Lyme Disease Diagnostics

What can we use now
What do we need for the future?

Anja Garritsen, Innatoss Laboratories, NL

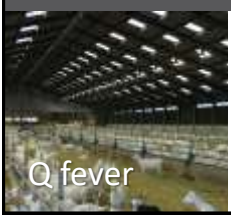
Today's presentation

Innatoss

Diagnostics for Lyme Disease
The present
- Diagnostic Concepts
- Available Tools
- Use of diagnostic tests in different stages of disease

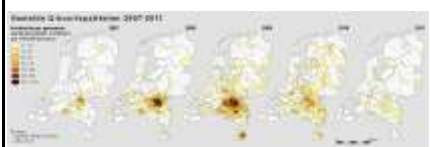
The future?
- Precision Diagnostics for Lyme Disease

Focus of Innatoss



Lyme disease

Location in the middle of Q fever epidemic



innatoss

Many ticks in the Netherlands

Tick bites	1,5 million
EM	30,000 EM
persistent	2500

GP visits for erythema migrans in the Netherlands 1994-2014 (source: RIVM)

Mission Innatoss

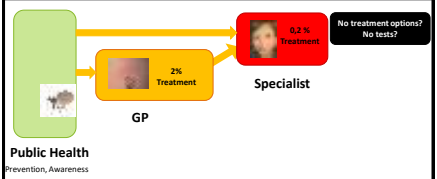
Catch it early!

And significantly reduce healthcare issues associated with infectious diseases such as Q fever and Lyme Disease

Impact

Early diagnosis of Lyme Borreliosis results in more effective treatment

Lyme in Time (the simple view)



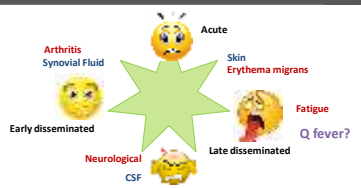
How do we diagnose?

Clinical signs & symptoms

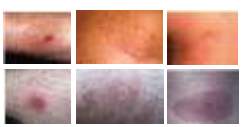
Indirect Detection
- Antibodies
- Memory T cell
- Innate Memory Cells

Direct Detection
- Culture
- PCR
- Mass Spectrometry
- Antigen detection

Different Faces of Lyme Disease



Even the simple signs are not easy



Different tests are needed

The ideal test
- is 95 % sensitive
- Is 98% specific

The ideal test
- gives a yes-no answer
- covers all stages of the disease

The ideal test
- costs € 15 or less

Serology

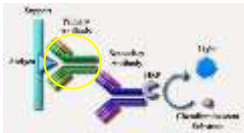
Clinical signs & symptoms

Indirect Detection
- Antibodies
- Memory T cell
- Innate Memory Cells

Direct Detection
- Culture
- PCR
- Mass Spectrometry
- Antigen detection

ELISA
Indirect Immune Fluorescence Assay
Western Blot (whole cell lysates)
Immunoblot (recombinant)
Protein Arrays

One principle

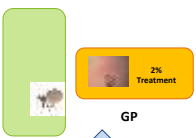


Antigen is coated to solid support
Antibodies in serum bind to antigen

Can be used with
- Blood
- Liquor
- Synovial fluid

Antibody tests work, sometimes….

Lyme over time



GP

Localized disease
When in doubt, **test!**

Antibody generation
Takes time

No antibodies?
Signs & symptoms?

What if antibodies are not generated?

Clinical signs & symptoms

Indirect detection
- Antibodies
- **Memory T cell**
- **Innate memory cells**

Direct detection
- Culture
- PCR
- Mann Spectrometry
- Antigen detection

Marketed CMI tests

Mantoux test (skin prick test)
Quantiferon – TB (Qiagen)
T-Spot-TB (Oxford Immunotec)

Quantiferon - CMV
T-Spot CMV

Q fever skin prick test
Q-detect (Innatoss)

Antibodies | Memory T cell | Innate immunity

CMI – the other side of the immune system

Humoral immunity
Antibodies tests
B-cells

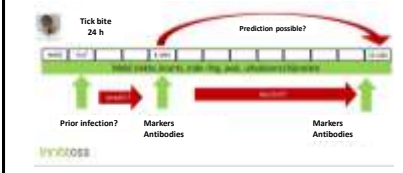
Cellular immunity
Biomarker tests
T-cells

Will a cell-based assay detect more infections and/or detect them sooner

Available CMI tests for Lyme

	AID	LTT (IMD)	Invitalab (MELISA)	Armin	BCA	Qiagen	Spirofind revised	Ixodes
Cells	PBMC	PBMC	PBMC	PBMC	PBMC	Whole blood	PBMC	Whole blood
Antigens	B31 Osp-mix			= AID	= AID plus LFA1	Flagellin p66 DbpB OspC	B. afzelii B. garinii B. burg	Early antigens
Material	lysate peptides	lysate peptides	many	= AID	= AID	peptides	lysates	Recomb proteins
Read-out	ELISpot IFNγ	Cell proliferation	Cell proliferation	Elispot IFNγ	Elispot IFNγ/IL2	IFNγ	Elispot IL-1β	IFNγ + others
Intended use	Exposure	Lyme	Lyme	Lyme	Lyme	Early	Early	Early
Regulatory	CE	LDT	LDT			Pre-market	Pre-market	Developme nt

