Laboratory Diagnostics:
Utility of Different Test Systems

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- Basic epidemiology
- Direct detection
- Indirect detection
- New tests
- Tests not to use
- Test Quality
- Summary

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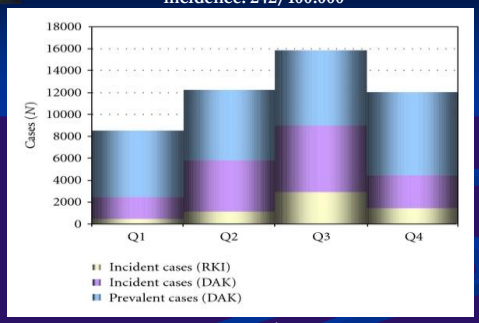
Annual Epidemiology

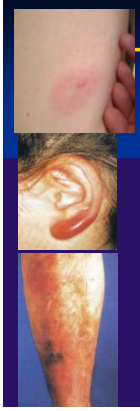
incidence: 242/100.000



median: 2007/2008

Müller I. & Freitag M.H. , Clin Develop Immunol, (2012)

The Clinical Picture



erythema migrans	65-80%
neuroborreliosis	10-12%
Lyme arthritis	8% (EU)
	30% (USA)
lymphadenosis cutis benigna	1-3%
acrodermatitis chronica atrophicans	1-2%
carditis	0.2-3%
ophthalmoborreliosis	0.2%

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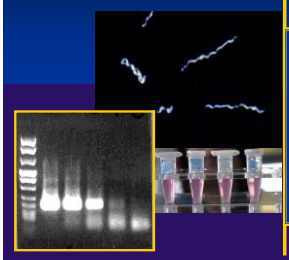
Direct Detection Methods



PCR/ Culture:	Sensitiviy
Skin biopsy:	app. 70/ 70%
Blood (highly variable):	app. 10/ 1-50%
CSF:	app. 20/ 30 %
Synovial fluid/ biopsy	app. 70/ 1%

Hunfeld KP et al., Borreliosis. In: Thomas L: Laboratory & Diagnosis (2012)

Indirect Detection Methods

Serology

- IHA+
- IFT +
- ELISA +,*
- Immunoblot +,*
- Luminex-Tests*

+whole cell lysate, *recombinant antigens

Serology: Two-tier Testing

screening test: EIA (IgG / IgM)

- positive / borderline → confirmatory test
- negative → negative Lyme-serology, follow-up

confirmatory test: immunoblot (IgG / IgM)

- positive → positive serology
- borderline → Back-up assay, follow-up
- negative → negative serology

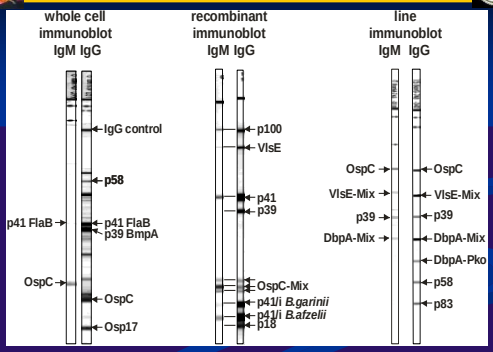
Hunfeld K.P. et al., Current Probl Dermatol (2009)

Serodiagnostics

Stage-dependent antibody kinetics
(mean seroprevalence (EU): 15-20%)

stage	seropositive patients [%]	IgM [%]	IgG [%]
I	20 - 80	bis 90	bis 70
II	50 - 90	30 – 80	65 – 100
III	95 - 100	5 - 48	100

Immunoblot: Why?



Hunfeld K.P. et al., Current Probl Dermatol, (2009)

Antigen-specific Antibody Kinetics

proteine	phase	specificity	cross reactivity
p83/100	late	+++	(+)
p58	early/late	++	(+)
p43	early/late	++	(+)
p41 (flagellin)	early/late	+	+++
p39 (BmpA)	late	++	(+)
OspA	late	+	(+)
OspC	early/late	+++	(+)
Osp17 (DbpA)	late	++	(+)
VlsE	early/late	++	(+)
p41 (int)	early/late	++	(+)

Immunoblot

Pros & Cons

- high sensitivity & specificity
- antigen- & antibody-specific analysis
- banding pattern essential for interpreting results
- follow-up (special indications only!)
- integrative report (EIA+BLOT) offers substantial information!

