

Point-of-care detection of recent *Borrelia* infections using Sofia 2 Lyme+ as Tier 1 assay

Background and objective

- Laboratory diagnosis of Lyme disease consists of a two-tier testing algorithm for antibodies against the *Borrelia* bacterium
- Enzyme immunoassays (EIA) are used as Tier 1 assays to detect either IgM or IgG antibodies; positive/borderline samples are subsequently tested on corresponding Tier 2 immunoblots for confirmation
- Sofia 2 Lyme+ fluorescent immunoassay (FIA) is a point-of-care Tier 1 assay, developed for rapid detection of both IgM and IgG antibodies against European *Borrelia* strains from a single sample

Objective: To determine if Sofia 2 Lyme+ has adequate sensitivity to detect recent *Borrelia* infections in the Netherlands and to monitor seroconversion in longitudinal patient samples



Methods: Feasibility study

Testing of longitudinal sample sets: Tier 1 method comparison

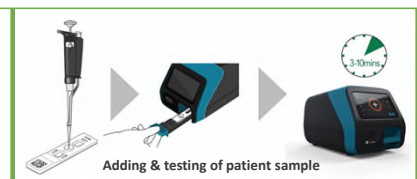
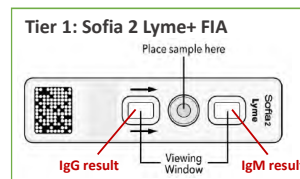
- Serum samples tested for *Borrelia* antibody titers using classic Tier 1 EIAs
 - ✓ Euroimmun Anti-Borrelia IgM ELISA
 - ✓ Euroimmun Anti-Borrelia plus VlsE IgG ELISA
 - ✓ Immunicities C6 ELISA
- Serum samples tested for *Borrelia* antibody titers using Sofia 2 Lyme+ FIA

Immunoblots performed for all samples for verification of Tier 1 results

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



Comparison



Results

Sequential serum samples from two Dutch cohorts

- Outdoor workers participating in annual Lyme screening program (n=25)**
 - Yearly follow-up measurement of *Borrelia* serology after tick season
 - Subjects selected with seroconversion between two sequential measurements (detected by 3 EIAs in parallel)
 - Archived serum samples before and after seroconversion tested

- Subjects participating in Ixodes tick bite study (n=24)**
 - Borrelia* serology performed directly after tick bite and after 4 and 12 weeks
 - Subjects selected with seroconversion between measurements, or increased titers (detected by 3 EIAs in parallel)
 - Archived serum samples before and after seroconversion/titer increase tested

Serological detection of recent *Borrelia* infections

Table 1. Longitudinal sample sets by cohort and serological profile

<i>Borrelia</i> antibody development	Annual Lyme screening	Tick bite study
Seroconversion from negative to positive	25	19
Positive titers increased at follow-up testing	0	5
Total	25	24

Figure 1. Tier 1 method comparison: Detection of recent infections by cohort

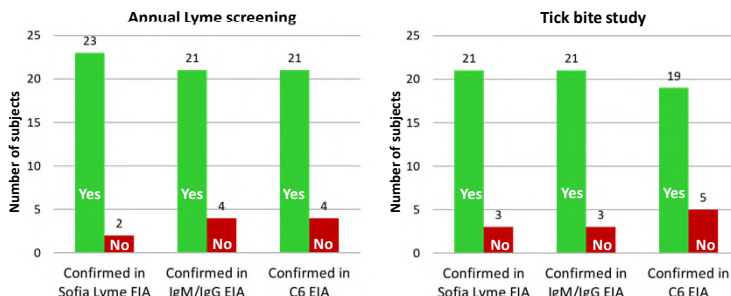


Table 2. Tier 1 method comparison: Detection of recent infections by serological profile

<i>Borrelia</i> antibody development	Confirmed in Sofia Lyme FIA	Confirmed in IgM/IgG EIA	Confirmed in C6 EIA
Seroconversion from negative to positive	41 of 44	37 of 44	36 of 44
Positive titers increased at follow-up testing	3 of 5	5 of 5	4 of 5
Total	44 of 49	42 of 49	40 of 49

Figure 2. Confirmation of recent infections: Overall sensitivity of Tier 1 tests

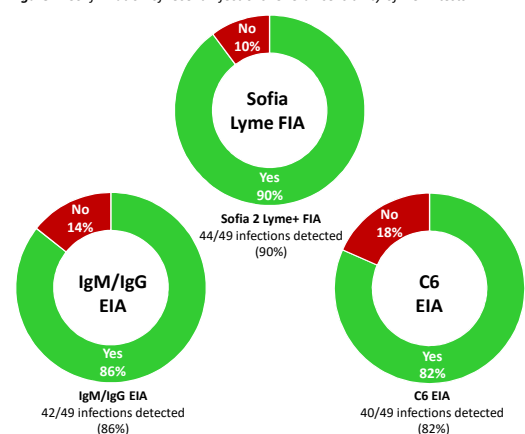


Table 3. Example of serological data collected for each longitudinal sample set tested

Longitudinal sample set	IgM EIA (RU/mL)	IgG EIA (RU/mL)	C6 EIA (Lyme Index)	Sofia Lyme IgM (S/CO)	Sofia Lyme IgG (S/CO)	ViraStripe IgM blot	ViraStripe IgG blot
First sample	7	11	0.2	0.60	0.96	Neg (no bands)	Neg (no bands)
Follow-up sample	275	51	5.7	8.35	2.60	Pos (OspC, p41)	Pos (VlsE, OspC)

IgM/IgG EIA borderline: 16-22 RU/ml; C6 EIA borderline: 0.9-1.1; Sofia IgG/IgM positive $I_f \geq 1.0$ (S/CO = signal divided by cut-off value)

Conclusions

- Sofia 2 Lyme+ FIA is a **sensitive assay** for detection of recent *Borrelia* infections and identifies a **higher percentage of seroconversions** than by testing with either IgM & IgG EIA or C6 EIA alone
- For patients with a doubtful erythema migrans or non-specific complaints after a tick bite, Sofia 2 Lyme+ FIA can provide rapid test results to **support the clinical diagnosis**
- Sofia 2 Lyme+ can be applied for **screening purposes** among populations with frequent tick bite exposure in highly endemic areas
- As synthetic and recombinant antigens are combined in Sofia 2 Lyme+ FIA, the assay fits within the tendency to modify two-tier testing by including a second EIA instead of an immunoblot