FACULTÉ de MÉDECINE
de STRASBOURG

UNIVERSITÉ DE STRASBOURG

Les Hôpitaux Universitaires de STRASBOURG

SYNDICAT DES BIOLOGISTES
Le syndicat de tous les biologistes médicaux

# Les outils diagnostics de la borréliose de Lyme

Laboratoire de Bactériologie, CHU de Strasbourg
EA 7290 Groupe borréliose de Lyme

Frédéric Schramm – SDB – 2e Journée scientifique 10 avril 2017

# Liens d'intérêt

SYNDICAT DES BIOLOGISTES
Le syndicat de tous les biologistes médicaux

**Aucun lien à déclarer**

# Plan de la présentation

- **Borréliose de Lyme : qq bases pour comprendre**
- **Diagnostic clinique & biologique**
  - les outils “validés” du diagnostic biologique
  - les tests “alternatifs” non validés
  - contexte clinique & indications des examens biologiques

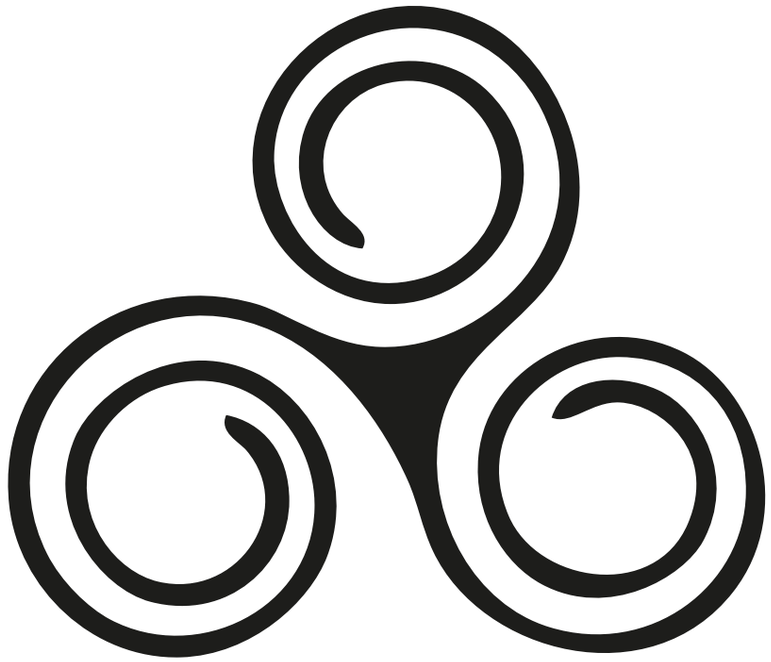
SYNDICAT DES BIOLOGISTES
Le syndicat de tous les biologistes médicaux

# LES BASES

# Maladies à transmission vectorielle

SYNDICAT DES BIOLOGISTES
Le syndicat de tous les biologistes médicaux

Hôte



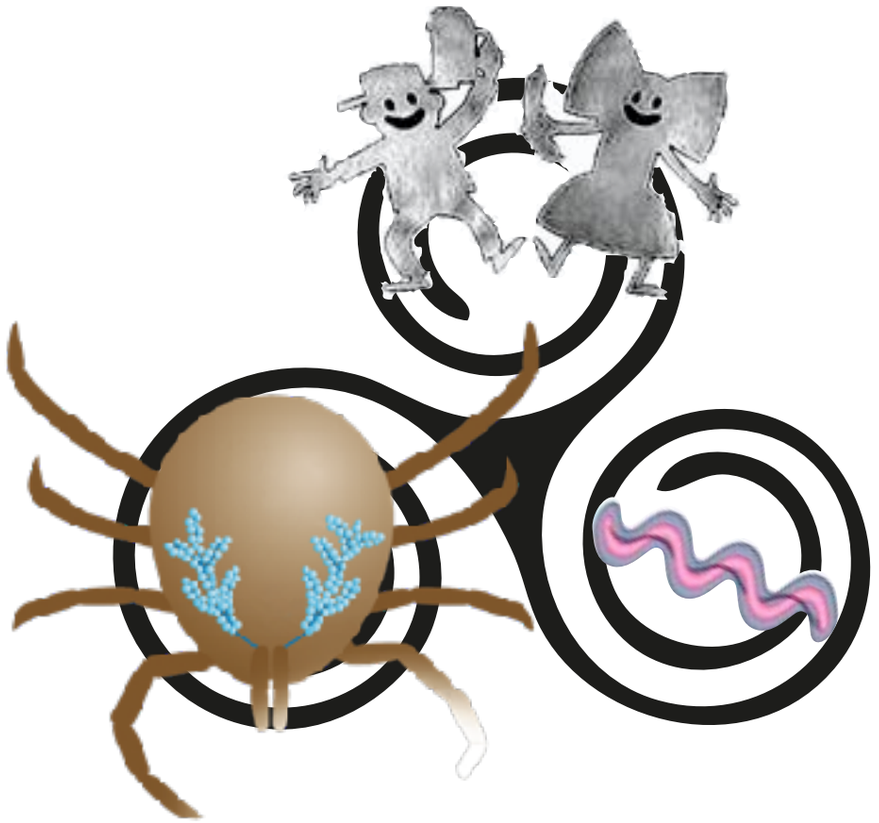
Vecteur

Pathogène

# Maladies à transmission vectorielle

SYNDICAT DES BIOLOGISTES
Le syndicat de tous les biologistes médicaux

Hôte



Vecteur

*Borrelia*

Pathogène

**Borréliose de Lyme**

# *Borreliella* : position taxonomique

SYNDICAT DES BIOLOGISTES
Le syndicat de tous les biologistes médicaux

**Classe** — **Spirochètes**

**Ordre** — ***Spirochaetales***

**Famille** — ***Borreliaceae*** ***Spirochaetaceae*** ***Brachyspiraceae*** ***Leptospiraceae***

**Genre** — ***Borreliella*** ***Borrelia*** ***Treponema*** ***Brachyspira*** ***Leptospira*** ***Leptonema*** ***Cristispira***

**Espèces**

***Borreliella*** *(B. burgdorferi* sensu lato)

| | | |
|---|---|---|
| *B.b.* sensu stricto | *B. andersonii* | *B. japonica* |
| *B. garinii* | *B. californiensis* | *B. tanukii* |
| *B. bavariensis* | *B. carolinensis* | *B. turdi* |
| *B. afzelii* | *B. americana* | *B. sinica* |
| *B. spielmanii* | *B. kurtenbachii* | *B. yangtze* |
| *B. valaisiana* | *B. chilensis* | *B. lanei* |
| *B. lusitaniae* | *B. finlandensis* | |
| *B. bissettii* | *B. genomospecies 2* | |
| *B. mayonii* | | |

***Borrelia*** **(Fièvres récurrentes)**

| | |
|---|---|
| *B. miyamotoi* | *B. crocidurae* |
| *B. recurrentis* | *B. hispanica* |
| *B. duttonii* | *B. hermsii* |
| *B. turicatae* | ... |

**Autres *Borrelia***

*B. lonestari*
*B. anserina*
...

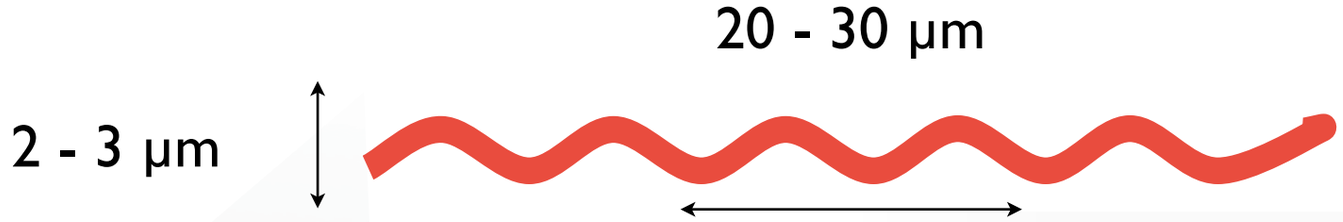
2018

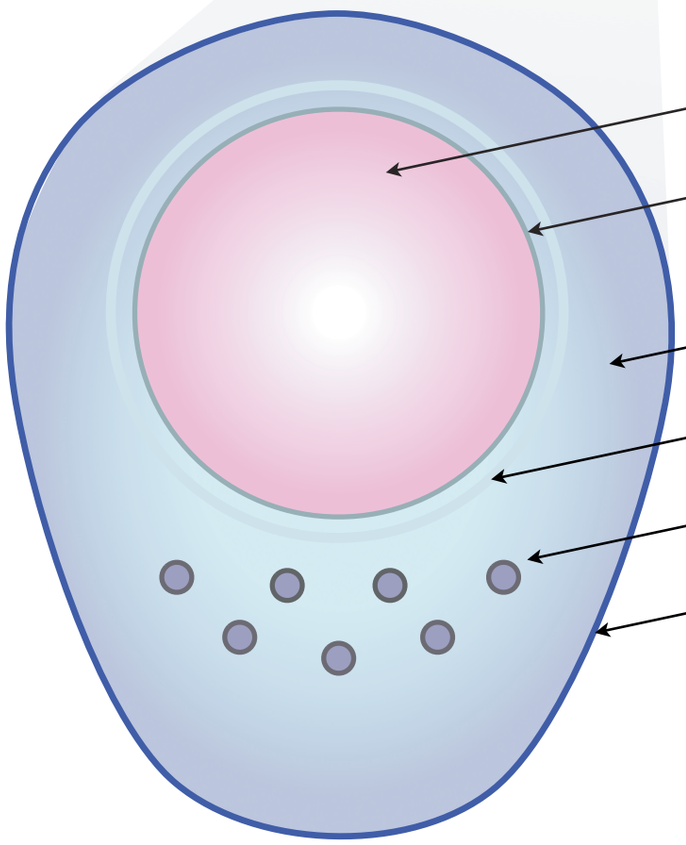
**_Borreliella_ impliquées en pathologie humaine (Lyme)**

D'après REVUE FRANCOPHONE DES LABORATOIRES - DÉCEMBRE 2013 - N°457

# *Borrelia* : structure générale

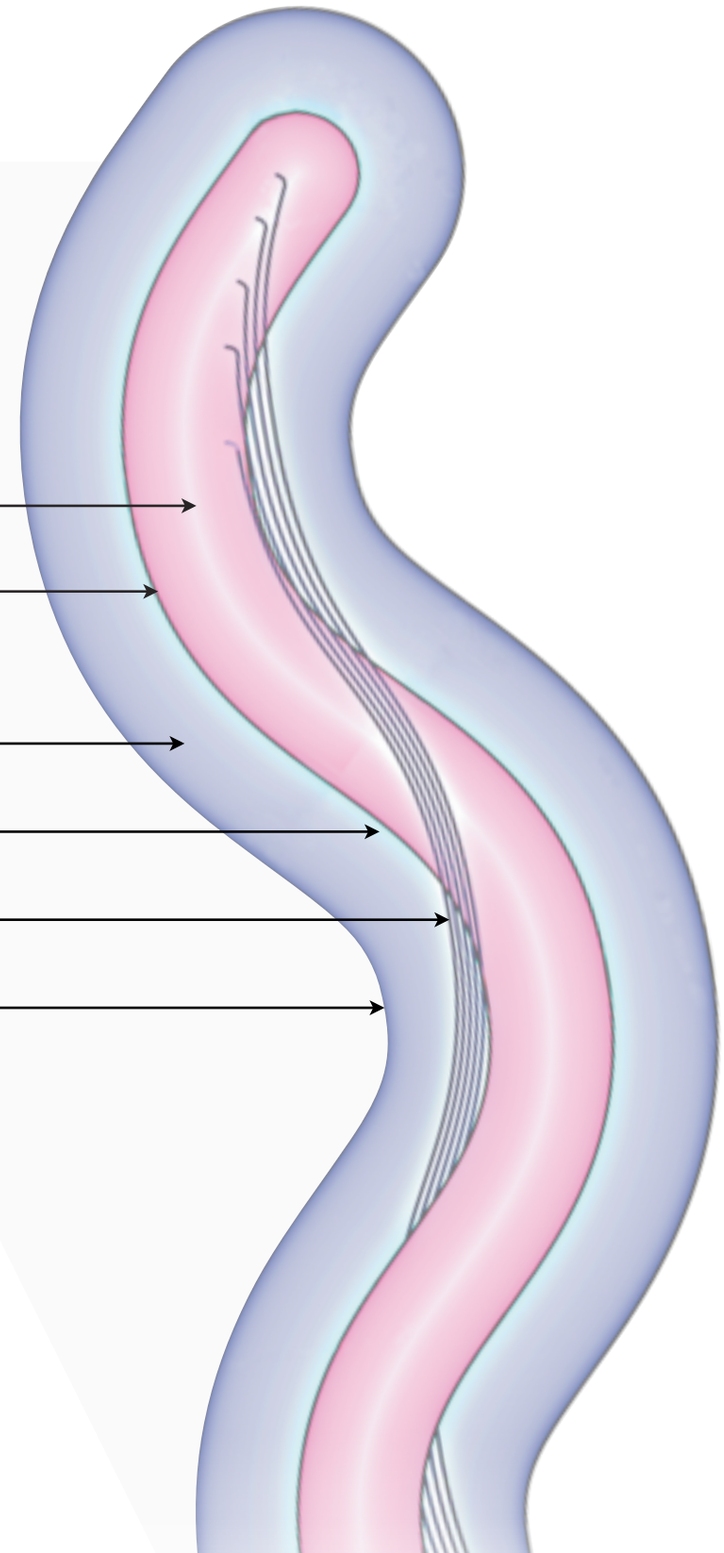
SYNDICAT DES BIOLOGISTES
Le syndicat de tous les biologistes médicaux



- cylindre protoplasmique
- membrane interne
- espace périplasmique
- peptidoglycanne
- flagelles
- membrane externe

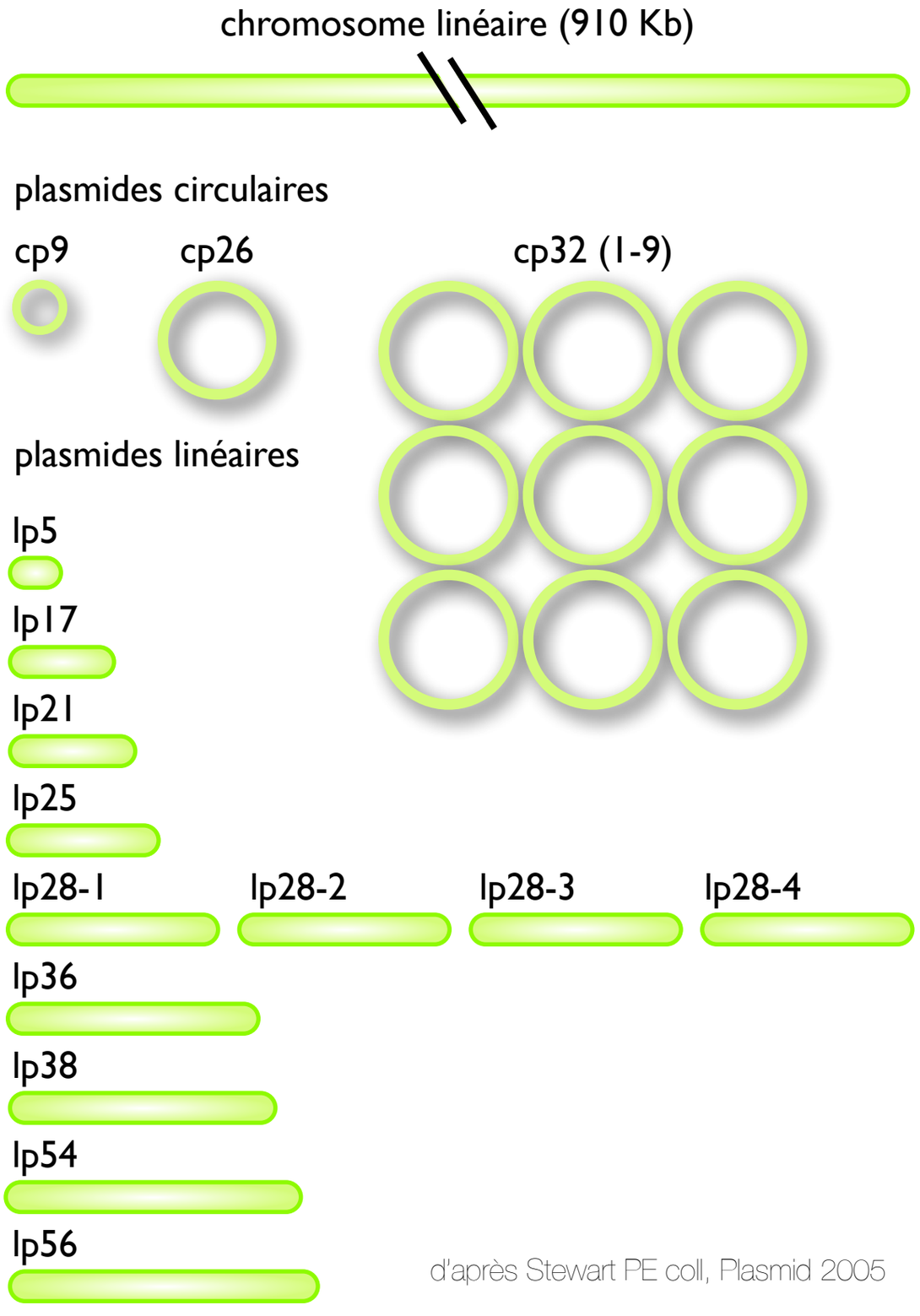
(**lipoprotéines**, absence de LPS)



D'après REVUE FRANCOPHONE DES LABORATOIRES - DÉCEMBRE 2013 - N°457

# *Borrelia* : un génome très particulier

génome de petite taille (1,5 Mb)

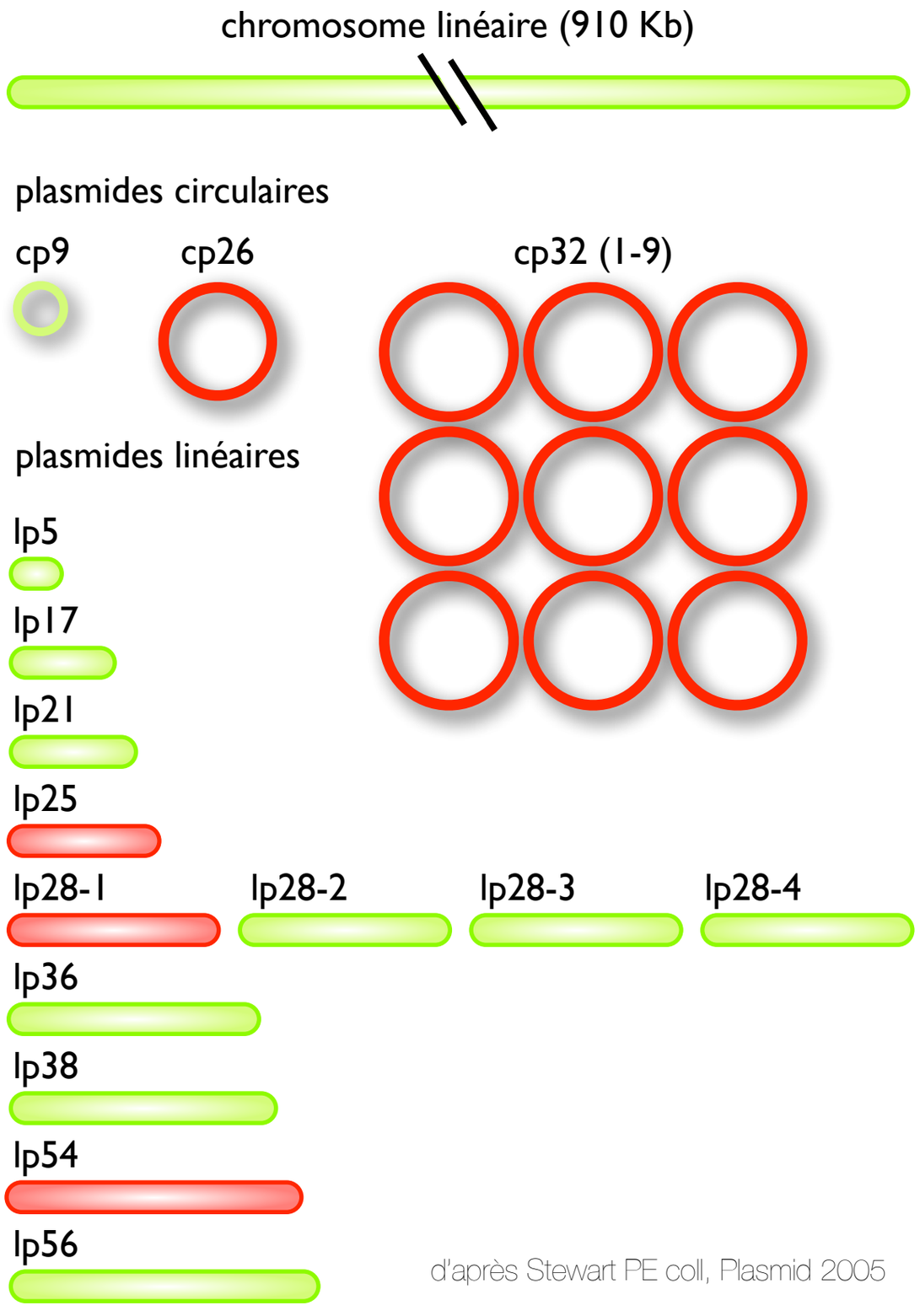



d'après Stewart PE coll, Plasmid 2005

- chromosome **linéaire**
- plasmides **linéaires**
- 23 plasmides (linéaires et circulaires)
  - bactérie connue avec **+ grand nb** de plasmide
  - plasmides = 40 % du génome

# *Borrelia* : un génome très particulier

génome de petite taille (1,5 Mb)




d'après Stewart PE coll, Plasmid 2005

- chromosome **linéaire**
- plasmides **linéaires**
- 23 plasmides (linéaires et circulaires)
  - bactérie connue avec **+ grand nb** de plasmide
  - plasmides = 40 % du génome
  - certains plasmides essentiels à la virulence

## Autres caractéristiques

- absence de LPS Takayama K et coll., Infect Immun 1987
- nbx lipoprot. surface (Osp, Vlse ...) : 5% génome
- pas de toxines / pas de protéases
- très peu de protéines sécrétées

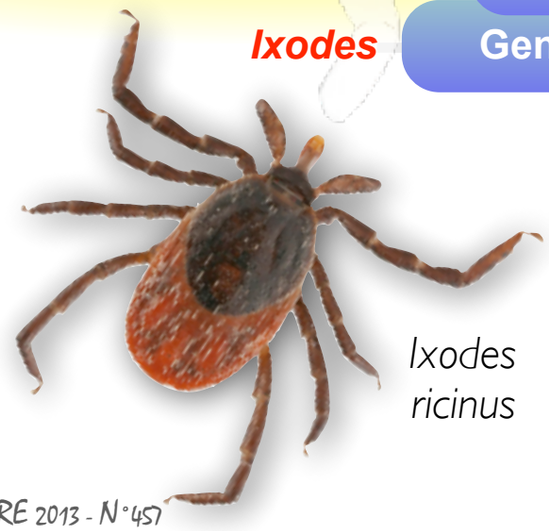
# Position taxonomique des tiques du genre *Ixodes*

SYNDICAT DES BIOLOGISTES
Le syndicat de tous les biologistes médicaux

Phylum — Arthropodes
Sous-phylum — Chélicérates — Mandibulates (Antennates)
Classe — Arachnides — Insectes
Sous-classe — Acariens
Super-ordre — Parasitiformes
Ordre — *Ixodida*
Super-famille — *Ixodoidea* (tiques)
Famille — ***Argasidae*** (tique molles) — ***Nuttalliellidae*** — ***Ixodidae*** (tiques dures) — Famille — ***Culicidae*** (moustiques)
***Ixodinae*** — Sous-famille
***Ixodes*** — Genre

© Frédéric Schramm

*Ornithodoros*

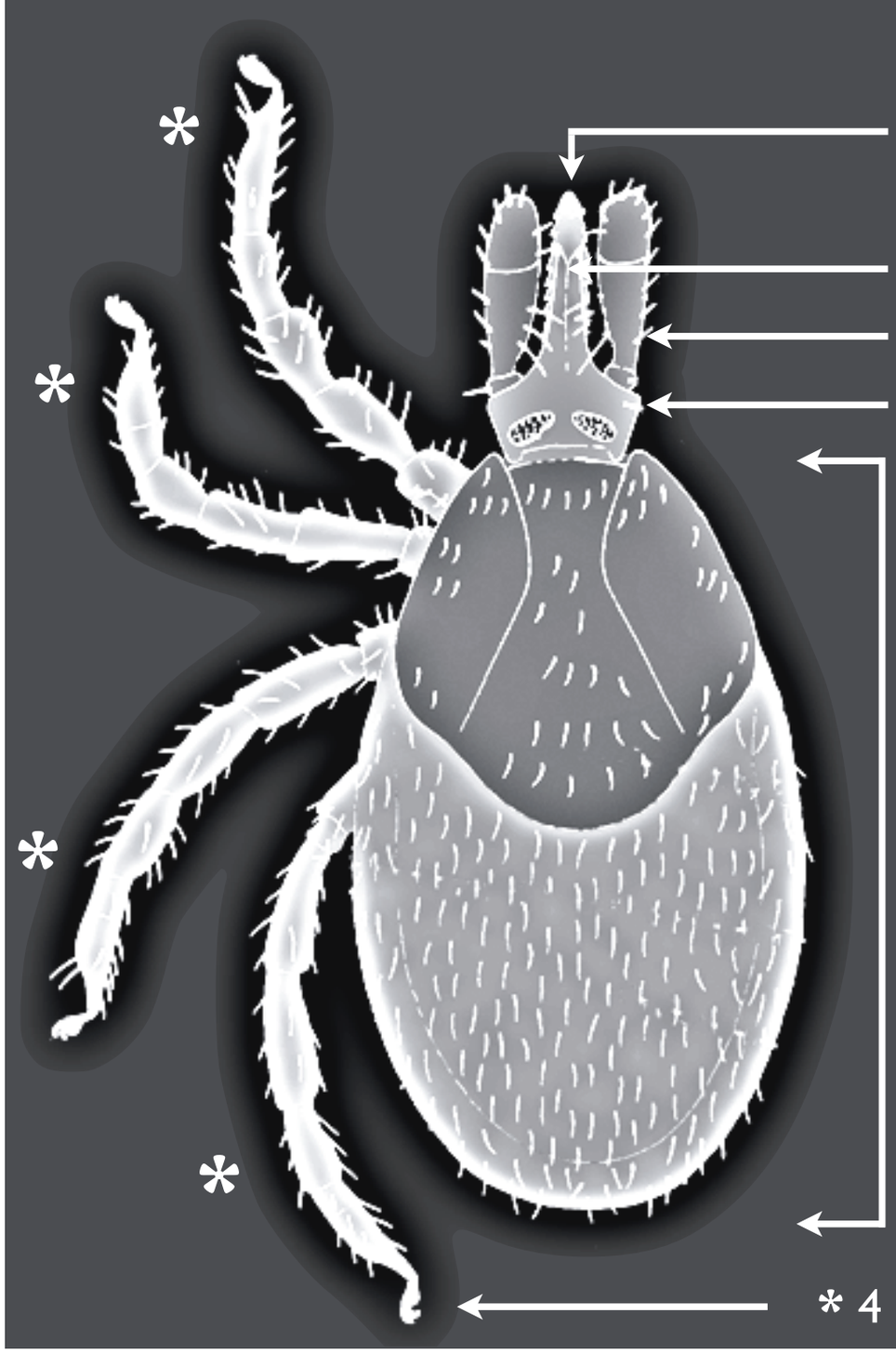


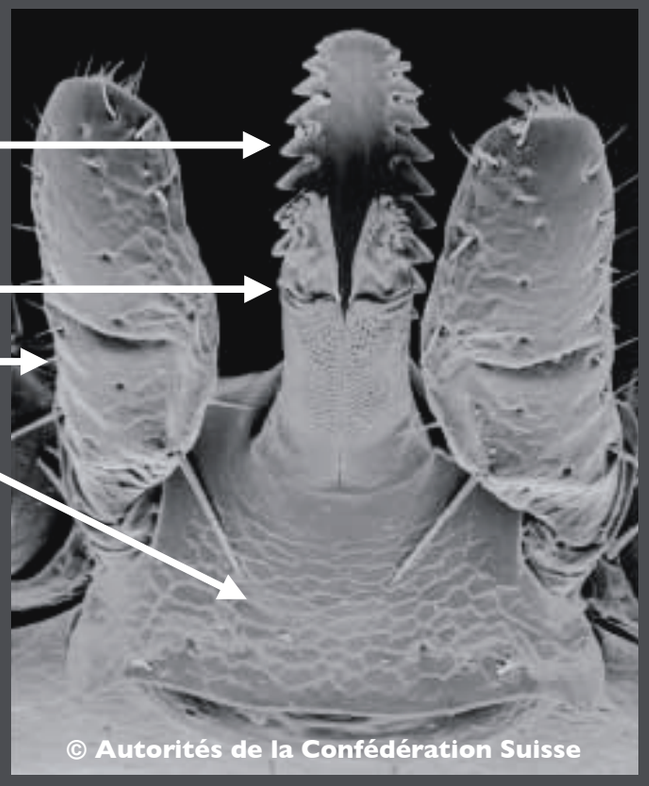
*Ixodes ricinus*

D'après REVUE FRANCOPHONE DES LABORATOIRES - DÉCEMBRE 2013 - N°457

# Anatomie des tiques du genre *Ixodes*

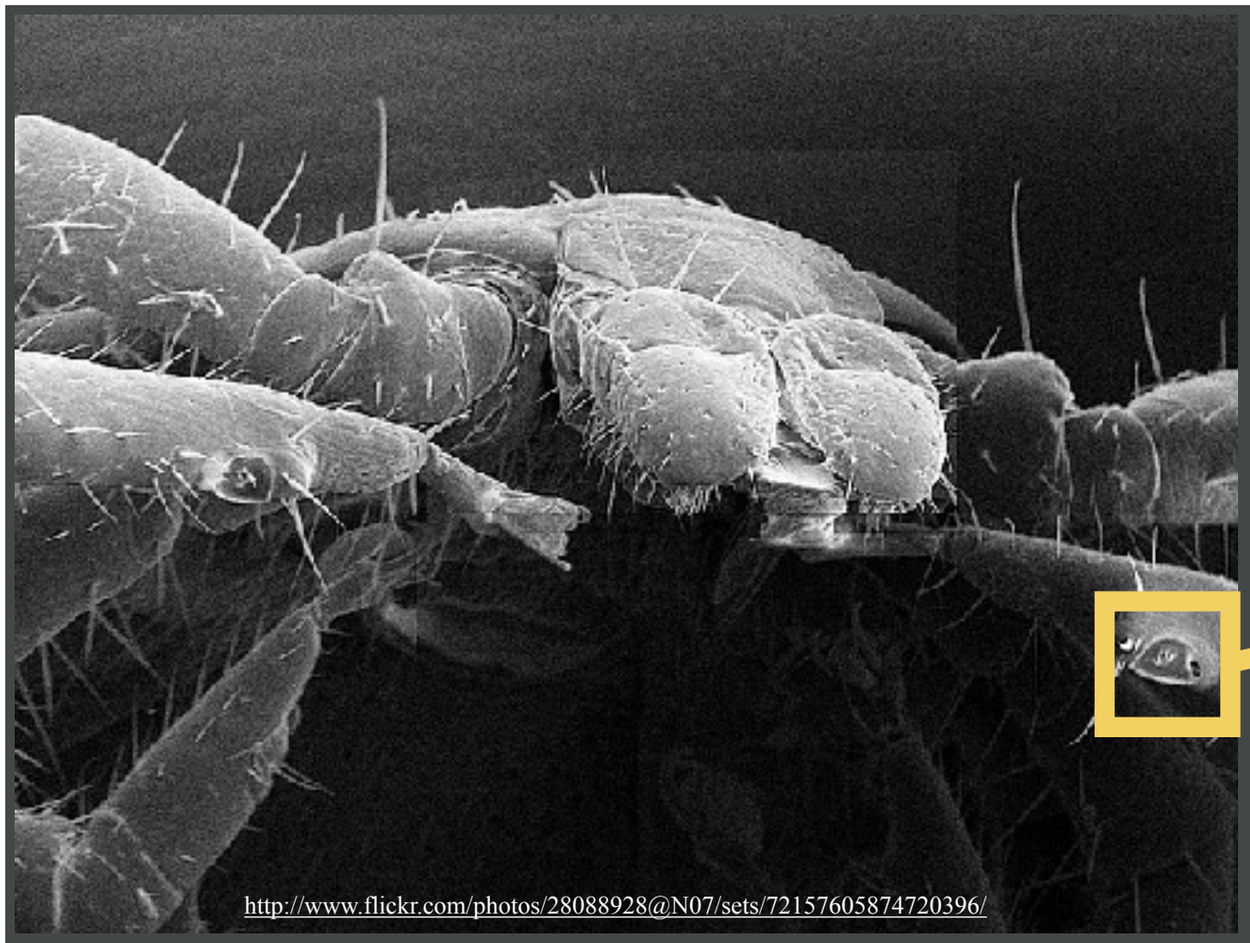
## *Ixodes ricinus* : adulte femelle

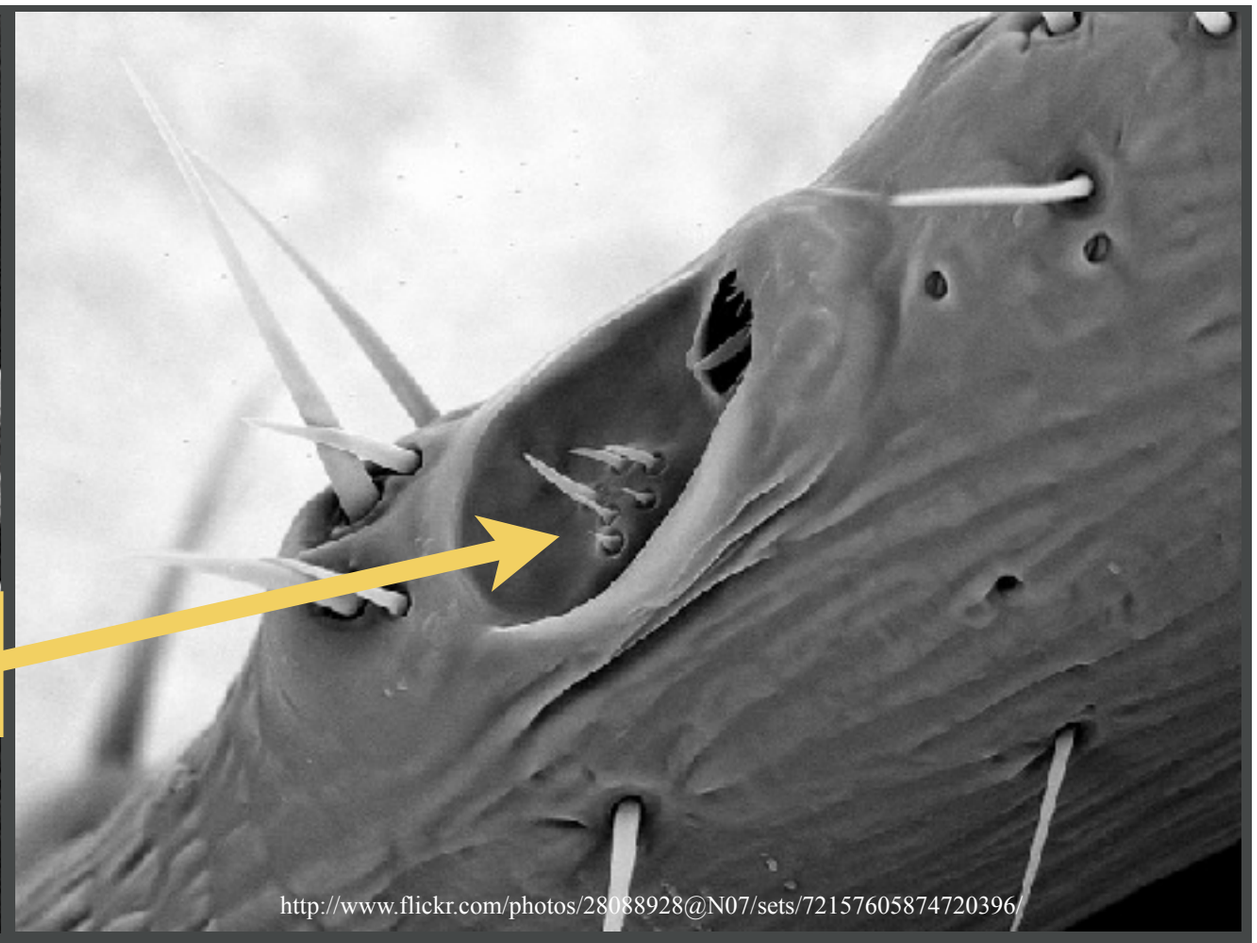




# Organes sensoriels




## repérage des hôtes cibles

- pédipalpes
- soies
  - ★ distribuées sur l'ensemble de la surface du tégument
- organe de Haller
  - ★ localisé à la partie dorsale du tarse de la 1re paire de pattes

