

Lyme Disease Diagnostics

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

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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



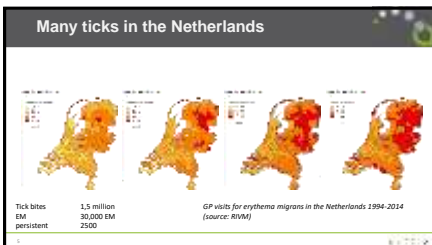
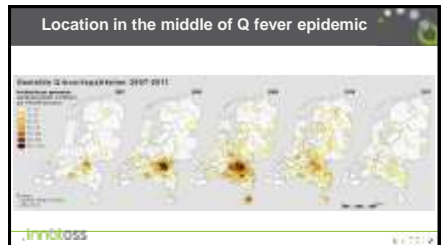
Focus of Innatoss



Q fever





Lyme disease



Mission Innatoss

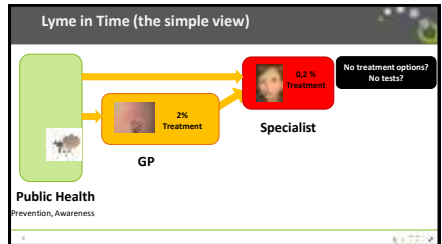
Catch it early!

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



How do we diagnose?

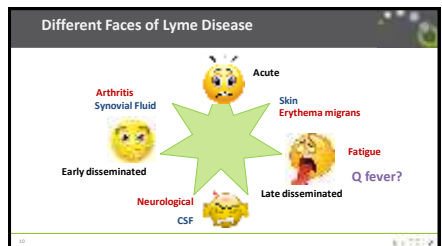
Clinical signs & symptoms

Indirect Detection

- Antibodies
- Memory T cell
- Innate Memory Cells

Direct Detection

- Culture
- PCR
- Mass Spectrometry
- Antigen detection



Even the simple signs are not easy

A grid of six small images showing various skin rashes, likely erythema migrans, which are characteristic of Lyme disease.

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

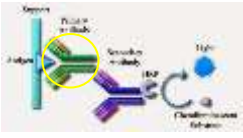
Indirect Detection
Antibodies
 Memory T cell
 Innate Memory Cells

Direct Detection
 Culture
 PCR
 Mass Spectrometry
 Antigen detection



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

One principle

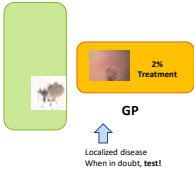


Antigen is coated to solid support
 Antibodies in serum bind to antigen

Can be used with
 Blood
 Liquor
 Synovial fluid

Antibody tests work, sometimes....

Lyme over time



Antibody generation
 Takes time

No antibodies?
 Signs & symptoms?

GP
 Localised disease
 When in doubt, test!

2% Treatment

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)

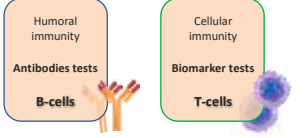
Quantiferon - CMV
 T-Spot CMV

Q fever skin prick test
 Q-detect (Innatoss)



Antibodies Memory T cell Innate Immunity

CMI – the other side of the immune system



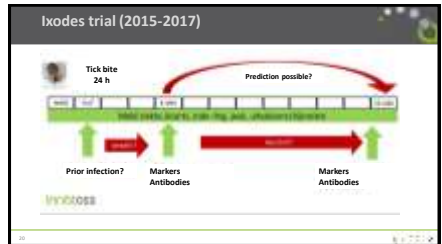
Humoral immunity
 Antibodies tests
B-cells

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 (IMC)	InvitLab (MELISA)	Armin	BCA	Oligen	Spirofind revised	ixodes
Cells	PBMC	PBMC	PBMC	PBMC	PBMC	Whole blood	PBMC	Whole blood
Antigens	B.31 Osp-mix			= AID	= AID plus IFA2	Flagellin p66; OspB; OspC	B. afzelii B. garinii B. burg	Early antigens
Maternal	Iyate peptides	Iyate peptides	many	= AID	= AID	peptides	Iyates	Recomb proteins
Read-out	ELISpot IFN γ	Cell proliferation	Cell proliferation	ELISpot IFN γ	ELISpot IFN γ /IL2	IFN γ	ELISpot IL-1 β	IFN γ + others
Intended use	Exposure	Lyme	Lyme	Lyme	Lyme	Early	Early	Early
Regulatory	CE	LDT	LDT			Pre-market	Pre-market	Development



