

# Update on the diagnosis of Lyme disease



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# CLINICAL MANIFESTATIONS of LYME BORRELIOSIS

## SKIN

Borreliolymphocytoma



Multiple erythema migrans



Erythema migrans



Acrodermatitis chronica atrophicans

## CARDIAC INVOLVEMENT

A-V block,  
other



## MUSCULOSKELETAL SYSTEM

## NEUROLOGICAL INVOLVEMENT

Meningitis,  
Cranial neuritis,  
Sy Bannwarth,  
other



fatigue,  
malaise, myalgia,  
arthralgia etc.

## OTHER

EYES

# Diagnosis of borrelial infection

## DIAGNOSIS



CLINICAL



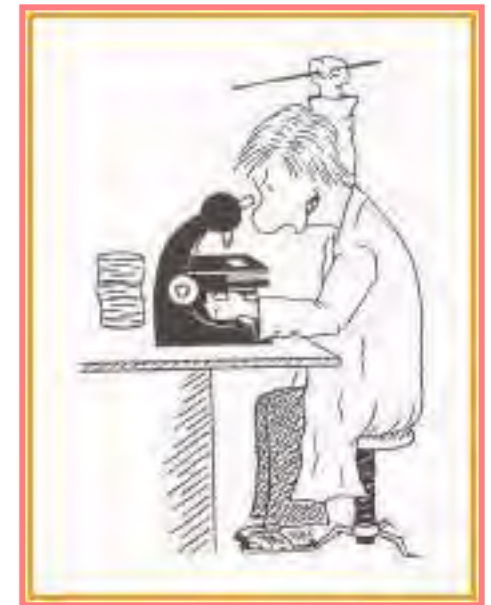
MICROBIOLOGICAL



typical clinical manifestation



- *erythema migrans*



# Diagnosis of borrelial infection



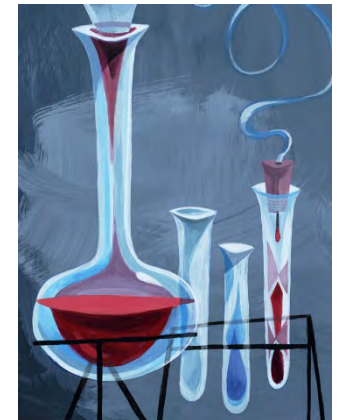
## mikrobiological methods

### DIRECT

- culture, isolation
- confirmation of borrelial DNA

### INDIRECT

- serological tests



# CULTIVATION → ISOLATION

**SAMPLES:** skin, CSF, blood, synovial fluid, other from different clinical manifestations

\* Sample collection before antibiotic therapy

\* Sterile conditions



Update?

→ Sample quantity  
(e.g. 2,0 mL CSF)

Update?

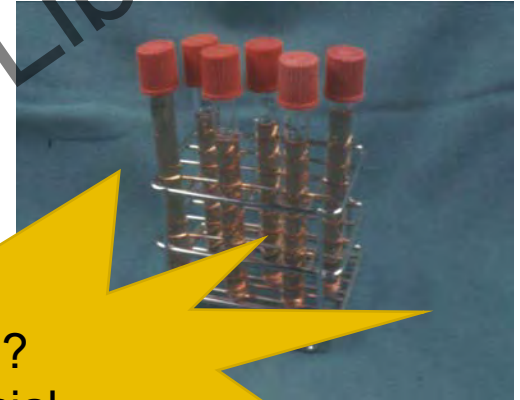
→ Sample inoculation into the medium at the patient's site



\* Transport to the laboratory as soon as possible at room temperature

# CULTIVATION → ISOLATION

**MEDIUM:** modified Kelly medium  
(MKP, BSK-II, BSK-H, BSK-Hi)



Isolation **is dependent** on:

- \* selected medium for cultivation  
(MKP, BSK-II, BSK-H, BSK-Hi)
- \* medium complements  
(origin of particular component, quantity of particular component, antibiotic supplement, etc.)
- \* pH of the medium
- \* medium maintenance  
(storage, transport, duration time)

Update ???  
→ commercial  
medium



# CULTIVATION → ISOLATION

**CONDITIONS for cultivation:** 33° C, at least 9 weeks

Isolation **is dependent** on:

- \* sample quantity (*Borrelia* density)
- \* culture maintenance (transfer to the laboratory, temperature of incubation, shaking, centrifugation, microscopy)

Update ???  
→ knowledge about culture procedure

*B. burgdorferi* - tendency to overgrowth *B. afzelii* and *B. garinii* in mix culture; *B. garinii* - tendency to overgrowth *B. afzelii* in mix culture)

**So, method is requested, time consuming, and low yield!**



# CULTIVATION → ISOLATION



## Borreliae were isolated:

- from different samples (e.g. skin, blood and/or CSF) in *erythema migrans* patients  
→ **early dissemination**
- from different samples (e.g. skin, blood and/or CSF) in *acrodermatitis chronica atrophicans* or Lyme neuroborreliosis patients  
→ **persistent, systemic infection**
- from different samples simultaneously  
→ **infection with different strains**



# CULTIVATION → ISOLATION

## Borreliae were isolated:

- in patients with high antibody titers  
→ role of antibodies

Update ???  
→ vaccine development

- in patients previously treated for Lyme borreliosis  
→ reinfection ?



# *Borrelia* species identification?

Borreliae are:

- Slow growing
- Requested for cultivation
- Metabolic inactive



How can we perform *Borrelia* identification?

→ analysis of borrelial DNA

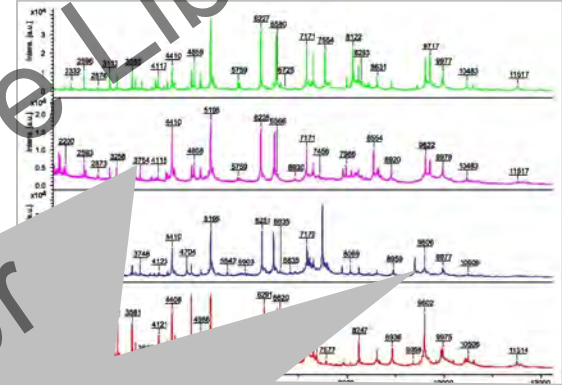




# Borrelia species identification?

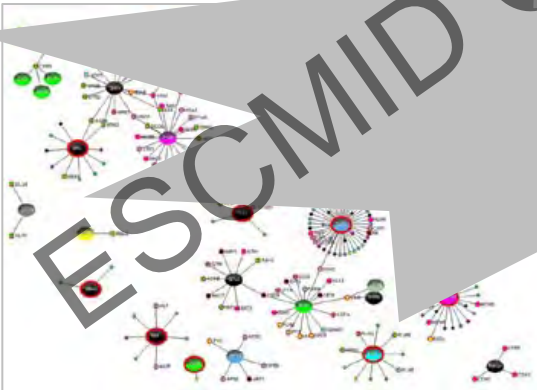
**PFGE** (pulsed-field gel electrophoresis):

- restriction of whole borrelial DNA with MseI
- electrophoresis: 10 sec, 24 h



UPDATE ?!

- New molecular technics
- New methods (MALDI-TOF ??)



MseI

- OspC, other → sequencing, restriction, SSCP (single strand conformation polymorphism)
- MLST (multi locus sequence typing) – of house keeping genes

# CULTIVATION → ISOLATION



- \* Confirmation of infection
- \* Strain identification (species, types within species)
- \* Epidemiological studies

*Borrelia* distributions regarding to:

- geographic regions (Europe, North America, Asia)
- biological material (humans, animals, ticks)
- clinical manifestation (skin, nervous system, etc.)

# *Borrelia burgdorferi* sensu lato global distribution

## North America

*B. burgdorferi* ss

## Europe

*B. afzelii*

*B. garinii*

*B. burgdorferi* ss

*B. spielmanii*

***B. bavariensis***

## Asia

*B. afzelii*

*B. garinii*

Species not in author

*B. andersonii*

*B. bissoni*

*B. an.*

*B.*

*B. carolinensis*

*B. kurtenbachii*

*B. chilensis*

Update?