## stimuli

- ★ thermiques
- ★ mécaniques
- ★ chimiques

# “Quête” des hôtes cibles



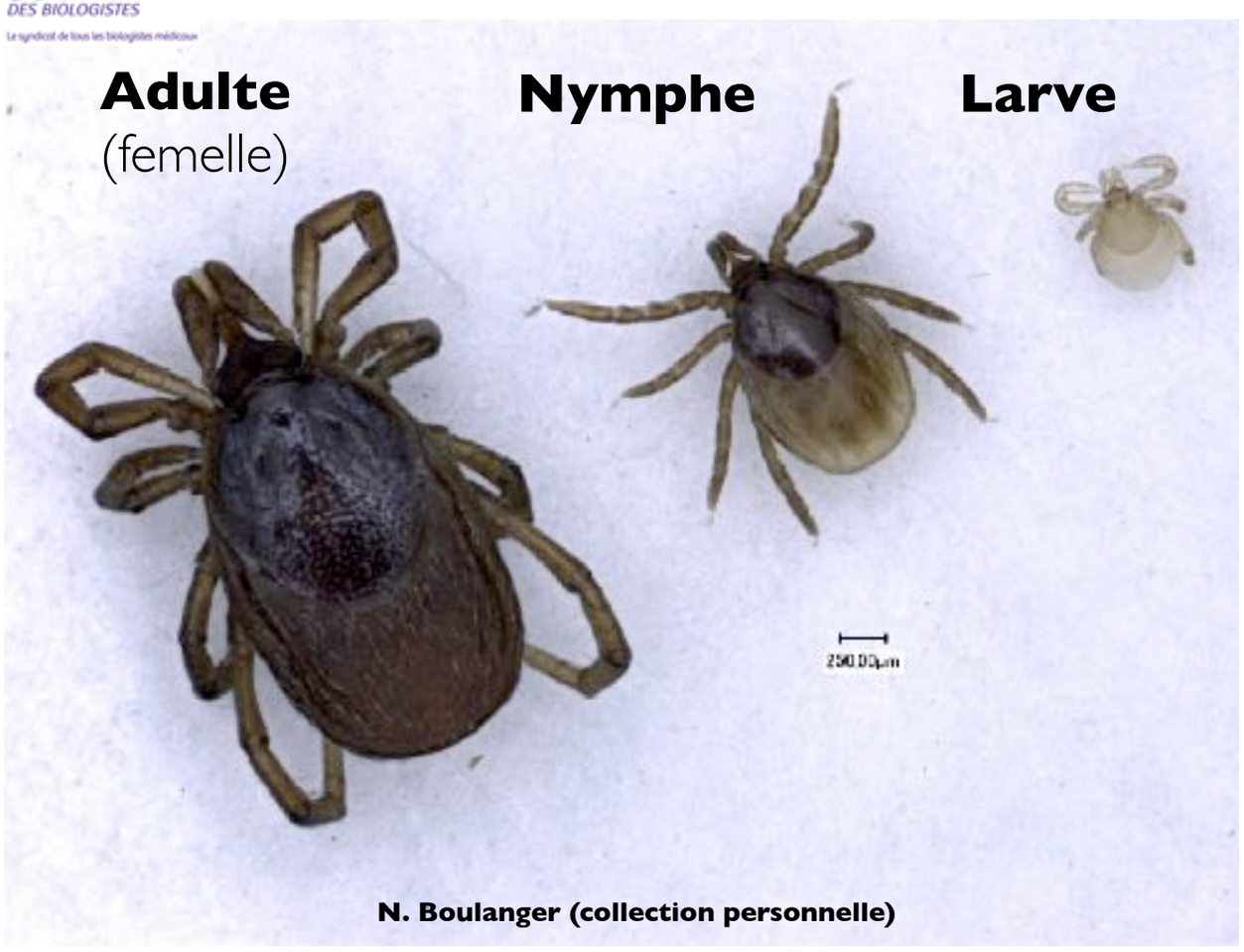
- “quête” au-dessus de la state herbacée
- attente “à l’affût” du passage d’un hôte

**Prise d’un repas sanguin sur un hôte vertébré**

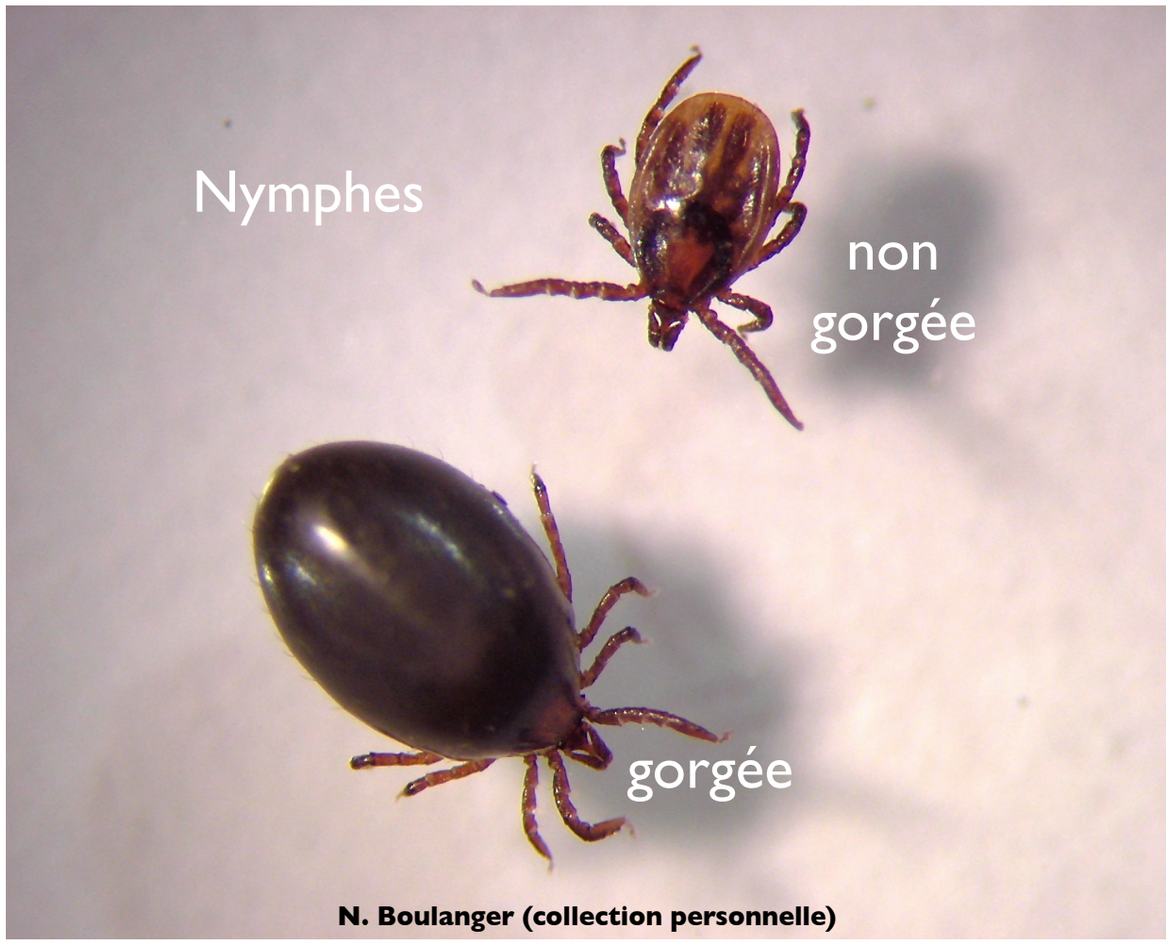
# Stases de développement de la tique *I. ricinus*


| **Larve** | **Nymphe** | **Adulte** (mâle) | **Adulte** (femelle) |
|---|---|---|---|
| 3 paires de pattes | 4 paires de pattes | 4 paires de pattes | 4 paires de pattes |

# Stases de développement de la tique *I. ricinus*




N. Boulanger (collection personnelle)

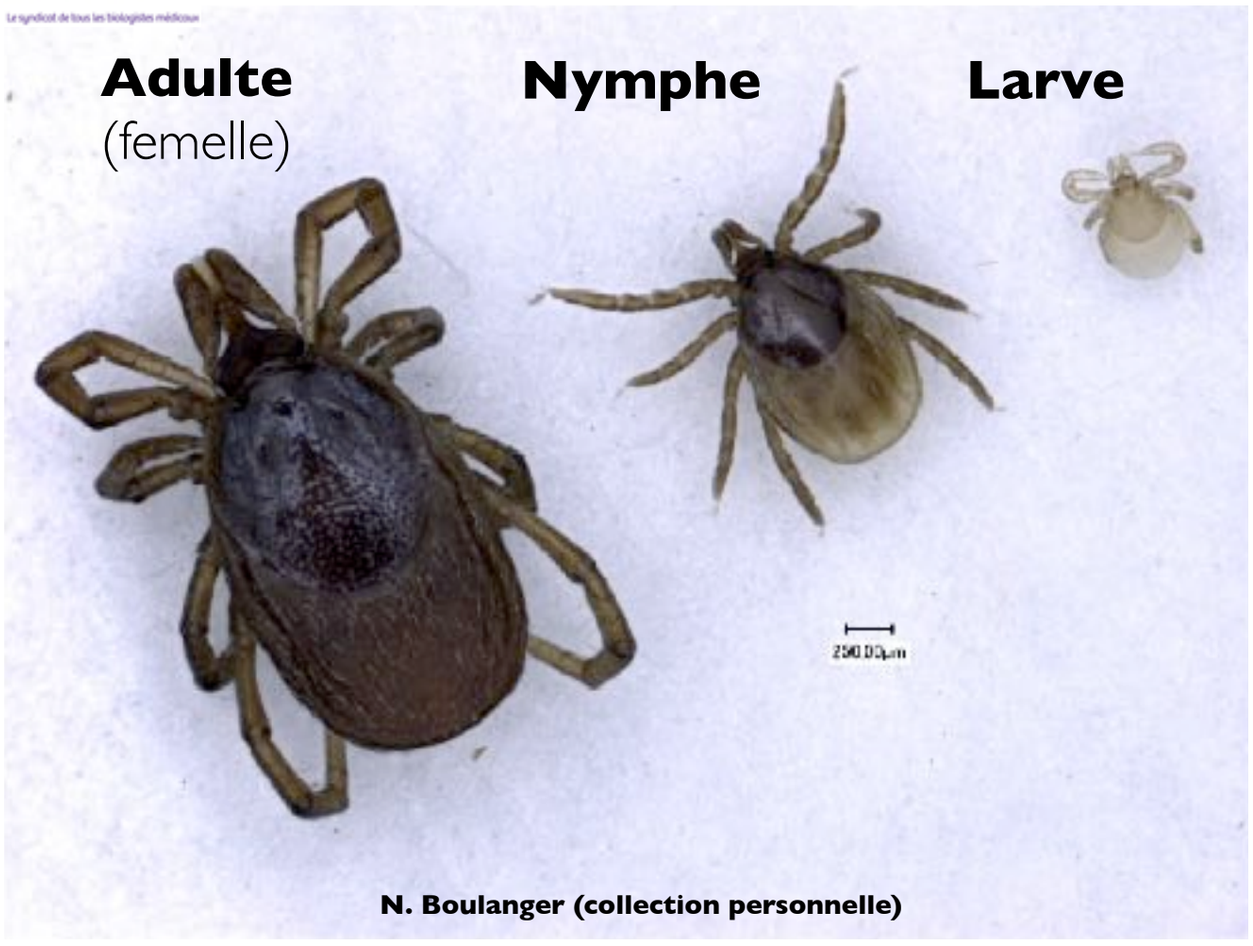



N. Boulanger (collection personnelle)

**métamorphose entre chaque stase après un repas sanguin**

- Cycle complet
  - durée = 2 à 3 ans

# Stases de développement de la tique *I. ricinus*




N. Boulanger (collection personnelle)

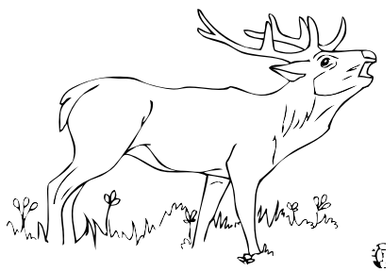
**métamorphose entre chaque stase après un repas sanguin**

- Cycle complet
  - durée = 2 à 3 ans
- Repas sanguin **2-10 jours**
  - durée variable (fonction stase)

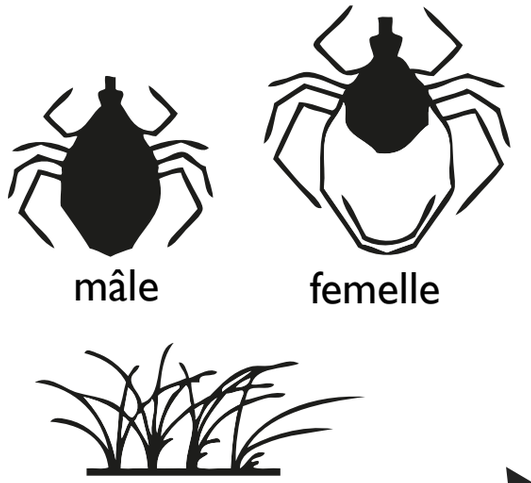
# Le cycle des tiques du genre *Ixodes*

SYNDICAT DES BIOLOGISTES
Le syndicat de tous les biologistes médicaux

**télotropes**



## Adultes

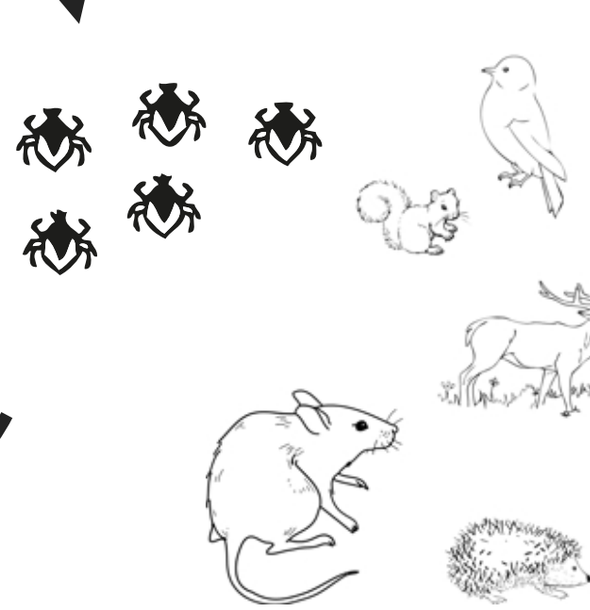


mâle femelle

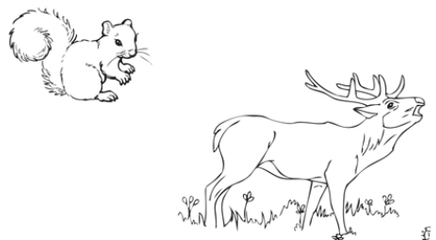


## Oeufs

## Larves



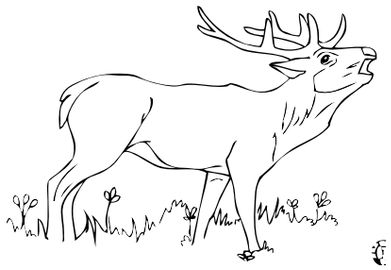
## Nymphes



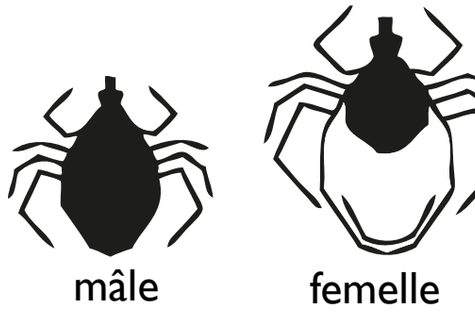
# Le cycle des tiques du genre *Ixodes*

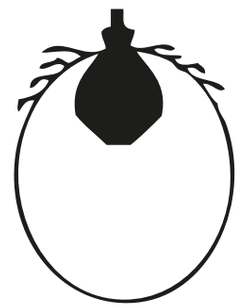
SYNDICAT DES BIOLOGISTES
Le syndicat de tous les biologistes médicaux

**télotropes**



## Adultes

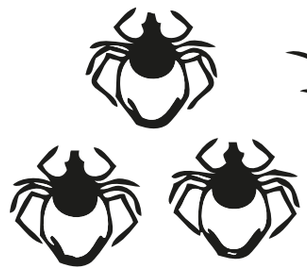


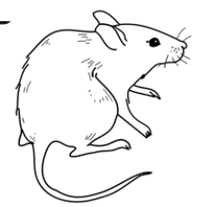



## Oeufs

**transmission de *Borrelia***

## Nymphes



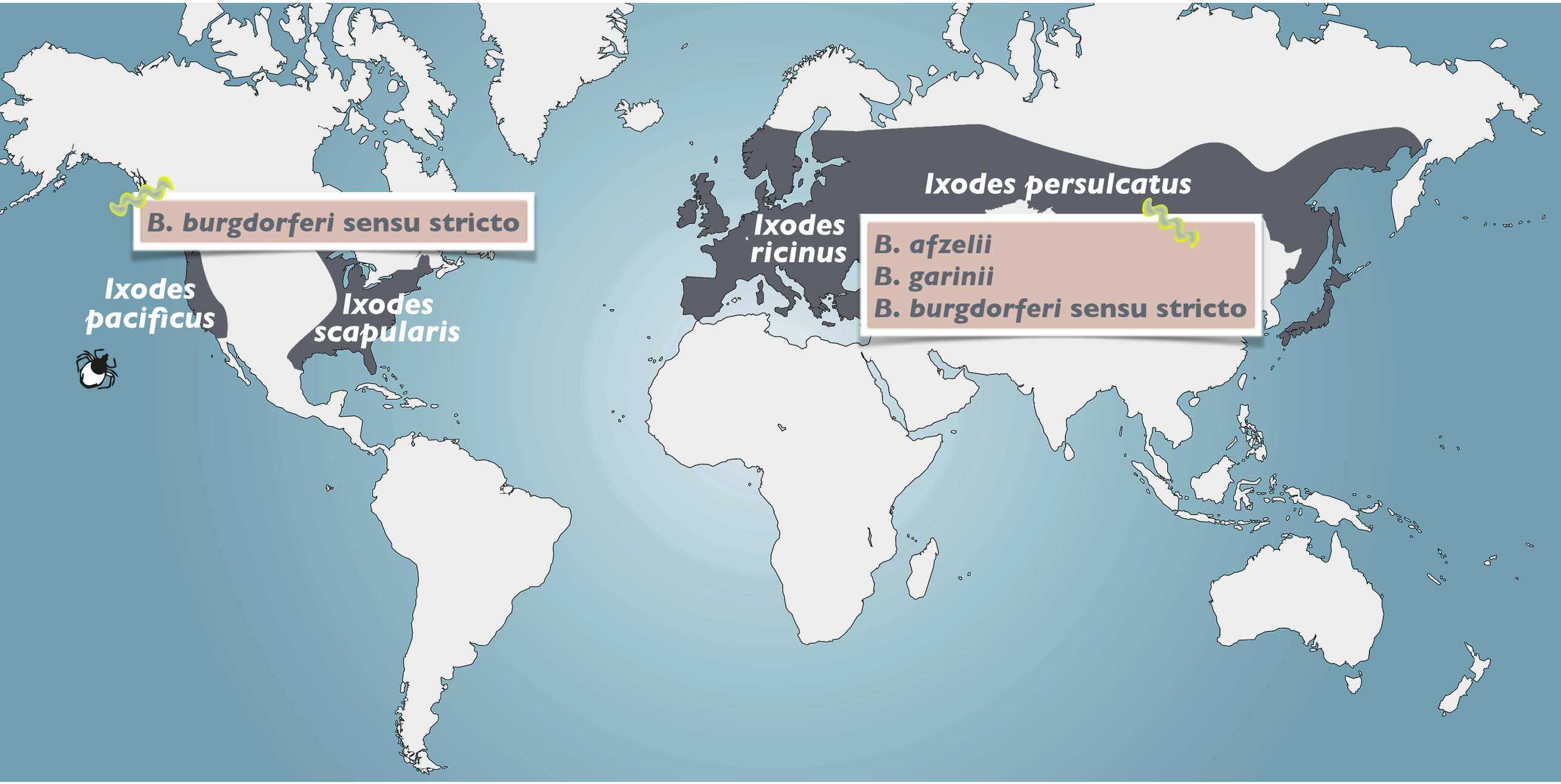


## Larves

# Répartition géographique

SYNDICAT DES BIOLOGISTES
Le syndicat de tous les biologistes médicaux

## Les vecteurs

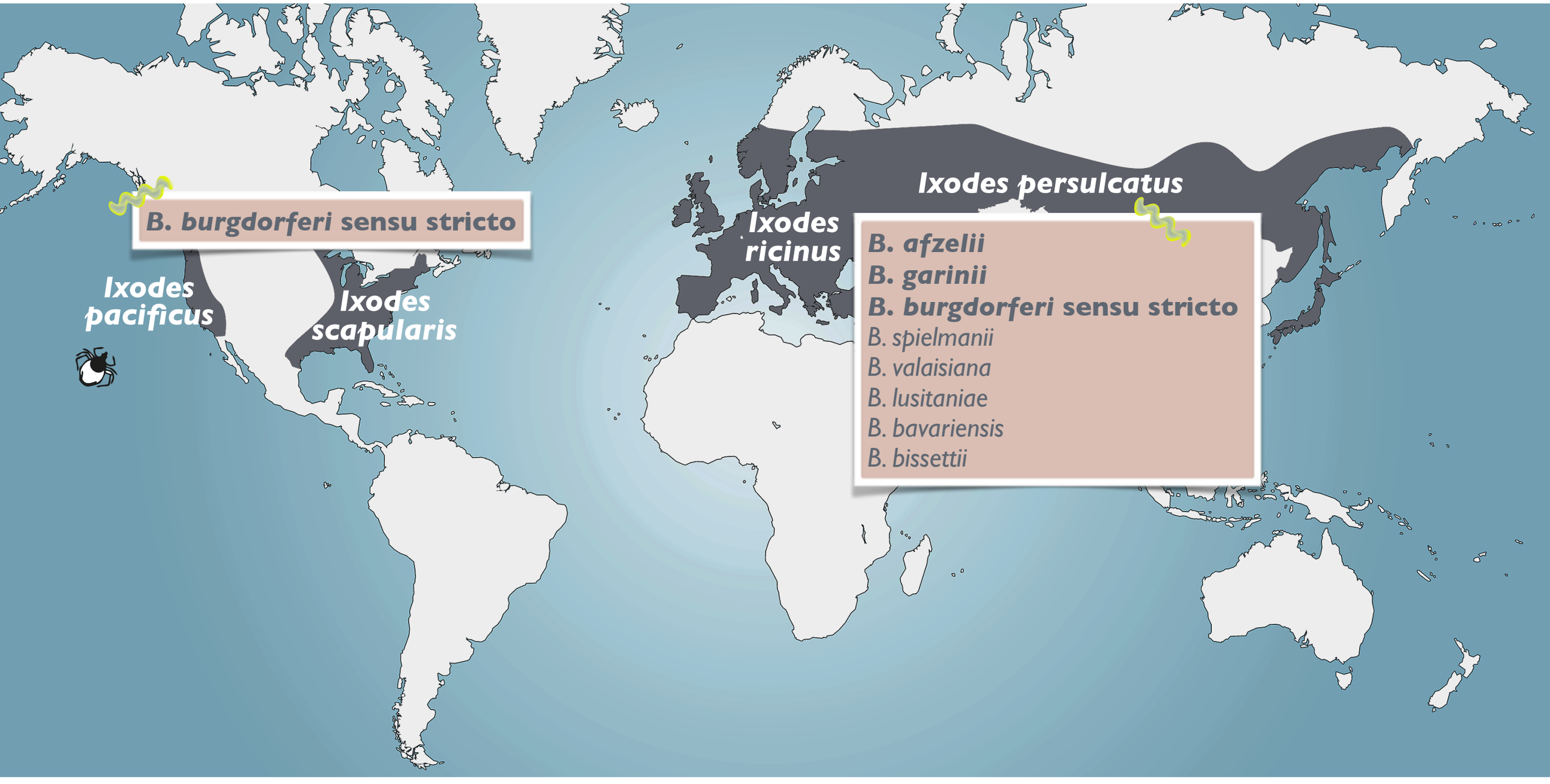



**_Borrelia_ et son vecteur : espèces impliquées en pathologie humaine**

# Répartition géographique

SYNDICAT DES BIOLOGISTES
Le syndicat de tous les biologistes médicaux

## Les vecteurs

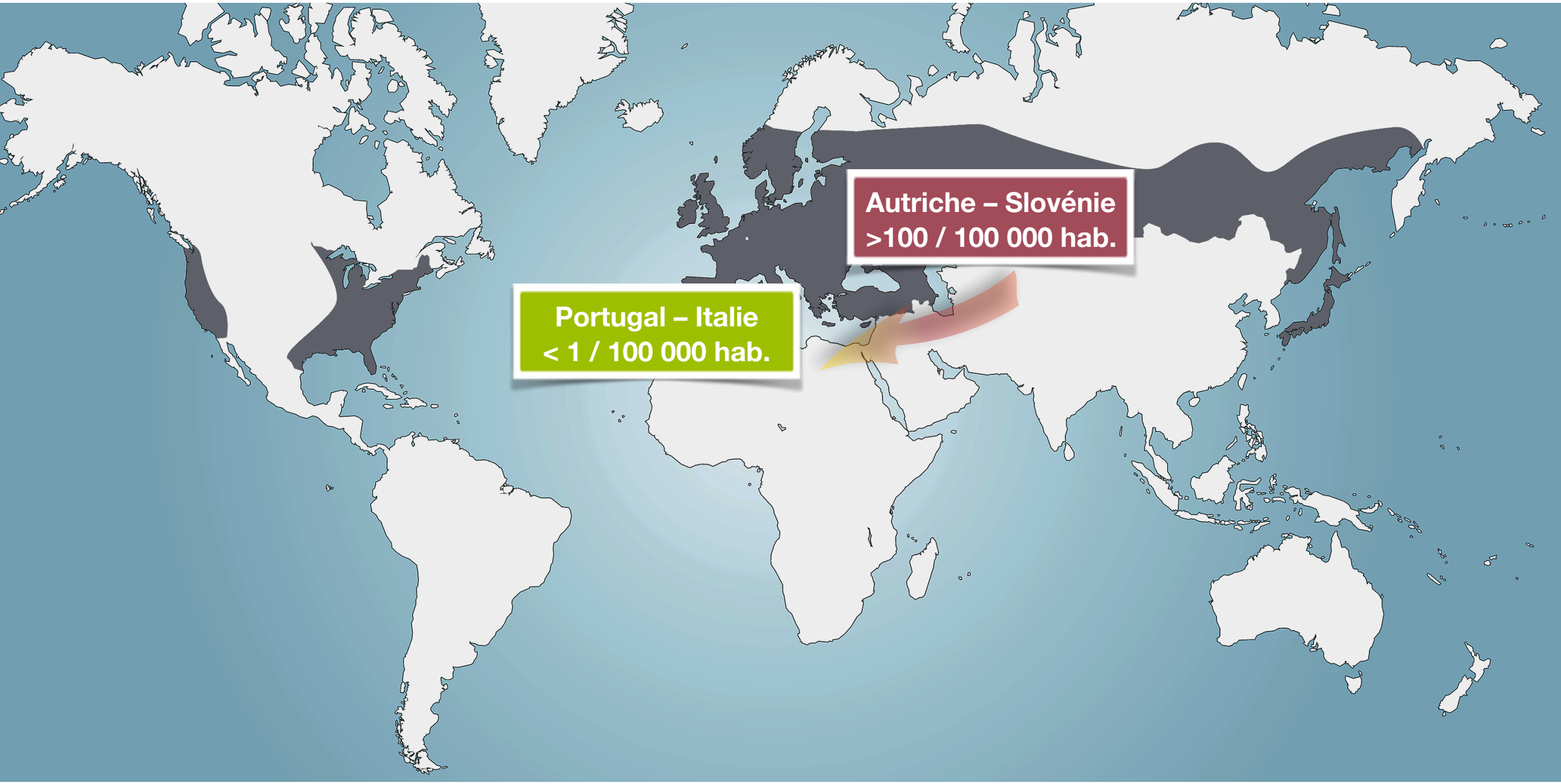



**_Borrelia_ et son vecteur : espèces impliquées en pathologie humaine**

# Répartition géographique

SYNDICAT DES BIOLOGISTES
Le syndicat de tous les biologistes médicaux

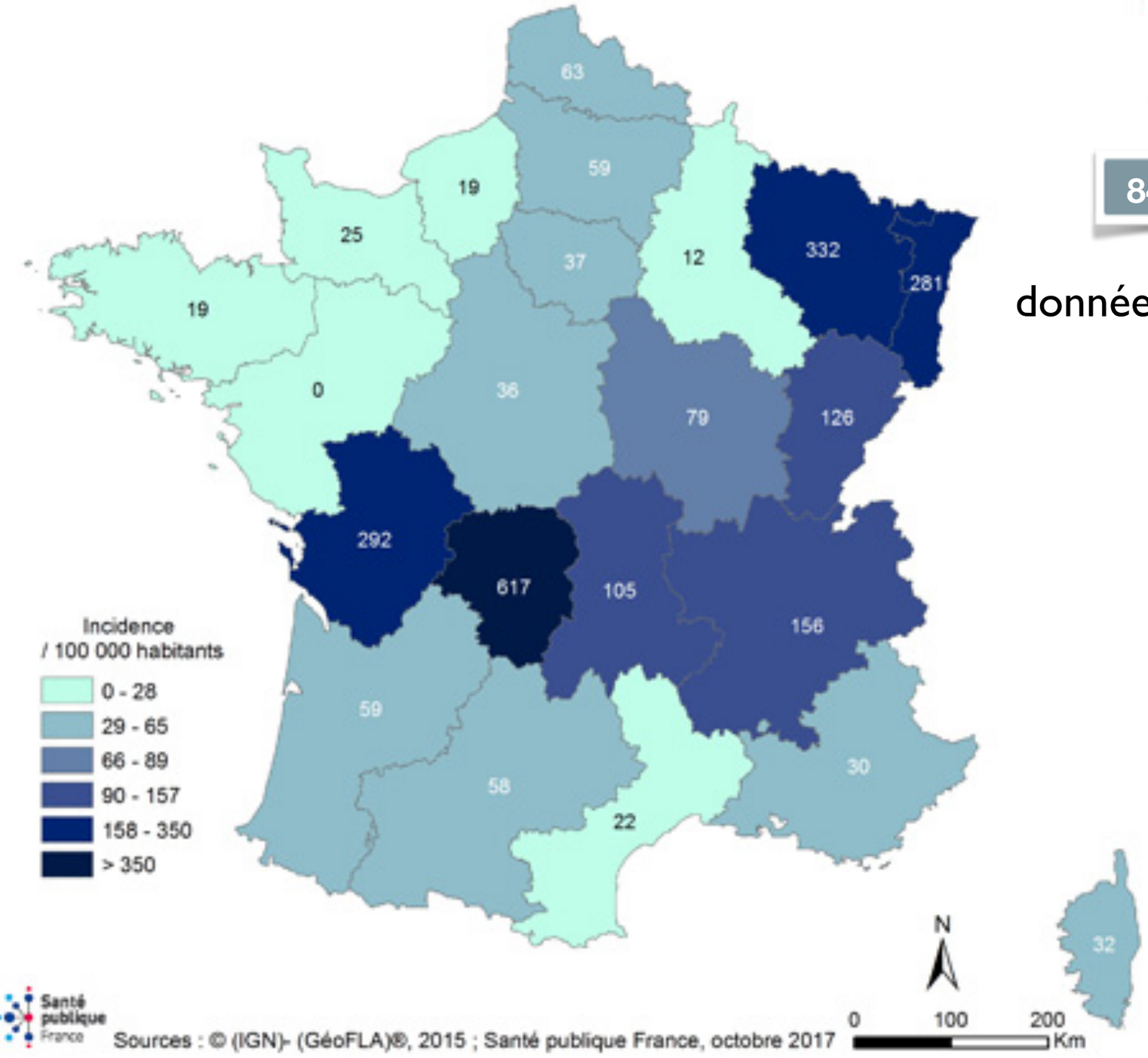
## Superposition géographique : vecteur – maladie



# Répartition géographique

SYNDICAT DES BIOLOGISTES
Le syndicat de tous les biologistes médicaux

## France : estimations pour l'année 2016




**55 000 cas**

**84 / 100 000 hab.**

données réseau Sentinelles

- France
  - incidence intermédiaire, mais disparités régionales : Centre de la France et Est

# Données françaises récentes

SYNDICAT DES BIOLOGISTES
Le syndicat de tous les biologistes médicaux

## Augmentation réelle du nombre de cas ?

**55 000 cas**

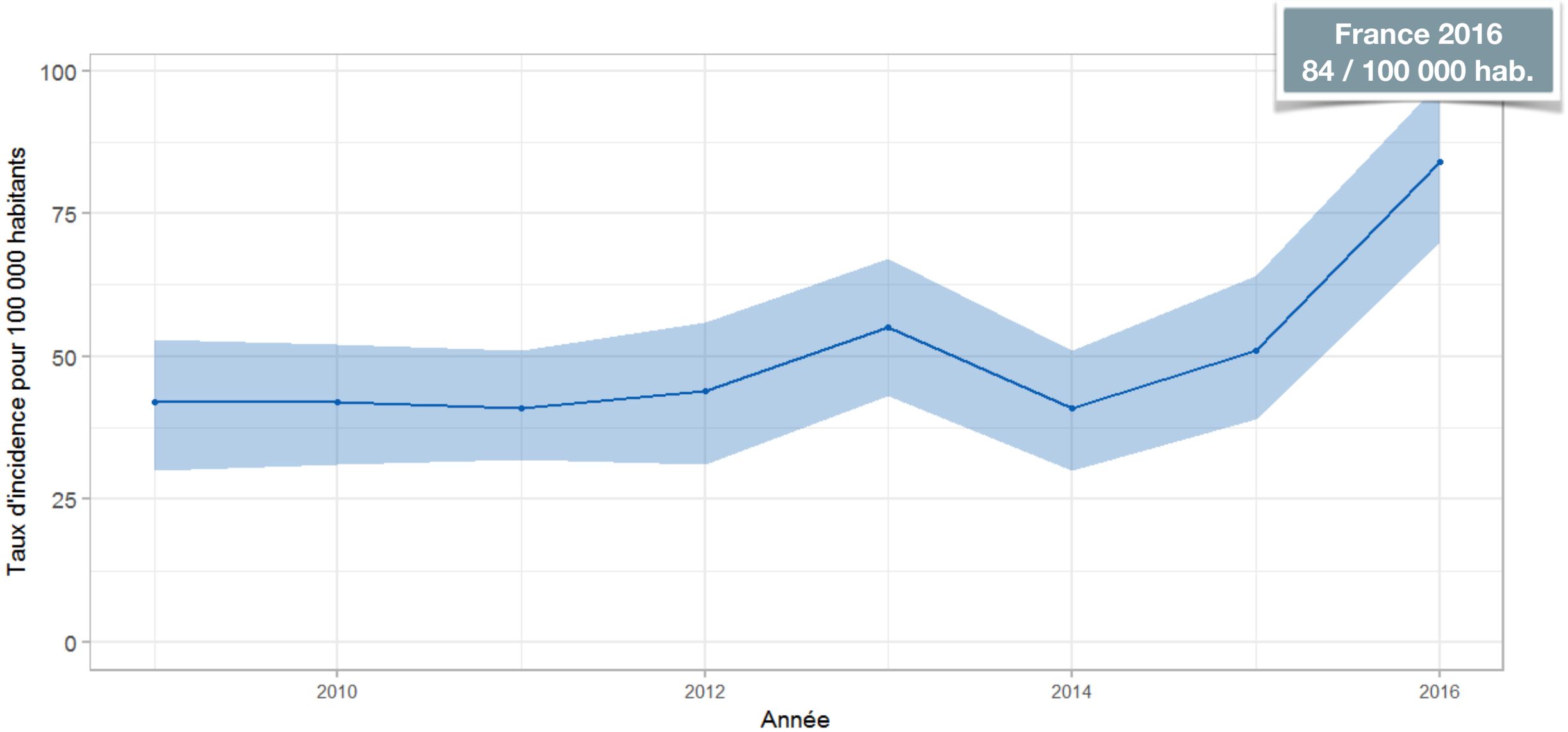



Figure 13.1 : Evolution du taux d'incidence annuel des cas de borréliose de Lyme vus en consultation de médecine générale en France métropolitaine de 2009 à 2016 (intervalle de confiance à 95%)

- Nombre de cas validés : 194, dont 194 (100 %) individuellement décrits
  - 184 érythèmes migrants (94,8%) et 10 formes disséminées (5,2%)

**très faible nb de cas (2016 n=194)**

Données 2016 Réseau Sentinelles

# Histoire naturelle de la maladie


http://vincentperrocheau.files.wordpress.com/2013/02/foret.jpg

## Risque de transmission à l'homme

# Histoire naturelle de la maladie

- Risque d'exposition aux piqûres infestantes de tique
  - intensité d'exposition au vecteur (loisirs, professionnels exposés)
  - densité des tiques infectées (nymphes +++) dans le biotope fréquenté
- Risque de transmission à l'homme après piqûre de tique
  - le risque ↗ avec de la durée d'attachement
  - risque global estimé : 1-4 % ➙ très faible si < 24h poss dès 8h (Europe) ou < 48h (US)

## Données expérimentales de la transmission

*I. scapularis + Bbss*

**hamster**

Transmission dès **24h**

Efficacité max à **72h**

Transmission dès **17h**

Efficacité max à **47h**

*I. ricinus + B. afzelii*

**gerbille**

d'après Piesman et coll., JCM 1987; Hojgaard et coll., JME 2008 ; Kahl et coll., IJMM 1998, Crippa et coll., VBZD 2002

# Histoire naturelle de la maladie

**3 - 25 % tiques infectées en Europe**

**Piqûre de tique infectée**

→ **Pas de transmision**

**Transmission** →

**Infection avortée**

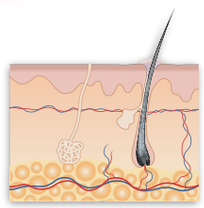
**1 - 4 %**

**Borréliose de Lyme**

absence de TT

**10 %**

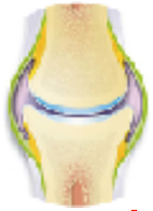
**phase précoce localisée**



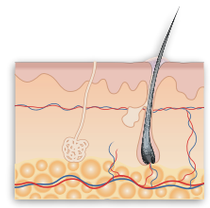
Érythème migrant

**phase précoce disséminée**

neuro



articulaire

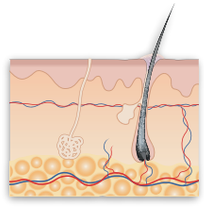


Lymphocytome

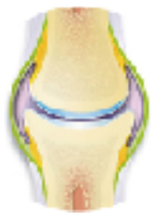
cardiaque

oculaire

**phase tardive**



**A**crodermatite
**C**hronique
**A**trophiante



chroniques

# Pathotypes

| **Europe**<br>**Strle F, Stanek G. Curr Probl Dermatol 2009. Lipsker D, Jaulhac B, eds.** | **fréquence** | **remarque** | ***B. afzelii*** | ***B. garinii*** | ***Bb ss*** |
|---|---|---|---|---|---|
| Erythème migrant | 70 - 90 % | | 70-90 % | 10-20 % | rare |
| Neuroborréliose | 15 - 25 % | | + | ++ | rare |
| Arthrite | 2 - 7 % | | données discordantes en fonction des études | | |
| ACA | 5 - 6 % | fréq ♀, sujet âgé | ++ | rare | très rare |
| Lymphocytome | 2 - 5 % | fréq enfant | ++ | rare | rare |
| Atteinte cardiaque, oculaire | <1 % | | pas assez de données | | |

| **USA**<br>**(données CDC, 2001-2010)**<br>**213 500 patients** | **fréquence** | **remarque** | ***Bb ss*** |
|---|---|---|---|
| Erythème migrant | 70 % | | 100 % |
| Neuroborréliose | 14 % | | " |
| Arthrite | 30 % | < 10 % séries récentes | " |
| ACA | - | rarissime aux USA | - |
| Lymphocytome | - | rarissime aux USA | - |
| Atteinte cardiaque, oculaire | 1 % | | " |

