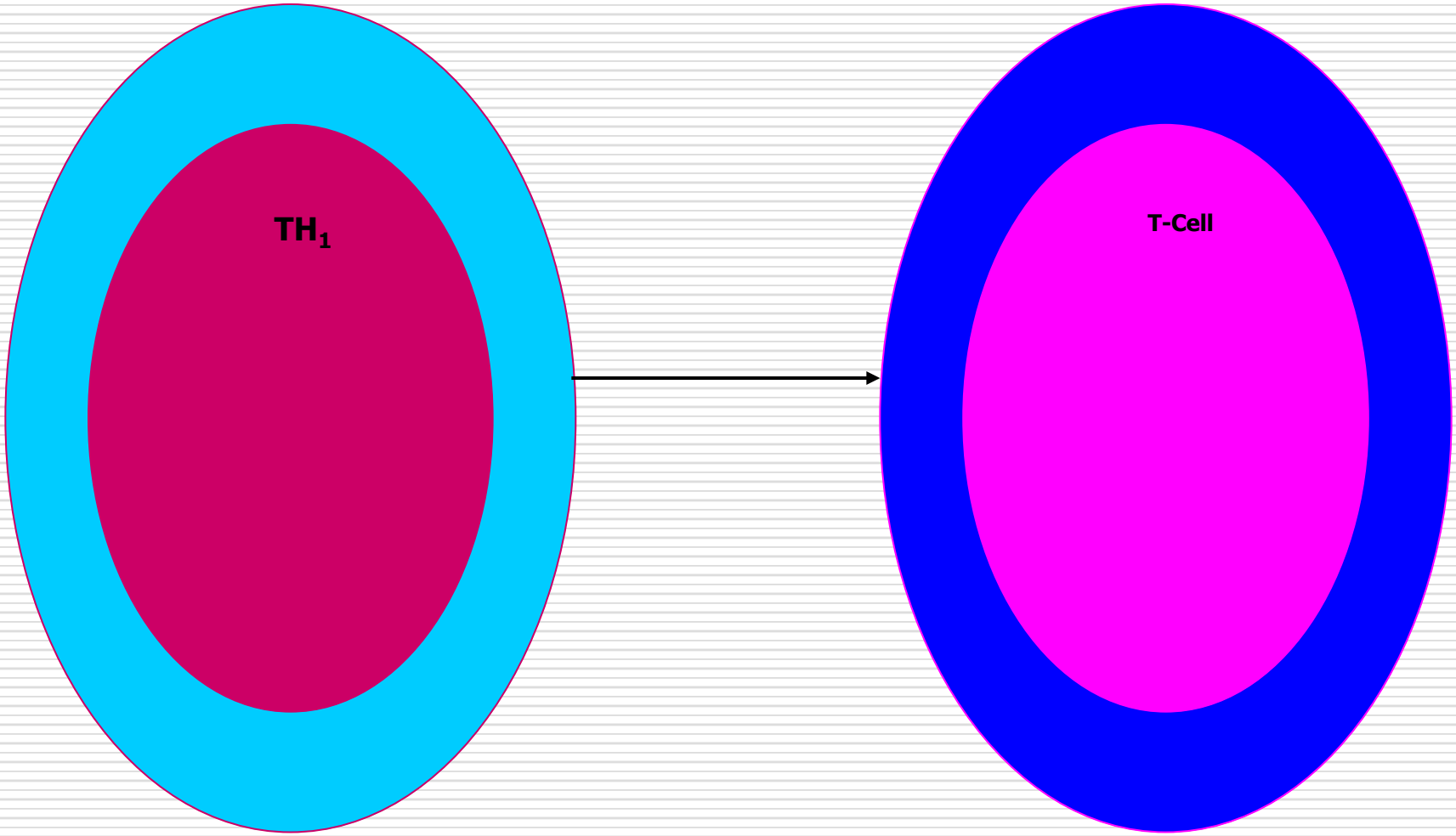
T-cells: EliSpot and CD57 cells



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CD3-/CD57+ T-lymphocytes

1. Subpopulation of the CD56+ NK cells
2. Reduction may indicate chronic Lyme disease (symptoms > 1 year)
3. Reduction in untreated and inadequately treated Lyme disease
4. Not highly specific: Also low in other bacterial infections, esp. Chlamydia pneumoniae and Mycoplasma pneumoniae

Reference range

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

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Example Borrelia EliSpot laboratory test report

arminlabs
DIAGNOSING TICK-BORNE DISEASES

Armin Schwarzbach MD PhD
Specialist for laboratory medicine

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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

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DIAGNOSING TICK-BORNE DISEASES

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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...