→ thanks to molecular  
methods, more and more  
*Borrelia* species appears

*B. sinica*

*B. turdi*

*B. tanukii*

*B. japonica*

***B. yangtze***

*Borrelia* species regarding to biological origin  
(data from Slovenia)

Species	<i>B.afzelii</i>	<i>B.garini</i>	<i>B.burgdorferi</i> sensu stricto
Biol.origin			
Humans	75-82 %	9-17 %	1-5 %
Ticks	39-53 %	34-56 %	12.5-33 %
Reservoir Animals	82 %	17.7 %	41.8 %
Birds	13.6-19 %	45.5-59.5 %	9-13.5 %

## Borrelia species regarding to clinical manifestations (data from Slovenia)

Species	<i>B. afzelii</i>	<i>B. garinii</i>	<i>B. burgdorferi</i> sensu stricto
Clinical manifestations			
Erythema migrans	78%	21%	1%
Acrodermatitis chronica atrophicans	80%	16%	4%
Neuroborreliosis	35%	65%	-

# Diagnosis of borrelial infection

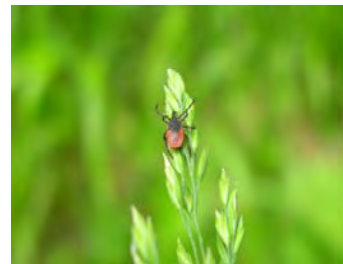
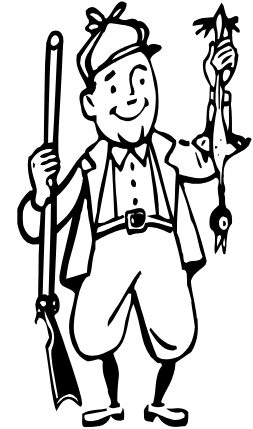
## AMPLIFICATION of DNA

### Samples:

- skin, CSF, synovial fluid, blood, others

..... DIFFERENT CLINICAL MANIFESTATIONS .....

- samples from animals, birds, ticks





# Diagnosis of borrelial infection

## AMPLIFICATION of DNA

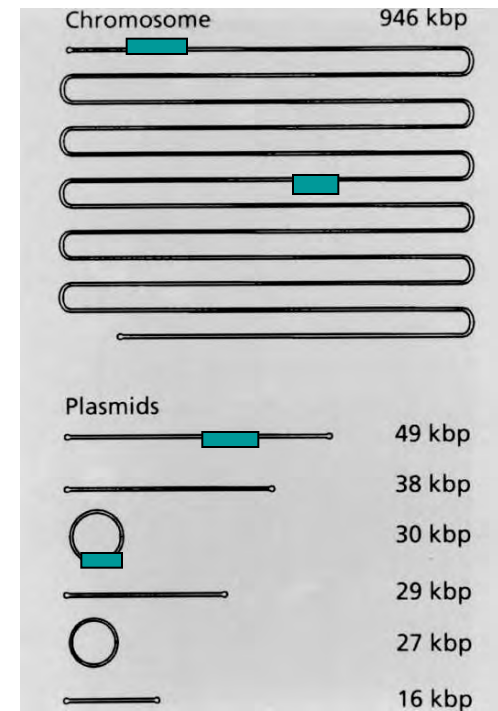
**METHOD:** polymerase chain reaction, **PCR**

↓  
amplification of target DNA

TARGET: borrelial chromosomal or plasmid DNA  
gene for: flagellin, OspA, OspC, 5S-23S, dbp, hbb, recA, 16S rRNA, hsp, others } >100 gens

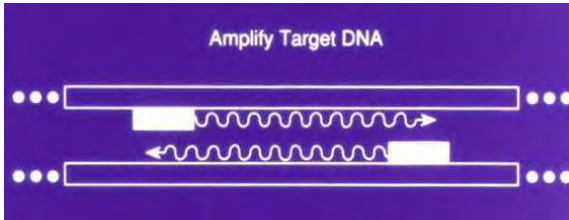
↓  
**PCR product:**

- \* gel electrophoresis
- \* SSCP
- \* sequencing
- \* automatic (RT-PCR)
- \* restriction
- \* hybridisation
- \* T<sub>m</sub> determination