Ixodes trial (2015-2017)

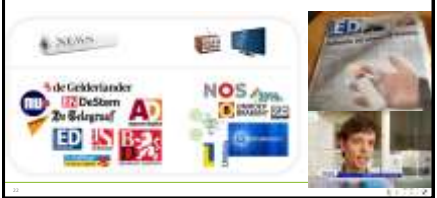


Ixodes study - challenges

Getting access to people with tick bites

Logistics

Media attention



Ixodes study – results to date

Recruitment
610 participants recruited
500 with tick bite, uninfected
30 with tick bite, infected subjects
80 without tick bite

Biomarkers
Strong responders in the ***healthy*** control group
Likely due to some of the antigens used
Working on a solution
Repeating part of the study

Addressing logistics: ID-LYME

Spin-off from Ixodes study improved serological test strategy

Observation

There are patients with Lyme-like symptoms who test negative in ELISA but are positive in an immunoblot

Question

Is this true positive or cross-reactivity

Opportunity

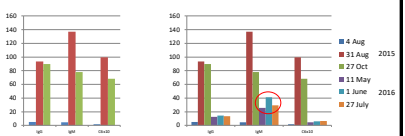
Ixodes study provided well-defined cases

Case 1

No Erythema Migrans or other symptoms

Case 1

No Erythema Migrans or other symptoms



Case 1 – IgM after 3 months

Case 1 – IgM after 9 months

Case 1 - IgG after 3 months

Case 1 – IgG after 9 months

IgG ELISA negative

VlsE garinii, flagellin and BmpA antibodies remaining

Regular pattern for prior infections

Case 2: Value of Immunoblot for reinfections

C6 0,8
IgG 40
IgM 8

Case 2: Value of immunoblot for reinfections

C6 1,9
IgG 43
IgM 11

Recommendation

- Use available diagnostic tools as well as you can

- 3 ELISAs that are really different (C6 and Euroimmun IgG/IgM)
- 2-3 Immunoblots that have unique features (Mikrogen, Euroimmun)
- Combine them

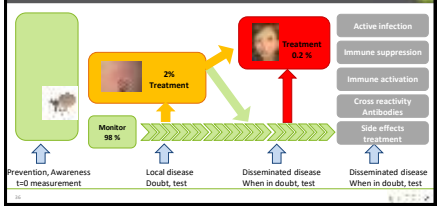
Will not solve the problem of people that make not antibodies

Serological lessons

- Use diverse antigens, do not rely on one test
- Use immunoblots as well as ELISAs (one tier)
- Quantify ELISA and immunoblot
- Repeat measurements
- Baseline measurement is useful to detect infection

- Anyone can do this, can be implemented around the world
- At least 50% more cases identified

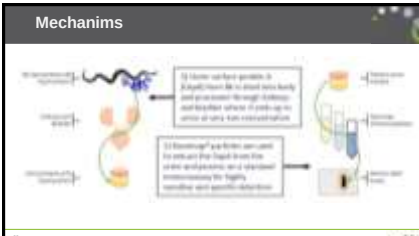
Lyme Testing Strategy over Time



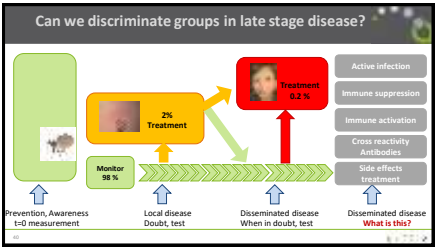
Direct Detection of Borrelia

Nanotrap®

Mechanims

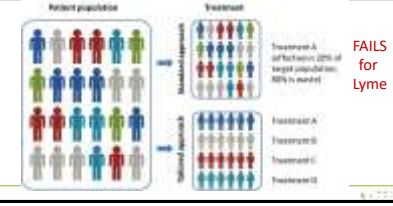


Can we discriminate groups in late stage disease?



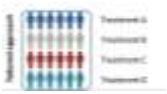
Precision Medicine for Lyme Disease

Precision Medicine



Precision Medicine for Lyme

Your knowledge is required



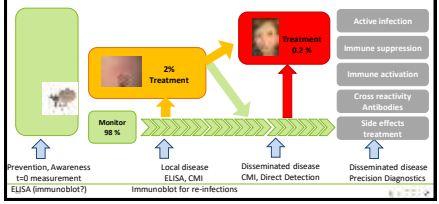
Which groups exist

What treatments are effective

How can we distinguish these groups

Right intervention for the right people

Lyme Testing Strategy (the complicated view)



What is needed to make this happen

COLLABORATION

FUNDING