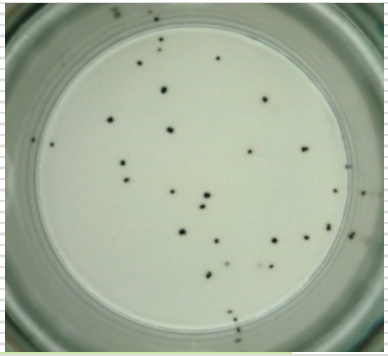
... ELISPOT can be retested to gain additional information in follow-up assays...

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

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

94 % Specificity of Borrelia-Elispot-LTT

84 % Sensitivity of Borrelia Elispot-LTT



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EliSpot Test Results Compared to "Standard" Laboratory

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

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

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

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

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

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

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Currently the EliSpot is available for:

- Borrelia burgdorferi (B.b. sensu stricto + garinii + afzelii)
- Borrelia myamotoi
- Ehrlichia/Anaplasma
- Bartonella henselae EliSpot
- Babesia microti EliSpot
- Chlamydia pneumoniae
- Chlamydia trachomatis
- Mycoplasma pneumoniae
- Yersinia species
- Epstein Barr Virus (EBV)
- Cytomegalovirus (CMV)
- Herpes Simplex Virus 1 / 2 (HSV 1 / 2)
- Varicella Zoster Virus (VZV)
- Candida
- Aspergillus

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Comparing Lyme Testing

Key terms:

ELISA – Enzyme Linked Immuno Sorbent Assay

Specificity - True negative rate

Sensitivity - True positive rate

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

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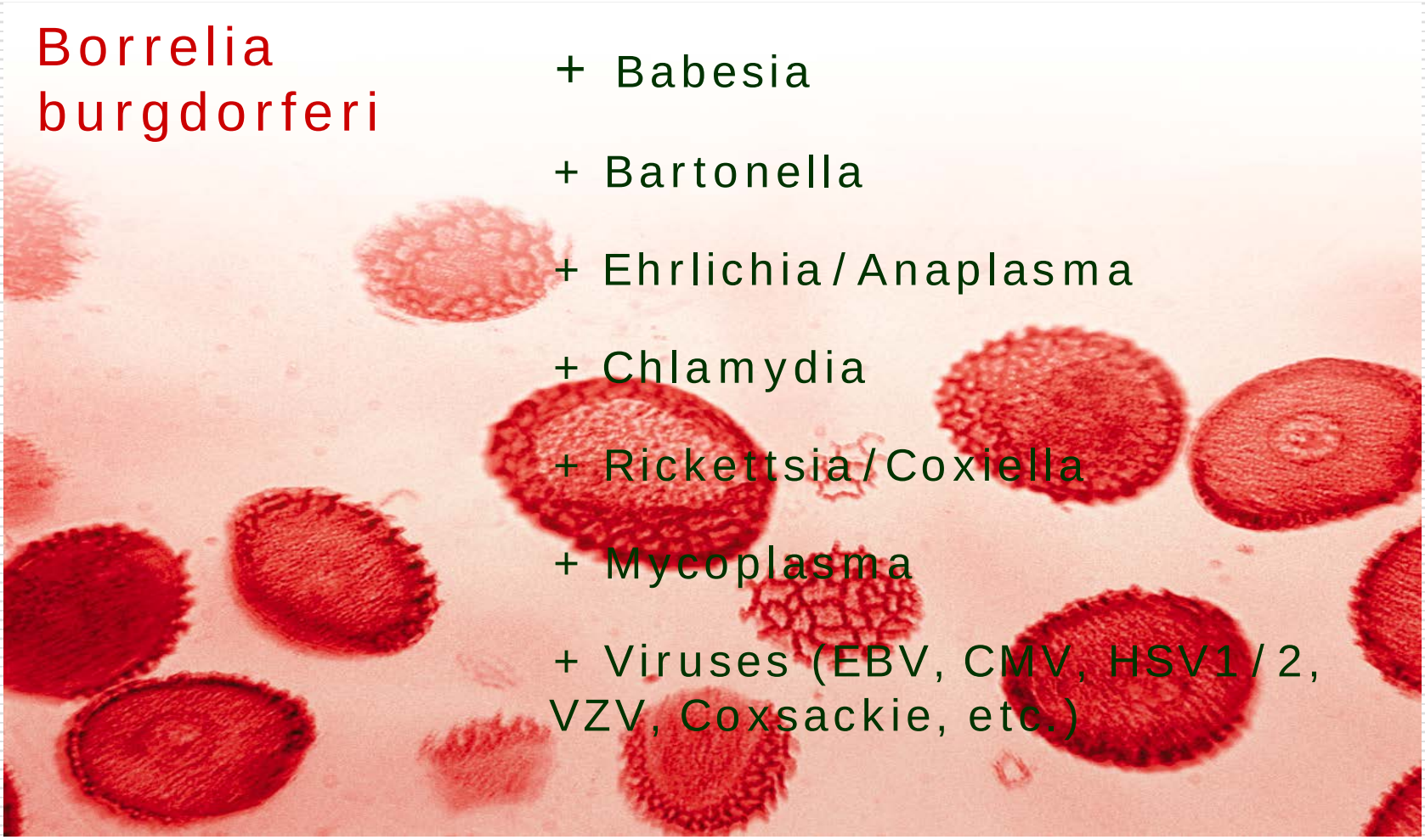
LYME BORRELIOSIS and CO-INFECTIONS