# Diagnosis of borrelial infection

## AMPLIFICATION of DNA



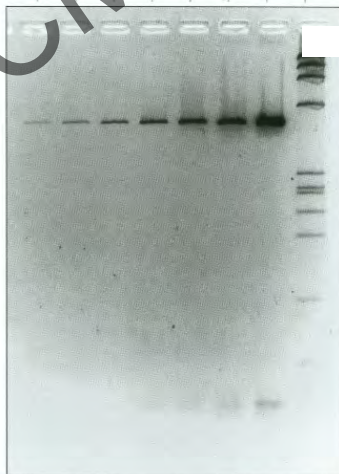
### Specificity

- target DNA

### Sensitivity

- amount of borrelial DNA (sample?)
- sample transport, storage, processing
- inhibition (host DNA, inhibitors)
- “nested” PCR

Cycle number 8 12 14 16 18 20 24  
φX 174 DNA marker



### Update ??????

- \* not standardized
- \* requested
- \* expensive
- \* false positive/negative

Fast



# PCR – our results

*erythema migrans*, skin biopsy (Ø 4 mm) – samples no. 150  
 PCR versus culture

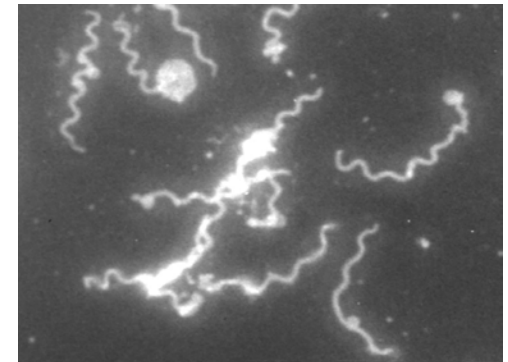
	PCR ( <u>flagellin</u> )	PCR ( <u>nested OspA</u> )	culture (MKP medium)
<b>POSITIV:</b>	42 (28 %)	92 (61 %)	75 (50 %)
<b>NEGATIV:</b>	108 (72 %)	58 (39 %)	75 (50 %)

Update?

→ we are still waiting for excellent PCR

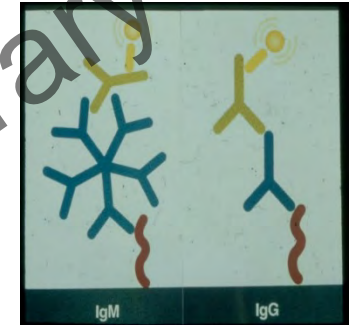
flagellin versus culture

POSITIVE: 25/150 (16.7 %)	} 62/150 (41 %)
NEGATIVE: 37/150 (25 %)	



# Diagnosis of borrelial infection

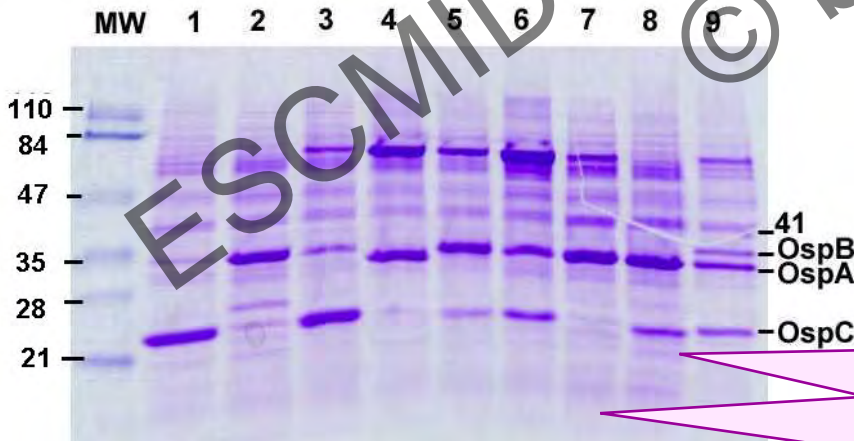
## SEROLOGICAL TESTS



**LOOKING FOR:** specific IgM and IgG

**SAMPLES:** blood, cerebrospinal fluid, synovial fluid

Antibodies are directed against borrelial antigens.



Which *Borrelia* antigens should we use for serological testing?