- two-tier testing: impaired sensitivity
- Lack of standardisation
- high hands on time
- Higher cost

Comparing IgG-EIAs With and Without VlsE
Multi-Center-Study (2005)

specifity

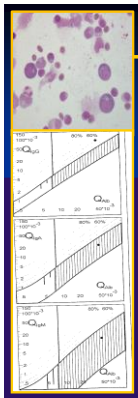
	samples	negative	EIA IgG N	EIA IgG [%]	EIA IgG/VlsE N	EIA IgG/VlsE [%]
bacterial infection	177	158	149	94,3	156	98,7
viral infection	324	287	280	97,6	284	99,0
autoimmune disease	158	123	120	97,6	123	100,0
Blood donors	687	628	623	99,2	624	99,4

sensitivity

Lyme borreliosis	samples	EIA IgG N	EIA IgG [%]	EIA IgG/VlsE N	EIA IgG/VlsE [%]
Stadium I	202	95	47,0	135	66,8
Stadium II	181	146	80,7	159	87,8
Stadium III	156	156	100	156	100

Hunfeld KP et al., 10. ICLB Wien, (2005)

Neuroborreliosis



impact of basic diagnostics

- lymphocytic pleocytosis
- activated B-lymphocytes
- blood-brain barrier dysfunction (Qalb: up to 50 x 10^{-3})
- intrathecal IgM-, IgG-, (IgA-) response

sensitivity: 70%; specificity: 98%

AI-calculation & cross match-blot

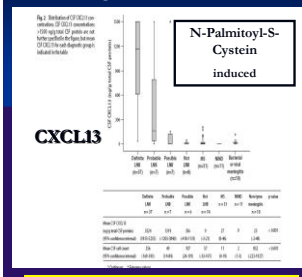
sensitivity: 75-95%; specificity: 97%

Cave: antibody production in CSF only: 5-25%

Blanc F et al., Neurology, (2007);
Oschmann P., et al., Neurology, (1998)

New Markers of Early Neuroborreliosis

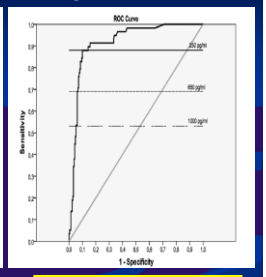
N=56 patients; N=33 controls



CI-sensitivity: 91-100%;
CI-specificity: 31-86% ,
normal after 4 mos.: 82%

Ljostad U, et al., J Neurol, (2007)

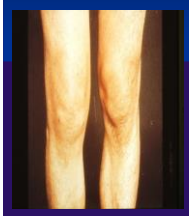
N=58 patients; N=303 controls



sensitivity: 88%;
specificity: 89%

Van Burgel et al., JCM, (2011)

Lyme-Arthritis



clinical diagnostic clues

Exposure or previous tick-bite
Previous erythema migrans
daktylitis, achillo-tendinitis, bursitis
errosions (very rarely) after several years

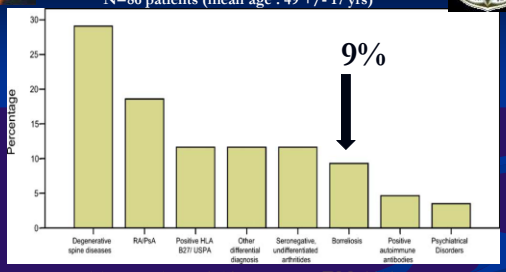
laboratory diagnostic clues

CRP not elevated
voluminous swelling with intraarticular swelling
granulo- (mono-) cytosis
Highly positive serology (!!)
Molecular detection of borrelia by PCR (70-90%)

Schnarr S, et al., Best Pract Res Clin Rheumatol, (2006)

Chronic Lyme Borreliosis??