# DIAGNOSTIC CLINIQUE & BIOLOGIQUE

# DIAGNOSTIC BIOLOGIQUE

# LES OUTILS “VALIDÉS”

# Diagnostic biologique direct

## Les outils du diagnostic direct

- **culture**
  - ☆ croissance lente / milieu BSK spécifique mais non sélectif / expérience ++

Fond noir

- **biologie moléculaire**
  - ☆ PCR "maison", peu de trousses commercialisées

PCR temps réel

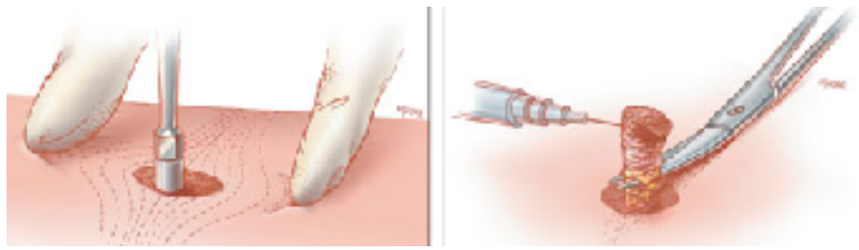
- **examen anatomopathologique**
  - ☆ infiltrat compatible mais non spécifique

Examens spécialisés

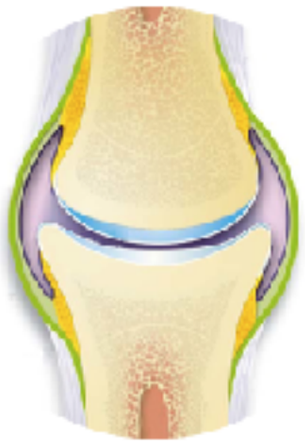
Prélèvements "invasifs"

**"deuxième intention"**

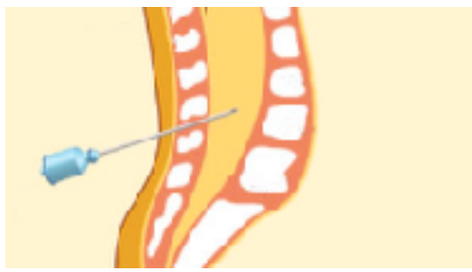
## Quels prélèvements ?



biopsie cutanée

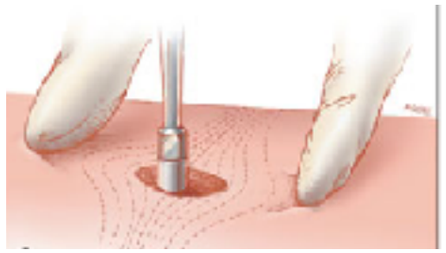


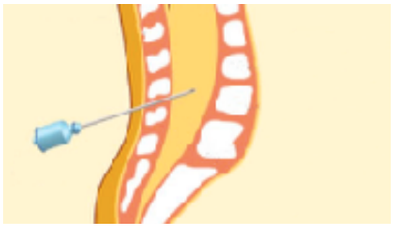
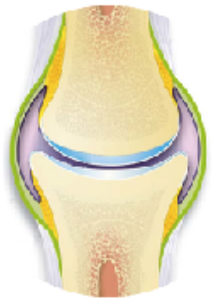
matériel articulaire

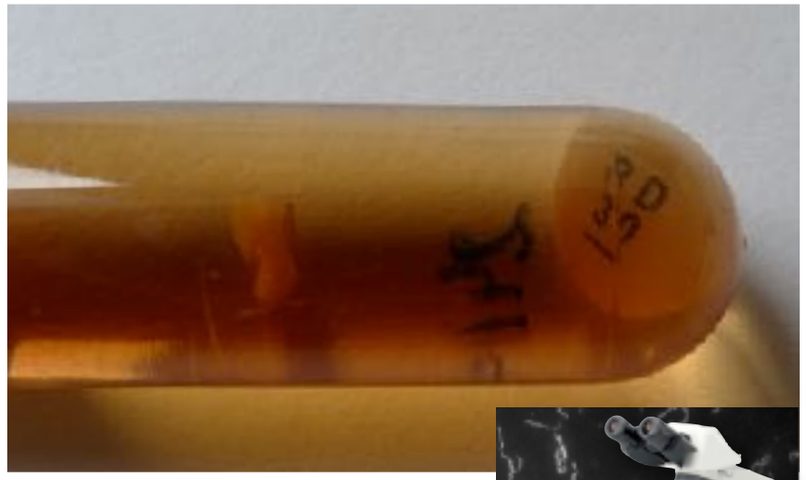


LCR

# Diagnostic biologique direct






## Culture

- aspsie stricte
- ensemencement immédiat en milieu BSK au lit du patient
- acheminement à T° ambiante
- + disque rifampicine à l'arrivée au labo (↘ risque de contamination)

## PCR

- acheminement à T° ambiante en milieu BSK si PCR + culture
- acheminement à -80 °C (tube stérile sans additif)
- acheminement à +4 °C possible si transport < 48h (dans eau φ si peau)

# Sérologie : techniques de dépistage

## Évolution des outils sérologiques

tests ELISA

IFI

1re génération

Ag cellulaires complets

2e génération

Ag purifiés

3e génération

+ Ag recombinants (VlsE)

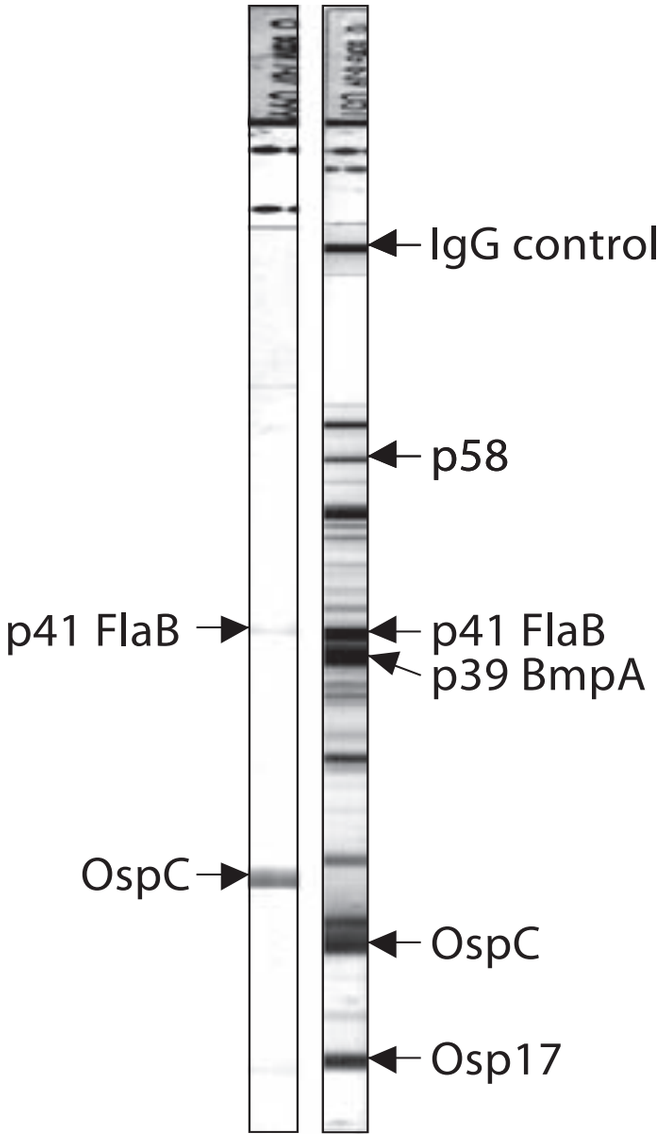
☆ Ac totaux ou détection séparée IgG/IgM (meilleure interprétation)

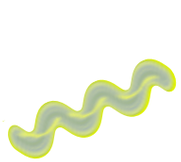
## Performances des tests ELISA

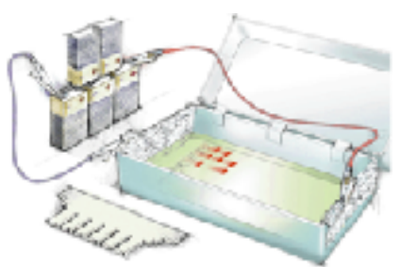
- sensibilité très bonne de la plupart des trousses
- marquage CE insuffisant pour garantir la qualité du réactif utilisé
- spécificité minimale de 90 % requise (critères EUCALB)

# Sérologie : techniques de confirmation

Whole cell immunoblot IgM IgG

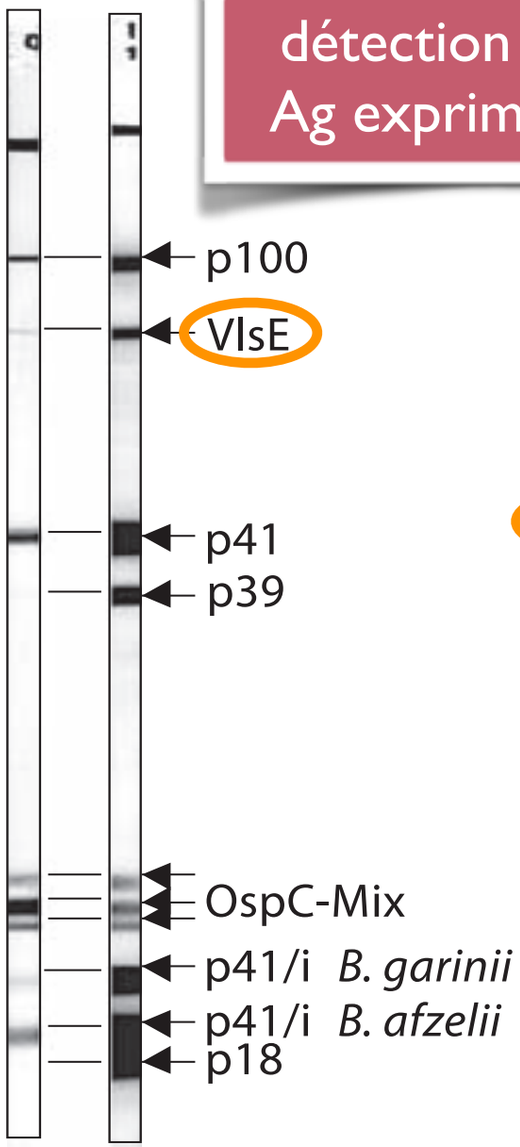


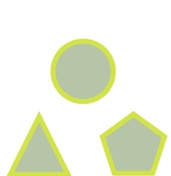



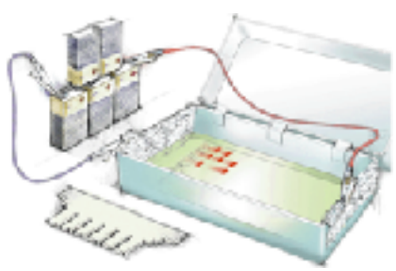


Recombinant immunoblot IgM IgG

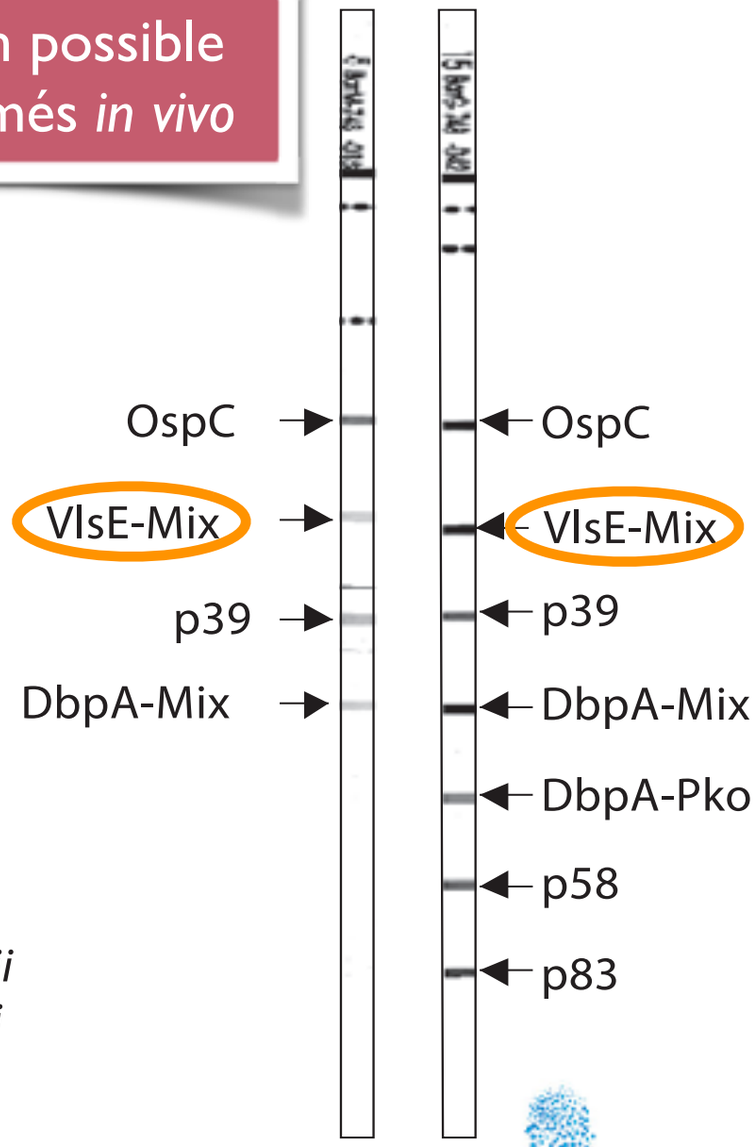
détection possible
Ag exprimés *in vivo*

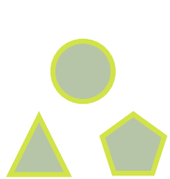




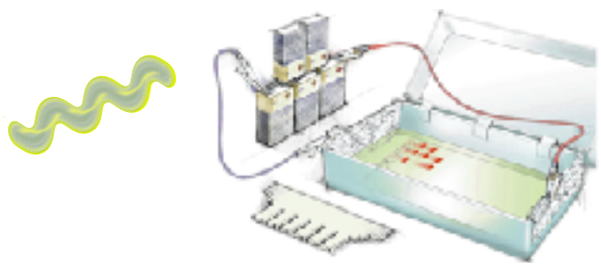
Line immunoblot IgM IgG

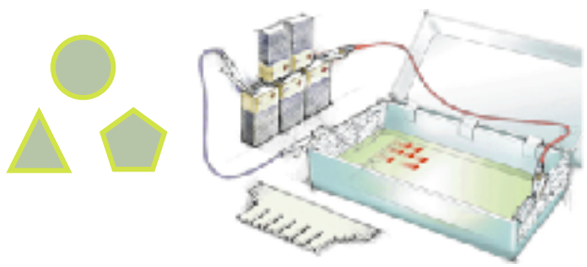


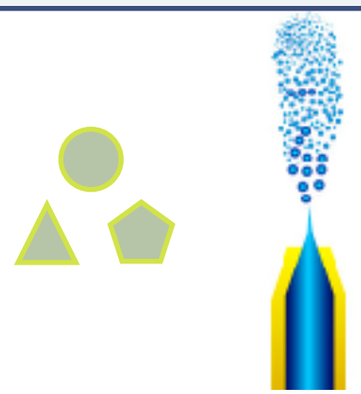



# Tests de confirmation

| Whole cell immunoblot | Recombinant immunoblot | Line immunoblot |
|---|---|---|
| **tous Ag "naturels" accessibles** | **sélection de protéines recombinantes purifiées** | |
| **Ag spé / Ag peu spé** | **choix d'Ag spé** | **choix d'AG spé** |
| **Ag exprimés en culture *in vitro*** | **possibilité incorporation d'Ag présents *in vivo* uniquement (VlsE)** | |
| **SDS-PAGE** | **SDS-PAGE** | **Ag "sprayés"** |
| Ag dénaturés | Ag dénaturés | détection possible d'Ac spé de l'Ag natif |
| Ag d'une seule espèce | possibilité Ag de pls espèces | possibilité Ag pls espèces<br>+ détection séparée d'Ag de même PM |
| **peu standardisés** | **meilleure standardisation** | |
| **expertise importante de lecture** | **lecture plus facile** | |
| **coût le plus faible** | **coût élevé** | **coût le plus élevé** |







# Diagnostic biologique indirect

## Les outils du diagnostic indirect : sérologie

**Stratégie en 2 temps**

**1. Test de première intention**

tests ELISA

**si - → sérologie négative**

automates

- sensibilité très bonne de la plupart des trousses
- qualité du réactif utilisé ? marquage CE insuffisant
- spécificité minimale de 90 % requise (critères EUCALB)

**on recherche la meilleure sensibilité possible, quitte à avoir une spécificité “faible” (>90%)**

# Diagnostic biologique indirect

## Les outils du diagnostic indirect : sérologie

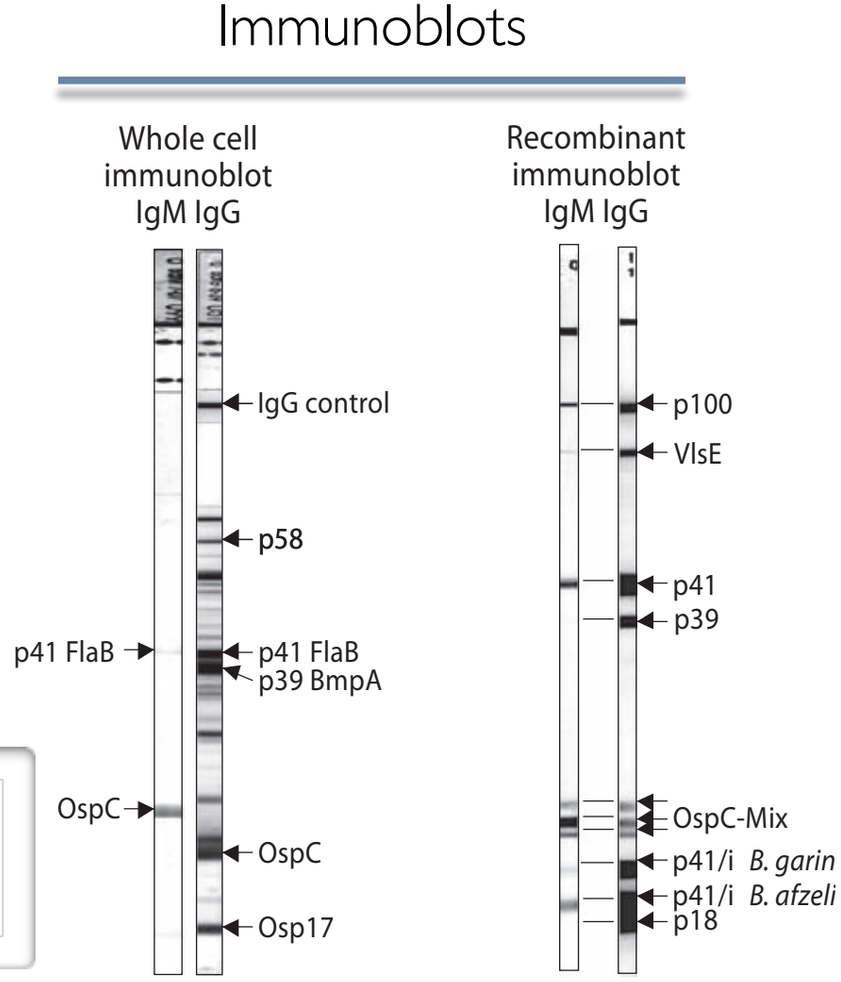
**Stratégie en 2 temps**

**1. Test de première intention** → tests ELISA

**si - → sérologie négative**

**2. Test de confirmation** → Immunoblots

- spécificité minimale de 95 % requise (critères EUCALB)
- confirme la spécificité des Ac détectés en ELISA
- large panel d'Ag de *Borrelia* → "profil Ac" du patient




**on s'assure avec ce test spécifique de confirmer que les Ac détectés sont bien dirigés contre *Borrelia***

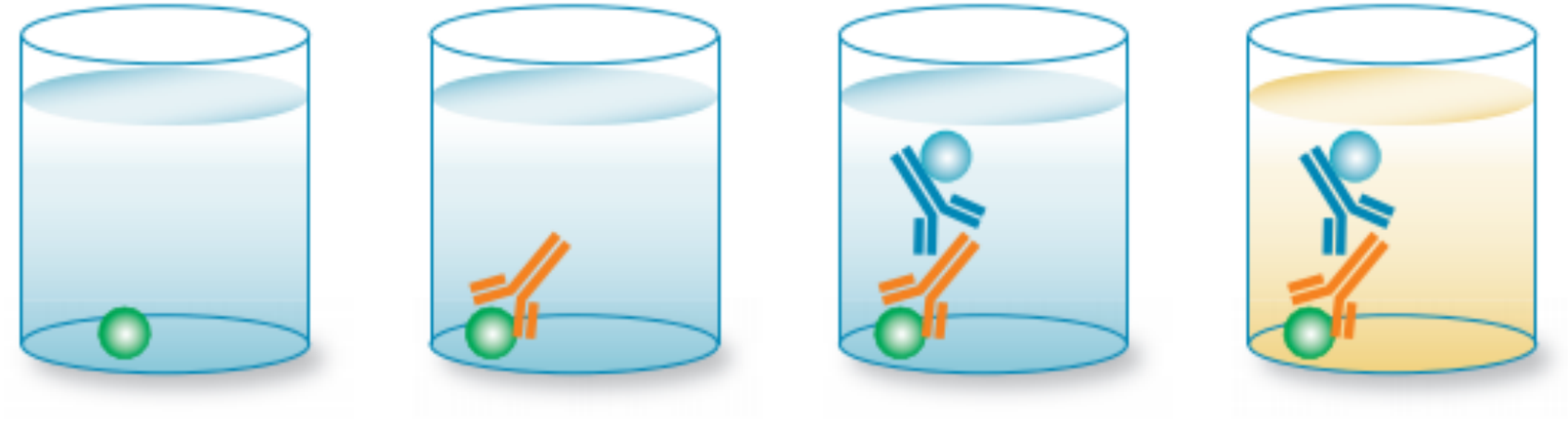
# Tests ELISA

**ELISA positif : Ac du patient ont « accroché » des épitopes des Ag présents**

**Les anticorps détectés sont-ils bien dirigés contre *Borrelia* (spécifiques) ???**

**Exemple**

**Test ELISA**

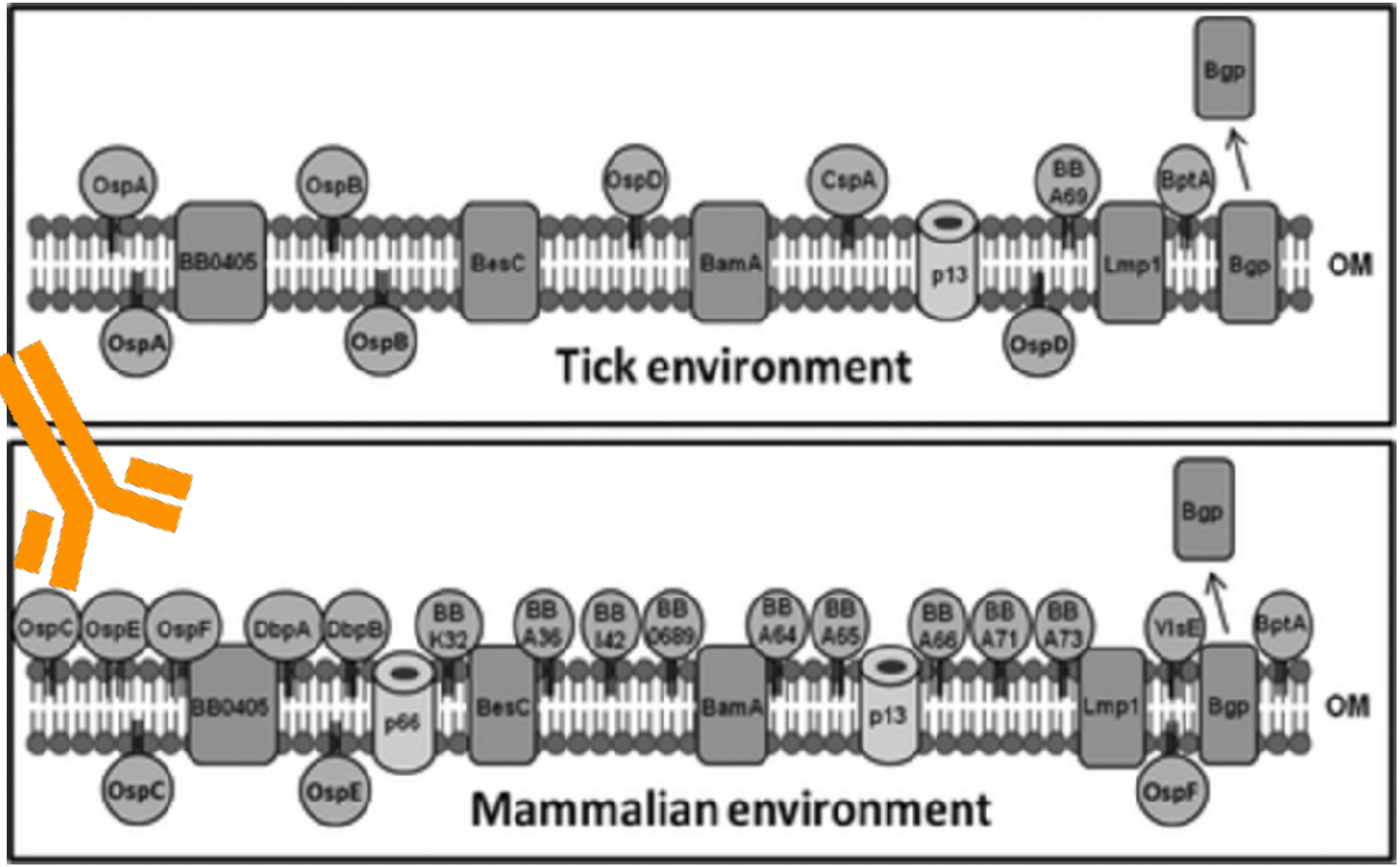


- **Test IgG**
  - Ag souche B. afzelii Pko (OspC ++) enrichi en VlsE
- **Test IgM**
  - Idem avec préadsorption sérums (↘ f. rhumatoïde)

# Tests de confirmation

**ELISA positif : Ac du patient ont « accroché » des épitopes des Ag présents**

**Les anticorps détectés sont-ils bien dirigés contre *Borrelia* (spécifiques) ???**



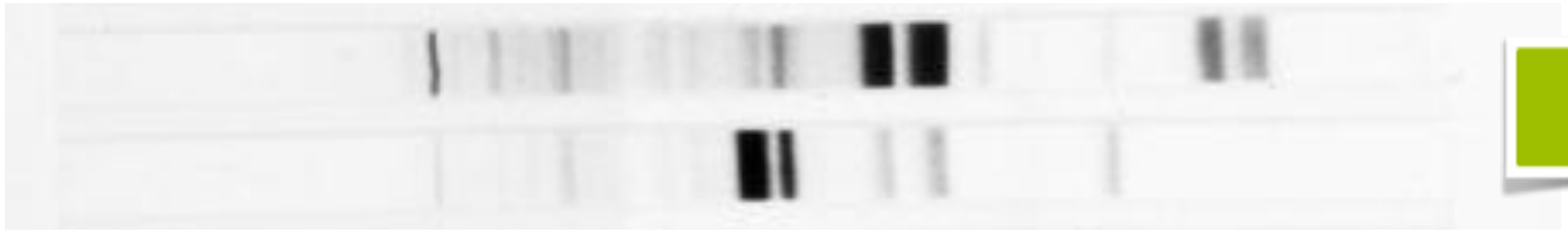

Kenedy et coll., FEMS Immunology & Medical Microbiology, Volume 66, Issue 1, October 2012

# Tests de confirmation

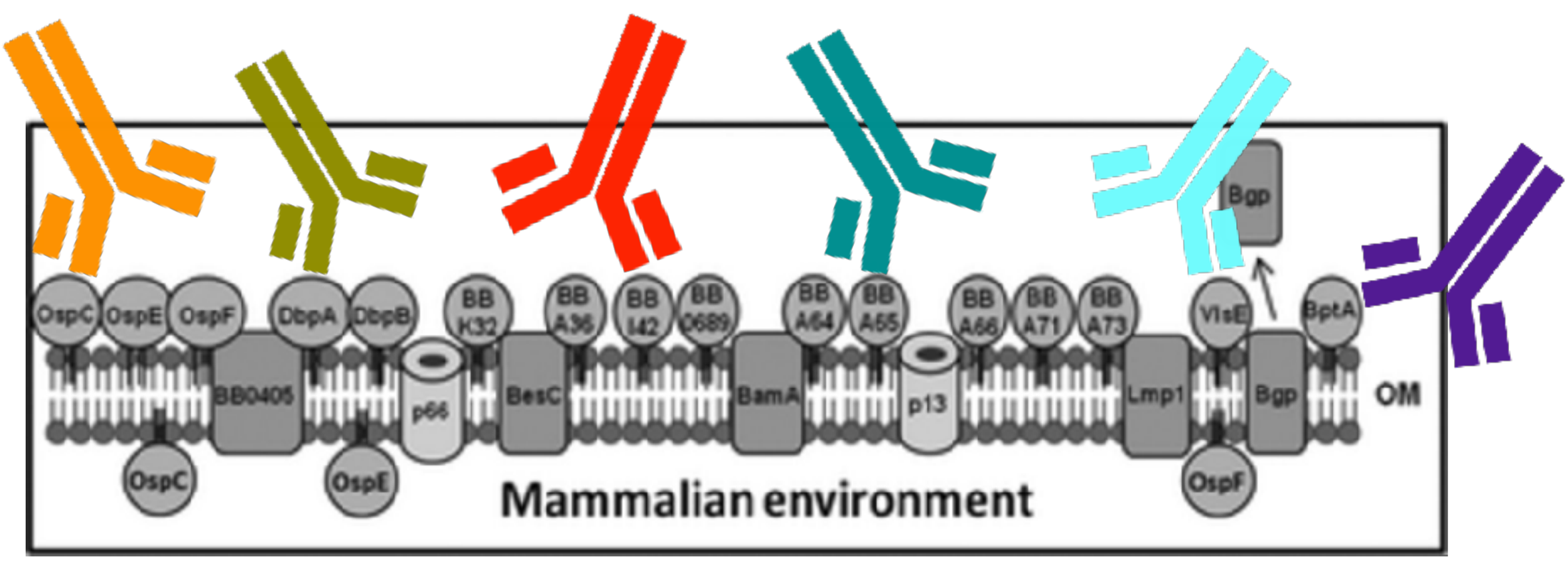
**ELISA positif : Ac du patient ont « accroché » des épitopes des Ag présents**

**Les anticorps détectés sont-ils bien dirigés contre *Borrelia* (spécifiques) ???**

\+



**Profil riche**
**Ac spécifiques**

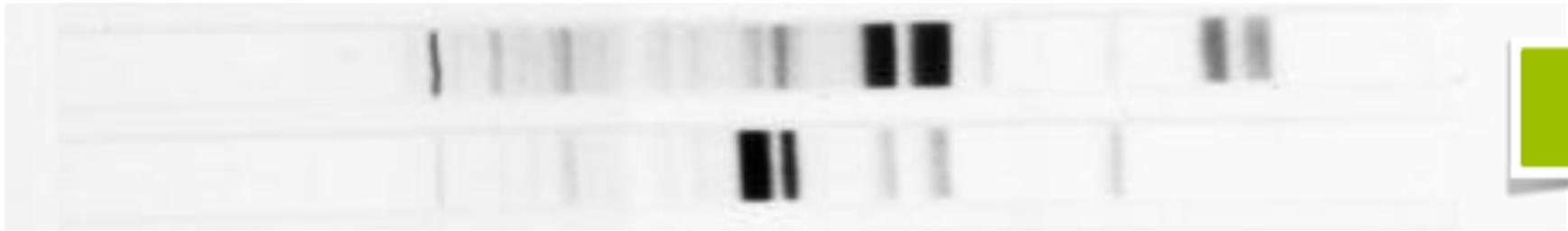


# Tests de confirmation

**ELISA positif : Ac du patient ont « accroché » des épitopes des Ag présents**

**Les anticorps détectés sont-ils bien dirigés contre *Borrelia* (spécifiques) ???**

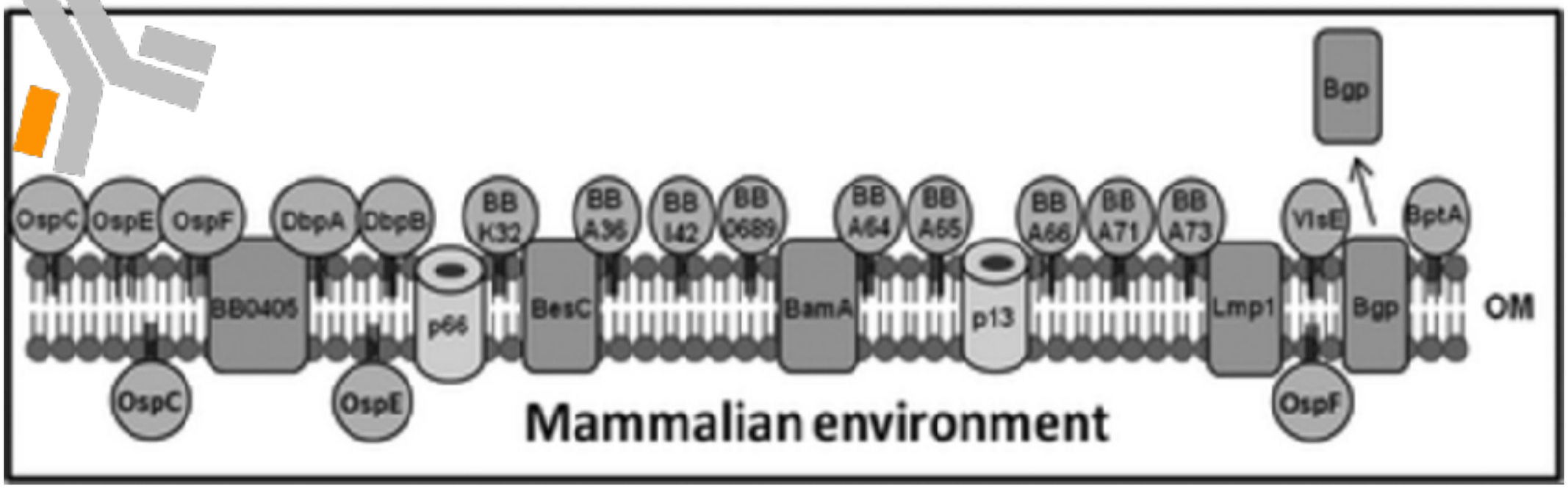
\+



**Profil riche**
**Ac spécifiques**

\-

**Profil pauvre**
**Ac non spé**



# Tests de confirmation

| Protéines | Phase précoce | Phases disséminée et tardive | Spécificité |
|---|---|---|---|
| p83/100 | | + | **forte** |
| p58 | + | + | moyenne |
| p43 | + | + | moyenne |
| p41 (**flagelline**) | + | + | faible |
| p39 (**BmpA**) | | + | moyenne |
| **OspA** | | + (arthrite) | moyenne |
| p21 **OspC** | + | + | **forte** |
| p17/p18 (**DbpA**) | | + | moyenne |
| **VlsE** | + | + | **forte** |

**absence de p41 rend très peu probable le diagnostic**

# Sérologie Lyme : un "pas à pas" rationnel

**Test de dépistage : ELISA (IgG/IgM)**

- positif
- négatif → **Résultat NEG**
  phase I/II : suivi à 3-6 sem si persistance suspicion clinique

**Test de confirmation : Immunoblot (IgG/IgM)**

| positif | douteux | négatif |
|---|---|---|
| Résultat POS | Tests complémentaires + suivi sérologique à 3-6 sem si persistance suspicion clinique | Résultat NEG |

**séroconversion : infection**

**faux pos ELISA**

# Évolution sérologique "classique"

## phase initiale de la maladie (au stade d'EM)

- **séropositivité** (≈ 20-60 % des patients)
  - ☆ 40-80 % "faux" négatifs
- **apparition initiale d'IgM (pas av 3 sem), puis apparition des IgG 2-3 sem plus tard**
  - ☆ séroconversion peut survenir
  - après TT efficace ? ☆ persistance possible d'Ac pls mois/années (y compris d'IgM)
  - ☆ absence totale d'Ac possible

## phases disséminée et tardive

- ↗ **progressive séropositivité** (≈ 90% lymphocytome borrélien ≈100% ACA / arthrite)

après TT efficace ? ☆ persistance possible d'Ac pls mois/années (y compris d'IgM)

**sérologie**

pas recommandée pour le diagnostic d'EM typique

pas recommandée pour le suivi de l'efficacité du TT

présence d'IgM pas synonyme d'une infection aiguë

# Sérologie positive ≠ borréliose de Lyme active

## Étude sérologique grand-Est France

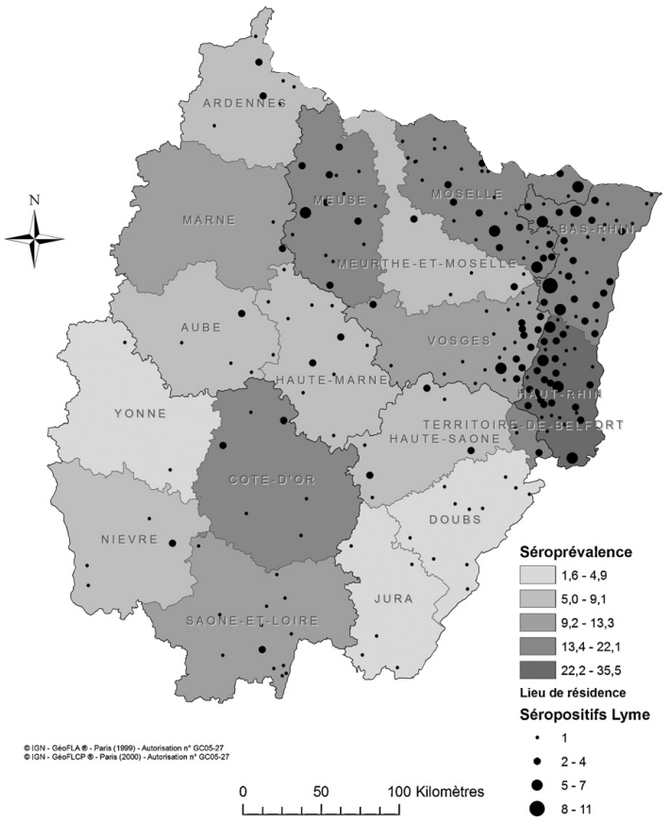
Thorin C et coll, Med Mal Inf 2008

- 2975 forestiers testés
- 17% séropositivité (27% en Alsace)

↓

- ☆ manifestations articulaires : 14%
- ☆ érythème migrant : 12%

Séroprévalence




## Persistance Ac

- Ac résiduels infection *Borrelia* passée

Cicatrice sérologique

## Réactions croisées (++ IgM)

Spécificité intrinsèque des tests sérologiques

- Ac syphilis, EBV/CMV/HSV … maladies de système, maladies dysimmunitaires, ...