# Diagnosis of borrelial infection

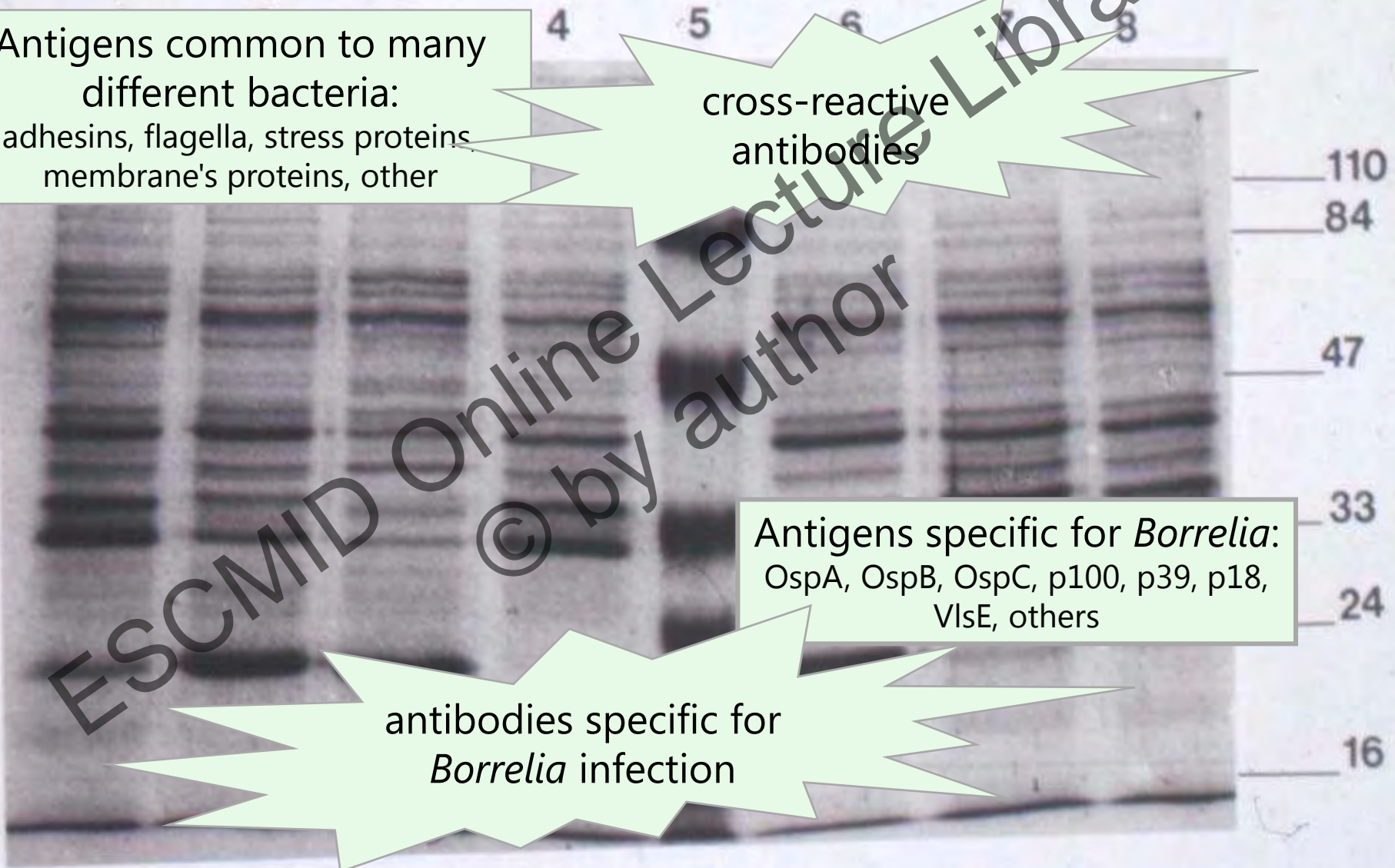
## SEROLOGICAL TESTS

Antigens common to many different bacteria:  
adhesins, flagella, stress proteins, membrane's proteins, other

cross-reactive antibodies

Antigens specific for *Borrelia*:  
OspA, OspB, OspC, p100, p39, p18, VlsE, others

antibodies specific for *Borrelia* infection



TOMORROW ???

- to develop test to could distinguish ongoing from past infection.

\* ANTIGENS in serological tests differ regarding to:

- \* *Borrelia* species (*B. afzelii*, *B. garinii*, *B. burgdorferi* sensu stricto, other)
- \* strain within the species (antigen heterogeneity)
- \* antigen preparation (whole cell, sonicated whole cell, purified antigens, recombinant antigens)
- \* combination of antigens (common antigens, *Borrelia* specific antigens)
- \* number of antigens (from one to more), etc.