Borrelia burgdorferi

+ Babesia

+ Bartonella

+ Ehrlichia/Anaplasma

+ Chlamydia

+ Rickettsia/Coxiella

+ Mycoplasma

+ Viruses (EBV, CMV, HSV1/2, VZV, Coxsackie, etc.)

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Laboratory tests Bartonella

Bartonella henselae IgG/IgM antibodies

Bartonella quintana IgG/IgM antibodies

Bartonella henselae EliSpot

Bartonella PCR in blood (EDTA)

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

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Laboratory tests for Babesia

Babesia microti IgG/IgM antibodies

Babesia microti EliSpot

Babesia DNA PCR or FISH in blood (EDTA blood)

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Laboratory tests for Chlamydia pneumoniae

Chlamydia pneumoniae EliSpot

Chlamydia pneumoniae IgA and Chlamydia pneumoniae IgG:

half-life of local IgA antibodies 2 weeks

New study on 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)

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Laboratory tests for Mycoplasma pneumoniae

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

Mycoplasma pneumoniae EliSpot

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Laboratory tests for Epstein Barr Virus (EBV)

Epstein Barr Virus IgG/IgM antibodies

Epstein Barr Virus Anti-EBNA antibodies

Epstein Barr Virus Early Antigen antibodies (reactivated or chronic)

Epstein Barr Virus Elispot

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

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Laboratory tests for CMV

CMV IgG/IgM antibodies

CMV Elispot

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Laboratory tests for 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

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Laboratory tests for Varicella Zoster Virus (VZV)

Varicella Zoster Virus (VZV) – IgG/IgA/IgM antibodies
(half-life time of local-standing IgA-antibodies: 2 weeks)

Varicella Zoster Virus (VZV) - Elispot

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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

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Laboratory tests Coxsackie Virus

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

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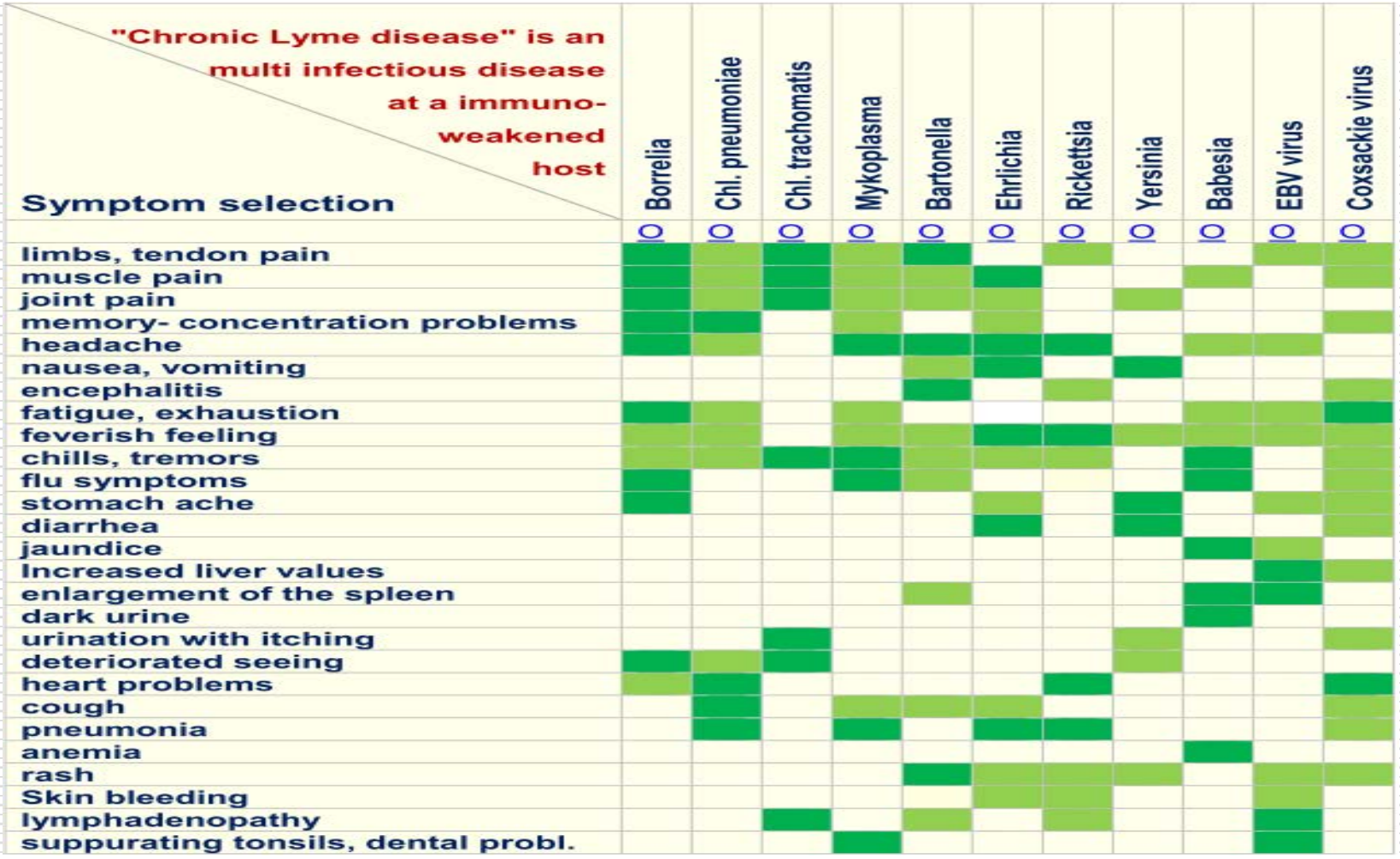
MULTIPLE SYMPTOMS = MULTIPLE INFECTIONS