# Investigation clinico-biologique

- éléments cliniques
  - données anamnestiques : exposition possible piqûre de tique, évolution clinique
  - type des manifestations cliniques observées
- données sérologiques (+ autres éléments biologiques)
  - classe des immunoglobulines détectées (IgM, IgG)
  - profil antigénique de la réaction sérologique (immunoempreintes)

**IgM isolées**
**Ag limités** (Fla, OspC)

**IgG prédominants**
**Ag variés** (p83/100, VlsE, OspA, DbpA, ...)

| | | | |
|---|---|---|---|
| ACA / arthrite exclues | manifestations précoces réaction croisée ? | manifestations tardives cicatrice sérologique ? | infection récente peu probable |

sauf réinfection !!!

# Performances intrinsèques/extrinsèques des tests

## Performances intrinsèques

- sensibilité (se) : % de test positif chez sujets malades
- spécificité (spé) : % de test négatif chez les sujets sains
- intrinsèques au test (pour une situation clinique donnée : ex arthrite de Lyme)
  - ☆ sont fixées par le seuil de positivité retenu (ne varient pas quand seuil fixé)
  - ☆ sont indépendantes de la prévalence de la maladie

## Performances extrinsèques

- valeur prédictive positive (VPP) : % de test sujets malades parmi les tests +
- valeur prédictive négative (VPN) : % de sujets sains parmi les tests -
- extrinsèques au test (pour une situation clinique donnée : ex arthrite de Lyme)
  - ☆ VPN et VPP varient en fonction du niveau de prévalence dans pop testée

# Performances intrinsèques/extrinsèques des tests

| Séro ELISA | Test + | Test - | Total |
|---|---|---|---|
| Lyme confirmé | Vrais positifs | Faux négatifs | ? |
| Sujet sain | Faux positifs | Vrais négatifs | ? |
| Total | ? | ? | 10 000 |

# Performances intrinsèques/extrinsèques des tests

Prévalence : 1%

Spécificité : 90%

Sensibilité : 90%

**Forte**

**Recos EUCALB**

**Moyenne**

| **Séro ELISA** | **Test +** | **Test -** | **Total** |
|---|---|---|---|
| **Lyme confirmé** | **?** Vrais positifs | **?** Faux négatifs | **?** |
| **Sujet sain** | **?** Faux positifs | **?** Vrais négatifs | **?** |
| **Total** | **?** | **?** | **10 000** |

# Performances intrinsèques/extrinsèques des tests

SYNDICAT DES BIOLOGISTES
Le syndicat de tous les biologistes médicaux

| Prévalence : 1% | Spécificité : 90% | Sensibilité : 90% |
|---|---|---|
| **Forte** | **Recos EUCALB** | **Moyenne** |

| **Séro ELISA** | **Test +** | **Test -** | **Total** |
|---|---|---|---|
| **Lyme confirmé** | **?** Vrais positifs | **?** Faux négatifs | **100** |
| **Sujet sain** | **?** Faux positifs | **?** Vrais négatifs | **9 900** |
| **Total** | **?** | **?** | **10 000** |

# Performances intrinsèques/extrinsèques des tests

SYNDICAT DES BIOLOGISTES
Le syndicat de tous les biologistes médicaux

Prévalence : 1%

Spécificité : 90%

Sensibilité : 90%

**Forte**

**Recos EUCALB**

**Moyenne**

| **Séro ELISA** | **Test +** | **Test -** | **Total** |
|---|---|---|---|
| **Lyme confirmé** | **?** Vrais positifs | **?** Faux négatifs | **100** |
| **Sujet sain** | **990** Faux positifs | **8 910** Vrais négatifs | **9 900** |
| **Total** | **?** | **?** | **10 000** |

# Performances intrinsèques/extrinsèques des tests

SYNDICAT DES BIOLOGISTES
Le syndicat de tous les biologistes médicaux

Prévalence : 1%

Spécificité : 90%

Sensibilité : 90%

**Forte**

**Recos EUCALB**

**Moyenne**

| **Séro ELISA** | **Test +** | **Test -** | **Total** |
|---|---|---|---|
| **Lyme confirmé** | **90**<br>Vrais positifs | **10**<br>Faux négatifs | **100** |
| **Sujet sain** | **990**<br>Faux positifs | **8 910**<br>Vrais négatifs | **9 900** |
| **Total** | **1 080** | **8 920** | **10 000** |

# Performances intrinsèques/extrinsèques des tests

SYNDICAT DES BIOLOGISTES
Le syndicat de tous les biologistes médicaux

| Prévalence : 1% | Spécificité : 90% | Sensibilité : 98% |
|---|---|---|
| **Forte** | **Recos EUCALB** | **Excellente** |

| **Séro ELISA** | **Test +** | **Test -** | **Total** |
|---|---|---|---|
| **Lyme confirmé** | **98** Vrais positifs | **2** Faux négatifs | **100** |
| **Sujet sain** | **990** Faux positifs | **8 910** Vrais négatifs | **9 900** |
| **Total** | **1 088** | **8 912** | **10 000** |

# Performances intrinsèques/extrinsèques des tests

SYNDICAT DES BIOLOGISTES
Le syndicat de tous les biologistes médicaux

| Prévalence : 1% | Spécificité : 95% | Sensibilité : 98% |
|---|---|---|
| **Forte** | **> Recos EUCALB** | **Excellente** |

| **Séro ELISA** | **Test +** | **Test -** | **Total** |
|---|---|---|---|
| **Lyme confirmé** | **98**<br>Vrais positifs | **2**<br>Faux négatifs | **100** |
| **Sujet sain** | **495**<br>Faux positifs | **9 405**<br>Vrais négatifs | **9 900** |
| **Total** | **593** | **9 407** | **10 000** |

# Performances intrinsèques/extrinsèques des tests

SYNDICAT DES BIOLOGISTES
Le syndicat de tous les biologistes médicaux

Prévalence : 1%

Spécificité : 95%

Sensibilité : 98%

**Forte**

**> Recos EUCALB**

**Excellente**

| **Séro ELISA** | **Test +** | **Test -** | **Total** |
|---|---|---|---|
| **Lyme confirmé** | **98** Vrais positifs | **2** Faux négatifs | **100** |
| **Sujet sain** | **495** Faux positifs | **9 405** Vrais négatifs | **9 900** |
| | **593** | **9 407** | **10 000** |

pop à forte prévalence (1%)

**VPN = ???**

**VPP = ???**

# Performances intrinsèques/extrinsèques des tests

SYNDICAT DES BIOLOGISTES
Le syndicat de tous les biologistes médicaux

Prévalence : 1%

Spécificité : 95%

Sensibilité : 98%

**Forte**

**> Recos EUCALB**

**Excellente**

| **Séro ELISA** | **Test +** | **Test -** | **Total** |
|---|---|---|---|
| **Lyme confirmé** | **98** Vrais positifs | **2** Faux négatifs | **100** |
| **Sujet sain** | **495** Faux positifs | **9 405** Vrais négatifs | **9 900** |
| | **593** | **9 407** | **10 000** |

pop à forte prévalence (1%)

**VPN = 99,98 %**

**VPP = ???**

# Performances intrinsèques/extrinsèques des tests

Prévalence : 1%

Spécificité : 95%

Sensibilité : 98%

**Forte**

**> Recos EUCALB**

**Excellente**

| Séro ELISA | Test + | Test - | Total |
|---|---|---|---|
| **Lyme confirmé** | **98** Vrais positifs | **2** Faux négatifs | **100** |
| **Sujet sain** | **495** Faux positifs | **9 405** Vrais négatifs | **9 900** |
| | **593** | **9 407** | **10 000** |

pop à forte prévalence (1%)

**VPN = 99,98 %**

**VPP = 16,5 %**

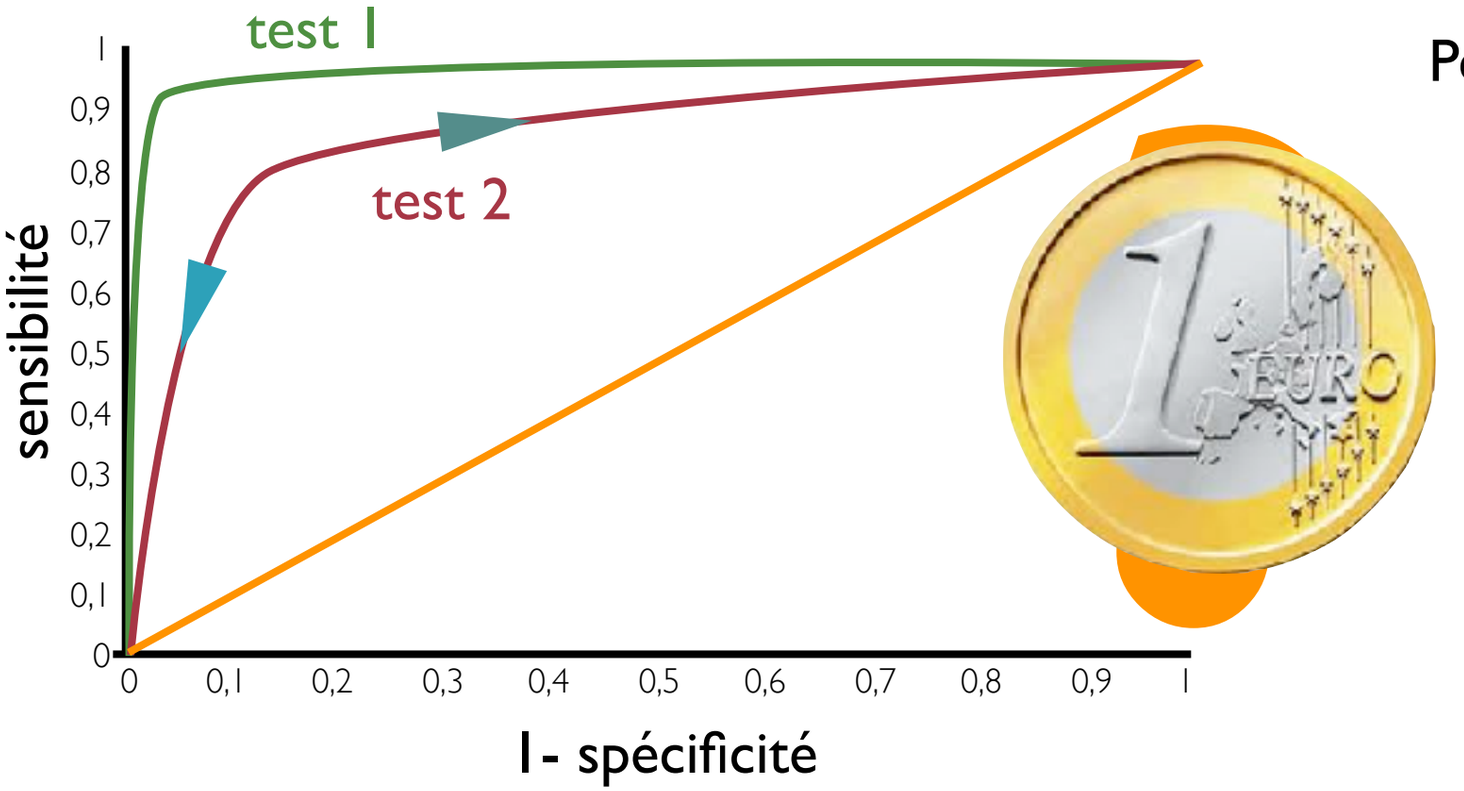
# Bon usage des trousses commerciales

SYNDICAT DES BIOLOGISTES
Le syndicat de tous les biologistes médicaux

Prévalence : 1%

Spécificité : 95%

Sensibilité : 98%







Performances intrinsèques du test

se/spé test 1 > se/spé test 2

Détermination seuil positivité

seuil bas : ↗ se ↘ spé

seuil haut : ↘ se ↗ spé

pop à forte prévalence (1%)

**VPN = 99,98 %**

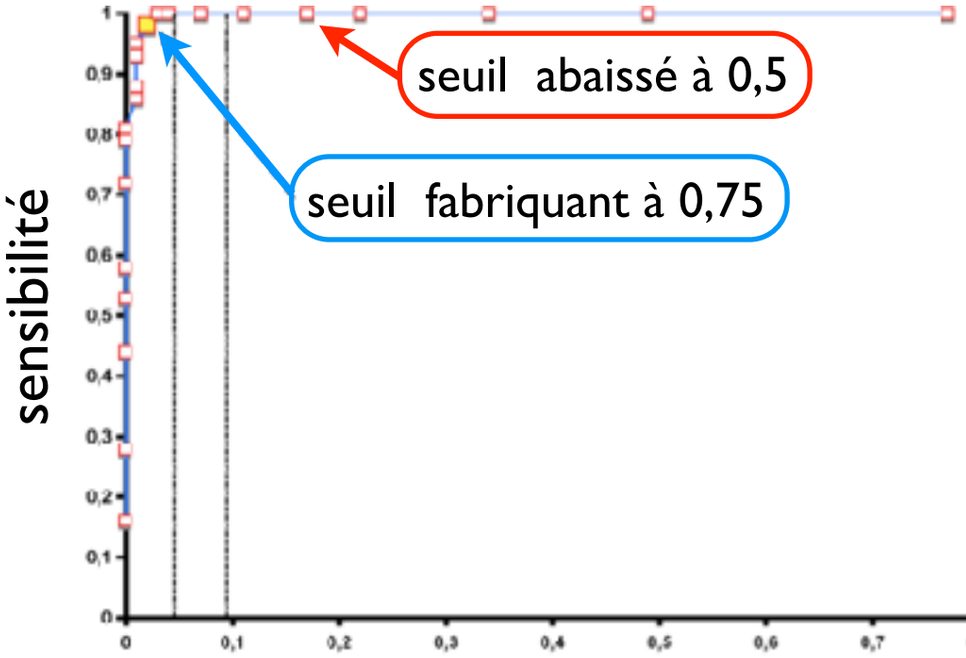
**VPP = 16,5 %**

# Bon usage des trousses commerciales

Prévalence : 1%

Spécificité : 95%

Sensibilité : 98%




37 sérums de sujets avec neuroborréliose confirmée

100 sérums de sujets indemnes de borréliose de Lyme

seuil fabriquant : se = 98% spé = 95%

pop à forte prévalence (1%)

**VPN = 99,98 %**

**VPP = 16,5 %**

pop à prévalence moy (0,1%)

**VPN = 99,99 %**

**VPP = 1,92 %**

seuil ↘ : se = 100% spé = 80%

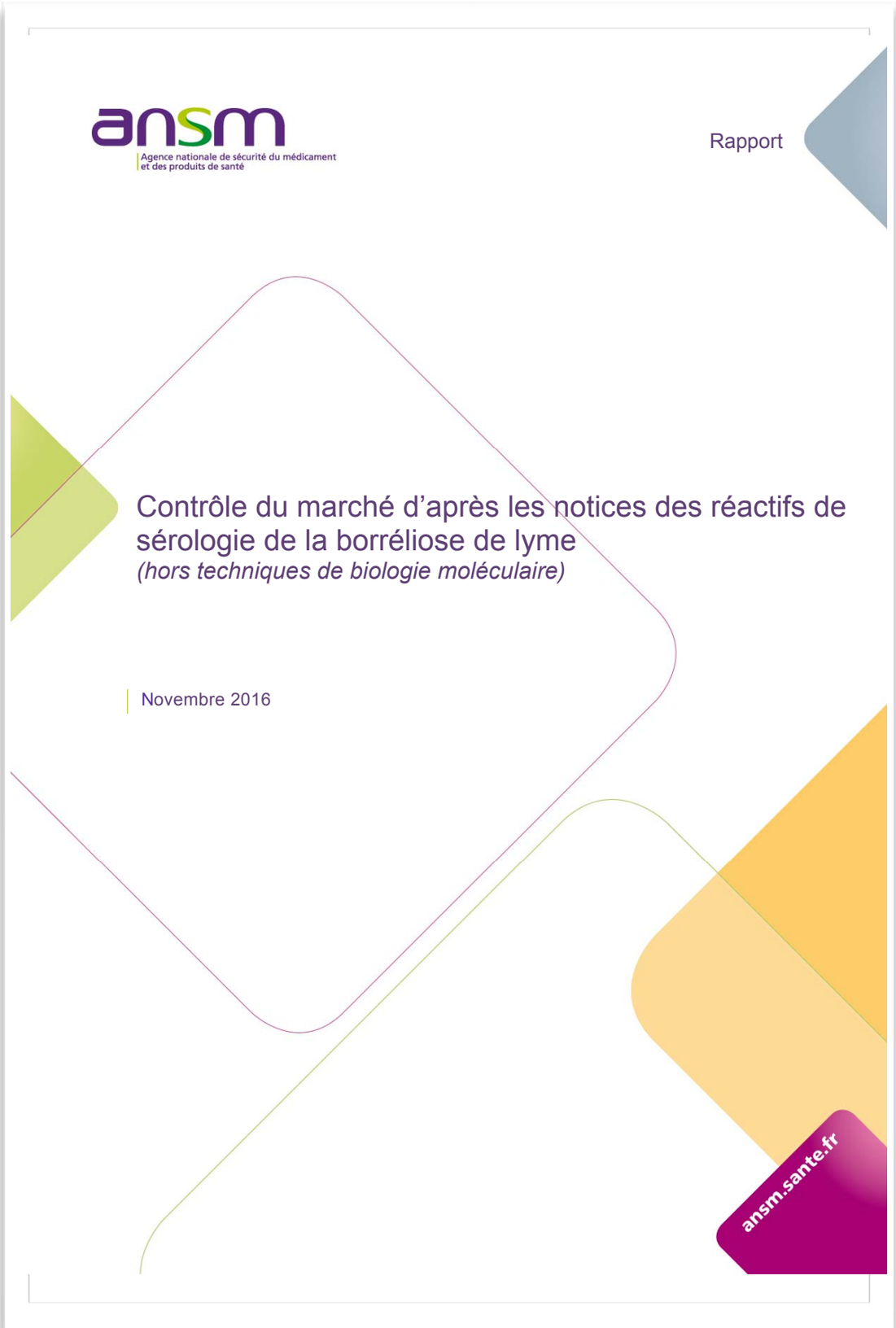
pop à faible prévalence (0,1%)

**VPN = 100 %**

**VPP = 0,5 %**

# Pour vous aider dans le choix des trousses

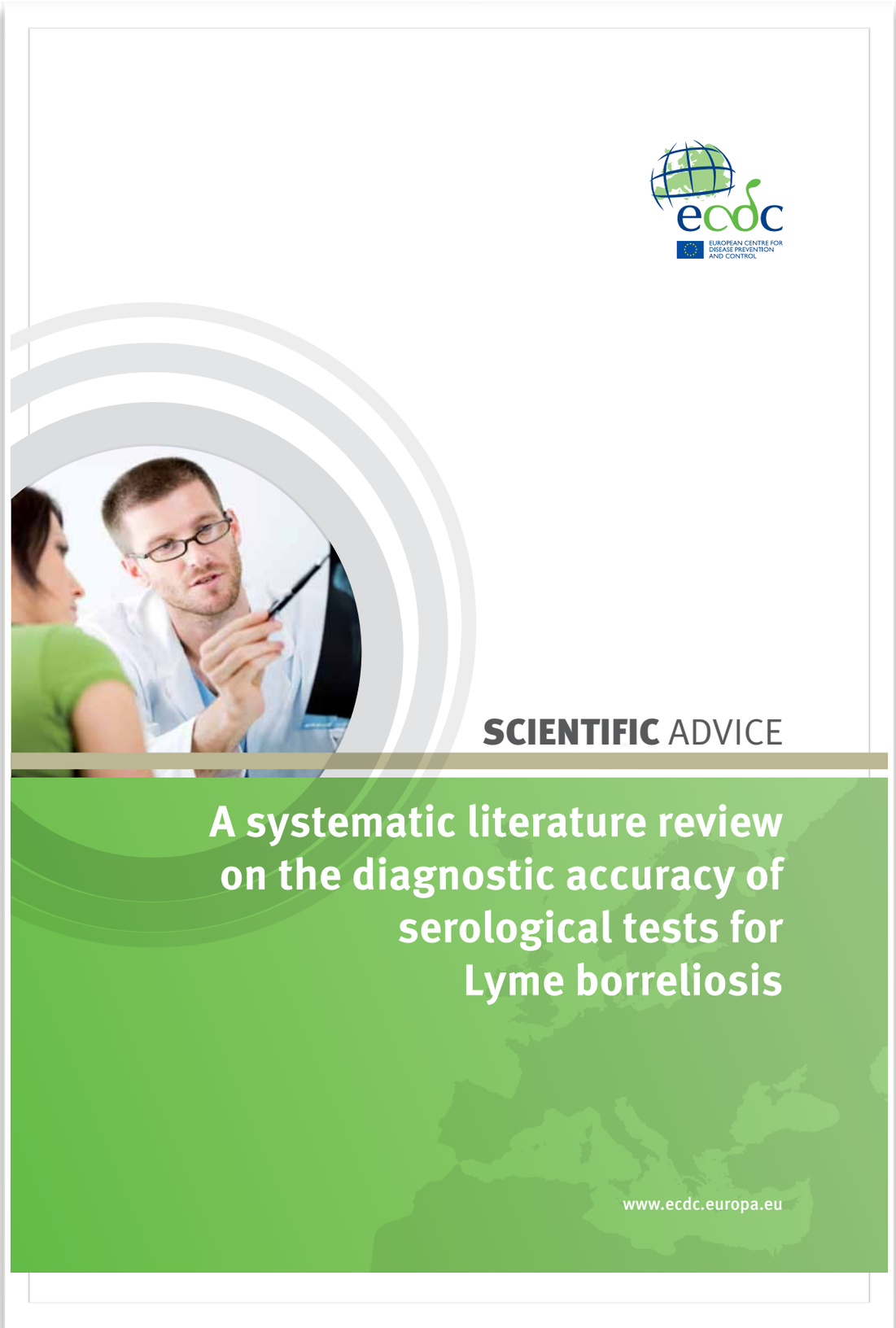
SYNDICAT DES BIOLOGISTES
Le syndicat de tous les biologistes médicaux




Analyses des notices

# Pour vous aider dans le choix des trousses

SYNDICAT DES BIOLOGISTES
Le syndicat de tous les biologistes médicaux




Analyses littérature

# Pour vous aider dans le choix des trousses

Analyses littérature

**Figure 9. ROC scatter plot (A) and fitted summary ROC curves (B) for NB case-control studies with healthy controls**

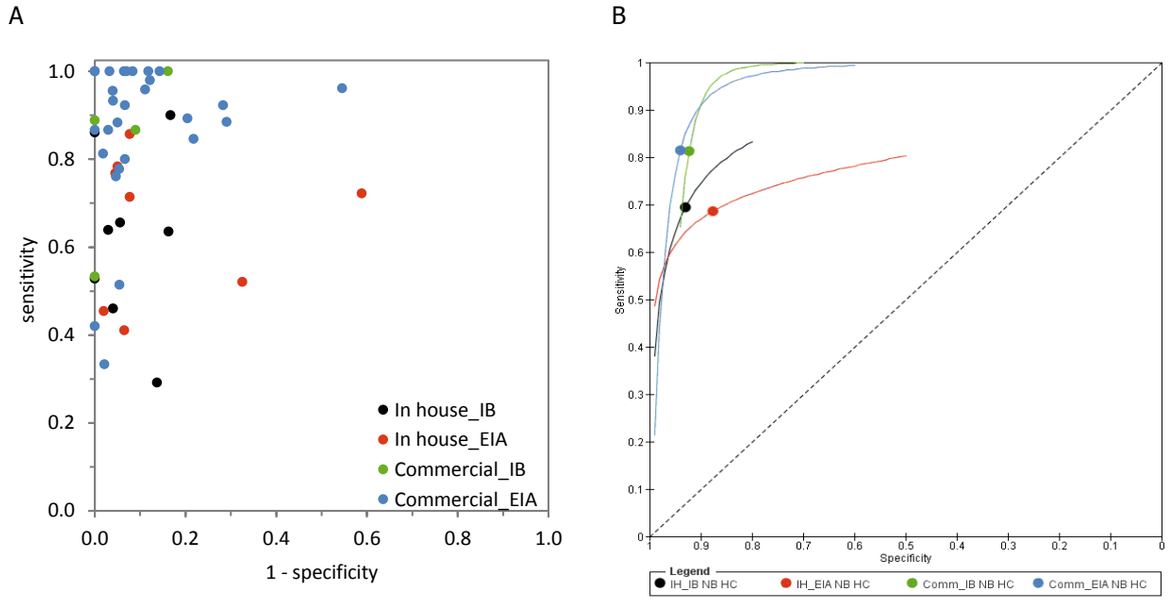
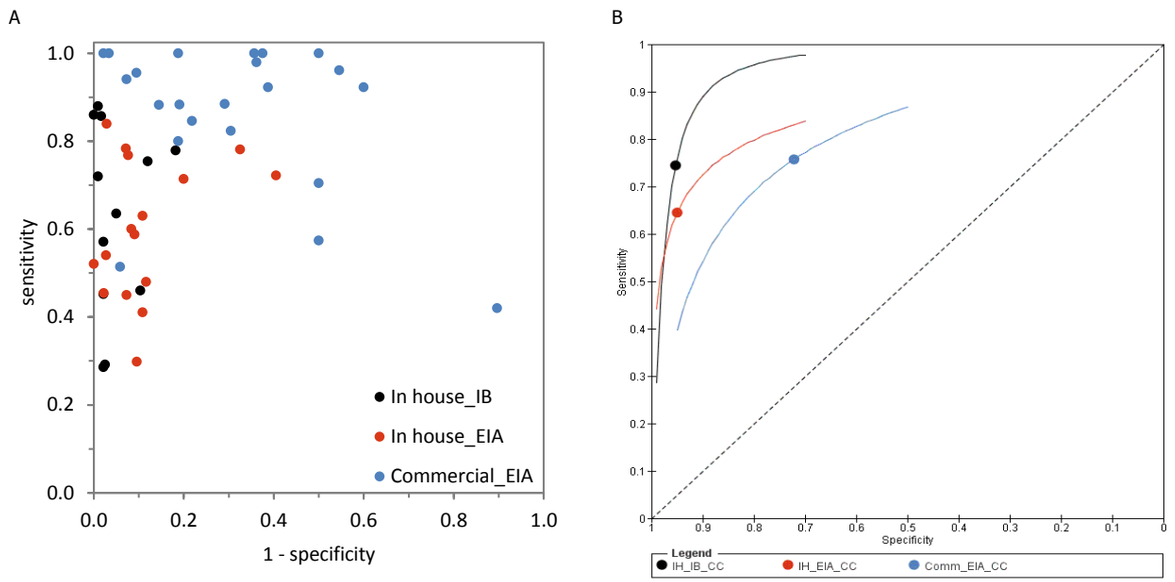
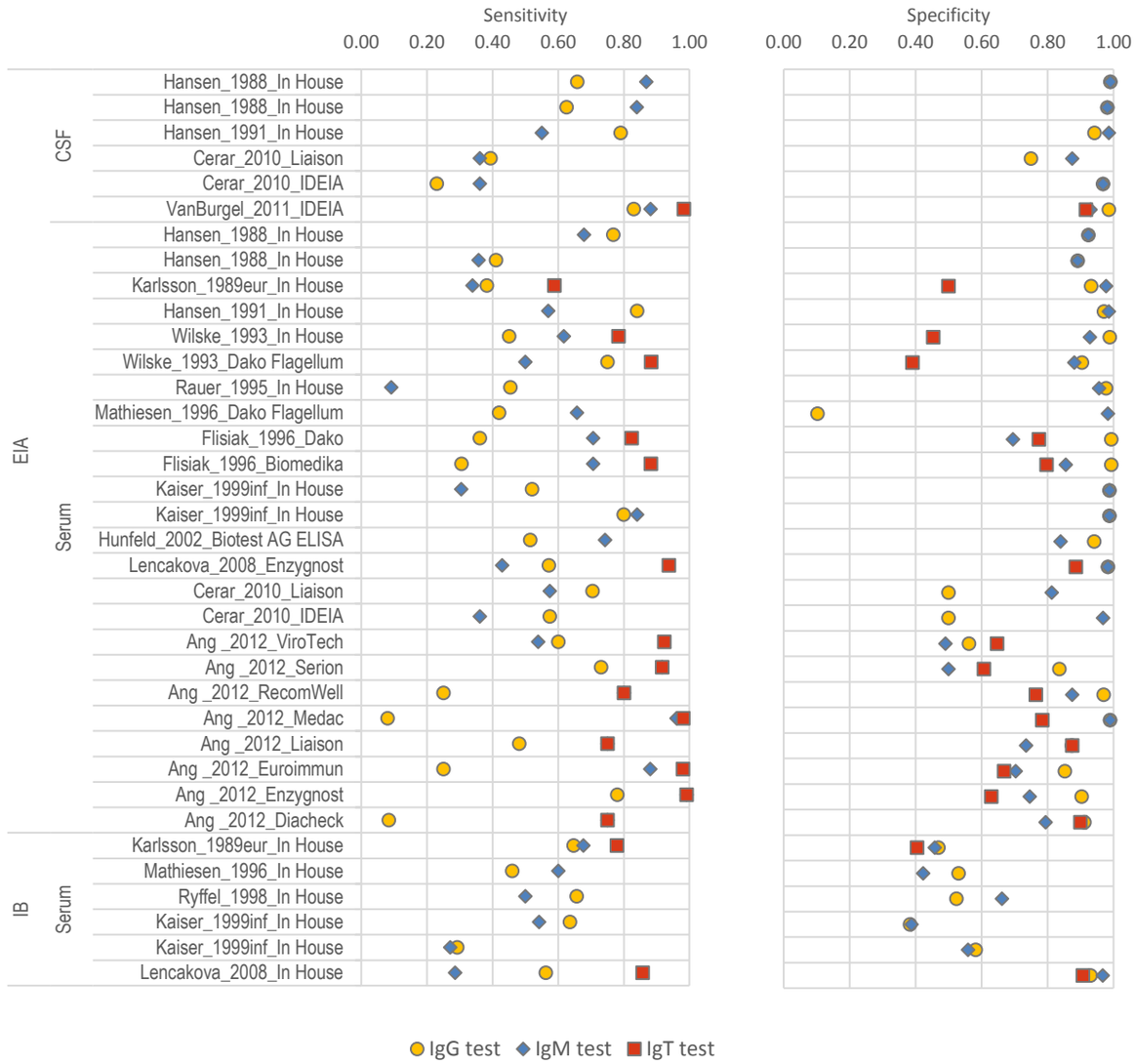



**Figure 13. ROC scatter plot (A) and fitted summary ROC curves (B) for NB case-control studies with cross-reacting controls**




## Annex 8. Neuroborreliosis: case-control studies with cross-reacting controls

Sensitivity and specificity of IgM, IgG and IgT tests for NB case-control studies with cross-reacting controls. Of the 36 tests, 30 are EIA and 6 are IB. Studies are sorted according to year of publication.



# Pour vous aider dans le choix des trousses

SYNDICAT DES BIOLOGISTES
Le syndicat de tous les biologistes médicaux

Etude comparative

Centre National Référence Borrelia

Les Hôpitaux Universitaires de STRASBOURG



## Evaluation des trousses de sérologie *Borrelia* par immuno-empreinte

**Drs S. De Martino, P. Zachary, Pr. Benoît Jaulhac**

CNR des *Borrelia,* CHU de Strasbourg

http://www.chru-strasbourg.fr/Les-centres-de-reference/Borrelia

# TESTS “ALTERNATIFS” NON VALIDÉS

SYNDICAT DES BIOLOGISTES
Le syndicat de tous les biologistes médicaux

# TESTS DE TRANSFORMATION LYMPHOCYTAIRES

# Test de transformation lymphocytaire (LTT)

Send Orders of Reprints at reprints@benthamscience.org

104 The Open Neurology Journal, 2012, 6, (Suppl 1-M5) 104-112

Open Access

**The Lymphocyte Transformation Test for Borrelia Detects Active Lyme Borreliosis and Verifies Effective Antibiotic Treatment**

Volker von Baehr[1], Cornelia Doebis[1], Hans-Dieter Volk[2], Rüdiger von Baehr[1,*]

[1]Institute for Medical Diagnostics, Immunology Department, Nicolaistrasse 22, 12247 Berlin

[2]Institute for Medical Immunology, Charité University Medicine Berlin, Campus Mitte, Charitéplatz 1, 10117 Berlin

**Abstract:** Borrelia-specific antibodies are not detectable until several weeks after infection and even if they are present, they are no proof of an active infection. Since the sensitivity of culture and PCR for the diagnosis or exclusion of borreliosis is too low, a method is required that detects an active Borrelia infection as early as possible. For this purpose, a lymphocyte transformation test (LTT) using lysate antigens of Borrelia burgdorferi sensu stricto, Borrelia afzelii and Borrelia garinii and recombinant OspC was developed and validated through investigations of seronegative and seropositive healthy individuals as well as of seropositive patients with clinically manifested borreliosis. The sensitivity of the LTT in clinical borreliosis before antibiotic treatment was determined as 89,4% while the specificity was 98,7%. In 1480 patients with clinically suspected borreliosis, results from serology and LTT were comparable in 79,6% of cases. 18% were serologically positive and LTT-negative. These were mainly patients with borreliosis after antibiotic therapy. 2,2% showed a negative serology and a positive LTT result. Half of them had an early erythema migrans. Following antibiotic treatment, the LTT became negative or borderline in patients with early manifestations of borreliosis, whereas in patients with late symptoms, it showed a regression while still remaining positive. Therefore, we propose the follow-up monitoring of disseminated Borrelia infections as the main indication for the Borrelia-LTT.

**Keywords:** Borrelia serology, borreliosis, diagnostics, immune response, lymphocyte transformation test, T cells.

## INTRODUCTION

Lyme borreliosis is the most common disease transmitted by tick bite. Lyme borreliosis first manifests locally on the skin at the site of the tick bite and then systemically, possibly affecting one or more organs such as the skin, joints, muscles, sense organs, nervous system and heart. In the latter case, early (stage I and II) and late (stage III) manifestations can be distinguished (1). Lyme borreliosis should be diagnosed by history and clinical symptoms. If the clinical symptoms are clear, laboratory diagnostics are of secondary importance only. The difficulty is, however, that the tick bite often goes unnoticed and the erythema migrans does not necessarily occur or is not noticed. In these cases, the requirement for early antibiotic treatment of borreliosis to prevent the complications of systemic dissemination of the pathogen, particularly of late borreliosis, cannot be met.

The symptoms associated with the systemic phase of Lyme borreliosis can be highly varied and ambiguous. In these cases, the detection of Borrelia-specific antibodies (serological laboratory diagnosis) becomes important for the diagnosis and treatment decision. The necessarily high quality demands cannot yet be completely fulfilled by Borrelia serology due to the following reasons: 1) Borrelia-specific IgM antibodies, and IgG antibodies in particular, cannot be detected until several weeks after infection [1, 2].Seronegative cases with late stage Lyme borreliosis have also been recently described [3]. But these are becoming more rare with the increasing quality of the assays following the introduction of recombinant Borrelia antigens. 2) The heterogeneity of Borrelia species and strains within a species requires a polymorphism of the Borrelia-specific protein antigens [4, 5]. This is a difficult problem for the sensitivity of Borrelia serology. 3) IgM antibodies against Borrelia OspC may be of the nonspecific type [4, 5]. 4) A positive serological finding alone is not proof of a current active Borrelia infection [1, 4, 5]. 5) Borrelia serology is not suitable for the monitoring of therapy and evaluation of progress as IgG and IgM antibodies may persist for years after borreliosis has been cured [6].

The direct detection of Borrelia by culture or PCR has a high diagnostic value in the case of a positive result, but a negative result does not rule out Lyme borreliosis [4, 5].

There is currently no method available which, in addition to the serology, answers the question as to whether a specific case is a status post Borrelia infection or active borreliosis.