N=86 patients (mean age : 49 +/- 17 yrs)



- positive serology: 47%
- past EM: 33%
- tick-bite: 60%
- antibiotic treatment: 86%

Seidel M et al., Eur J Clin Microbiol Infect Dis, (2007)

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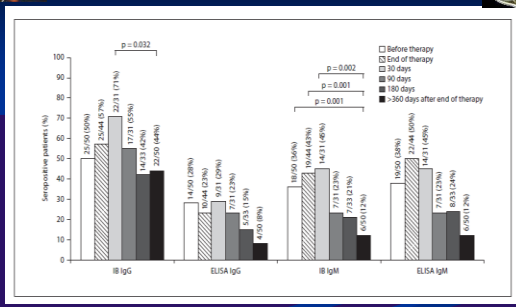
Lyme Serology: Diagnostic Comment

Be aware that:

- additional clinical information is always essential for interpreting of laboratory results !
- early treatment may avert seroconversion or regular IgM/IgG-switch in early manifestations of LB (e.g. EM, FP)!
- specific AB (also IgM) may persist for months or even years after a past infection !
- a regular follow-up is not recommended !
- Do not comment on treatment issues!

Follow-up: Does it Make Sense?



Serological follow-up in 50 patients with erythema migrans

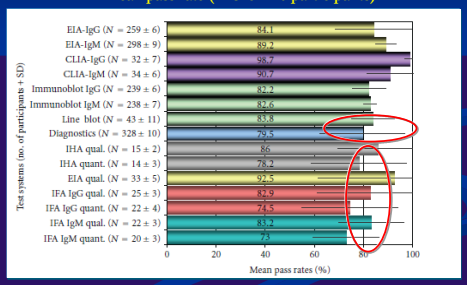
Glatz M et al., Dermatology, (2008)

Follow-up: Are There Indications ??

- seronegative or borderline results in patients with atypical early manifestations (e.g. atypical erythema, FP)
- IgM positivity only (suspicion of nonspecific reactions):
  - detection of IgG-seroconversion
- early neuroborreliosis (e.g. FP) and negative CSF result
- be aware:
  - single IgM-positivity speaks against long-lasting manifestations of LB!
  - a significant change can be stated only if samples are tested in parallel (serum stock!)

External Quality Control (INSTAND) Lyme Serology Proficiency Testing

results of 8 surveys (2006-2009)
Mean pass rate (n=328 ± 10 participants)



Müller I. & Freitag M.H. , Clin Develop Immunol, (2012)

Not Recommended Diagnostic Methods

- direct detection of borrelia from ticks
- lymphocyte transformation test (LTT)
- detection of so called „cystic forms“ of *B. burgdorferi*
- VCS-test (Visual contrast sensitivity test)

Extrapolated Cost Germany

DAK: 6 Mio. insured
→ 7.4 % of the german population (82.1 mio)

annual cost (GILEAD) p.a. for Germany:

→ patients tested (N):	~ 1.397.635
→ Diagnostic tests (N):	~ 3.068.932
→ cost of testing (+10% priv. insured)	~ 41.253.240 €
→ antibiotic treatments (N):	~ 283.912
→ treatment cost:	~ 9.961.865 €
→ total cost:	~ 51.215.150 €

Müller I. & Freitag M.H. , Clin Develop Immunol, (2012)

Summary

- Diagnostics in Lyme disease is not easy but feasable
- Interpretation requires additional clinical information
- Serological testing is the method of choice in most cases
- Well established and evaluated methods should always be used
- Drawbacks in serology:
  - False negatives early on in the course of disease
  - Heterogeneity of different genospecies and strains in Europe
  - Clear cut activity marker is missing!
  - Lack of standardization and test quality in commercial assays
- Culture and PCR:
  - For suitable sample material (biopsies, CSF, synovia) only
  - Mainly for tricky cases (lab experience important)
- Follow-up very rarely indicated!
- No treatment recommendations based on serology only!