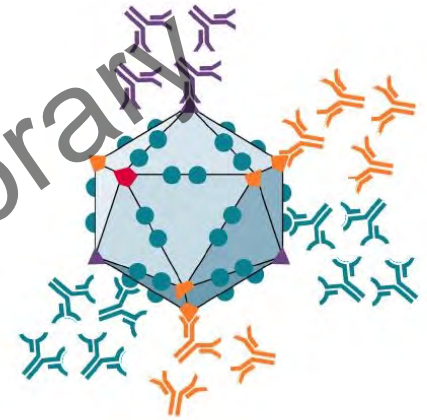
Update?

- prepare commercially available tests for confirming patients antibodies against specific borrelial antigens.



# Diagnosis of borrelial infection

## SEROLOGICAL TESTS

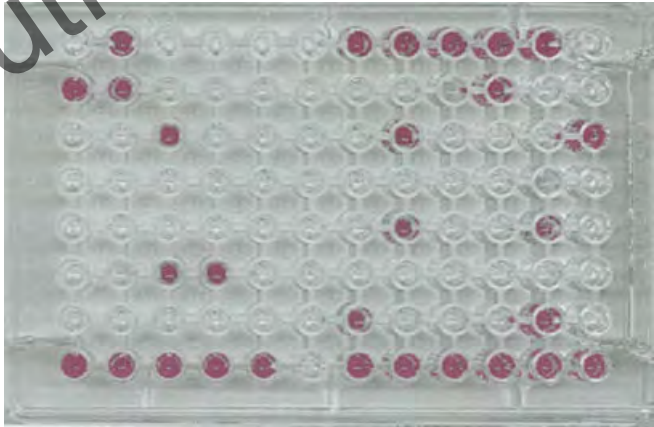


\* Antibodies against many borrelial antigens (cumulative)

IgM, IgG



**IFT** imunofluorescent test



**ELISA**

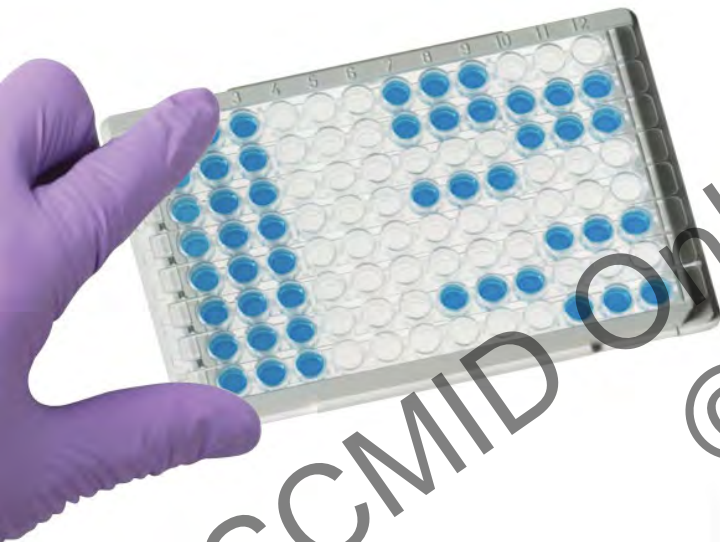
enzyme-immuno test

How do we measure antibody quantities in IFT and how in ELISA tests?

# Diagnosis of borrelial infection

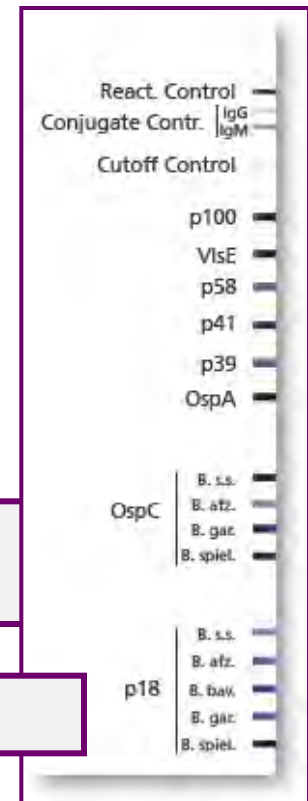
## SEROLOGICAL TESTS

\* Tests which prove antibodies against specific *Borrelia* antigens (one or more)



\* Particular, *Borrelia* specific, recombinant antigens (e.g. OspC, VlsE, OspC, other)

\* Tests which prove immune fingerprint of borrelial infection





# Diagnosis of borrelial infection

## SEROLOGICAL TESTS

### Diagnostic guidelines:

- **Two steps approach** – two test, screening (at least 90% specificity) and confirmatory (>95% specificity)
- **One step approach** – single assay with both specificity and sensitivity >98%
- **follow-up is recommended**

Underestimation ?