Each humoral immune response to an infection requires a specific cellular immune response with clonal proliferation of various antigen-specific lymphocyte subpopulations. Of central importance here are antigen-specific T helper lymphocytes ($CD4^+$ $T_H$ cells). In addition to effector T cells, long-lived T and B memory lymphocytes are formed. In the presence of antigen-presenting cells and protein antigens,

*Address correspondence to this author at the Institut für Medizinische Diagnostik Berlin, Nicolaistraße 22, 12247 Berlin, Germany;
Tel: +49-30-77001220; Fax: +49-30-77001230;
E-mail: v.baehr@imd-berlin.de

1876-2035/12 2012 Bentham Open

**Distinction infection active vs cicatrice ?**

**Vérification de l'efficacité du TT ?**

**Se : 89,4 %**
**Spé : 98,7 %**

# Test de transformation lymphocytaire (LTT)

The Lymphocyte Transformation Test for Borrelia Detects Active Lyme Borreliosis and Verifies Effective Antibiotic Treatment

Volker von Baehr[1], Cornelia Doebis[1], Hans-Dieter Volk[2], Rüdiger von Baehr[1,*]

[1]Institute for Medical Diagnostics, Immunology Department, Nicolaistrasse 22, 12247 Berlin

[2]Institute for Medical Immunology, Charité University Medicine Berlin, Campus Mitte, Charitéplatz 1, 10117 Berlin

Abstract: Borrelia-specific antibodies are not detectable until several weeks after infection and even if they are present, they are no proof of an active infection. Since the sensitivity of culture and PCR for the diagnosis or exclusion of borreliosis is too low, a method is required that detects an active Borrelia infection as early as possible. For this purpose, a lymphocyte transformation test (LTT) using lysate antigens of Borrelia burgdorferi sensu stricto, Borrelia afzelii and Borrelia garinii and recombinant OspC was developed and validated through investigations of seronegative and seropositive healthy individuals as well as of seropositive patients with clinically manifested borreliosis. The sensitivity of the LTT in clinical borrelioses before antibiotic treatment was determined as 89,4% while the specificity was 98,7%. In 1480 patients with clinically suspected borreliosis, results from serology and LTT were comparable in 76,4% of cases. 18% were serologically positive and LTT-negative. These were mainly patients with borrelioses after antibiotic therapy. 2,2% showed a negative serology and a positive LTT result. Half of them had an early erythema migrans. Following antibiotic treatment, the LTT became negative or borderline in patients with early manifestations of borreliosis, whereas in patients with late symptoms, it showed a regression while still remaining positive. Therefore, we propose the follow-up monitoring of disseminated Borrelia infections as the main indication for the Borrelia-LTT.

Keywords: Borrelia serology, borreliosis, diagnostics, immune response, lymphocyte transformation test, T cells.

INTRODUCTION

Lyme borreliosis is the most common disease transmitted by tick bite. Lyme borreliosis first manifests locally on the skin at the site of the tick bite and then systemically, possibly affecting one or more organs such as the skin, joints, muscles, sense organs, nervous system and heart. In the latter case, early (stage I and II) and late (stage III) manifestations can be distinguished (1). Lyme borreliosis should be diagnosed by history and clinical symptoms. If the clinical symptoms are clear, laboratory diagnostics are of secondary importance only. The difficulty is, however, that the tick bite often goes unnoticed and the erythema migrans does not necessarily occur or is not noticed. In these cases, the requirement for early antibiotic treatment of borreliosis to prevent the complications of systemic dissemination of the pathogen, particularly of late borreliosis, cannot be met.

The symptoms associated with the systemic phase of Lyme borreliosis can be highly varied and ambiguous. In these cases, the detection of Borrelia-specific antibodies (serological laboratory diagnosis) becomes important for the diagnosis and treatment decision. The necessarily high quality demands cannot yet be completely fulfilled by Borrelia serology due to the following reasons: 1) Borrelia-specific IgM antibodies, and IgG antibodies in particular, cannot be detected until several weeks after infection [1, 2].Seronegative cases with late stage Lyme borreliosis have also been recently described [3]. But these are becoming more rare with the increasing quality of the assays following the introduction of recombinant Borrelia antigens. 2) The heterogeneity of Borrelia species and strains within a species requires a polymorphism of the Borrelia-specific protein antigens [4, 5]. This is a difficult problem for the sensitivity of Borrelia serology. 3) IgM antibodies against Borrelia OspC may be of the nonspecific type [4, 5]. 4) A positive serological finding alone is not proof of a current active Borrelia infection [1, 4, 5]. 5) Borrelia serology is not suitable for the monitoring of therapy and evaluation of progress as IgG and IgM antibodies may persist for years after borreliosis has been cured [6].

The direct detection of Borrelia by culture or PCR has a high diagnostic value in the case of a positive result, but a negative result does not rule out Lyme borreliosis [4, 5].

There is currently no method available which, in addition to the serology, answers the question as to whether a specific case is a status post Borrelia infection or active borreliosis.

Each humoral immune response to an infection requires a specific cellular immune response with clonal proliferation of various antigen-specific lymphocyte subpopulations. Of central importance here are antigen-specific T helper lymphocytes (CD4+ Th cells). In addition to effector T cells, long-lived T and B memory lymphocytes are formed. In the presence of antigen-presenting cells and protein antigens,

*Address correspondence to this author at the Institut für Medizinische Diagnostik Berlin, Nicolaistraße 22, 12247 Berlin, Germany;
Tel: +49-30-77001220; Fax: +49-30-77001226;
E-mail: v.baehr@imd-berlin.de

1874-2815/12 2012 Bentham Open

**Se : 89,4 %**
**Spé : 98,7 %**

## Design de l'étude

- 94 Lyme+ : critères pour affirmer le diagnostic étiologique non précisés
- 160 contrôles : présélectionnés séro neg = biais sélection (corr LTT/séro)
- 1480 "clinical diagnosis of suspected Lyme borreliosis" : pas définition clinique

## Capacité du TTL à détecter une infection active ?

- design de l'étude : pas preuve infection active (clinique ? PCR ? Culture ?)

## Capacité du TTL à vérifier l'efficacité du TT ?

- design de l'étude : pas de suivi prospectif avec un groupe contrôle

**Ni le design de l'étude, ni les données présentées ne justifient le contenu du titre ou des CC de l'article**

## Autres remarques

- pas d'*ethic statement* et pas de conflits d'intérêt déclarés …
- mais lien avec un labo commercialisant et recommandant les tests ELISPOT
- http://www.imd-berlin.de/en/special-areas-of-competence/lymphocyte-transformation-test-ltt.html

# Test de transformation lymphocytaire (LTT)



The Open Neurology Journal, 2012, 6, (Suppl 1-M5) 104-112

Open Access

## The Lymphocyte Transformation Test for Borrelia Detects Active Lyme Borreliosis and Verifies Effective Antibiotic Treatment

Volker von Baehr[1], Cornelia Doebis[1], Hans-Dieter Volk[2], Rüdiger von Baehr[1,*]

[1]Institute for Medical Diagnostics, Immunology Department, Nicolaistrasse 22, 12247 Berlin

[2]Institute for Medical Immunology, Charité University Medicine Berlin, Campus Mitte, Charitéplatz 1, 10117 Berlin

**Abstract:** Borrelia-specific antibodies are not detectable until several weeks after infection and even if they are present, they are no proof of an active infection. Since the sensitivity of culture and PCR for the diagnosis or exclusion of borreliosis is too low, a method is required that detects an active Borrelia infection as early as possible. For this purpose, a lymphocyte transformation test (LTT) using lysate antigens of Borrelia burgdorferi sensu stricto, Borrelia afzelii and Borrelia garinii and recombinant OspC was developed and validated through investigations of seronegative and seropositive healthy individuals as well as of seropositive patients with clinically manifested borreliosis. The sensitivity of the LTT in clinical borreliosis before antibiotic treatment was determined as 89,4% while the specificity was 98,7%. In 1480 patients with clinically suspected borreliosis, results from serology and LTT were comparable in 79,4% of cases. 18% were serologically positive and LTT-negative. These were mainly patients with borreliosis after antibiotic therapy. 2,2% showed a negative serology and a positive LTT result. Half of them had an early erythema migrans. Following antibiotic treatment, the LTT became negative or borderline in patients with early manifestations of borreliosis, whereas in patients with late symptoms, it showed a regression while still remaining positive. Therefore, we propose the follow-up monitoring of disseminated borrelia infections as the main indication for the Borrelia-LTT.

**Keywords:** Borrelia serology, borreliosis, diagnostics, immune response, lymphocyte transformation test, T cells.

### INTRODUCTION

Lyme borreliosis is the most common disease transmitted by tick bite. Lyme borreliosis first manifests locally on the skin at the site of the tick bite and then systemically, possibly affecting one or more organs such as the skin, joints, muscles, sense organs, nervous system and heart. In the latter case, early (stage I and II) and late (stage III) manifestations can be distinguished (1). Lyme borreliosis should be diagnosed by history and clinical symptoms. If the clinical symptoms are clear, laboratory diagnostics are of secondary importance only. The difficulty is, however, that the tick bite often goes unnoticed and the erythema migrans does not necessarily occur or is not noticed. In these cases, the requirement for early antibiotic treatment of borreliosis to prevent the complications of systemic dissemination of the pathogen, particularly of late borreliosis, cannot be met.

The symptoms associated with the systemic phase of Lyme borreliosis can be highly varied and ambiguous. In these cases, the detection of Borrelia-specific antibodies (serological laboratory diagnosis) becomes important for the diagnosis and treatment decision. The necessarily high quality demands cannot yet be completely fulfilled by Borrelia serology due to the following reasons: 1) Borrelia-specific IgM antibodies, and IgG antibodies in particular, cannot be detected until several weeks after infection [1, 2].Seronegative cases with late stage Lyme borreliosis have also been recently described [3]. But these are becoming more rare with the increasing quality of the assays following the introduction of recombinant Borrelia antigens. 2) The heterogeneity of Borrelia species and strains within a species requires a polymorphism of the Borrelia-specific protein antigens [4, 5]. This is a difficult problem for the sensitivity of Borrelia serology. 3) IgM antibodies against Borrelia OspC may be of the nonspecific type [4, 5]. 4) A positive serological finding alone is not proof of a current active Borrelia infection [1, 4, 5]. 5) Borrelia serology is not suitable for the monitoring of therapy and evaluation of progress as IgG and IgM antibodies may persist for years after borreliosis has been cured [6].

The direct detection of Borrelia by culture or PCR has a high diagnostic value in the case of a positive result, but a negative result does not rule out Lyme borreliosis [4, 5].

There is currently no method available which, in addition to the serology, answers the question as to whether a specific case is a status post Borrelia infection or active borreliosis.

Each humoral immune response to an infection requires a specific cellular immune response with clonal proliferation of various antigen-specific lymphocyte subpopulations. Of central importance here are antigen-specific T helper lymphocytes ($CD4^+$ $T_H$ cells). In addition to effector T cells, long-lived T and B memory lymphocytes are formed. In the presence of antigen-presenting cells and protein antigens,

*Address correspondence to this author at the Institut für Medizinische Diagnostik Berlin, Nicolaistraße 22, 12247 Berlin, Germany;
Tel: +49-30-77001220; Fax: +49-30-77001226;
E-mail: v.baehr@imd-berlin.de

1876-2859/12 2012 Bentham Open

LETTER PARASITOLOGY

## The lymphocyte transformation test for the diagnosis of Lyme borreliosis has currently not been shown to be clinically useful

**R. B. Dessau[1], V. Fingerle[2], J. Gray[3], K.-P. Hunfeld[4], B. Jaulhac[5], O. Kahl[6], W. Kristoferitsch[7], G. Stanek[8] and F. Strle[9]**

1) Department of Clinical Microbiology, Slagelse Hospital, Slagelse, Denmark; 2) German Health and Food Safety Authority, Munich, Germany; 3) UCD School of Biology and Environmental Science, University College Dublin, Dublin, Ireland; 4) Institute for Laboratory Medicine, Microbiology & Infectious Control, Northwest Medical Centre, Academic Teaching Hospital, School of Medicine, The Johann Wolfgang Goethe-University, Frankfurt, Germany; 5) National Reference Centre for Borrelia and Groups d'Etude de la Borréliose de Lyme, Laboratory of Bacteriology, Hôpitaux Universitaires de Strasbourg et Université de Strasbourg, Strasbourg, France; 6) Labor Kahl GmbH, Berlin, Germany; 7) Karl Landsteiner Institute for Neuroimmunological and Neurodegenerative Diseases, SMZ-Ost Donaustadt, 8) Institute for Hygiene and Applied Immunology, Medical University of Vienna, Vienna, Austria and 9) Department of Infectious Diseases, University Medical Centre Ljubljana, Ljubljana, Slovenia

This letter is a comment on a study using the lymphocyte transformation test (LTT) for the diagnosis of active Lyme borreliosis caused by Borrelia burgdorferi sensu lato [1]. This LTT study reports the findings derived from a validation panel containing 120 blood donors seronegative for Borrelia, 40 seronegative patients with autoimmune diseases, 40 healthy seropositive controls, and 94 seropositive patients with clinical signs of Lyme borreliosis. Furthermore, 1480 samples were investigated with both serology (*Borrelia* IgG and IgM ELISA, and western blot; Mikrogen, Munich, Germany) and the LTT.

The study has several major shortcomings. Concerning inclusion criteria, it was not clearly specified how the 94 patients with clinical Lyme borreliosis were defined. For example, it was not specified whether the six patients with Bannwarth's syndrome had spinal pleocytosis and a positive antibody index, as required by the European case definitions for Lyme borreliosis, and it remains unclear how it was determined that the 94 patients with erythema migrans were suffering from Lyme borreliosis [2]. The 160 controls for the LTT were preselected as being seronegative for Borrelia-specific antibodies, and this could introduce a selection bias, because serology and LTT results tend to correlate. The specificity of the LTT could therefore be overestimated. Concerning the selection criteria for the large group of 1480 patients, it is not clear what is meant by 'clinical diagnosis of suspected Lyme borreliosis', among what appears to be a mixture of protean disorders. The clinical spectrum of these patients was not described. Concerning the methods, it is confusing to the reader that a cut-off for a positive stimulation index may be both >5 and >3. In the results section, the selection of subsets in the tables numbered 2–5 was not explained and the numbers do not add up. For example, 392 of the 1480 patients were reported to be LTT-positive; however, only 340 reappear in Table 3, without an explanation of how this subset was selected. A flow diagram would have been helpful. Forty per cent of the 1480 patients suspected of having Lyme borreliosis were LTT-positive, and 60% were serology-positive. This is a high percentage of positive results as compared with a series of consecutive patients suspected of having Lyme borreliosis in Denmark, where 9.2% were found to be IgM-positive and 3.3% IgG-positive. This indicates either selection bias or specificity problems in the LTT and/or the serology assay.

The main point of the article as taken from the title is the delay of the LTT to detect active infection and the effect of antibiotic treatment. However, owing to the study design, evidence of active infection is lacking. Clinical features indicating follow-up and/or detection of the organism by culture or PCR are absent. Also, the conclusion that the Borrelia LTT may be used for follow-up monitoring of disseminated B. burgdorferi sensu lato infections and provide indications for antibiotic treatment is not supported by the study design, as this would require a prospective trial with a control group. Thus, the LTT paper contains methodological shortcomings with a risk of selection bias, and the study design and the data do not support the contents of the title or the

**Corresponding author:** R. Dessau, Department of Clinical Microbiology, Slagelse Hospital, Ingerslev Boulevard 18, 4200 Slagelse, Denmark;
**E-mail: raoul.dessau@regionsjaelland.dk**

The authors of this publication are members of the ESCMID study group for Lyme borreliosis: www.escmid.org/ESGBOR



SYNDICAT DES BIOLOGISTES
Le syndicat de tous les biologistes médicaux

*Cells* **2012**, *1*, 153-167; doi:10.3390/cells1020153

OPEN ACCESS

*cells*

ISSN 2073-4409
www.mdpi.com/journal/cells

*Article*

## Can ELISPOT Be Applied to A Clinical Setting as A Diagnostic Utility for Neuroborreliosis?

**Marika Nordberg [1,2,*], Pia Forsberg [1,3], Dag Nyman [2], Barbro H. Skogman [4], Clara Nyberg [2], Jan Ernerudh [5,6], Ingvar Eliasson [7] and Christina Ekerfelt [5]**

**Abstract:** The aim of this prospective study was to investigate the diagnostic performance of *Borrelia* (Bb)-induced interferon (IFN)-γ secretion assessed by ELISPOT modified to its feasibility for clinical laboratories as a supplementary tool to the laboratory diagnosis of Lyme neuroborreliosis (LNB) in an endemic setting. Between 2002 and 2004, patients with symptoms of suspected clinical LNB were included in a study conducted on the Åland islands in the Finnish archipelago, which is a hyperendemic area for Lyme borreliosis (LB). Fourteen patients with confirmed LNB and 103 patients with non-LNB were included, and the numbers of spontaneous and *Bb*-activated IFN-γ-secreting cells were assessed by the ELISPOT assay. The ELISPOT assay showed a weak diagnostic performance

*Received: 1 April 2012; in revised form: 7 May 2012 / Accepted: 8 May 2012 / Published: 8 June 2012*

**Se : 89,4 %**
**Spé : 98,7 %**

**Erreurs méthodologiques majeures**

**Neuroborrélioses**

**Se : 36 %**
**Spé : 82 %**

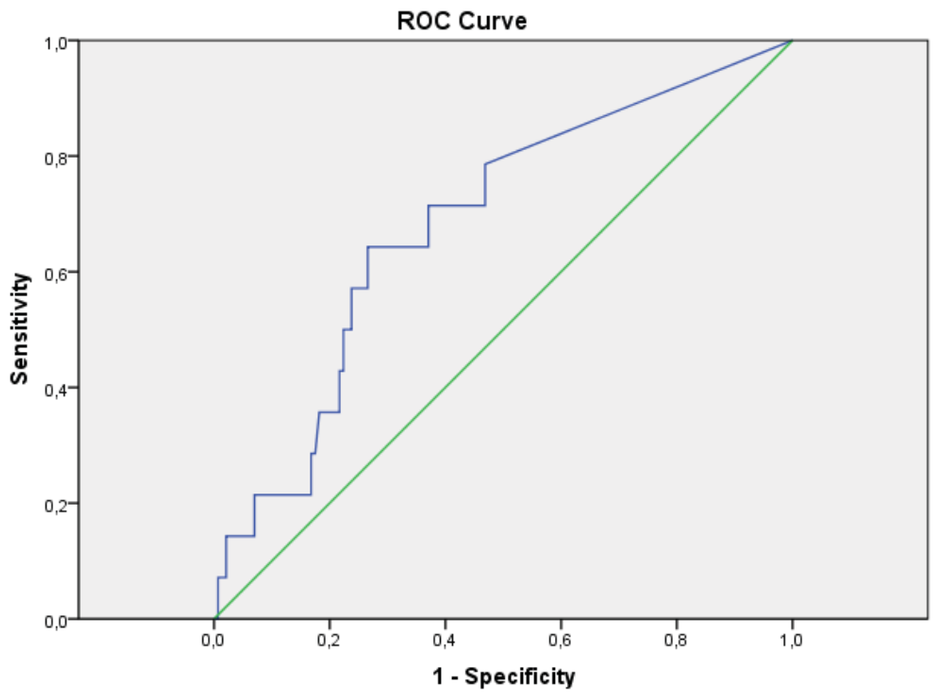
**étude contrôlée cas/T**

# Test de transformation lymphocytaire (LTT)

## Design de l'étude

- 14 neuroborrélioses : clinique + LCR ≥5 MNC/µL + SIT IgG
- 103 contrôles : symptomatiques non-Lyme (neuro, art, …séro sg et SIT neg)

| Diagnostic groups | ELISPOT cut-off ≥5 spots | | ELISPOT cut-off ≥10 spots | |
|---|---|---|---|---|
| | Positive | Negative | Positive | Negative |
| Lyme neuroborreliosis (n = 14) | 5 (36%) | 9 (64%) | 3 (21%) | 11 (79%) |
| Non-Lyme borreliosis (n = 103) | 19 (18%) | 84 (82%) | 8 (8%) | 95 (92%) |




**Performances très faibles**
**Spécificité médiocre (nbx faux + si test sur large pop)**

SYNDICAT DES BIOLOGISTES
Le syndicat de tous les biologistes médicaux

*Cells* **2012**, *1*, 153-167; doi:10.3390/cells1020153

OPEN ACCESS

*cells*

ISSN 2073-4409
www.mdpi.com/journal/cells

*Article*

**Can ELISPOT Be Applied to A Clinical Setting as A Diagnostic Utility for Neuroborreliosis?**

**Marika Nordberg [1,2,*], Pia Forsberg [1,3], Dag Nyman [2], Barbro H. Skogman [4], Clara Nyberg [2], Jan Ernerudh [5,6], Ingvar Eliasson [7] and Christina Ekerfelt [5]**

**Neuroborrélioses**

**Se : 36 %**
**Spé : 82 %**

**étude contrôlée cas/T**

# Test de transformation lymphocytaire (LTT)

SYNDICAT DES BIOLOGISTES
Le syndicat de tous les biologistes médicaux

| Prévalence : 1% | Spécificité : 82% | Sensibilité : 36% |
|---|---|---|
| **Forte** | **<< Recos EUCALB** | **Très faible** |

| **LTT** | **Test +** | **Test -** | **Total** |
|---|---|---|---|
| **Neuroborréliose confirmée** | **36** Vrais positifs | **64** Faux négatifs | **100** |
| **Sujet sain** | **1 782** Faux positifs | **8 118** Vrais négatifs | **9 900** |
| **Total** | **1 818** | **8 182** | **10 000** |

# Test de transformation lymphocytaire (LTT)

SYNDICAT DES BIOLOGISTES
Le syndicat de tous les biologistes médicaux

AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology®

BACTERIOLOGY

Check for updates

An Enzyme-Linked Immunosorbent Spot Assay Measuring *Borrelia burgdorferi* B31-Specific Interferon Gamma-Secreting T Cells Cannot Discriminate Active Lyme Neuroborreliosis from Past Lyme Borreliosis: a Prospective Study in the Netherlands

T. van Gorkom,[a,f] S. U. C. Sankatsing,[b] W. Voet,[c] D. M. Ismail,[a] R. H. Muilwijk,[a] M. Salomons,[a] B. J. M. Vlaminckx,[d] A. W. J. Bossink,[e] D. W. Notermans,[f] J. J. M. Bouwman,[a]* K. Kremer,[f] S. F. T. Thijsen[a]

April 2018 Volume 56 Issue 4 e01695-17 Journal of Clinical Microbiology jcm.asm.org 1

AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology®

BACTERIOLOGY

Check for updates

An Enzyme-Linked Immunosorbent Spot Assay Measuring *Borrelia burgdorferi* B31-Specific Interferon Gamma-Secreting T Cells Cannot Discriminate Active Lyme Neuroborreliosis from Past Lyme Borreliosis: a Prospective Study in the Netherlands

T. van Gorkom,[a,f] S. U. C. Sankatsing,[b] W. Voet,[c] D. M. Ismail,[a] R. H. Muilwijk,[a] M. Salomons,[a] B. J. M. Vlaminckx,[d] A. W. J. Bossink,[e] D. W. Notermans,[f] J. J. M. Bouwman,[a]* K. Kremer,[f] S. F. T. Thijsen[a]

April 2018 Volume 56 Issue 4 e01695-17 Journal of Clinical Microbiology

**Pas de distinction infection active vs passée**

# Test de transformation lymphocytaire (LTT)

SYNDICAT DES BIOLOGISTES
Le syndicat de tous les biologistes médicaux

AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology®

BACTERIOLOGY

Check for updates

An Enzyme-Linked Immunosorbent Spot Assay Measuring *Borrelia burgdorferi* B31-Specific Interferon Gamma-Secreting T Cells Cannot Discriminate Active Lyme Neuroborreliosis from Past Lyme Borreliosis: a Prospective Study in the Netherlands

T. van Gorkom,[a,f] S. U. C. Sankatsing,[b] W. Voet,[c] D. M. Ismail,[a] R. H. Muilwijk,[a] M. Salomons,[a] B. J. M. Vlaminckx,[d] A. W. J. Bossink,[e] D. W. Notermans,[f] J. J. M. Bouwman,[a]* K. Kremer,[f] S. F. T. Thijsen[a]

ABSTRACT Two-tier serology testing is most frequently used for the diagnosis of Lyme borreliosis (LB); however, a positive result is no proof of active disease. To establish a diagnosis of active LB, better diagnostics are needed. Tests investigating the cellular immune system are available, but studies evaluating the utility of these tests on well-defined patient populations are lacking. Therefore, we investigated the utility of an enzyme-linked immunospot spot (ELISpot) assay to diagnose active Lyme neuroborreliosis. Peripheral blood mononuclear cells (PBMCs) of various study groups were stimulated by using *Borrelia burgdorferi* strain B31 and various recombinant antigens, and subsequently, the number of *Borrelia*-specific interferon gamma (IFN-γ-secreting) T cells was measured. We included 33 active and 37 treated Lyme neuroborreliosis patients, 28 healthy individuals treated for an early manifestation of LB in the past, and 145 untreated healthy individuals. The median numbers of *B. burgdorferi* B31-specific IFN-γ-secreting T cells/2.5 × $10^5$ PBMCs did not differ between active Lyme neuroborreliosis patients (8.0; interquartile range [IQR], 0.3 to 14.0), treated Lyme neuroborreliosis patients (4.5; IQR, 2.0 to 18.6), and treated healthy individuals (7.4; IQR, 2.3 to 14.8) ($P = 1.000$); however, the median number of *B. burgdorferi* B31-specific IFN-γ-secreting T cells/2.5 × $10^5$ PBMCs among untreated healthy individuals was lower (2.0; IQR, 0.5 to 3.9) ($P \leq 0.016$). We conclude that the *Borrelia* ELISpot assay, measuring the number of *B. burgdorferi* B31-specific IFN-γ-secreting T cells/2.5 × $10^5$ PBMCs, correlates with exposure to the *Borrelia* bacterium but cannot be used for the diagnosis of active Lyme neuroborreliosis.

KEYWORDS *Borrelia burgdorferi*, ELISpot, Lyme borreliosis, Lyme neuroborreliosis, T-cell activation, active disease, antibodies, cytokines, diagnostics, interferon gamma

In the Netherlands, Lyme borreliosis (LB) poses a considerable threat to human health. A study among general practitioners (GPs) found a threefold increase of patients reporting tick bites and diagnoses of erythema migrans (EM), an early, localized skin rash, in the period between 1994 and 2009 (1). Between 2009 and 2014, the incidence of reported tick bites ranged between 486 and 564 consultations per 100,000 inhabitants and the number of GP-reported diagnoses of EM ranged between 134 and 140 per 100,000 inhabitants (2). The true incidence rate is probably higher, since only a

Received [illegible] Returned for modification [illegible] Accepted [illegible]
Accepted manuscript posted online [illegible]
Citation [illegible]
Editor [illegible]
Copyright [illegible]
*[illegible]

April 2018 Volume 56 Issue 4 e01695-17 Journal of Clinical Microbiology jcm.asm.org 1

## Design de l'étude

- 33 NB actives : clinique + LCR ≥5 MNC/µL +/- SIT IgG (n=25)
- 37 NB traitées : idem, mais ≥4 mois après arrêt du TT
- 28 contrôles sains (asympto), TT dans le passé pour Lyme (5/28 séro sg +)
- 145 contrôles sains (asympto) sans ATCD de Lyme (18/145 séro sg +)

# Test de transformation lymphocytaire (LTT)

SYNDICAT DES BIOLOGISTES
Le syndicat de tous les biologistes médicaux

AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology®

BACTERIOLOGY

Check for updates

**An Enzyme-Linked Immunosorbent Spot Assay Measuring *Borrelia burgdorferi* B31-Specific Interferon Gamma-Secreting T Cells Cannot Discriminate Active Lyme Neuroborreliosis from Past Lyme Borreliosis: a Prospective Study in the Netherlands**

T. van Gorkom,[a,f] S. U. C. Sankatsing,[b] W. Voet,[c] D. M. Ismail,[a] R. H. Muilwijk,[a] M. Salomons,[a] B. J. M. Vlaminckx,[d] A. W. J. Bossink,[e] D. W. Notermans,[f] J. J. M. Bouwman,[a]* K. Kremer,[f] S. F. T. Thijsen[a]

ABSTRACT Two-tier serology testing is most frequently used for the diagnosis of Lyme borreliosis (LB); however, a positive result is no proof of active disease. To establish a diagnosis of active LB, better diagnostics are needed. Tests investigating the cellular immune system are available, but studies evaluating the utility of these tests on well-defined patient populations are lacking. Therefore, we investigated the utility of an enzyme-linked immunospot assay (ELISpot) assay to diagnose active Lyme neuroborreliosis. Peripheral blood mononuclear cells (PBMCs) of various study groups were stimulated by using *Borrelia burgdorferi* strain B31 and various recombinant antigens, and subsequently, the number of *Borrelia*-specific interferon gamma (IFN-γ)-secreting T cells was measured. We included 33 active and 37 treated Lyme neuroborreliosis patients, 28 healthy individuals treated for an early manifestation of LB in the past, and 145 untreated healthy individuals. The median numbers of *B. burgdorferi* B31-specific IFN-γ-secreting T cells/2.5 × 10⁵ PBMCs did not differ between active Lyme neuroborreliosis patients (8.0; interquartile range [IQR], 0.3 to 14.0), treated Lyme neuroborreliosis patients (4.5; IQR, 2.0 to 18.6), and treated healthy individuals (7.9; IQR, 2.3 to 14.8) ($P = 1.000$); however, the median number of *B. burgdorferi* B31-specific IFN-γ-secreting T cells/2.5 × 10⁵ PBMCs among untreated healthy individuals was lower (2.0; IQR, 0.5 to 3.9) ($P = 0.016$). We conclude that the *Borrelia* ELISpot assay, measuring the number of *B. burgdorferi* B31-specific IFN-γ-secreting T cells/2.5 × 10⁵ PBMCs, correlates with exposure to the *Borrelia* bacterium but cannot be used for the diagnosis of active Lyme neuroborreliosis.

KEYWORDS *Borrelia burgdorferi*, ELISpot, Lyme borreliosis, Lyme neuroborreliosis, T-cell activation, active disease, antibodies, cytokines, diagnostics, interferon gamma

In the Netherlands, Lyme borreliosis (LB) poses a considerable threat to human health. A study among general practitioners (GPs) found a threefold increase of patients reporting tick bites and diagnoses of erythema migrans (EM), an early, localized skin rash, in the period between 1994 and 2009 (1). Between 2009 and 2014, the incidence of reported tick bites ranged between 488 and 564 consultations per 100,000 inhabitants and the number of GP-reported diagnoses of EM ranged between 134 and 140 per 100,000 inhabitants (2). The true incidence rate is probably higher, since only a

Received [redacted] Returned for modification [redacted] Accepted [redacted]
Accepted manuscript posted online [redacted]
Citation [redacted]
Editor [redacted]

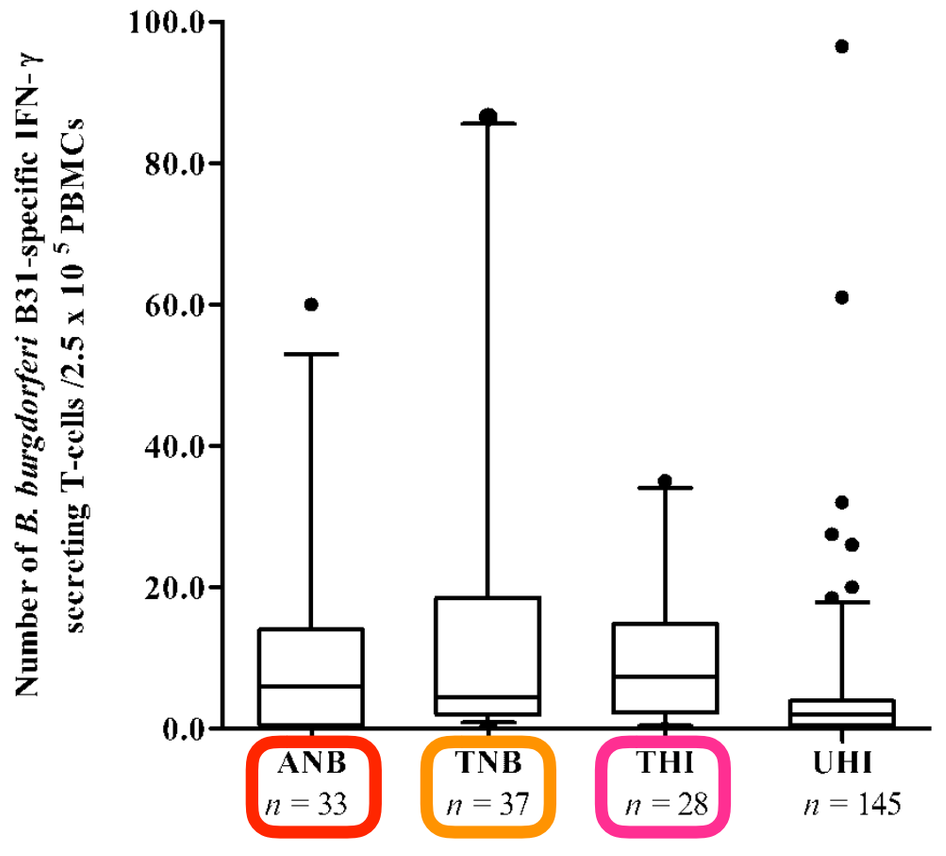


*[redacted]



## Design de l'étude

- 33 NB actives : clinique + LCR ≥5 MNC/µL +/- SIT IgG (n=25)
- 37 NB traitées : idem, mais ≥4 mois après arrêt du TT
- 28 contrôles sains (asympto), TT dans le passé pour Lyme (5/28 séro sg +)
- 145 contrôles sains (asympto) sans ATCD de Lyme (18/145 séro sg +)




**aucune ≠ entre 3 groupes**

# Test de transformation lymphocytaire (LTT)

BACTERIOLOGY

Journal of Clinical Microbiology®

**An Enzyme-Linked Immunosorbent Spot Assay Measuring *Borrelia burgdorferi* B31-Specific Interferon Gamma-Secreting T Cells Cannot Discriminate Active Lyme Neuroborreliosis from Past Lyme Borreliosis: a Prospective Study in the Netherlands**

T. van Gorkom,[a,f] S. U. C. Sankatsing,[b] W. Voet,[c] D. M. Ismail,[a] R. H. Muilwijk,[a] M. Salomons,[a] B. J. M. Vlaminckx,[d] A. W. J. Bossink,[e] D. W. Notermans,[f] J. J. M. Bouwman,[a]* K. Kremer,[f] S. F. T. Thijsen[a]

ABSTRACT Two-tier serology testing is most frequently used for the diagnosis of Lyme borreliosis (LB); however, a positive result is no proof of active disease. To establish a diagnosis of active LB, better diagnostics are needed. Tests investigating the cellular immune system are available, but studies evaluating the utility of these tests on well-defined patient populations are lacking. Therefore, we investigated the utility of an enzyme-linked immunospot assay (ELISpot) assay to diagnose active Lyme neuroborreliosis. Peripheral blood mononuclear cells (PBMCs) of various study groups were stimulated by using *Borrelia burgdorferi* strain B31 and various recombinant antigens, and subsequently, the number of *Borrelia*-specific interferon gamma (IFN-γ)-secreting T cells was measured. We included 33 active and 37 treated Lyme neuroborreliosis patients, 28 healthy individuals treated for an early manifestation of LB in the past, and 145 untreated healthy individuals. The median numbers of *B. burgdorferi* B31-specific IFN-γ-secreting T cells/2.5 × 10⁵ PBMCs did not differ between active Lyme neuroborreliosis patients (8.0; interquartile range [IQR], 0.3 to 14.0), treated Lyme neuroborreliosis patients (4.5; IQR, 2.0 to 18.6), and treated healthy individuals (7.9; IQR, 2.3 to 14.8) ($P = 1.000$); however, the median number of *B. burgdorferi* B31-specific IFN-γ-secreting T cells/2.5 × 10⁵ PBMCs among untreated healthy individuals was lower (2.0; IQR, 0.5 to 3.9) ($P = 0.016$). We conclude that the *Borrelia* ELISpot assay, measuring the number of *B. burgdorferi* B31-specific IFN-γ-secreting T cells/2.5 × 10⁵ PBMCs, correlates with exposure to the *Borrelia* bacterium but cannot be used for the diagnosis of active Lyme neuroborreliosis.