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

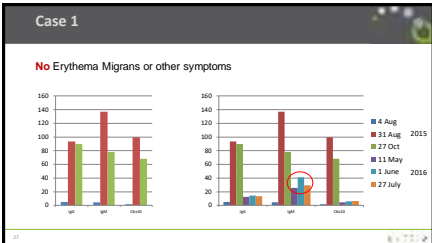
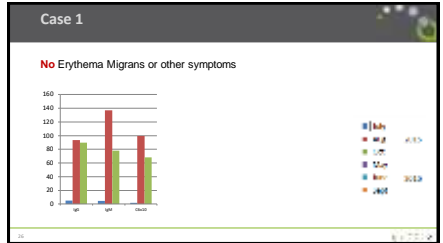
This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101018888

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 – IgM after 3 months

Case No.	DOB	Sex	Referral Date	Referral Source
1	1985-08-04	M	2015-08-04	GP
2	1985-08-31	M	2015-08-31	GP
3	1985-10-27	M	2015-10-27	GP
4	1985-11-11	M	2015-11-11	GP
5	1985-06-03	M	2015-06-03	GP
6	1985-07-27	M	2015-07-27	GP

Case 1 – IgM after 9 months

Case No.	DOB	Sex	Referral Date	Referral Source
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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

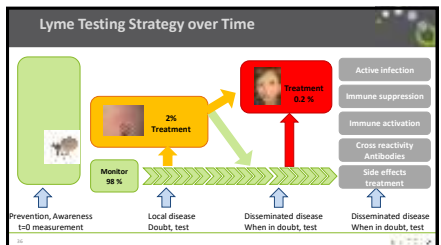
- Use available diagnostic tools as well as you can
- 3 ELISAs that are really different (C6 and Euroimmun IgG/IgM)
- 2-3 Immunoblots that have unique features (Mikrogen, Euroimmun)
- Combine them

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



Direct Detection of Borrelia

CERES Nanosciences
 CERES Nanosciences
 Nanotrap® Technology

The diagram shows a central globe with arrows pointing to various stages of Borrelia detection. Below the globe are three circular icons representing different detection methods: **Direct**, **Indirect**, and **Antigen**.

Nanotrap®

The flowchart illustrates the Nanotrap® process. It starts with **Antigen** and **Antibody** components. The process involves **Antigen** binding to **Antibody**, which is then captured by **Nanotrap®**. This leads to **Antigen** detection and **Antibody** detection. The final step is **Antigen** detection, which is used for **Antigen** detection and **Antibody** detection.

Mechanims

The diagram illustrates the mechanism of Borrelia infection and detection. It shows **Borrelia** (a wavy line) entering a cell and interacting with **Antigen** and **Antibody**. The process involves **Borrelia** binding to **Antigen** and **Antibody**, leading to **Antigen** detection and **Antibody** detection. The final step is **Antigen** detection, which is used for **Antigen** detection and **Antibody** detection.

Can we discriminate groups in late stage disease?

The flowchart shows the progression of disease from **Prevention, Awareness** to **Local disease** to **Disseminated disease**. It includes a **Monitor 98%** box and a **Treatment 2%** box. A **Treatment 0.2%** box is highlighted in red. A list of effects includes: **Active infection**, **Immune suppression**, **Immune activation**, **Cross reactivity**, **Antibodies**, and **Side effects treatment**. A red box asks: **What is this?**

Precision Medicine for Lyme Disease

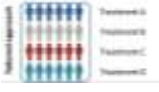
The diagram shows the transition from **Standard of care** to **Identified medicine** to **Precision medicine**. It includes a **Stratification** box and a **Personalization** box. The **Stratification** box lists: **Patients and groups**, **No disease**, **Subtypes**, **Comorbidities**, **Clinical features**, and **Genomics**. The **Personalization** box lists: **Patient individual**, **Profession**, **Clinical features**, **Mechanism of action**, **Environment**, **Relativistic & Public**, and **Biomechanics**.

Precision Medicine

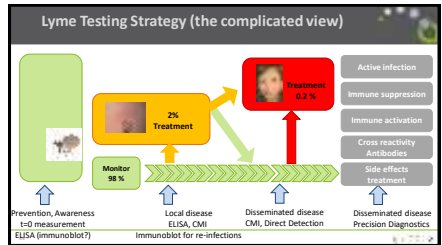
The diagram shows a **Patient population** of 100 people (represented by icons) being divided into **Stratification** groups. These groups are then treated with **Treatment A**, **Treatment B**, **Treatment C**, and **Treatment D**. A red box indicates: **FAILS for Lyme**.

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



What is needed to make this happen

COLLABORATION

FUNDING