OVERRATING ?

... a single test ...?

It is necessary to take in consideration the seroprevalence in certain region.

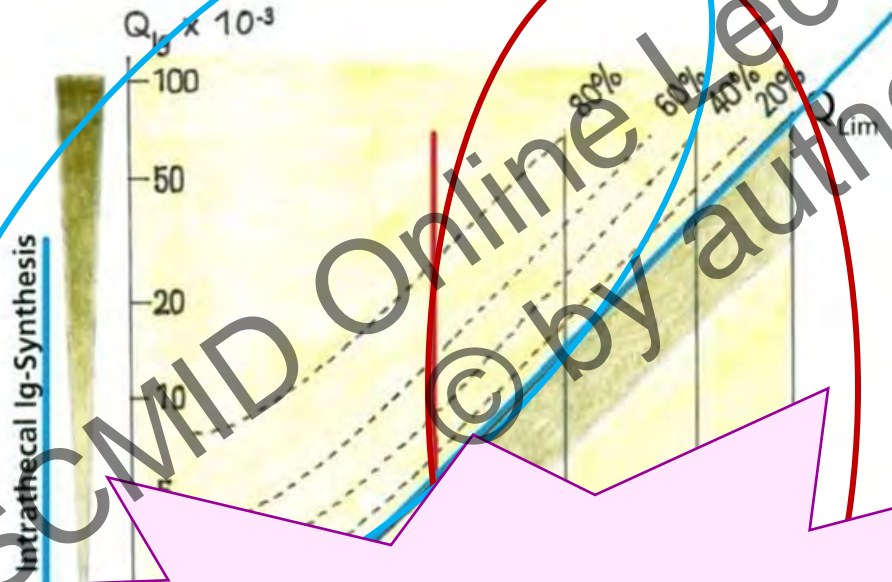
# Diagnosis of borrelial infection

## SEROLOGICAL TESTS

### NEUROBORRELIOSIS

Intrathecal presence of specific antibodies

intrathecal synthesis



### CSF:BLOOD ratio

- Albumin
- Total immunoglobulins (IgM, IgG, IgA)
- Specific antibodies (IgM, IgG, IgA)

UPDATE ???  
- Reiber formula

barrier disorder

# Specificity of immune response

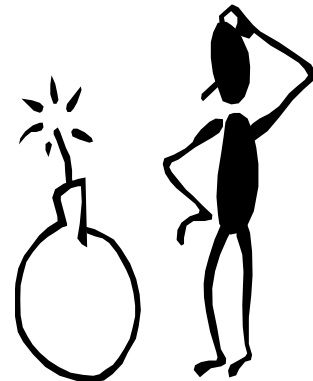
## Immune response:

- \* is developing slowly (IgM: 3 - 6 weeks, IgG: 1 - 3 months)
- \* may be weak or absent
- \* with the chronicity of the infection percentage of seropositive patients arise



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clinical manifestations	seropositive
early, localized (e.g. EM)	<b>20</b> - 50 %
early, disseminated (e.g. neuroborreliosis)	50 - 90 %
chronic infection (e.g. ACA)	90 - <b>100</b> %



# Specificity of immune response

## \* not protective

in spite of immune response:

- *Borreliae* could be isolated from clinical material
- patient can be reinfected after the tick bite

UPDATE ???

- Vaccine ???

## \* present at healthy persons

in high-endemic areas

## \* antibody cross reaction

- OTHER INFECTIONS: relapsing fever, syphilis, other bacteria/virus infection (EBV, CMV, *Mycoplasma pneumoniae*, other)
- OTHER DISEASES: autoimmune disorders (rheumatoid arthritis, lupus, other), other



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