KEYWORDS *Borrelia burgdorferi*, ELISpot, Lyme borreliosis, Lyme neuroborreliosis, T-cell activation, active disease, antibodies, cytokines, diagnostics, interferon gamma

In the Netherlands, Lyme borreliosis (LB) poses a considerable threat to human health. A study among general practitioners (GPs) found a threefold increase of patients reporting tick bites and diagnoses of erythema migrans (EM), an early, localized skin rash, in the period between 1994 and 2009 (1). Between 2009 and 2014, the incidence of reported tick bites ranged between 488 and 564 consultations per 100,000 inhabitants and the number of GP-reported diagnoses of EM ranged between 134 and 140 per 100,000 inhabitants (2). The true incidence rate is probably higher, since only a

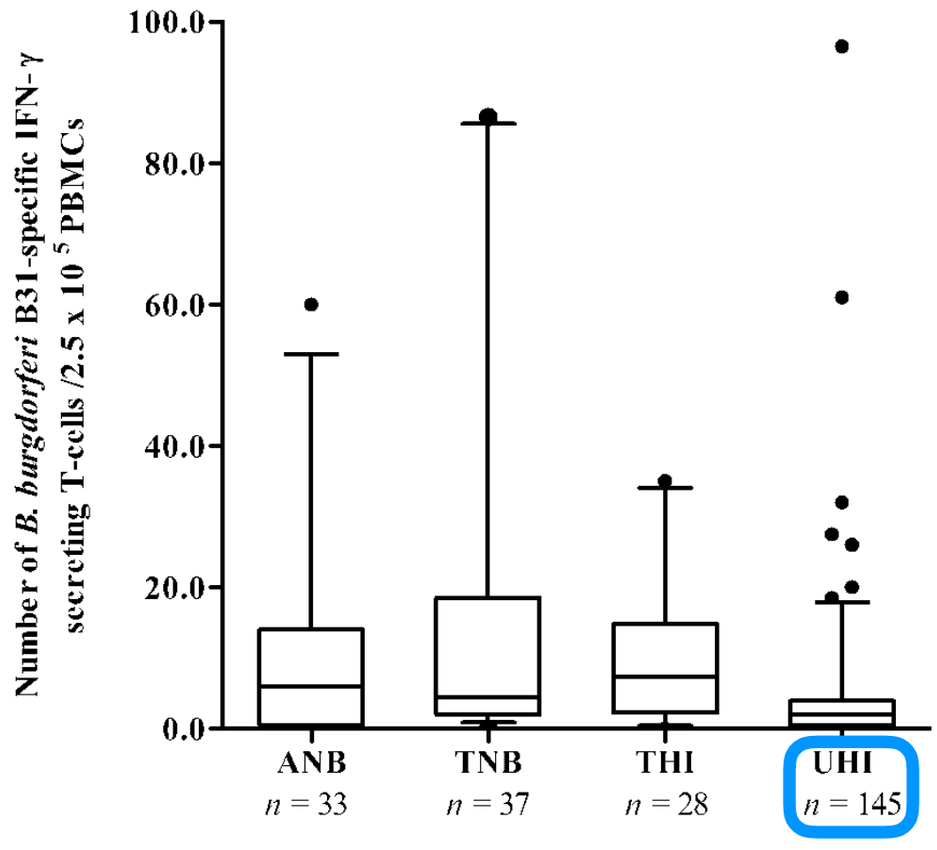
Received [illegible] Returned for modification [illegible] Accepted [illegible]
Accepted manuscript posted online [illegible]
Citation [illegible]
Editor [illegible]
Copyright [illegible]

*Present address [illegible]



## Design de l'étude

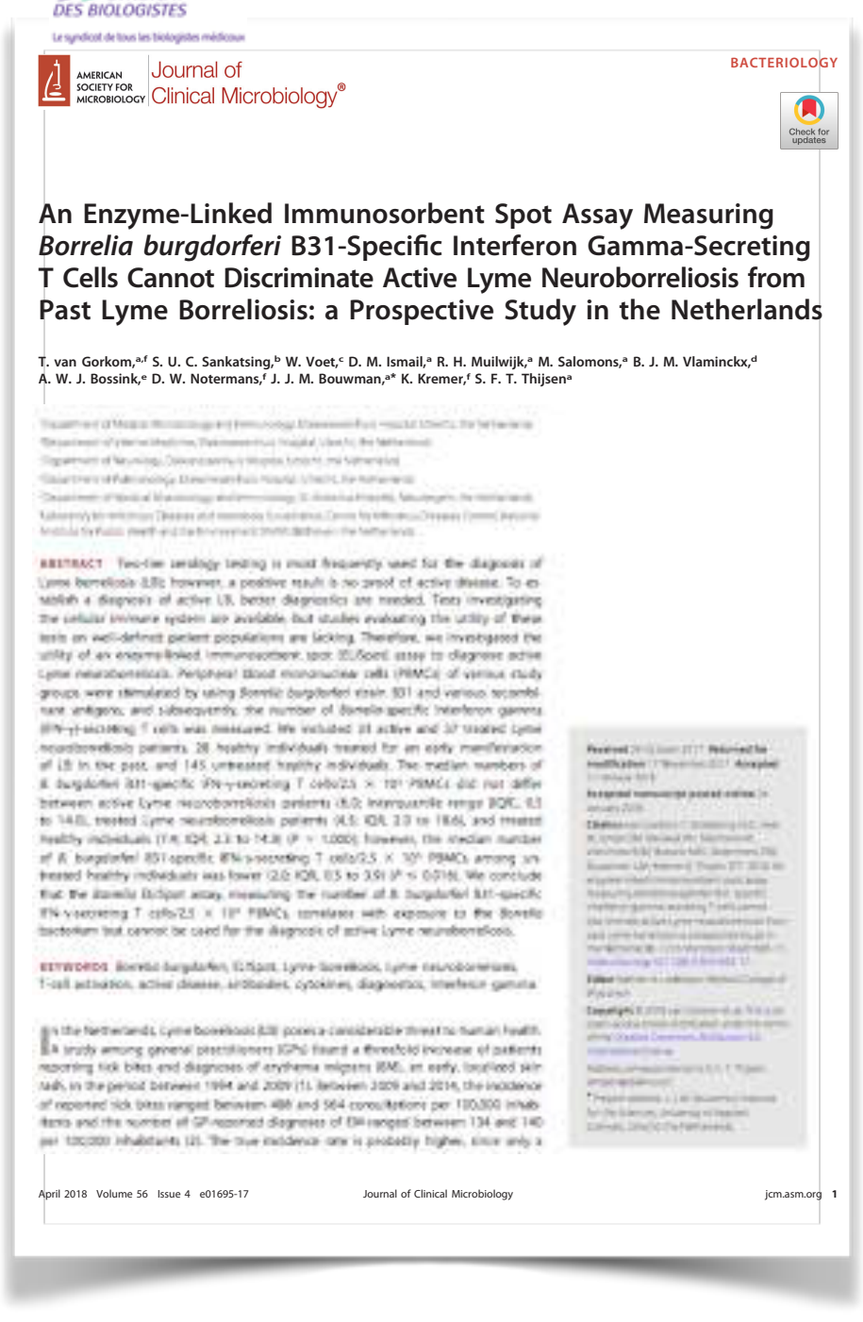
- 33 NB actives : clinique + LCR ≥5 MNC/µL +/- SIT IgG (n=25)
- 37 NB traitées : idem, mais ≥4 mois après arrêt du TT
- 28 contrôles sains (asympto), TT dans le passé pour Lyme (5/28 séro sg +)
- 145 contrôles sains (asympto) sans ATCD de Lyme (18/145 séro sg +)




**aucune ≠ entre 3 groupes**

**sujets sains non-TT : taux plus bas**

# Test de transformation lymphocytaire (LTT)



SYNDICAT DES BIOLOGISTES
Le syndicat de tous les biologistes médicaux

AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology®

BACTERIOLOGY

Check for updates

**An Enzyme-Linked Immunosorbent Spot Assay Measuring *Borrelia burgdorferi* B31-Specific Interferon Gamma-Secreting T Cells Cannot Discriminate Active Lyme Neuroborreliosis from Past Lyme Borreliosis: a Prospective Study in the Netherlands**

T. van Gorkom,[a,f] S. U. C. Sankatsing,[b] W. Voet,[c] D. M. Ismail,[a] R. H. Muilwijk,[a] M. Salomons,[a] B. J. M. Vlaminckx,[d] A. W. J. Bossink,[e] D. W. Notermans,[f] J. J. M. Bouwman,[a]* K. Kremer,[f] S. F. T. Thijsen[a]

ABSTRACT Two-tier serology testing is most frequently used for the diagnosis of Lyme borreliosis (LB); however, a positive result is no proof of active disease. To establish a diagnosis of active LB, better diagnostics are needed. Tests investigating the cellular immune system are available, but studies evaluating the utility of these tests on well-defined patient populations are lacking. Therefore, we investigated the utility of an enzyme-linked immunospot spot (ELISpot) assay to diagnose active Lyme neuroborreliosis. Peripheral blood mononuclear cells (PBMCs) of various study groups were stimulated by using *Borrelia burgdorferi* strain B31 and various recombinant antigens, and subsequently, the number of *Borrelia*-specific interferon gamma (IFN-γ)-secreting T cells was measured. We included 28 active and 37 treated Lyme neuroborreliosis patients, 28 healthy individuals treated for an early manifestation of LB in the past, and 145 untreated healthy individuals. The median numbers of *B. burgdorferi* B31-specific IFN-γ-secreting T cells/2.5 × 10⁵ PBMCs did not differ between active Lyme neuroborreliosis patients (8.0; interquartile range [IQR], 0.3 to 14.0), treated Lyme neuroborreliosis patients (4.5; IQR, 2.0 to 18.6), and treated healthy individuals (7.4; IQR, 2.3 to 14.8) ($P = 1.000$); however, the median number of *B. burgdorferi* B31-specific IFN-γ-secreting T cells/2.5 × 10⁵ PBMCs among untreated healthy individuals was lower (2.0; IQR, 0.5 to 3.5) ($P = 0.016$). We conclude that the *Borrelia* ELISpot assay, measuring the number of *B. burgdorferi* B31-specific IFN-γ-secreting T cells/2.5 × 10⁵ PBMCs, correlates with exposure to the *Borrelia* bacterium but cannot be used for the diagnosis of active Lyme neuroborreliosis.

KEYWORDS *Borrelia burgdorferi*, ELISpot, Lyme borreliosis, Lyme neuroborreliosis, T-cell activation, active disease, antibodies, cytokines, diagnostics, interferon gamma

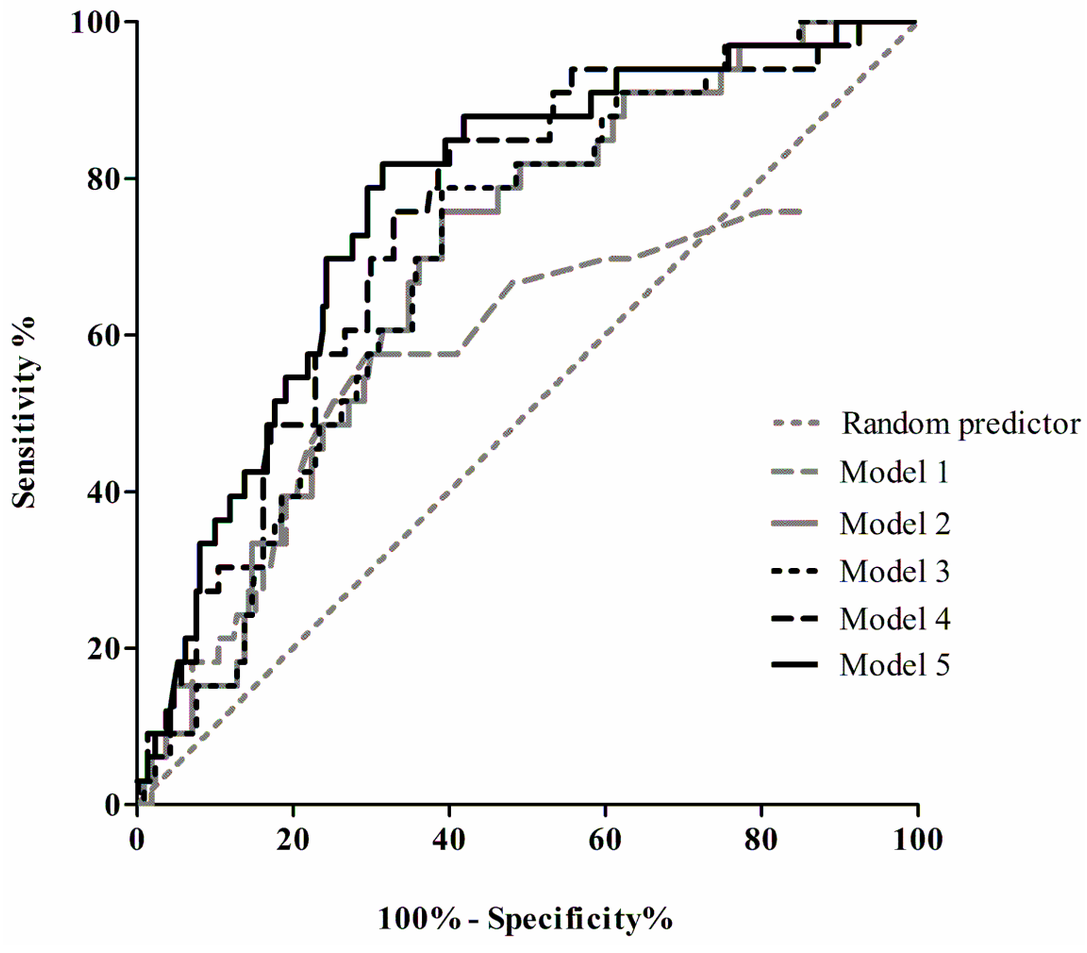
In the Netherlands, Lyme borreliosis (LB) poses a considerable threat to human health. A study among general practitioners (GPs) found a threefold increase of patients reporting tick bites and diagnoses of erythema migrans (EM), an early, localized skin rash, in the period between 1994 and 2009 (1). Between 2009 and 2014, the incidence of reported tick bites ranged between 488 and 564 consultations per 100,000 inhabitants and the number of GP-reported diagnoses of EM ranged between 134 and 140 per 100,000 inhabitants (2). The true incidence rate is probably higher, since only a

Received [illegible] Returned for modification [illegible] Accepted [illegible]
Accepted manuscript posted online [illegible]
Citation [illegible]
Editor [illegible]
Copyright [illegible]
Address correspondence to [illegible]
* Present address [illegible]



## Courbes ROC

- Model 1 : performances de l'ELISPOT
- Model 2 : performances facteurs de risques (piqûre tique et âge)
- Model 3 : Model 2 + ELISPOT




**ELISPOT performances médiocres**

**ELISPOT moins bon que F. risques**

**ELISPOT + F. risques à peine mieux**

# Test de transformation lymphocytaire (LTT)

SYNDICAT DES BIOLOGISTES
Le syndicat de tous les biologistes médicaux

**Les données scientifiques actuelles ne permettent pas de recommander ce test diagnostique dans les borrélioses de Lyme tardives en raison de son manque de spécificité**

SYNDICAT DES BIOLOGISTES

Le syndicat de tous les biologistes médicaux

# MARQUAGE NK CD57

# Marquage CD57

SYNDICAT DES BIOLOGISTES
Le syndicat de tous les biologistes médicaux

## ↘ NK CD57 associée aux formes tardives de borréliose ?
## Normalisation NK CD57 pourrait permettre de vérifier l'efficacité du TT ?

PubMed | cd57 lyme

**Format**: Summary **Sort by**: Most Recent **Per page**: 20

**Search results**

**Items: 6**

1. [Cellular Borrelia tests].
Tveten Y, Noraas S, Aase A.
Tidsskr Nor Laegeforen. 2014 Jan 28;134(2):146-7. doi: 10.4045/tidsskr.13.1053. Norwegian. No abstract available.
PMID: 24477143 **Free Article**

2. [Indolent lymphoma of the ear].
Valois A, Bastien C, Granel-Broca F, Cuny JF, Barbaud A, Schmutz JL.
Ann Dermatol Venereol. 2012 Dec;139(12):818-23. doi: 10.1016/j.annder.2012.10.578. Epub 2012 Nov 26. French.
PMID: 23237280

3. Natural killer cell counts are not different between patients with post-**Lyme** disease syndrome and controls.
Marques A, Brown MR, Fleisher TA.
Clin Vaccine Immunol. 2009 Aug;16(8):1249-50. doi: 10.1128/CVI.00167-09. Epub 2009 Jun 10.
PMID: 19515868 **Free PMC Article**

4. Musical hallucinations in patients with **Lyme** disease.
Stricker RB, Winger EE.
South Med J. 2003 Jul;96(7):711-5.
PMID: 12940329

5. Longterm decrease in the **CD57** lymphocyte subset in a patient with chronic **Lyme** disease.
Stricker RB, Burrascano J, Winger E.
Ann Agric Environ Med. 2002;9(1):111-3.
PMID: 12088407

6. Decreased **CD57** lymphocyte subset in patients with chronic **Lyme** disease.
Stricker RB, Winger EE.
Immunol Lett. 2001 Feb 1;76(1):43-8.
PMID: 11222912

# Marquage CD57

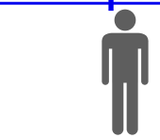
**↘ NK CD57 associée aux formes tardives de borréliose ?**
**Normalisation NK CD57 pourrait permettre de vérifier l'efficacité du TT ?**

Decreased **CD57** lymphocyte subset in patients with chronic **Lyme** disease.
Stricker RB, Winger EE.
Immunol Lett. 2001 Feb 1;76(1):43-8.

n=73 "Lyme chronique" … mais signes non spé et mal définis
choix des sujets contrôle inappropriés (10 Lyme "aigu" + 22 AIDS)

Longterm decrease in the **CD57** lymphocyte subset in a patient with chronic **Lyme** disease.
Stricker RB, Burrascano J, Winger E.
Ann Agric Environ Med. 2002;9(1):111-3.

n=1



Musical hallucinations in patients with **Lyme** disease.
Stricker RB, Winger EE.
South Med J. 2003 Jul;96(7):711-5.

n=1

# Marquage CD57

frontiers in IMMUNOLOGY

MINI REVIEW ARTICLE
published: 09 December 2013
doi: 10.3389/fimmu.2013.00422

## Functional significance of CD57 expression on human NK cells and relevance to disease

***Carolyn M. Nielsen, Matthew J. White, Martin R. Goodier and Eleanor M. Riley****

*Department of Immunology and Infection, London School of Hygiene and Tropical Medicine, London, UK*

***Edited by:***
*Yenan Bryceson, Karolinska Institutet, Sweden*

***Reviewed by:***
*William Garrow Kerr, SUNY Upstate Medical University, USA*
*Björn Önfelt, Karolinska Institutet, Sweden*

****Correspondence:***
*Eleanor M. Riley, Department of Immunology and Infection, London School of Hygiene and Tropical Medicine, Room 236, Keppel Street, London WC1E 7HT, UK*
*e-mail: eleanor.riley@lshtm.ac.uk*

Historically, human NK cells have been identified as $CD3^{-}CD56^{+}CD16^{\pm}$ lymphocytes. More recently it has been established that CD57 expression defines functionally discrete sub-populations of NK cells. On T cells, CD57 expression has been regarded as a marker of terminal differentiation and (perhaps wrongly) of anergy and senescence. Similarly, CD57 expression seems to identify the final stages of peripheral NK cell maturation; its expression increases with age and is associated with chronic infections, particularly human cytomegalovirus infection. However, $CD57^{+}$ NK cells are highly cytotoxic and their presence seems to be beneficial in a number of non-communicable diseases. The purpose of this article is to review our current understanding of CD57 expression as a marker of NK cell function and disease prognosis, as well as to outline areas for further research.

**Keywords: CD57, NK cells, HCMV infection, ageing, chronic infection, cancer, autoimmune diseases, T cells**

### CD57 IS A MARKER OF NK CELL DIFFERENTIATION

CD57 was first identified on cells with natural killer activity using the murine monoclonal antibodies Human Natural Killer-1 (HNK-1) (1) and Leu-7 (2) and was subsequently assigned the cluster of differentiation (CD) designation, CD57, at the fourth International Workshop of Human Leukocyte Antigens in 1989. HNK-1/Leu-7/CD57 was initially believed to be uniquely expressed on NK cells – and was used to define this population (1, 3) – although it was soon apparent that CD57 was expressed only on a subset of functionally distinct NK cells (4). CD57 was subsequently identified on $CD8^{+}$ T cells (5–7) as well as cells of neural crest origin (1, 8–13). Indeed, it was the neuroscience community that ultimately defined CD57 as a terminally sulfated carbohydrate epitope (glucuronic acid 3-sulfate) (14–16). In neural cells, the CD57 epitope is predominantly restricted to adhesion molecules (17) but little attention has been paid to the precise identity of the molecules expressing the CD57 epitope on NK cells and T cells, precluding a full understanding of the relationship between CD57 expression and lymphocyte function. Although one study identified the CD57 epitope on the IL-6 receptor gp130 of resting lymphocytes (18), the cells expressing CD57/gp130 were not identified and no comprehensive analysis of CD57-expressing molecules on T cells or NK cells has been reported.

While first characterized as an NK cell marker, CD57 has been more widely explored as a marker of replicative senescence on T cells (19). Under conditions of persistent immune stimulation, memory T cells convert from $CD28^{+}CD57^{-}$ to $CD28^{-}CD57^{+}$ (20); $CD57^{+}$ cells have short telomeres, low telomerase activity, low expression of cell-cycle associated genes and limited proliferative capacity (20, 21). However, $CD57^{+}CD28^{-}CD8^{+}$ T cells can proliferate given an appropriate cytokine milieu (22), their sensitivity to apoptosis is disputed (23, 24), they are highly cytotoxic (25, 26) and express natural killer receptors (27). $CD57^{+}CD8^{+}$ T cells should thus be regarded as terminally differentiated, oligoclonal populations of cytotoxic cells generated in response to chronic antigen stimulation.

In light of the T cell data it was suggested that CD57 may also be a marker of NK cells with poor proliferative capacity and, perhaps, a degree of immunosenescence (21, 23, 28). Indeed, acquisition of CD57 on NK cells – following stimulation with IL-2 or co-culture with target cells – correlates with maturation of the $CD16^{dim}$ NK cell subset, with lower expression of NKp46, NKp30, NKG2D, and NKG2A, and higher expression of CD16, LIR-1, and killer cell immunoglobulin-like receptors (KIRs) (29). Similarly, in hematopoietic stem cell transplant recipients exposed to human cytomegalovirus (HCMV) infection, differentiation of $CD56^{dim}$ NK cells involves acquisition of CD57, loss of NKG2A, gain of KIRs, and changing expression of homing molecules (30). These studies, together with experiments in $Rag1^{-/-}\gamma c^{-/-}$ mice reconstituted with human hematopoietic stem cells and treated with IL-15 (30), and the observation that fetal and newborn NK cells lack CD57 (31), indicate that $CD57^{+}$ NK cells differentiate from $CD56^{dim}CD57^{-}$ NK cells in an irreversible process with highly stable expression of CD57 likely being the final step in maturation (30, 32). This differentiation is accompanied by functional changes (29, 30): compared with $CD57^{-}$ cells, $CD57^{+}$ NK cells proliferate less well in response to IL-2 and IL-15 and produce less IFN-γ in response to IL-12 and IL-18, consistent with their lower levels of IL-12Rβ mRNA (29) and reduced surface expression of IL-2Rβ and IL-18Rα (30). On the other hand, $CD57^{+}$ NK cells retain their cytolytic potential (30) and a proportion of $CD57^{+}$ NK cells are able to produce IFN-γ after crosslinking of CD16 [Ref. (29); White et al. submitted] indicating that $CD57^{+}$ NK cells are intrinsically able to produce IFN-γ but that they may have different activation requirements.

In summary, therefore, progression from $CD56^{bright}$ to $CD56^{dim}CD57^{-}$ to $CD56^{dim}CD57^{+}$ reflects a maturation pathway for NK cells (33, 34) and rather than being a marker of anergy or



Cellules NK : lymphocytes CD3−CD56+CD16±

CD57 exprimé par une ss-pop de NK

CD57 aussi exprimé par ss-pop LT CD8+ (marqueur diff terminale)

CD57 aussi exprimé par cellules dérivées des crête neurales

# Marquage CD57

| Cancer type | Observations | Reference |
|---|---|---|
| Acute lymphoblastic leukemia | Increased NK cell activity and increased numbers of $CD57^+$ and $CD16^+$ NK cells in bone marrow associated with complete remission | Sorskaar et al. (57) |
| Hodgkin's disease | Absence/low number of $CD57^+$ NK cells in tumor tissue (by immunohistochemistry) associated with relapse | Ortaç et al. (58) |
| Non-Hodgkin's lymphoma | Higher numbers of intratumoral $CD57^+$ NK cells are associated with relapse free survival in pediatric cases | Ortaç et al. (58) |
| Metastatic tumors in the brain | $CD57^+$ NK cells infiltrate brain metastases of various origins (lung, breast, and renal carcinomas; melanoma) but no correlation between numbers of infiltrating $CD57^+$ NK cells and apoptosis of malignant cells | Vaquero et al. (59) |
| Colorectal cancer | Increased $CD57^+$ NK cells in germinal centers of draining lymph nodes, but rarely in primary or metastatic lesions; $CD57^+$ NK cells may prevent establishment of tumor in lymph nodes? | Adachi et al. (60) |
| Bladder carcinoma | Lower frequency of $CD56^+$ and $CD57^+$ PBMC in patients with invasive and non-invasive tumors is correlated with reduced cytotoxicity against T24 bladder cancer cell line | Hermann et al. (61) |
| Breast carcinoma | Survival is positively correlated with the number of tumor infiltrating $CD57^+$ NK cells and with expression of CX3CL1 (a known NK cell chemoattractant) by the tumor cells | Park et al. (62) |
| Gastric carcinoma | $CD57^+$ NK cell infiltration associated with a lower clinical grade tumor, reduced venous invasion, fewer lymph node metastases, less lymphocytic invasion, and increased 5 year survival outcome | Ishigami et al. (63) |
| Oral squamous cell carcinoma | Low density of tumor infiltrating $CD57^+$ NK cells and high numbers of $TNF^+$ cells associated with higher clinical staging | Turkseven and Oygur (64) |
| Esophageal squamous cell carcinoma | Tumor infiltrating $CD57^+$ NK cells positively associated with increased survival over 80 months | Lv et al. (87) |
| Squamous cell lung carcinoma | Tumor infiltrating $CD57^+$ NK cells positively correlated with increased survival 2 years after surgery | Villegas et al. (88) |
| Pulmonary adenocarcinoma | Higher absolute numbers of tumor infiltrating $CD57^+$ NK cells correlated with tumor regression | Takanami et al. (89) |
| Various | Low numbers of $CD57^+$ NK cells in peripheral blood are associated with carcinomas of colon, lung, breast, and neck; no association was with melanoma or sarcoma | Balch et al. (90) |

# Marquage CD57

| | Observations | Reference |
|---|---|---|
| **AUTOIMMUNE DISEASE** | | |
| Alopecia areata | $CD57^+$ NK cells are significantly reduced in peripheral blood of patients with multiple foci of alopecia | Imai et al. (91) |
| Atopic dermatitis | Reduced frequencies of $CD57^+$ NK cells in peripheral blood of patients compared to healthy controls, with greatest reduction in the most severe cases | Wehrmann et al. (126) and Matsumura (127) |
| Sjögren's syndrome | Decreased numbers of $CD57^+$ NK cells observed in peripheral blood of patients compared to controls | Struyf et al. (128) |
| IgA nephropathy | Decreased proportion of $CD57^+$ $CD16^+$ lymphocytes in the peripheral blood of patients compared to healthy controls | Antonaci et al.(129) |
| Psoriasis | NK cells infiltrating skin lesions – but also unaffected skin – are predominantly CD57low | Batista et al. (85) |
| **INFECTION** | | |
| HCMV | Increased proportions of $CD57^+$ NK cells in infected individuals; CD57 expression limited to the $NKG2C^+$ subset | Gratama et al. (110), Lopez-Vergès et al. (99) and Foley et al. (111, 112) |
| HIV | In chronic infections, there is a loss of CD57-/dim NK cells, but the absolute number of $CD57^+$ NK cells remains constant | Hong et al. (100) |
| Chikungunya virus | Increased proportions of $CD57^+$ NK cells after infection in $HCMV^+$ patients | Petitdemange et al. (115) |
| Hantavirus | $NKG2C^+$ NK cell subset expanded during infection in $HCMV^+$ patients and the majority of these cells are $CD57^+$ | Björkström et al. (114) |
| Hepatitis B and Hepatitis C | $NKG2C^+$ NK cell population is expanded in chronic infections, and these are predominantly $CD57^+$, but co-infection with HCMV appears to be the driver of this effect | Béziat et al. (97) |
| Lyme disease | Conflicting evidence on whether chronic disease leads to a reduced proportion of $CD57^+$ NK cells in peripheral blood | Stricker et al. (117), Stricker and Winger (118), and Marques et al. (119) |

# Marquage CD57

CLINICAL AND VACCINE IMMUNOLOGY, Aug. 2009, p. 1249–1250 Vol. 16, No. 8
1556-6811/09/$08.00+0 doi:10.1128/CVI.00167-09

## Natural Killer Cell Counts Are Not Different between Patients with Post-Lyme Disease Syndrome and Controls▽

Adriana Marques,[1]* Margaret R. Brown,[2] and Thomas A. Fleisher[2]

*Laboratory of Clinical Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland,[1] and Immunology Service, Department of Laboratory Medicine, Clinical Center, National Institutes of Health, Bethesda, Maryland[2]*

Received 16 April 2009/Accepted 1 June 2009

**It has been reported that patients with "chronic Lyme disease" have a decreased number of natural killer cells, as defined by the CD57 marker. We performed immunophenotyping in 9 individuals with post-Lyme disease syndrome, 12 who recovered from Lyme disease, and 9 healthy volunteers. The number of natural killer cells was not significantly different between the groups.**

Lyme disease, the most common vector-borne illness in the United States, is caused by *Borrelia burgdorferi* and transmitted by the bite of the *Ixodes* sp. tick (the deer tick). The disease usually begins with erythema migrans, an expanding skin lesion at the site of the tick bite. Within several days or weeks, there is hematogenous dissemination of the spirochetes, and patients may present with dermatologic, neurological, cardiac, and rheumatologic involvement (7). "Chronic Lyme disease" is a controversial term applied to a broad spectrum of patients, including individuals with Lyme disease and those with post-Lyme disease syndrome (PLDS), as well as patients with no evidence of current or past *B. burgdorferi* infection (5, 6). PLDS is defined as the persistence or relapse of nonspecific symptoms (such as fatigue, musculoskeletal pain, and cognitive complaints) in patients who have had Lyme disease and have received an adequate course of antibiotic therapy.

It has been reported that patients diagnosed with chronic Lyme disease have a decreased number of natural killer cells, as defined by the CD57 marker, and that the changes in the number of $CD57^+$ cells can be monitored as evidence of response to therapy (9–10). CD57 was initially used as a marker for NK cells, but it is not expressed by all NK cells and is also expressed by T-cell subpopulations. It is thought that CD57 is a marker of terminally differentiated cells (4). Currently, the most common approach for identifying NK cells utilizes a combination of CD56 and CD16 surface markers used together with CD3 to exclude T cells expressing NK markers (NK T cells). The CD57 test is offered in some clinical laboratories and is being used by some health practitioners to evaluate and follow patients diagnosed with chronic Lyme disease. To further evaluate the utility of NK cell numbers in evaluating and/or monitoring this patient group, we performed immunophenotyping in 9 patients with PLDS, 12 individuals who recovered from Lyme disease, and 9 healthy volunteers.

Patients with PLDS had a past history of Lyme disease according to the Centers for Disease Control and Prevention clinical definition (1, 2), a prior positive serologic analysis confirmed by immunoglobulin G Western blotting (3), received at least one course of recommended antibiotic therapy (11), and had persistent or intermittent symptoms for at least 6 months after appropriate antibiotic therapy for Lyme disease. Common symptoms included widespread musculoskeletal pain and fatigue, memory and/or concentration impairment, and radicular pain, paresthesias, or dysesthesias. The onset of symptoms was coincident with or within 6 months of initial *B. burgdorferi* infection, symptoms were severe enough to interfere with daily life activities, and other causes were excluded. Individuals who recovered from Lyme disease also had a past history of Lyme disease according to the Centers for Disease Control and Prevention clinical definition and received recommended antibiotic therapy but had no complaints attributed to the disease. Controls included healthy volunteers from areas of endemicity ($n = 5$) with no previous history compatible with Lyme disease and who were seronegative for *B. burgdorferi*. The study was approved by the National Institute of Allergy and Infectious Diseases Institutional Review Board, and all individuals signed informed consent forms.

Peripheral blood specimens were obtained by phlebotomy on site. Anticoagulated (EDTA) samples were stained using the whole-blood lysis method and analyzed concurrently on a dual-laser FACSCalibur (BD Biosciences) using CellQuest software (BD Biosciences). Directly conjugated mouse anti-human monoclonal antibodies against CD3, CD4, CD8, CD20, CD16, CD56, and CD57 were used. Irrelevant, directly conjugated, mouse anti-human monoclonal antibodies were used to define background staining. All monoclonal antibodies were obtained from BD Biosciences and Beckman Coulter and used as recommended by the manufacturers. Lymphocytes were identified by forward and side scatter, and the lymphocyte gate was confirmed using the CD45/CD14 LeucoGate reagent (BD Biosciences). To calculate the absolute numbers of each lymphocyte subset, the percentage of positive cells was multiplied by the absolute peripheral blood lymphocyte count obtained using an automated hematology instrument on the same blood sample. Results were compared by Kruskal-Wallis test or Mann-Whitney test. The Spearman rank correlation coefficient was used to calculate quantitative correlations. All $P$ values

* Corresponding author. Mailing address: Clinical Studies Unit, Laboratory of Clinical Infectious Diseases, Building 10, Room 11N234, 10 Center Drive, MSC 1808, Bethesda, MD 20892. Phone: (301) 435-7568. Fax: (301) 402-5933. E-mail: amarques@niaid.nih.gov.

▽ Published ahead of print on 10 June 2009.



## Design de l'étude

- 9 PLDS (post- Lyme disease syndrome) : déf CDC, séro sg+, >6 mois postTT
- 12 REC (recovered) = Lyme guéris : déf CDC, asympto ap TT
- 9 HV (healthy volunteers) : pas d'ATCD Lyme et séro sg neg

# Marquage CD57

CLINICAL AND VACCINE IMMUNOLOGY, Aug. 2009, p. 1249–1250 Vol. 16, No. 8
1556-6811/09/$08.00+0 doi:10.1128/CVI.00167-09

Natural Killer Cell Counts Are Not Different between Patients with Post-Lyme Disease Syndrome and Controls▽

Adriana Marques,[1]* Margaret R. Brown,[2] and Thomas A. Fleisher[2]

*Laboratory of Clinical Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland,[1] and Immunology Service, Department of Laboratory Medicine, Clinical Center, National Institutes of Health, Bethesda, Maryland[2]*

Received 16 April 2009/Accepted 1 June 2009

**It has been reported that patients with "chronic Lyme disease" have a decreased number of natural killer cells, as defined by the CD57 marker. We performed immunophenotyping in 9 individuals with post-Lyme disease syndrome, 12 who recovered from Lyme disease, and 9 healthy volunteers. The number of natural killer cells was not significantly different between the groups.**

Lyme disease, the most common vector-borne illness in the United States, is caused by *Borrelia burgdorferi* and transmitted by the bite of the *Ixodes* sp. tick (the deer tick). The disease usually begins with erythema migrans, an expanding skin lesion at the site of the tick bite. Within several days or weeks, there is hematogenous dissemination of the spirochetes, and patients may present with dermatologic, neurological, cardiac, and rheumatologic involvement (7). "Chronic Lyme disease" is a controversial term applied to a broad spectrum of patients, including individuals with Lyme disease and those with post-Lyme disease syndrome (PLDS), as well as patients with no evidence of current or past *B. burgdorferi* infection (5, 6). PLDS is defined as the persistence or relapse of nonspecific symptoms (such as fatigue, musculoskeletal pain, and cognitive complaints) in patients who have had Lyme disease and have received an adequate course of antibiotic therapy.

It has been reported that patients diagnosed with chronic Lyme disease have a decreased number of natural killer cells, as defined by the CD57 marker, and that the changes in the number of $CD57^+$ cells can be monitored as evidence of response to therapy (9–10). CD57 was initially used as a marker for NK cells, but it is not expressed by all NK cells and is also expressed by T-cell subpopulations. It is thought that CD57 is a marker of terminally differentiated cells (4). Currently, the most common approach for identifying NK cells utilizes a combination of CD56 and CD16 surface markers used together with CD3 to exclude T cells expressing NK markers (NK T cells). The CD57 test is offered in some clinical laboratories and is being used by some health practitioners to evaluate and follow patients diagnosed with chronic Lyme disease. To further evaluate the utility of NK cell numbers in evaluating and/or monitoring this patient group, we performed immunophenotyping in 9 patients with PLDS, 12 individuals who recovered from Lyme disease, and 9 healthy volunteers.

Patients with PLDS had a past history of Lyme disease according to the Centers for Disease Control and Prevention clinical definition (1, 2), a prior positive serologic analysis confirmed by immunoglobulin G Western blotting (3), received at least one course of recommended antibiotic therapy (11), and had persistent or intermittent symptoms for at least 6 months after appropriate antibiotic therapy for Lyme disease. Common symptoms included widespread musculoskeletal pain and fatigue, memory and/or concentration impairment, and radicular pain, paresthesias, or dysesthesias. The onset of symptoms was coincident with or within 6 months of initial *B. burgdorferi* infection, symptoms were severe enough to interfere with daily life activities, and other causes were excluded. Individuals who recovered from Lyme disease also had a past history of Lyme disease according to the Centers for Disease Control and Prevention clinical definition and received recommended antibiotic therapy but had no complaints attributed to the disease. Controls included healthy volunteers from areas of endemicity ($n = 9$) with no previous history compatible with Lyme disease and who were seronegative for *B. burgdorferi*. The study was approved by the National Institute of Allergy and Infectious Diseases Institutional Review Board, and all individuals signed informed consent forms.

Peripheral blood specimens were obtained by phlebotomy on site. Anticoagulated (EDTA) samples were stained using the whole-blood lysis method and analyzed concurrently on a dual-laser FACSCalibur (BD Biosciences) using CellQuest software (BD Biosciences). Directly conjugated mouse anti-human monoclonal antibodies against CD3, CD4, CD8, CD20, CD16, CD56, and CD57 were used. Irrelevant, directly conjugated, mouse anti-human monoclonal antibodies were used to define background staining. All monoclonal antibodies were obtained from BD Biosciences and Beckman Coulter and used as recommended by the manufacturers. Lymphocytes were identified by forward and side scatter, and the lymphocyte gate was confirmed using the CD45/CD14 LeucoGate reagent (BD Biosciences). To calculate the absolute numbers of each lymphocyte subset, the percentage of positive cells was multiplied by the absolute peripheral blood lymphocyte count obtained using an automated hematology instrument on the same blood sample. Results were compared by Kruskal-Wallis test or Mann-Whitney test. The Spearman rank correlation coefficient was used to calculate quantitative correlations. All $P$ values

* Corresponding author. Mailing address: Clinical Studies Unit, Laboratory of Clinical Infectious Diseases, Building 10, Room 11N234, 10 Center Drive, MSC 1808, Bethesda, MD 20892. Phone: (301) 435-7668. Fax: (301) 402-5933. E-mail: amarques@niaid.nih.gov.

