# Annual serological testing for Lyme in a population with high occupational tick bite exposure

**Marlies van Lent, Anke Smits, Kim van den Berg, Anja Garritsen**
Innatoss Laboratories BV, Oss, Netherlands

## Background: Occupational risk of Lyme disease

- Outdoor workers have an increased risk of tick bites and thereby *Borrelia* infections, leading to Lyme disease
- If left untreated, late stage symptoms can develop including neurological and joint manifestations
- Disseminated Lyme disease leads to high costs for healthcare systems and for society by loss of productivity
- Systematic serological testing among high risk populations in *Borrelia* endemic areas enables early diagnosis and treatment of infections

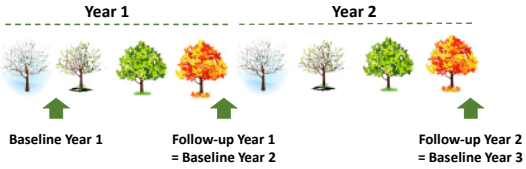
**Objective:**

**Development of an annual screening program for detection of new *Borrelia* infections**

## Approach: Longitudinal follow-up of *Borrelia* serology

### Screening program for outdoor workers

- Start: Baseline measurement prior to tick season to determine existing serology status
- Follow-up: Sequential measurement after each tick season
- Survey on tick bite exposure, previous Lyme diagnosis, and use of antibiotics




### Stringent two-tier testing

- Initial testing of all samples in 3 ELISAs
- Confirmation of positive or dubious results by immunoblot
- Individual tests performed and interpreted according to manufacturers instructions

### Systematic interpretation of results

Overall results, compared to previous testing

- Antibody levels stable or decreased = **no (recent) infection**
- Antibody levels increased, not (yet) immunoblot confirmed = **early infection or crossreactivity;** re-testing after ± 6 weeks
- Antibody levels increased, immunoblot confirmed = **new infection;** consider antibiotic treatment

## Results: Winter 2018-2019 Follow-up measurements

### Participants

- 1.358 outdoor workers from 46 organizations in all parts of The Netherlands
  - 46% water management (n=625)
  - 36% green maintenance (n=494)
  - 18% ecologists, biologists, engineers (n=239)
- Employees without Lyme related complaints
- Baseline measurement before 2018 tick season; follow-up between Oct 2018 - March 2019

### Serological tests

**ELISAs**
***Whole cell sonicate + recombinant antigens***

- Euroimmun Anti-Borrelia IgM ELISA
- Euroimmun Anti-Borrelia plus VlsE IgG ELISA
- Immunetics C6 ELISA

**Immunoblots**
***Native + recombinant antigens***

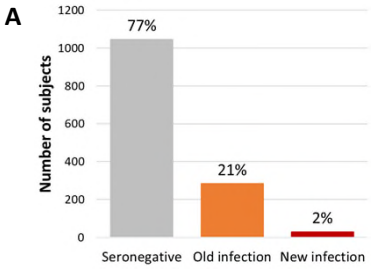
- Viramed Borrelia ViraStripe IgG blot
- Viramed Borrelia ViraStripe IgM blot

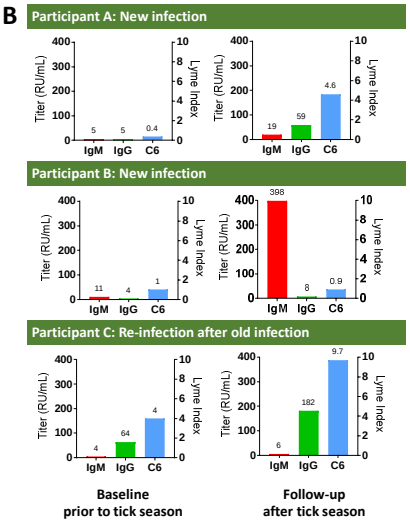
ELISA & immunoblot: detection of antibodies against various *B. burgdorferi* s.l. genospecies

## 2018-2019 Annual screening: high seroprevalence and 2% new infections

### Key findings

- In **1 in 50** outdoor workers: new *Borrelia* infection detected after 2018 tick season, of which subjects were not aware
- **65%** of subjects with a new infection did not recall tick bites in the 2018 tick season
- For **16 of 18 subjects** with a new infection who were tested after antibiotic treatment, antibody levels decreased after 6 months
- Seroprevalence of **23%** found in high risk group, compared to 4-8% in general Dutch population[1]
- Prevalence & incidence of *Borrelia* infections not affected by type of outdoor work, age, gender, and region



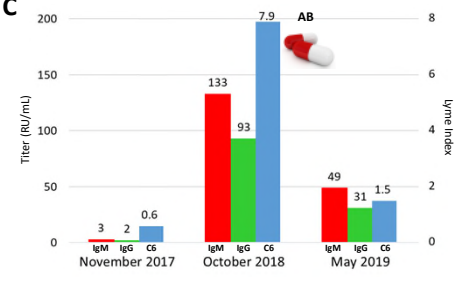




***Figure 1. Borrelia infections detected by annual screening***

***Panel A:*** *Total number and proportion of old (n=283) and new (n=29) infections among 1.358 individuals with follow-up measurement in Oct 2018 - March 2019.*

***Panel B:*** *ELISA data for 3 subjects with a recent infection. Positive ELISA results were confirmed by immunoblot for all 3 donors. New infections were identified based on either seroconversion from seronegative to seropositive, or on already present antibodies that strongly increased (= re-infection, including new antigen bands on immunoblot).*
***IgM/IgG*** *titers in RU/mL [cut-off: 16-22 RU/ml];* ***C6*** *as Lyme Index [cut-off: 0,9-1,1].*

***Panel C:*** *ELISA data for subject with new infection, detected in Oct 2018. Subject was subsequently treated with antibiotics (AB) in Nov 2018.*

***Reference:*** *[1] Dutch CBO Guideline Lyme disease, 2013.*

## Conclusion & outlook

- Longitudinal follow-up of *Borrelia* serology after each tick season can discriminate between old and new infections
- *Borrelia* infections in absence of early clinical symptoms can be identified and treated in a timely and appropriate fashion
- **Future perspective:** For populations with high tick bite exposure in *Borrelia* endemic areas, annual Lyme screening should be applied as addition to protective measures against tick bites