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Thank you very much for your attention !

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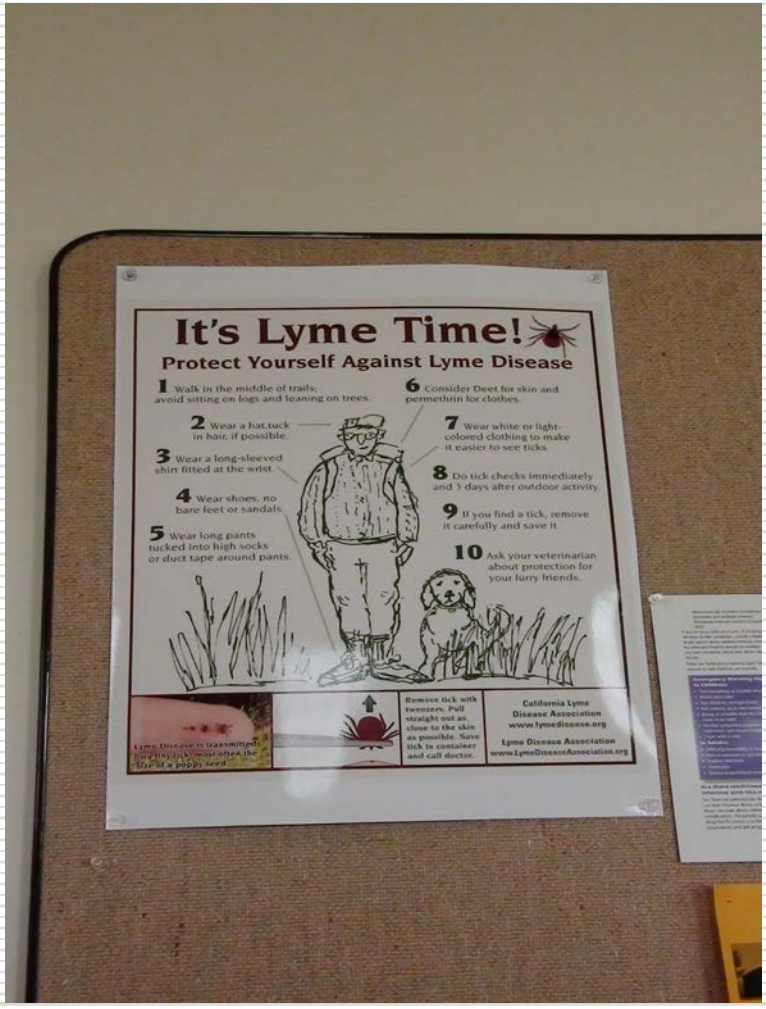

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LDN 2018 Conference Presentation

By Dr Armin Schwarzbach

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