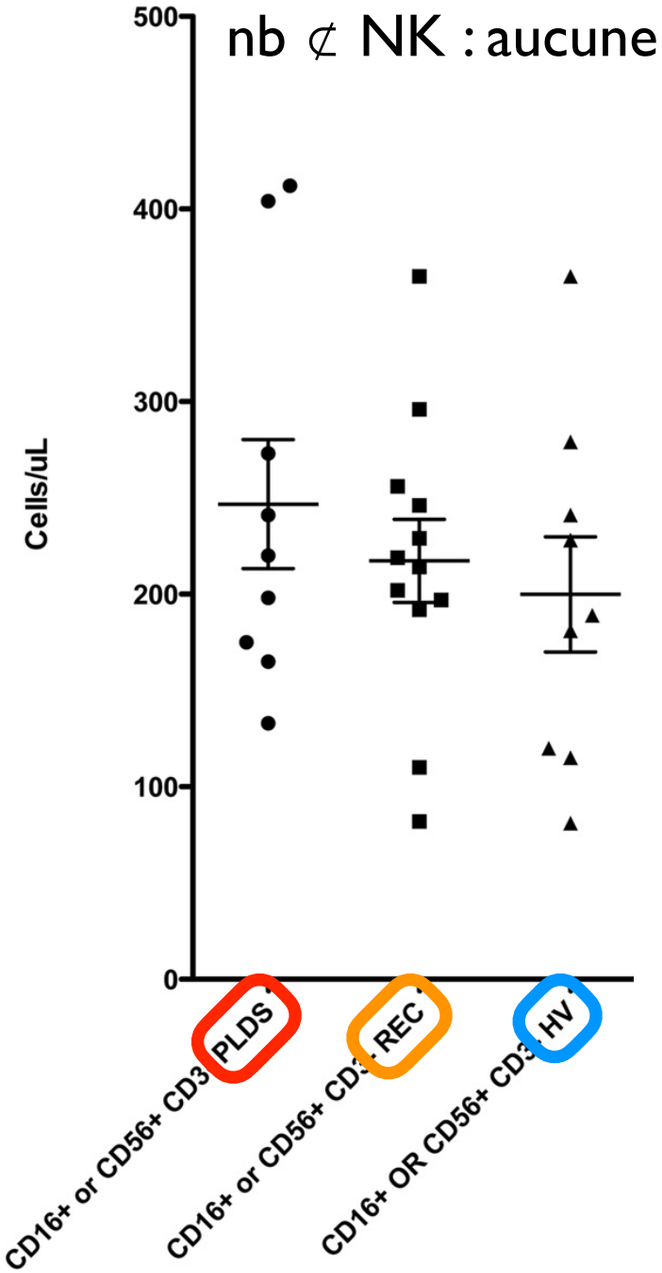
▽ Published ahead of print on 10 June 2009.



## Design de l'étude

- 9 PLDS (post- Lyme disease syndrome) : déf CDC, séro sg+, >6 mois postTT
- 12 REC (recovered) = Lyme guéris : déf CDC, asympto ap TT
- 9 HV (healthy volunteers) : pas d'ATCD Lyme et séro sg neg

nb ⊄ NK : aucune ≠ entre 3 groupes



# Marquage CD57

CLINICAL AND VACCINE IMMUNOLOGY, Aug. 2009, p. 1249–1250 Vol. 16, No. 8
1556-6811/09/$08.00+0 doi:10.1128/CVI.00167-09

Natural Killer Cell Counts Are Not Different between Patients with Post-Lyme Disease Syndrome and Controls▽

Adriana Marques,[1]* Margaret R. Brown,[2] and Thomas A. Fleisher[2]

*Laboratory of Clinical Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland,[1] and Immunology Service, Department of Laboratory Medicine, Clinical Center, National Institutes of Health, Bethesda, Maryland[2]*

Received 16 April 2009/Accepted 1 June 2009

**It has been reported that patients with "chronic Lyme disease" have a decreased number of natural killer cells, as defined by the CD57 marker. We performed immunophenotyping in 9 individuals with post-Lyme disease syndrome, 12 who recovered from Lyme disease, and 9 healthy volunteers. The number of natural killer cells was not significantly different between the groups.**

Lyme disease, the most common vector-borne illness in the United States, is caused by *Borrelia burgdorferi* and transmitted by the bite of the *Ixodes* sp. tick (the deer tick). The disease usually begins with erythema migrans, an expanding skin lesion at the site of the tick bite. Within several days or weeks, there is hematogenous dissemination of the spirochetes, and patients may present with dermatologic, neurological, cardiac, and rheumatologic involvement (7). "Chronic Lyme disease" is a controversial term applied to a broad spectrum of patients, including individuals with Lyme disease and those with post-Lyme disease syndrome (PLDS), as well as patients with no evidence of current or past *B. burgdorferi* infection (5, 6). PLDS is defined as the persistence or relapse of nonspecific symptoms (such as fatigue, musculoskeletal pain, and cognitive complaints) in patients who have had Lyme disease and have received an adequate course of antibiotic therapy.

It has been reported that patients diagnosed with chronic Lyme disease have a decreased number of natural killer cells, as defined by the CD57 marker, and that the changes in the number of $CD57^+$ cells can be monitored as evidence of response to therapy (9–10). CD57 was initially used as a marker for NK cells, but it is not expressed by all NK cells and is also expressed by T-cell subpopulations. It is thought that CD57 is a marker of terminally differentiated cells (4). Currently, the most common approach for identifying NK cells utilizes a combination of CD56 and CD16 surface markers used together with CD3 to exclude T cells expressing NK markers (NK T cells). The CD57 test is offered in some clinical laboratories and is being used by some health practitioners to evaluate and follow patients diagnosed with chronic Lyme disease. To further evaluate the utility of NK cell numbers in evaluating and/or monitoring this patient group, we performed immunophenotyping in 9 patients with PLDS, 12 individuals who recovered from Lyme disease, and 9 healthy volunteers.

Patients with PLDS had a past history of Lyme disease according to the Centers for Disease Control and Prevention clinical definition (1, 2), a prior positive serologic analysis confirmed by immunoglobulin G Western blotting (3), received at least one course of recommended antibiotic therapy (11), and had persistent or intermittent symptoms for at least 6 months after appropriate antibiotic therapy for Lyme disease. Common symptoms included widespread musculoskeletal pain and fatigue, memory and/or concentration impairment, and radicular pain, paresthesias, or dysesthesias. The onset of symptoms was coincident with or within 6 months of initial *B. burgdorferi* infection, symptoms were severe enough to interfere with daily life activities, and other causes were excluded. Individuals who recovered from Lyme disease also had a past history of Lyme disease according to the Centers for Disease Control and Prevention clinical definition and received recommended antibiotic therapy but had no complaints attributed to the disease. Controls included healthy volunteers from areas of endemicity ($n = 9$) with no previous history compatible with Lyme disease and who were seronegative for *B. burgdorferi*. The study was approved by the National Institute of Allergy and Infectious Diseases Institutional Review Board, and all individuals signed informed consent forms.

Peripheral blood specimens were obtained by phlebotomy on site. Anticoagulated (EDTA) samples were stained using the whole-blood lysis method and analyzed concurrently on a dual-laser FACSCalibur (BD Biosciences) using CellQuest software (BD Biosciences). Directly conjugated mouse anti-human monoclonal antibodies against CD3, CD4, CD8, CD20, CD16, CD56, and CD57 were used. Irrelevant, directly conjugated, mouse anti-human monoclonal antibodies were used to define background staining. All monoclonal antibodies were obtained from BD Biosciences and Beckman Coulter and used as recommended by the manufacturers. Lymphocytes were identified by forward and side scatter, and the lymphocyte gate was confirmed using the CD45/CD14 LeucoGate reagent (BD Biosciences). To calculate the absolute numbers of each lymphocyte subset, the percentage of positive cells was multiplied by the absolute peripheral blood lymphocyte count obtained using an automated hematology instrument on the same blood sample. Results were compared by Kruskal-Wallis test or Mann-Whitney test. The Spearman rank correlation coefficient was used to calculate quantitative correlations. All $P$ values

* Corresponding author. Mailing address: Clinical Studies Unit, Laboratory of Clinical Infectious Diseases, Building 10, Room 11N234, 10 Center Drive, MSC 1888, Bethesda, MD 20892. Phone: (301) 435-7668. Fax: (301) 402-5933. E-mail: amarques@niaid.nih.gov.

▽ Published ahead of print on 10 June 2009.

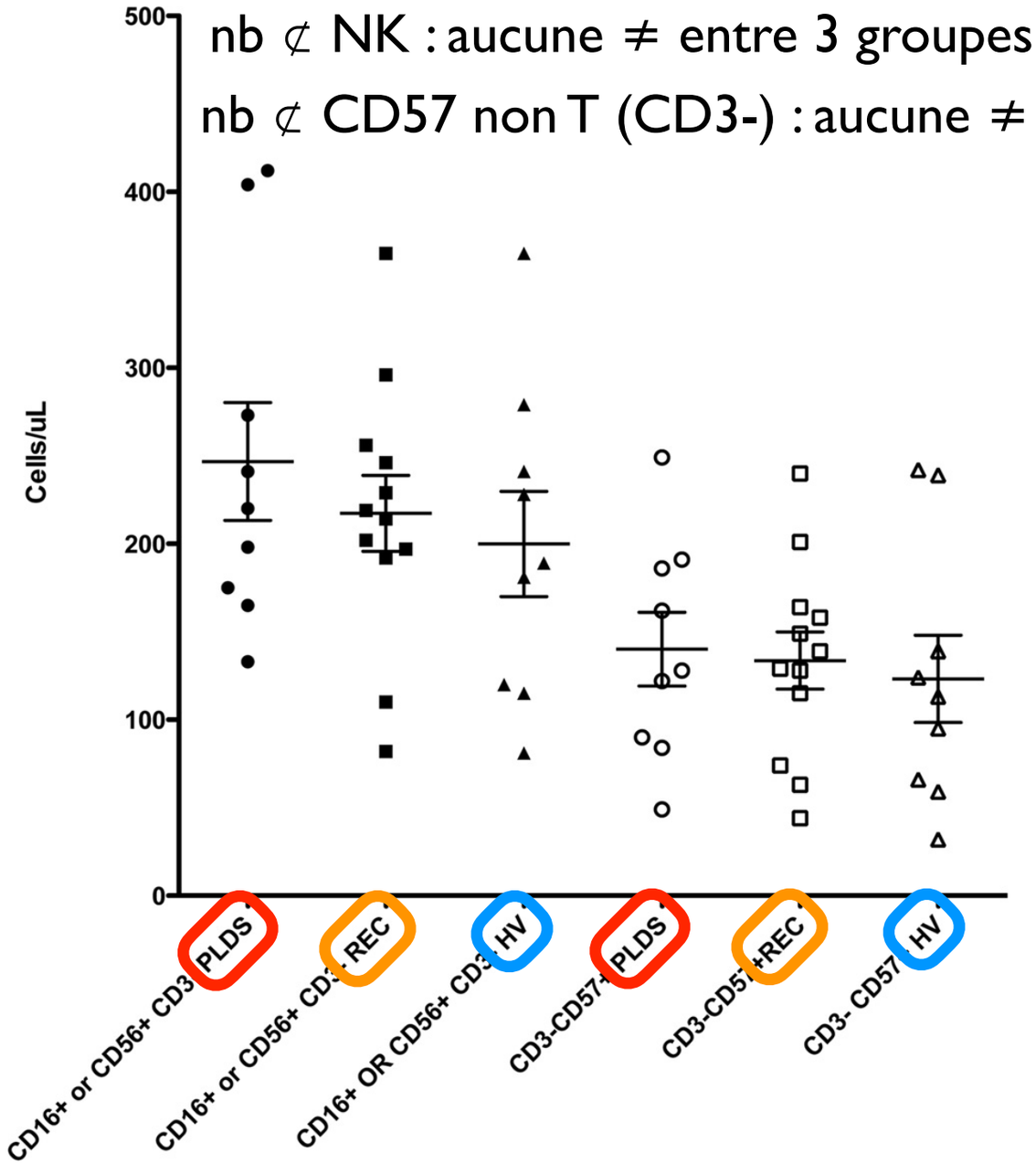


## Design de l'étude

- 9 PLDS (post- Lyme disease syndrome) : déf CDC, séro sg+, >6 mois postTT
- 12 REC (recovered) = Lyme guéris : déf CDC, asympto ap TT
- 9 HV (healthy volunteers) : pas d'ATCD Lyme et séro sg neg

nb ⊄ NK : aucune ≠ entre 3 groupes

nb ⊄ CD57 non T (CD3-) : aucune ≠ entre 3 groupes



# Marquage CD57

~~↘ NK CD57 associée aux formes tardives de borréliose ?~~

~~Normalisation NK CD57 pourrait permettre de vérifier l'efficacité du TT ?~~

**Les données scientifiques actuelles ne permettent pas de recommander ce test diagnostique dans les borrélioses de Lyme tardives**

SYNDICAT DES BIOLOGISTES

Le syndicat de tous les biologistes médicaux

# TESTS SUR TIQUE

# Test rapides sur tiques

SYNDICAT DES BIOLOGISTES
Le syndicat de tous les biologistes médicaux




**Performances intrinsèques ? Interprétation ?**

SYNDICAT DES BIOLOGISTES
Le syndicat de tous les biologistes médicaux

# AUTO-TESTS

## (vendus en pharmacie)

# Auto-tests

SYNDICAT DES BIOLOGISTES
Le syndicat de tous les biologistes médicaux

## Tests réalisés sur une goutte de sang




***Quelle est la fiabilité de l'AUTOTEST LYME ?***
Malgré la fiabilité de ce test, des résultats faussement positifs ou faussement négatifs sont possibles.
L'AUTOTEST LYME est fiable et est utilisé dans les milieux professionnels (hôpitaux, laboratoires). Les études réalisées montrent, sur des échantillons d'origine européenne, que l'AUTOTEST LYME permet de détecter les anticorps précoces (IgM) dirigés contre la bactérie *Borrélia* dans plus de 93% des cas mais il est toujours possible que la présence des anticorps IgM liés à une infection par *Borrélia* ne soit pas détectée par le test en raison du développement tardif de l'immunité chez certains sujets.

VEDA . LAB ...
Rue de l'Expansion - ZAT du Londeau - Cerisé
BP 181 - 61006 ALENCON Cedex (France)

**Autopiqueur stérile :** STERILE R

CE 0120

## Avantages

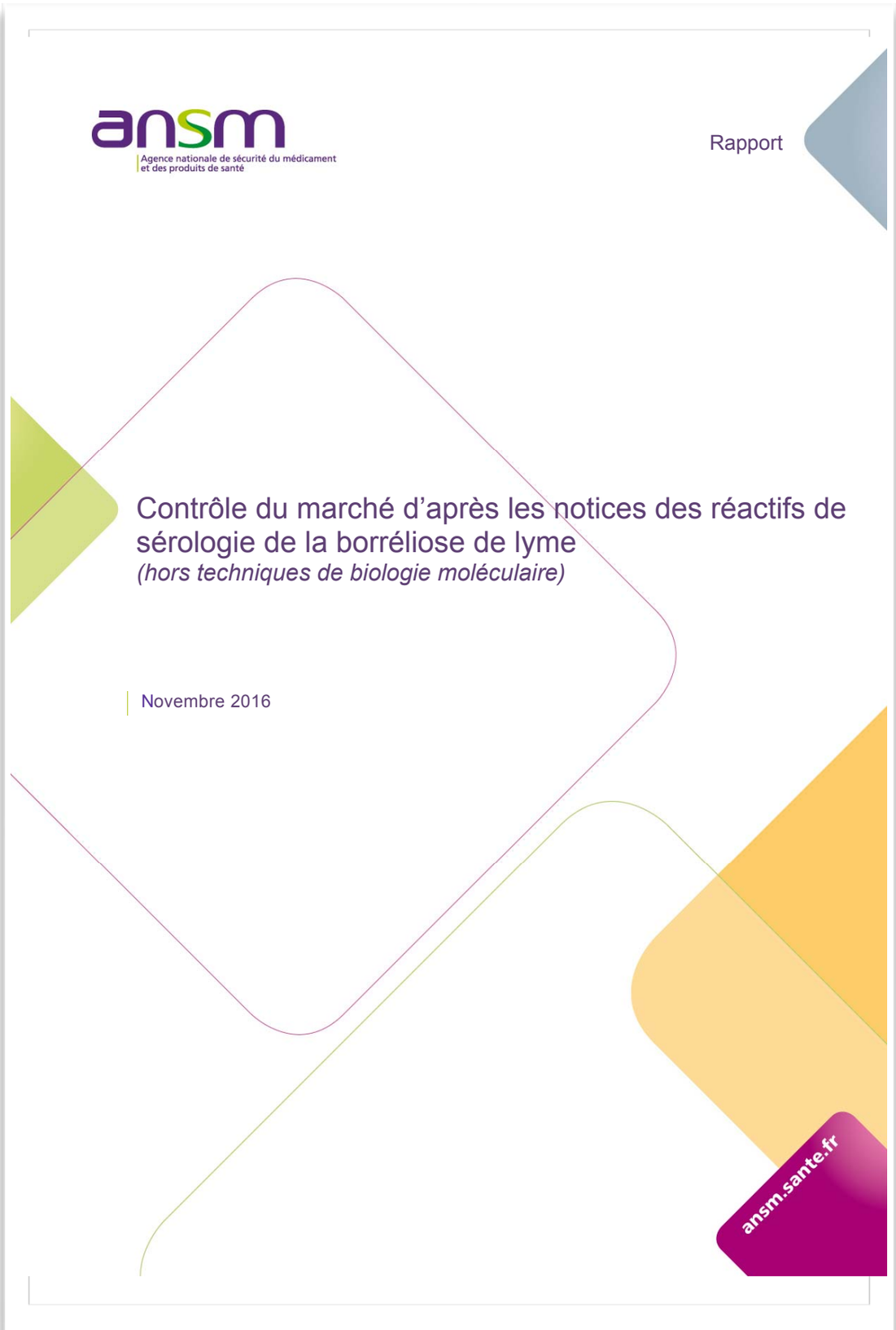
- Facilité d'utilisation : test réalisable par le patient lui-même
- Rapidité d'obtention des résultats
- Fiabilité des résultats
- Tests pouvant être effectués à tout moment
- Produits marqués CE

### Quelle est la fiabilité de MyTest Lyme ?

Malgré la fiabilité de ce test des résultats faussement positifs ou faussement négatifs sont possibles. Ce test est fiable et est utilisé dans les milieux professionnels (hôpitaux, laboratoires). Les études réalisées montrent, sur des échantillons d'origine européenne, que ce test permet de détecter les anticorps précoces (IgM) dirigés contre la bactérie Borrelia dans plus de 93% des cas. Cependant, il est toujours possible que la présence des anticorps IgM liés à une infection par Borrélia ne soit pas détectée par le test en raison du développement tardif de l'immunité chez certains sujets.

# Auto-tests

SYNDICAT DES BIOLOGISTES
Le syndicat de tous les biologistes médicaux



# Auto-tests

Rapport

| Fabricant /distributeur | Composition | Spécificité | Sensibilité |
|---|---|---|---|
| **All diag-Biosynex * (TDR)** (arrêt de commercialisation) | | | |
| **Nal von Minden (TDR)** | | | |
| **Veda Lab/Servibio* (TDR)** | | | |
| **Veda.Lab/Alere (autodiagnostic)** | | | |

* réactifs utilisés en France d'après les résultats du CNQ.

Recommandations remplies, remplies de façon partielle, insuffisamment remplies.

# Auto-tests

Rapport

- **Concernant plus spécifiquement le test d'autodiagnostic :**
  - **les informations destinées aux utilisateurs ont en partie été modifiées dans le projet de notice -à noter que la directive 98/79/CE permet de limiter les informations fournies aux utilisateurs profanes à celles qui leur sont compréhensibles- ;**
  - **les modalités d'évaluation des performances, notamment sur le sang total sont encore insuffisantes ;**
  - **ce test ne dose que les IgM, eux-mêmes détectés dans 40 à 60% des érythèmes migrants – ceci en raison de la faible réponse immunitaire à ce stade et dans un pourcentage plus faible de cas encore pour les stades disséminés de la maladie - ;**
  - **dans le cas d'un érythème migrant, le diagnostic clinique est suffisamment évocateur et la sérologie n'est pas assez sensible, par contre un traitement antibiotique immédiat est justifié et recommandé.**
  - **la détection dans le sang total ou le sérum, immédiatement après une piqure de tique (« dépistage ») est inutile en raison de la variabilité, de la faiblesse et de la lenteur de la mise en place de l'immunité individuelle, conduisant alors à une possible fausse sécurité devant un résultat négatif ;**

**Compte tenu des particularités cliniques de la Borréliose de Lyme et de l'insuffisance de ses performances, l'utilisation de ce test d'autodiagnostic est difficile à justifier.**

# Auto-tests

SYNDICAT DES BIOLOGISTES
Le syndicat de tous les biologistes médicaux

## Académie nationale de Pharmacie

**Rapport**

de l'Académie nationale de Pharmacie

***Autotests-TROD***

***Rôle du pharmacien d'officine***

***Décembre 2017***

Rapport adopté par le Conseil de l'Académie nationale de Pharmacie le 13 décembre 2017
Les auteurs déclarent ne pas avoir de conflits d'intérêts en relation avec ce rapport

# Auto-tests

## 2.3. Autotests

**Académie nationale de Pharmacie**

### 2.3.1. Définition et types d'autotests

Il s'agit d'un test, recueil ou traitement de signal biologique utilisé par l'usager ou son entourage et pour son seul usage, qui ne constitue ni un TROD, ni un examen de biologie médicale. Un autotest n'apporte qu'une orientation diagnostique et pas un diagnostic comme peut le faire un examen de biologie médicale.

- **Type A** : les systèmes prescrits par un médecin, qui utilisent un dispositif de mesure : il s'agit en particulier des glucomètres (recueil de sang capillaire ou capteur sous-cutané) ou des appareils de mesures de l'INR. Ces systèmes répondent à un besoin d'autosurveillance thérapeutique, sont actuellement validés sur le plan clinique et sont remboursés en France par l'Assurance Maladie.
- **Type B** : les tests de détection ou de recherche d'un signal biologique marqués « CE » et vendus en pharmacie d'officine, avec un objectif de dépistage ou d'orientation diagnostique. En pratique, les principaux autotests de ce type utilisés en France sont les suivants : test de grossesse, tests d'ovulation, « bandelettes » urinaires, éthylotests (bien que n'étant pas un DMDIV à proprement parler), VIH. Ces tests relèvent en général du monopole pharmaceutique et ne peuvent être vendus qu'en pharmacie, à l'exception des tests de grossesse, d'ovulation (hors pharmacies d'officine) et de détection des maladies infectieuses transmissibles (centres sanitaires).
- **Type C** : il s'agit de tests non marqués « CE » et vendus sans aucun contrôle, y compris sur Internet. La fiabilité de ces tests ne peut être vérifiée.

Concernant ces tests des types A et B, la réglementation de la vente est fondée aujourd'hui sur le marquage « CE » [I]. Celui-ci prévoit que l'évaluation du test est faite par le fabricant sur un mode d'auto-certification, sans qu'un organisme notifié ne vienne vérifier les allégations de la société.

# Auto-tests

| Tests sanguin | Avis |
|---|---|
| **VIH** | |
| **Cholestérol** | |
| **Carence en fer** | |
| **Thyroïde (TSH)** | |
| **Prostate (PSA)** | |
| **H. Pylori** | |
| **Allergie IgE** | |
| **Ac tétaniques** | |
| **Lyme (IgM)** | |
| **Test sur selles** | |
| **Cancer colorectal** | |
| **Tests urinaires** | |
| **Infection/albumine/Glucose** | |
| **Ménopause (FSH)** | |
| **Ovulation** | |

# Auto-tests

| Tests sanguin | Avis | |
|---|---|---|
| VIH | ✓ | utile |
| Cholestérol | | |
| Carence en fer | | |
| Thyroïde (TSH) | | |
| Prostate (PSA) | | |
| H. Pylori | | |
| Allergie IgE | | |
| Ac tétaniques | ✓ | utile |
| Lyme (IgM) | | |
| **Test sur selles** | **Avis** | |
| Cancer colorectal | | |
| **Tests urinaires** | **Avis** | |
| Infection/albumine/Glucose | ✓ | utile |
| Ménopause (FSH) | | |
| Ovulation | | |

# Auto-tests

| Tests sanguin | Avis | |
|---|---|---|
| **VIH** | ✓ | **utile** |
| **Cholestérol** | ≈ | **utilité faible** |
| **Carence en fer** | ≈ | **utilité faible** |
| **Thyroïde (TSH)** | ≈ | **utilité faible** |
| **Prostate (PSA)** | | |
| **H. Pylori** | | |
| **Allergie IgE** | | |
| **Ac tétaniques** | ✓ | **utile** |
| **Lyme (IgM)** | | |
| **Test sur selles** | **Avis** | |
| **Cancer colorectal** | | |
| **Tests urinaires** | **Avis** | |
| **Infection/albumine/Glucose** | ✓ | **utile** |
| **Ménopause (FSH)** | ≈ | **utilité faible** |
| **Ovulation** | ≈ | **utilité faible** |

# Auto-tests

| Tests sanguin | Avis | |
|---|---|---|
| **VIH** | ✓ | **utile** |
| **Cholestérol** | ≈ | **utilité faible** |
| **Carence en fer** | ≈ | **utilité faible** |
| **Thyroïde (TSH)** | ≈ | **utilité faible** |
| **Prostate (PSA)** | ✗ | **à éviter** |
| **H. Pylori** | ✗ | **à éviter** |
| **Allergie IgE** | ✗ | **à éviter** |
| **Ac tétaniques** | ✓ | **utile** |
| **Lyme (IgM)** | ✗ | **à éviter** |

Nous ne considérons pas comme favorable la balance bénéfice/risque de l'accès de tout usager à un autotest isolé de détection des anticorps sanguins IgM anti-*Borrelia*, compte tenu du risque majeur d'interprétation inadéquate.

| | | |
|---|---|---|
| **Infection/albumine/Glucose** | ✓ | **utile** |
| **Ménopause (FSH)** | ≈ | **utilité faible** |
| **Ovulation** | ≈ | **utilité faible** |

SYNDICAT DES BIOLOGISTES
Le syndicat de tous les biologistes médicaux

# CXCL-13 SUR LCR

# Dosage du CXCL-13 dans le LCR

JOURNAL OF CLINICAL MICROBIOLOGY, May 2011, p. 2027–2030 Vol. 49, No. 5
0095-1137/11/$12.00 doi:10.1128/JCM.00084-11


## Discriminating Lyme Neuroborreliosis from Other Neuroinflammatory Diseases by Levels of CXCL13 in Cerebrospinal Fluid▽†

N. D. van Burgel,[1]* F. Bakels,[2] A. C. M. Kroes,[1] and A. P. van Dam[3]

*Department of Medical Microbiology, Centre of Infectious Diseases, Leiden University Medical Centre, P.O. Box 9600 RC, Leiden, Netherlands[1]; Department of Neurology, Leiden University Medical Centre, P.O. Box 9600 RC, Leiden, Netherlands[2]; and Department of Medical Microbiology, OLVG Hospital, P.O. Box 95500, 1090 HM Amsterdam, Netherlands[3]*

Received 14 January 2011/Returned for modification 15 February 2011/Accepted 19 February 2011

**CXCL13 in cerebrospinal fluid (CSF) could be an important component for diagnosing Lyme neuroborreliosis (LNB). Levels of intrathecal CXCL13 were determined for 58 LNB patients and 210 controls; sensitivity was 88% and specificity was 89% (cutoff, 250 pg of CXCL13/ml of CSF). Elevated levels of CXCL13 can aid in the diagnosis of LNB, but levels should be interpreted with care.**

Diagnosing Lyme neuroborreliosis (LNB) is difficult because one of the most specific markers, the antibody index (AI), is negative in 22 to 45% of patients (1). Intrathecal levels of CXCL13 have been suggested to be a potential biomarker for LNB. CXCL13 is produced by antigen-presenting cells and is a selective chemoattractant for B cells and B-helper T cells. It has been shown that CXCL13 is expressed at high levels in cerebrospinal fluid (CSF) from LNB patients, while levels were barely detectable in CSF from subjects with noninflammatory neurological disease. Overall sensitivity for LNB ranged from 96 to 100%, and specificity ranged from 63 to 98% (3, 6, 11, 12). Case reports describing early diagnosis of LNB using CXCL13 levels in CSF have already been published (5, 9).

Our aim was to determine the diagnostic potential of levels of intrathecal CXCL13 to distinguish acute and late LNB from other central nervous system diseases in the pediatric and adult population.

Patients were identified retrospectively using the OLVG Hospital laboratory information management system. CSF and serum samples from 58 LNB patients before treatment were included. Criteria for diagnosing LNB patients were that their meningitis had no other cause and that they had three of the following four characteristics: positive serology at presentation, pleocytosis, positive AI with an Idetia Lyme neuroborreliosis kit (Idetia, Cambridge, United Kingdom), and objective neurological complaints with clinical improvement after treatment. From this group, definite LNB patients (n = 45) were those who had a pleocytosis and a positive AI, and probable LNB patients (n = 13) were those who had either pleocytosis (n = 12) or a positive AI (n = 1) (4). Ninety-one percent of the LNB patients presented within 6 months of the start of complaints; the range was 7 days to 48 months. Forty-one percent of LNB patients were children. Most LNB patients presented with a facial nerve paralysis (86%) or meningoradiculitis (26%). Seventeen percent of LNB patients reported experiencing erythema migrans (EM) before or at presentation.

As controls, we included 36 patients with Lyme borreliosis that did not meet the criteria for LNB, 93 patients with an infectious cause of meningitis/encephalitis, 62 patients with neurological inflammatory diseases, and 12 patients with non-inflammatory neurological complaints. Furthermore, seven HIV patients with no neurological complaints or evidence of an intrathecal infection were tested. For patient characteristics, see Table S1 in the supplemental material.

CSF samples were tested with a Quantikine human CXCL13/BLC/BCA-1 immunoassay (R&D Systems, Minneapolis, MN).

Results of the levels of CXCL13 in CSF are shown in Fig. 1. Median levels of CXCL13 were significantly elevated in LNB patients compared to those in the Lyme neuroborreliosis controls (medians, 1,183 and 5 pg of CXCL13/ml of CSF, respectively; $P < 0.001$).

Receiver operating characteristic (ROC) analysis revealed an optimal cutoff of 250 pg/ml, which resulted in 88% sensitivity and 89% specificity (Fig. 2). Results of intrathecal CXCL13 using the cutoff of 250 pg/ml for LNB patients and controls are shown in Table 1. CSF levels of CXCL13 correlated with the amount of intrathecal leukocytes in the CSF at presentation ($R = 0.172$; $P < 0.001$), but the seven LNB samples with CXCL13 levels of <250 pg/ml did not have exceptionally low CSF leukocyte counts (see Fig. S1 in the supplemental material).

Previously, a sensitivity of 96 to 100% was reported for CXCL13 in cases of LNB, but two studies did not define a cutoff (3, 6). One study defined a cutoff for CXCL13 levels expressed as ng of CXCL13/g of total protein in CSF (11). ROC curve analysis for the amount of CXCL13 per milliliter compared to the amount of CXCL13 per gram of total protein showed a similar area under the curve (AUC) in our population (0.91 to 0.90, respectively). Analysis using the cutoff of 337 ng/g in our population led to a decrease in sensitivity and specificity to 82% and 88%, respectively. One study defined a cutoff of 142 pg/ml of CSF. In our study, such a cutoff led to a sensitivity of 90% and a specificity of 84% (12).

* Corresponding author. Mailing address: Department of Medical Microbiology, Leiden University Medical Centre, P.O. Box 9600, 2300 RC, Leiden, Netherlands. Phone: (31) 71 526 3931. Fax: (31) 71 524 8148. E-mail: N.D.van_Burgel@LUMC.nl.

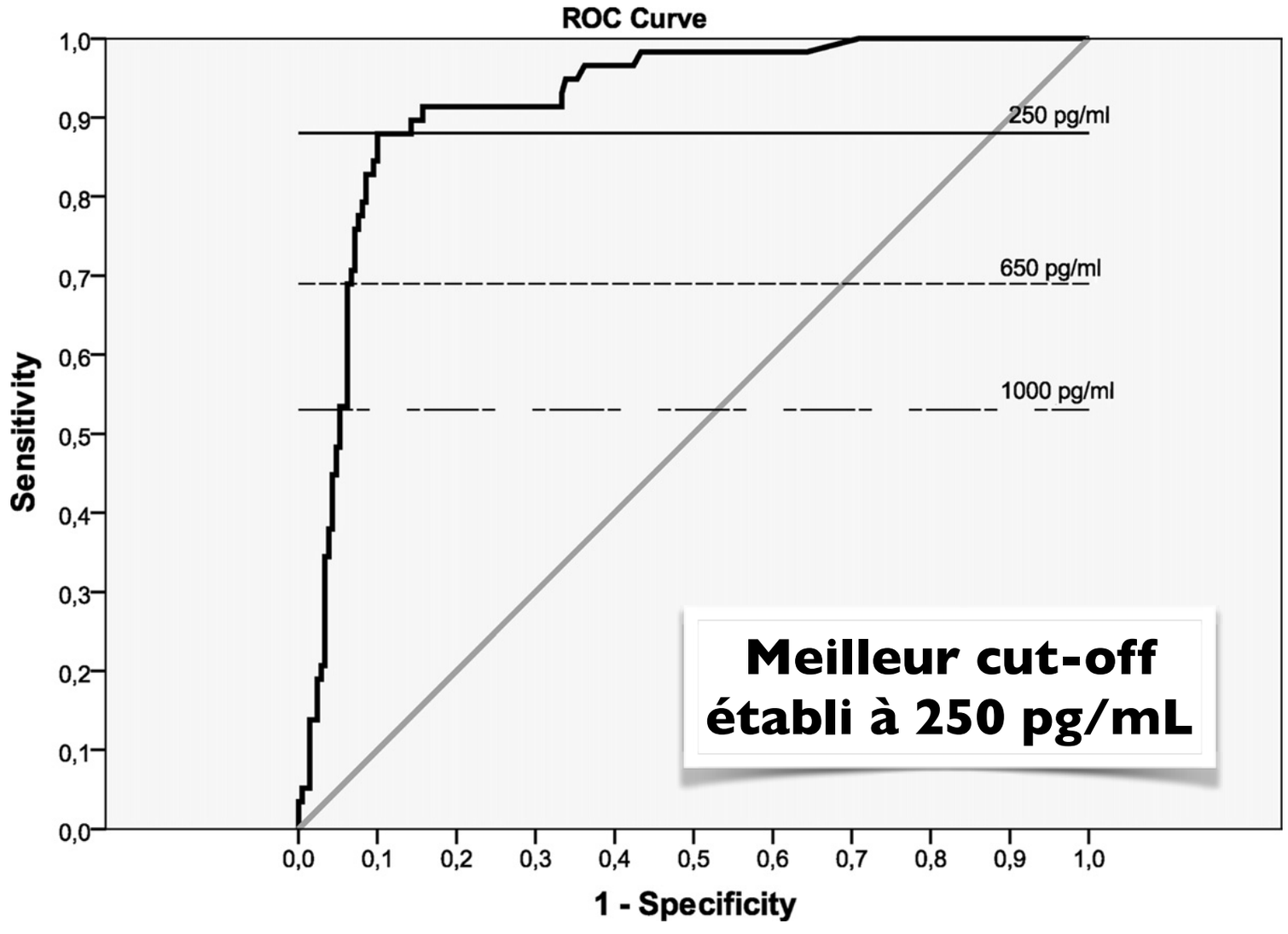
† Supplemental material for this article may be found at http://jcm.asm.org/.

▽ Published ahead of print on 2 March 2011.



## Design de l'étude

- 58 NB (avant TT) sans autre cause méningite avec 3/4 éléments suivants
  - ☆ sérologie pos / SIT pos / pléïocytose / signes typiques améliorés ss TT
- 210 contrôles :
  - ☆ 36 Lyme (non-NB), 93 méningites, 62 inflamm, 12 prb neuros, 7 HIV




**Meilleur cut-off établi à 250 pg/mL**

**Se : 88 %**
**Spé : 89 %**

# Dosage du CXCL-13 dans le LCR

JOURNAL OF CLINICAL MICROBIOLOGY, May 2011, p. 2027–2030 Vol. 49, No. 5
0095-1137/11/$12.00 doi:10.1128/JCM.00084-11


Discriminating Lyme Neuroborreliosis from Other Neuroinflammatory Diseases by Levels of CXCL13 in Cerebrospinal Fluid▽†

N. D. van Burgel,[1]* F. Bakels,[2] A. C. M. Kroes,[1] and A. P. van Dam[3]

*Department of Medical Microbiology, Centre of Infectious Diseases, Leiden University Medical Centre, P.O. Box 9600 RC, Leiden, Netherlands[1]; Department of Neurology, Leiden University Medical Centre, P.O. Box 9600 RC, Leiden, Netherlands[2]; and Department of Medical Microbiology, OLVG Hospital, P.O. Box 95500, 1090 HM Amsterdam, Netherlands[3]*

Received 14 January 2011/Returned for modification 15 February 2011/Accepted 19 February 2011

**CXCL13 in cerebrospinal fluid (CSF) could be an important component for diagnosing Lyme neuroborreliosis (LNB). Levels of intrathecal CXCL13 were determined for 58 LNB patients and 210 controls; sensitivity was 88% and specificity was 89% (cutoff, 250 pg of CXCL13/ml of CSF). Elevated levels of CXCL13 can aid in the diagnosis of LNB, but levels should be interpreted with care.**

Diagnosing Lyme neuroborreliosis (LNB) is difficult because one of the most specific markers, the antibody index (AI), is negative in 22 to 45% of patients (1). Intrathecal levels of CXCL13 have been suggested to be a potential biomarker for LNB. CXCL13 is produced by antigen-presenting cells and is a selective chemoattractant for B cells and B-helper T cells. It has been shown that CXCL13 is expressed at high levels in cerebrospinal fluid (CSF) from LNB patients, while levels were barely detectable in CSF from subjects with noninflammatory neurological disease. Overall sensitivity for LNB ranged from 96 to 100%, and specificity ranged from 63 to 98% (3, 6, 11, 12). Case reports describing early diagnosis of LNB using CXCL13 levels in CSF have already been published (5, 9).

Our aim was to determine the diagnostic potential of levels of intrathecal CXCL13 to distinguish acute and late LNB from other central nervous system diseases in the pediatric and adult population.

Patients were identified retrospectively using the OLVG Hospital laboratory information management system. CSF and serum samples from 58 LNB patients before treatment were included. Criteria for diagnosing LNB patients were that their meningitis had no other cause and that they had three of the following four characteristics: positive serology at presentation, pleocytosis, positive AI with an Idetia Lyme neuroborreliosis kit (Instol, Cambridge, United Kingdom), and objective neurological complaints with clinical improvement after treatment. From this group, definite LNB patients (*n* = 45) were those who had a pleocytosis and a positive AI, and probable LNB patients (*n* = 13) were those who had either pleocytosis (*n* = 12) or a positive AI (*n* = 1) (4). Ninety-one percent of the LNB patients presented within 6 months of the start of complaints; the range was 7 days to 48 months. Forty-one percent of LNB patients were children. Most LNB patients presented with a facial nerve paralysis (60%) or meningoradiculitis (20%). Seventeen percent of LNB patients reported experiencing erythema migrans (EM) before or at presentation.

As controls, we included 36 patients with Lyme borreliosis that did not meet the criteria for LNB, 93 patients with an infectious cause of meningitis/encephalitis, 62 patients with neurological inflammatory diseases, and 12 patients with non-inflammatory neurological complaints. Furthermore, seven HIV patients with no neurological complaints or evidence of an intrathecal infection were tested. For patient characteristics, see Table S1 in the supplemental material.

CSF samples were tested with a Quantikine human CXCL13/BLC/BCA-1 immunoassay (R&D Systems, Minneapolis, MN).

Results of the levels of CXCL13 in CSF are shown in Fig. 1. Median levels of CXCL13 were significantly elevated in LNB patients compared to those in the Lyme nonneuroborreliosis controls (medians, 1,183 and 3 pg of CXCL13/ml of CSF, respectively; $P < 0.001$).

Receiver operating characteristic (ROC) analysis revealed an optimal cutoff of 250 pg/ml, which resulted in 88% sensitivity and 89% specificity (Fig. 2). Results of intrathecal CXCL13 using the cutoff of 250 pg/ml for LNB patients and controls are shown in Table 1. CSF levels of CXCL13 correlated with the amount of intrathecal leukocytes in the CSF at presentation ($R = 0.172$; $P < 0.001$), but the seven LNB samples with CXCL13 levels of <250 pg/ml did not have unequivocally low CSF leukocyte counts (see Fig. S1 in the supplemental material).

Previously, a sensitivity of 96 to 100% was reported for CXCL13 in cases of LNB, but two studies did not define a cutoff (3, 6). One study defined a cutoff for CXCL13 levels expressed as ng of CXCL13/g of total protein in CSF (11). ROC curve analysis for the amount of CXCL13 per milliliter compared to the amount of CXCL13 per gram of total protein showed a similar area under the curve (AUC) in our population (0.91 to 0.90, respectively). Analysis using the cutoff of 337 ng/g in our population led to a decrease in sensitivity and specificity to 82% and 88%, respectively. One study defined a cutoff of 142 pg/ml of CSF. In our study, such a cutoff led to a sensitivity of 90% and a specificity of 84% (12).

* Corresponding author. Mailing address: Department of Medical Microbiology, Leiden University Medical Centre, P.O. Box 9600, 2300 RC, Leiden, Netherlands. Phone: (31) 71 526 3931. Fax: (31) 71 524 8148. E-mail: N.D.van_Burgel@LUMC.nl.

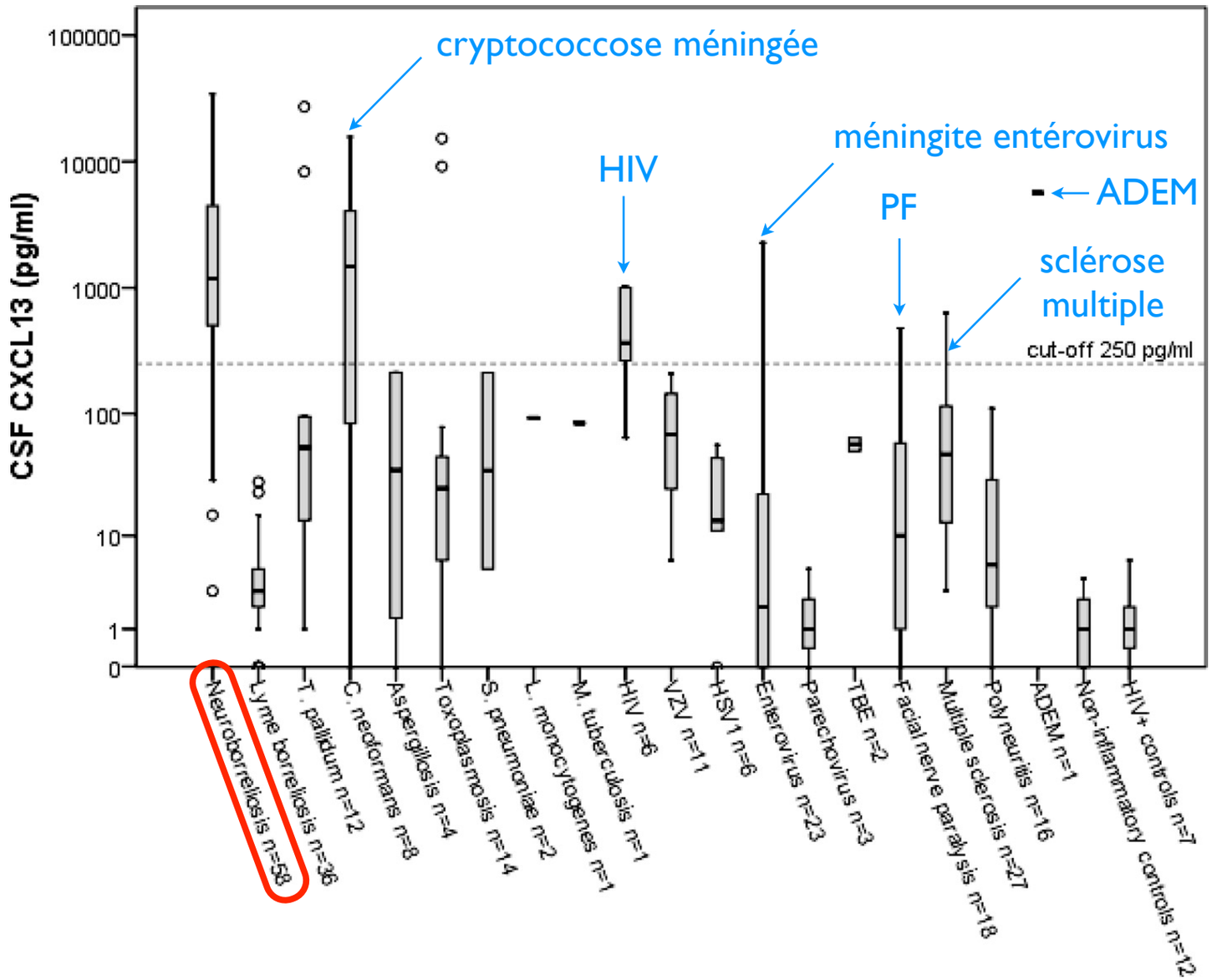
† Supplemental material for this article may be found at http://jcm.asm.org.

▽ Published ahead of print on 2 March 2011.



## Design de l'étude

- 58 NB (avant TT) sans autre cause méningite avec 3/4 éléments suivants
  - ☆ sérologie pos / SIT pos / pléïocytose / signes typiques améliorés ss TT
- 210 contrôles :
  - ☆ 36 Lyme (non-NB), 93 méningites, 62 inflamm, 12 prb neuros, 7 HIV




**Se : 88 %**
**Spé : 89 %**

# Dosage du CXCL-13 dans le LCR

JOURNAL OF CLINICAL MICROBIOLOGY, May 2011, p. 2027–2030 Vol. 49, No. 5
0095-1137/11/$12.00 doi:10.1128/JCM.00084-11


## Discriminating Lyme Neuroborreliosis from Other Neuroinflammatory Diseases by Levels of CXCL13 in Cerebrospinal Fluid▽†

N. D. van Burgel,[1]* F. Bakels,[2] A. C. M. Kroes,[1] and A. P. van Dam[3]

*Department of Medical Microbiology, Centre of Infectious Diseases, Leiden University Medical Centre, P.O. Box 9600 RC, Leiden, Netherlands[1]; Department of Neurology, Leiden University Medical Centre, P.O. Box 9600 RC, Leiden, Netherlands[2]; and Department of Medical Microbiology, OLVG Hospital, P.O. Box 95500, 1090 HM Amsterdam, Netherlands[3]*

Received 14 January 2011/Returned for modification 15 February 2011/Accepted 19 February 2011

**CXCL13 in cerebrospinal fluid (CSF) could be an important component for diagnosing Lyme neuroborreliosis (LNB). Levels of intrathecal CXCL13 were determined for 58 LNB patients and 210 controls; sensitivity was 88% and specificity was 89% (cutoff, 250 pg of CXCL13/ml of CSF). Elevated levels of CXCL13 can aid in the diagnosis of LNB, but levels should be interpreted with care.**

Diagnosing Lyme neuroborreliosis (LNB) is difficult because one of the most specific markers, the antibody index (AI), is negative in 21 to 45% of patients (1). Intrathecal levels of CXCL13 have been suggested to be a potential biomarker for LNB. CXCL13 is produced by antigen-presenting cells and is a selective chemoattractant for B cells and B-helper T cells. It has been shown that CXCL13 is expressed at high levels in cerebrospinal fluid (CSF) from LNB patients, while levels were barely detectable in CSF from subjects with noninflammatory neurological disease. Overall sensitivity for LNB ranged from 96 to 100%, and specificity ranged from 83 to 98% (3, 6, 11, 12). Case reports describing early diagnosis of LNB using CXCL13 levels in CSF have already been published (5, 9).

Our aim was to determine the diagnostic potential of levels of intrathecal CXCL13 to distinguish acute and late LNB from other central nervous system diseases in the pediatric and adult population.

Patients were identified retrospectively using the OLVG Hospital laboratory information management system. CSF and serum samples from 58 LNB patients before treatment were included. Criteria for diagnosing LNB patients were that their meningitis had no other cause and that they had three of the following four characteristics: positive serology at presentation, pleocytosis, positive AI with an Idexx Lyme neuroborreliosis kit (Idexx, Cambridge, United Kingdom), and objective neurological complaints with clinical improvement after treatment. From this group, definite LNB patients ($n = 45$) were those who had a pleocytosis and a positive AI, and probable LNB patients ($n = 13$) were those who had either pleocytosis ($n = 12$) or a positive AI ($n = 1$) (4). Ninety-one percent of the LNB patients presented within 6 months of the start of complaints; the range was 7 days to 48 months. Forty-one percent of LNB patients were children. Most LNB patients presented with a facial nerve paralysis (86%) or meningoradiculitis (26%). Seventeen percent of LNB patients reported experiencing erythema migrans (EM) before or at presentation.

As controls, we included 36 patients with Lyme borreliosis that did not meet the criteria for LNB, 93 patients with an infectious cause of meningitis/encephalitis, 62 patients with neurological inflammatory diseases, and 12 patients with non-inflammatory neurological complaints. Furthermore, seven HIV patients with no neurological complaints or evidence of an intrathecal infection were tested. For patient characteristics, see Table S1 in the supplemental material.

CSF samples were tested with a Quantikine human CXCL13/BLC/BCA-1 immunoassay (R&D Systems, Minneapolis, MN).

Results of the levels of CXCL13 in CSF are shown in Fig. 1. Median levels of CXCL13 were significantly elevated in LNB patients compared to those in the Lyme nonneuroborreliosis controls (medians, 1,183 and 3 pg of CXCL13/ml of CSF, respectively; $P < 0.001$).

Receiver operating characteristic (ROC) analysis revealed an optimal cutoff of 250 pg/ml, which resulted in 88% sensitivity and 89% specificity (Fig. 2). Results of intrathecal CXCL13 using the cutoff of 250 pg/ml for LNB patients and controls are shown in Table 1. CSF levels of CXCL13 correlated with the amount of intrathecal leukocytes in the CSF at presentation ($R = 0.172$; $P < 0.001$), but the seven LNB samples with CXCL13 levels of <250 pg/ml did not have exceptionally low CSF leukocyte counts (see Fig. S1 in the supplemental material).

Previously, a sensitivity of 96 to 100% was reported for CXCL13 in cases of LNB, but two studies did not define a cutoff (3, 6). One study defined a cutoff for CXCL13 levels expressed as ng of CXCL13/g of total protein in CSF (11). ROC curve analysis for the amount of CXCL13 per milliliter compared to the amount of CXCL13 per gram of total protein showed a similar area under the curve (AUC) in our population (0.91 to 0.90, respectively). Analysis using the cutoff of 337 ng/g in our population led to a decrease in sensitivity and specificity to 82% and 88%, respectively. One study defined a cutoff of 142 pg/ml of CSF. In our study, such a cutoff led to a sensitivity of 90% and a specificity of 84% (12).

* Corresponding author. Mailing address: Department of Medical Microbiology, Leiden University Medical Centre, P.O. Box 9600, 2300 RC, Leiden, Netherlands. Phone: (31) 71 526 3931. Fax: (31) 71 524 8148. E-mail: N.D.van_Burgel@LUMC.nl.

† Supplemental material for this article may be found at http://jcm.asm.org.

▽ Published ahead of print on 2 March 2011.



## Design de l'étude

- 58 NB (avant TT) sans autre cause méningite avec 3/4 éléments suivants
  - ☆ sérologie pos / SIT pos / pléïocytose / signes typiques améliorés ss TT
- 210 contrôles :
  - ☆ 36 Lyme (non-NB), 93 méningites, 62 inflamm, 12 prb neuros, 7 HIV

**Paramètre intéressant**
**mais manque de spécificité**

**Non recommandé pour le diagnostic**

**Se : 88 %**
**Spé : 89 %**

# INDICATIONS EN FONCTION DU CONTEXTE CLINIQUE

# Piqûre de tique


risque de transmission : 1-4 % ; ↗ avec durée attachement après 24h

*aucun examen complémentaire*

- Ce qu'il faut faire
  - **retirer la tique** (<24h, tire-tique)
  - **désinfection** cutanée locale
  - **surveiller** la zone piquée (> 1 mois)
  - mise à jour vaccins (tétanos)

- Et *SURTOUT* ...
  - **pas de sérologie**
  - **pas d'ATB systématique**
  - **pas de test sur la tique**

# Piqûre de tique


risque de transmission : 1-4 % ; ↗ avec durée attachement après 24h

## *Discuter une éventuelle antibioprophylaxie*

- À discuter si : zone endémie, piqûres multiples, attachement >72h
  - femmes enceintes : amox 3 g/j p.o. 10 à 14 j
  - enfant <8 ans : pas de recos, si ATB = amox 50 mg/kg/j p.o. 10 j
  - immunodéprimés : si ATB = 3 g/j p.o. ou doxycycline p.o. monodose 200 mg 10 à 21 j

# Érythème migrant

http://www.zeckenrollen.de/Zeckenschutz/Zecken-und-Krankheiten#

# Érythème migrant

## Caractéristiques épidémio-cliniques

- la + fréq des manifestations (≈ 80 % cas borréliose Lyme)
- intervalle libre après la piqûre (qq jours à qq sem)
- érythème maculopapuleux d'évolution annulaire centrifuge
- éclaircissement central inconstant, taille > 5 cm



**pathognomonique** de la borréliose de Lyme

## Diagnostic

EM typique

CLINIQUE

*aucun examen complémentaire*

PAS DE SÉROLOGIE !!

# Érythème migrant

## Caractéristiques épidémio-cliniques

- la + fréq des manifestations (≈ 80 % cas borréliose Lyme)
- intervalle libre après la piqûre (qq jours à qq sem)
- érythème maculopapuleux d'évolution annulaire centrifuge
- éclaircissement central inconstant, taille > 5 cm

**pathognomonique** de la borréliose de Lyme



## Diagnostic

EM typique → CLINIQUE → *aucun examen complémentaire*

PAS DE SÉROLOGIE !!

- si doute mesurer et revoir le patient à 48-72h : si ∅ ↗ = EM et traitement

# Érythème migrant

## Caractéristiques épidémio-cliniques

- la + fréq des manifestations (≈ 80 % cas borréliose Lyme)
- intervalle libre après la piqûre (qq jours à qq sem)
- érythème maculopapuleux d'évolution annulaire centrifuge
- éclaircissement central inconstant, taille > 5 cm

**pathognomonique** de la borréliose de Lyme



## Diagnostic

EM typique → CLINIQUE

EM **a**typique → AVIS DERMATO !!! → PCR / culture → biopsie cutanée

PAS DE SÉROLOGIE !!

# Érythème migrant

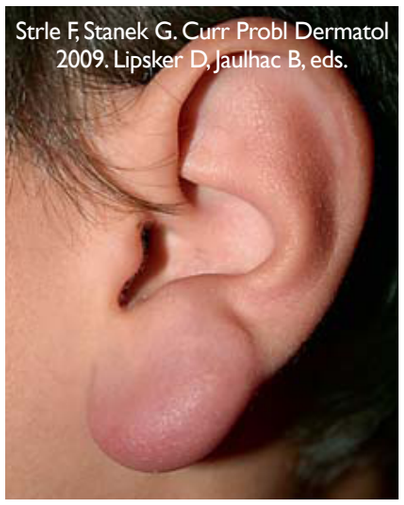
- Le message clé du dermatologue

"Toute **tache rouge ou rose** qui **grandit régulièrement et dépasse 5 cm** de diamètre chez quelqu'un qui est **exposé aux tiques** doit être considéré comme un **EM**, avec un **traitement antibiotique, sans test biologique** préalable ou ultérieur, mais avec **consultation de contrôle** entre 2 et 4 semaines plus tard. L'**absence de guérison doit conduire à une consultation chez un dermatologue** car ce n'est alors pas un EM"

# Lymphocytome borrélien

## Caractéristiques épidémio-cliniques

- manifestation rare (≈ 2 % cas) des borrélioses européennes, + fréq chez enfant
- lésion nodulaire : lobe oreille / région aréolo-mammelonnaire / scrotum ...


Strle F, Stanek G. Curr Probl Dermatol 2009. Lipsker D, Jaulhac B, eds.


Stanek G. et coll, Lancet 2003


Müllegger RR et coll, Am J Clin Dermatol 2008

## Diagnostic

CLINIQUE + Sérologie Lyme +

séro positive >90% des cas
si lésion récente, séro de contrôle possible

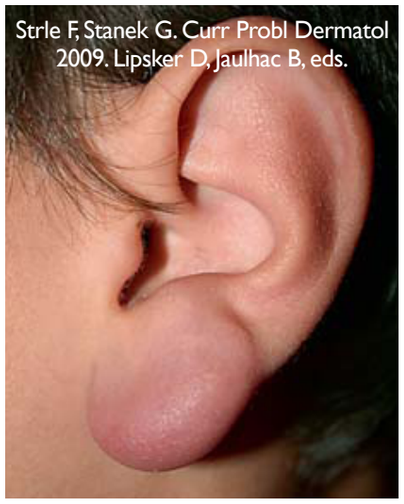
si doute

AVIS DERMATO !!!

# Lymphocytome borrélien

## Caractéristiques épidémio-cliniques

- manifestation rare (≈ 2 % cas) des borrélioses européennes, + fréq chez enfant
- lésion nodulaire : lobe oreille / région aréolo-mammelonnaire / scrotum ...


Strle F, Stanek G. Curr Probl Dermatol 2009. Lipsker D, Jaulhac B, eds.


Stanek G. et coll, Lancet 2003


Müllegger RR et coll, Am J Clin Dermatol 2008

## Diagnostic

CLINIQUE + Sérologie Lyme +

séro positive >90% des cas
si lésion récente, séro de contrôle possible

si doute : PCR / culture ; Histologie

biopsie cutanée

# Acrodermatite chronique atrophiante

## Caractéristiques épidémio-cliniques

- manifestation rare (≈ 5 % cas) des borrélioses européennes, + fréq chez sujet âgé
- survenue très tardive : pls mois – pls années ap piqûre
- lésions inflammatoires initiales puis lésions atrophiques irréversibles


Müllegger RR et coll, Am J Clin Dermatol 2008


Müllegger RR et coll, Am J Clin Dermatol 2008


Strle F, Stanek G. Curr Probl Dermatol 2009, Lipsker D, Jaulhac B, eds.


Stanek G. et coll, Lancet 2003


Müllegger RR et coll, Am J Clin Dermatol 2008

## Diagnostic

CLINIQUE + Sérologie Lyme +

séro négative ?
CHERCHER UNE AUTRE ÉTIOLOGIE !

si doute

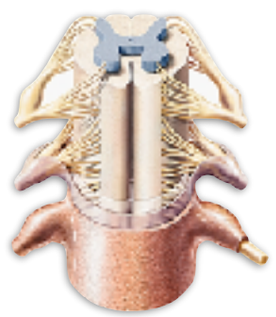
Histologie — biopsie cutanée

PCR / culture

# Neuroborréliose

## Neuroborréliose précoce

- 2e manifestation clinique la plus fréq. après l'érythème migrant
- méningoradiculites sensitives / paralysie nerf crâniens (VII ++ enfant)
- méningite "biologique" (↗ lymphos, ↗ protéines, ≈ glucose)



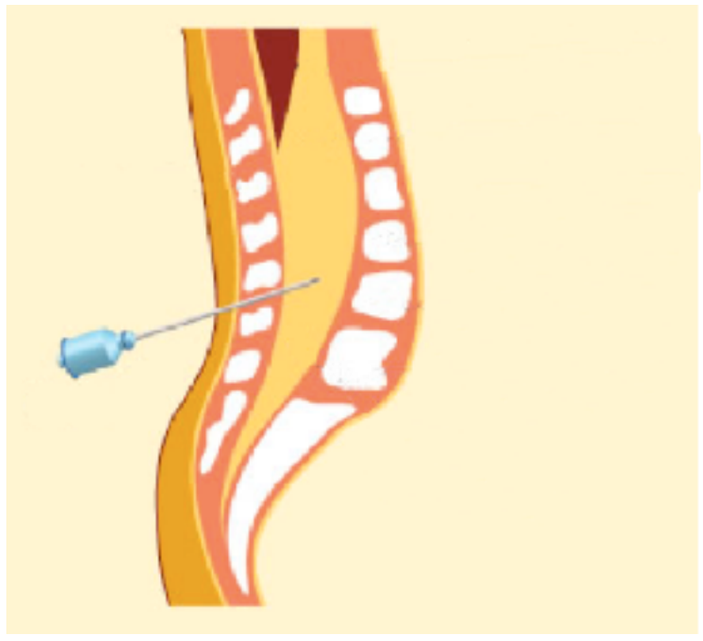
racines nerveuses

nerf facial

## Neuroborréliose tardive

- rare : atteintes encéphaliques/médullaires chroniques

## Diagnostic

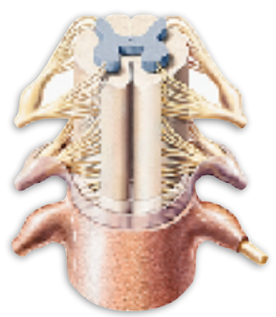


**ponction lombaire**

# Neuroborréliose

## Neuroborréliose précoce

- 2e manifestation clinique la plus fréq. après l'érythème migrant
- méningoradiculites sensitives / paralysie nerf crâniens (VII ++ enfant)
- méningite "biologique" (↗ lymphos, ↗ protéines, ≈ glucose)



racines nerveuses

nerf facial

## Neuroborréliose tardive

- rare : atteintes encéphaliques/médullaires chroniques

## Diagnostic

Méningite lymphocytaire

Peuvent manquer
si atteinte périphérique isolée ou phase initiale

Sérologie + LCR

Sérologie + sérum

Indice SIT

si doute

PCR / culture

LCR

Très faible sensibilité (<10%)
si pos diagnostic certain
Si nég pas possible de conclure

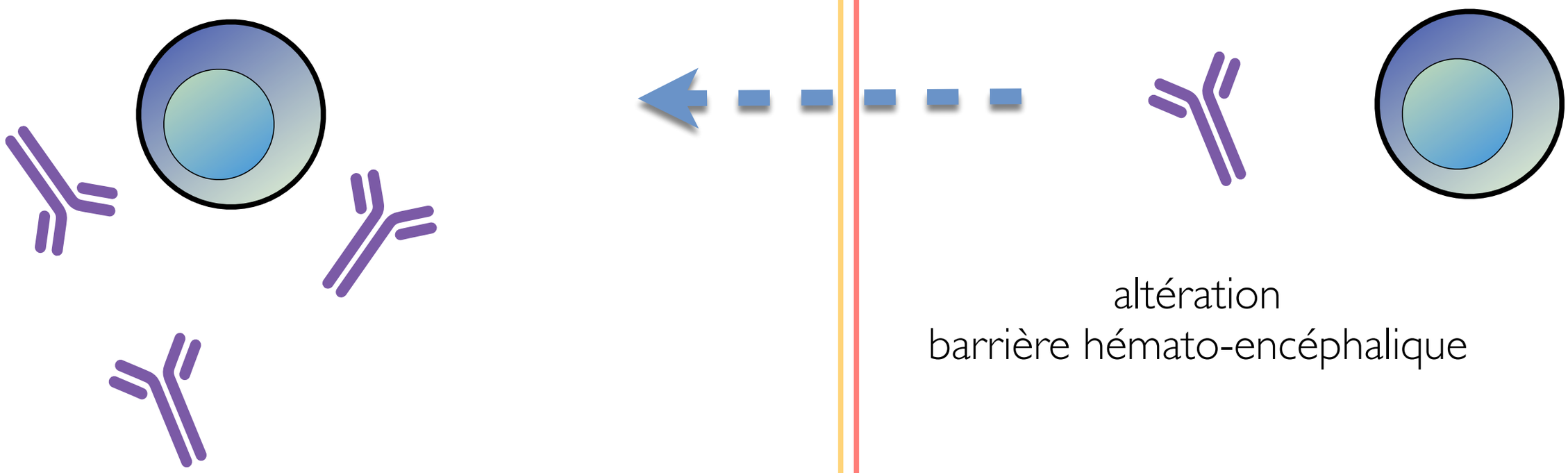
# Indice de synthèse intrathécale

**Recherche d'une synthèse intrathécale d'Ac anti-*Borrelia***

**Gold standard pour le diagnostic biologique des neuroborrélioses**

**production locale d'Ac**

**transfert passif d'Ac**



# Indice de synthèse intrathécale

**Recherche d'une synthèse intrathécale d'Ac anti-*Borrelia***

## échantillons à recueillir

- LCR : 1 mL
- sérum

LCR et sérum sont à prélever le même jour !!

## dosages

- labo de microbiologie : **sérologie Lyme ELISA** (sérum/LCR)
- labo de biochimie : **IgG totales** (sérum/LCR)

$$\text{Indice SIT} = \frac{\text{unités ELISA (LCR) x IgG totales (sérum)}}{\text{unités ELISA (sérum) x IgG totales (LCR)}}$$

**1,5 ≤ SIT < 2 : douteux** / **SIT ≥ 2 : positif**

sensibilité 75-95% ; spécificité 97% si SIT >2

# Atteintes articulaires

## Types d'atteintes

- Arthralgies – Arthrites

## Caractéristiques cliniques de l'arthrite

- mono/oligoarthrite – grosses articulations : genou ++
- délai survenue : qq sem à plusieurs mois
- **biologie sanguine peu perturbée** (GB, VS, CRP subnormales)
- **liquide synovial inflammatoire**

## Diagnostic

Faisceau d'arguments

Exclusion autres causes

manifestations associées
EM / neuroborréliose ...

Sérologie Lyme +

séro négative ?
CHERCHER UNE AUTRE ÉTIOLOGIE !

BONNE SENSIBILITÉ
85 %

si doute

SENSIBILITÉ
TRÈS FAIBLE

PCR / culture

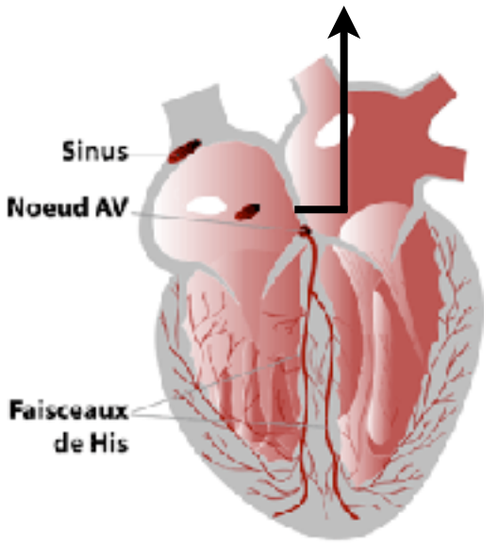
liq articulaire / biopsie synoviale

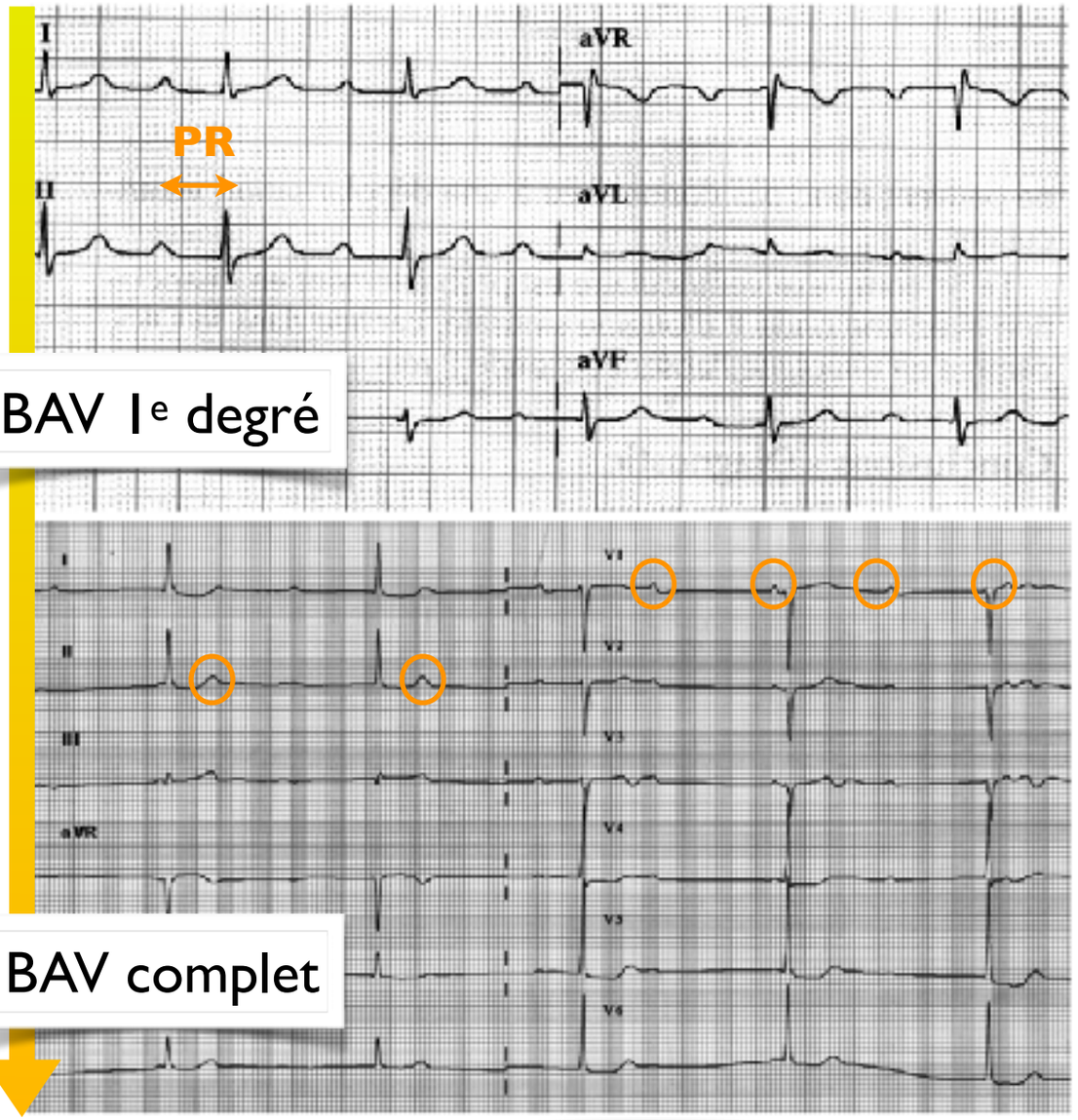
Biologie articulaire

# Atteinte cardiaque

## Troubles de la conduction

- supraventriculaires ++ (BAV de gravité variable)




## Atteintes des tuniques cardiaques

- **myopéricardites** endocardite (Hidri N et coll, CMI 2012)

ECG/imagerie

manifestations associées
EM / neuroborréliose ...

Sérologie Lyme +

Exclusion autres causes

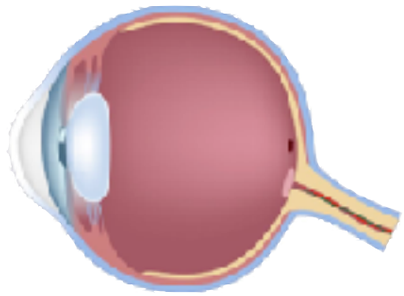
Faisceau d'arguments

PCR / culture

biopsie tissulaire cardiaque ...

# Atteinte oculaire

## Types d'atteinte



- toutes les structures anatomiques de l'oeil peuvent être concernées
- uvéite, kératite, rétinopathie, épisclérite, neuropathie ophtalmique …
- formes tardives : uvéite / neuropathie optique (rétro-bulbaire ou inflammatoire α aiguë)

Avis Ophtalmo spécialisé

Séro Lyme + IgG

si doute

PCR / culture

Sur humeur aqueuse et LCR

# Suivi

- Piqûre de tique
  - pas de sérologie (ni aucun autre examen complémentaire), pas de traitement antibiotique
  - suivi = surveillance (patient) de la zone piquée (> 1 mois) : cst si apparition EM ou signes gnrx
- Erythème migrant
  - pas de sérologie, traitement antibiotique
  - disparition EM parfois > 1 mois
  - suivi = clinique, vérifier disparition EM et non apparition manifestations disséminées
  - pas de sérologie pour suivre l'efficacité du TT : inutile !!!
- Manifestations disséminées de la Borréliose de Lyme
  - sérologie +/- examens complémentaires, traitement antibiotique
  - récupération lente des signes cliniques (ACA irréversible) : à expliquer au patient ++
  - suivi = clinique, 2 mois après la fin du TT, puis encore 2 mois plus tard
  - pas de sérologie pour suivre l'efficacité du TT : inutile !!!
  - si non réponse au TT : diagnostic erroné (autre étiologie) ? discuter nouveau TT (observance ?)

*Diapos B. Jaulhac*

# Diagnostic biologique Borréliose de Lyme

Document destiné aux biologistes - Décembre 2015

LE POINT SUR

RISQUES INFECTIEUX
> Zoonoses

## Borréliose de Lyme
## Diagnostic biologique

La borréliose de Lyme est :

- l'anthropozoonose la plus fréquente de l'hémisphère Nord,
- transmise par piqûre de tique avec un pic de fréquence d'avril à novembre,
- due à des spirochètes du genre *Borrelia* : les espèces pathogènes responsables sont regroupées dans le complexe *Borrelia burgdorferi* sensu lato (*B. burgdorferi* sl).

En Europe, on trouve essentiellement : *B. burgdorferi* sensu stricto (*B. burgdorferi* ss), *B. garinii* et *B. afzelii.*

Après une piqûre de tique infectante, 95% des sujets font une séroconversion sans signes cliniques.
Seuls 5% des sujets développent une infection active qui **peut** évoluer schématiquement en 3 phases :

### Manifestations cliniques de la borréliose de Lyme et diagnostic biologique

**→ 1 : Phase précoce localisée : érythème migrant (EM)**

- Délai d'apparition : entre 3 et 30 jours après la piqûre
- Seule manifestation de la maladie dans 80% des cas
- **La sérologie n'est pas indiquée à ce stade de la maladie**

**→ 2 : Phase précoce disséminée** (environ 15% des cas si absence de traitement antibiotique)

| Manifestations cliniques principales | | Sérologie | Examens complémentaires* |
|---|---|---|---|
| → neurologique | • méningoradiculites<br>• paralysie faciale<br>• syndrome méningé | Sang + LCR le même jour [synthèse intrathécale (SIT) IgG spécifiques] : sensibilité 75 à 95%, spécificité 97% si SIT>2 | LCR :<br>PCR uniquement si moins de 3 semaines d'évolution |
| → articulaire | • mono ou oligoarthrite<br>• grosse articulation (genou) | Positive IgG +++<br>(proche de 100%) | liquide articulaire :<br>PCR |

**→ 3 : Phase tardive** (plusieurs mois (> 6 mois) ou années après le début de l'infection non traitée)

| Manifestations cliniques principales | | Sérologie | Examens optionnels* |
|---|---|---|---|
| → cutanée | • Acrodermatite chronique atrophiante (ACA) | Positive (100%)<br>IgG +++ | biopsie cutanée :<br>PCR et histologie |

→ autres : **neurologiques** (encéphalomyélites chroniques, polyneuropathies sensitives axonales), **articulaires** (arthrites chroniques récidivantes) => examens biologiques identiques phase précoce disséminée.

* diagnostic direct par PCR => si positif : diagnostic certain ; si négatif : ne permet pas de conclure.

Liberté • Égalité • Fraternité
RÉPUBLIQUE FRANÇAISE
MINISTÈRE DES AFFAIRES SOCIALES, DE LA SANTÉ ET DES DROITS DES FEMMES

### → Renseignements cliniques à recueillir au moment du prélèvement

- Piqûre connue par une tique ? si oui : date de la dernière piqûre ?
- Signes cutanés : érythème migrant (EM)? si oui, date début de l'EM ?
  autres lésions cutanées ? → à préciser et date de début
- Signes neurologiques : méningo-radiculite ? Paralysie faciale ? si oui, date de début ?
  autres ? → à préciser et date de début
- Signes articulaires : arthrite ? arthralgies ? si oui, date de début ?

*Selon la norme NF EN ISO 15189 : 2012, la prescription doit fournir les informations cliniques pertinentes pour la réalisation de l'examen et l'interprétation des résultats*

**Situations pour lesquelles la sérologie n'a pas d'indication :**

- **Erythème migrant typique** (si EM atypique, ne pas faire de sérologie mais demander un avis dermatologique)
- **Sujet asymptomatique**
- **Piqûre de tique sans signes cliniques**
- **Dépistage des sujets exposés**
- **Contrôle sérologique des patients traités**

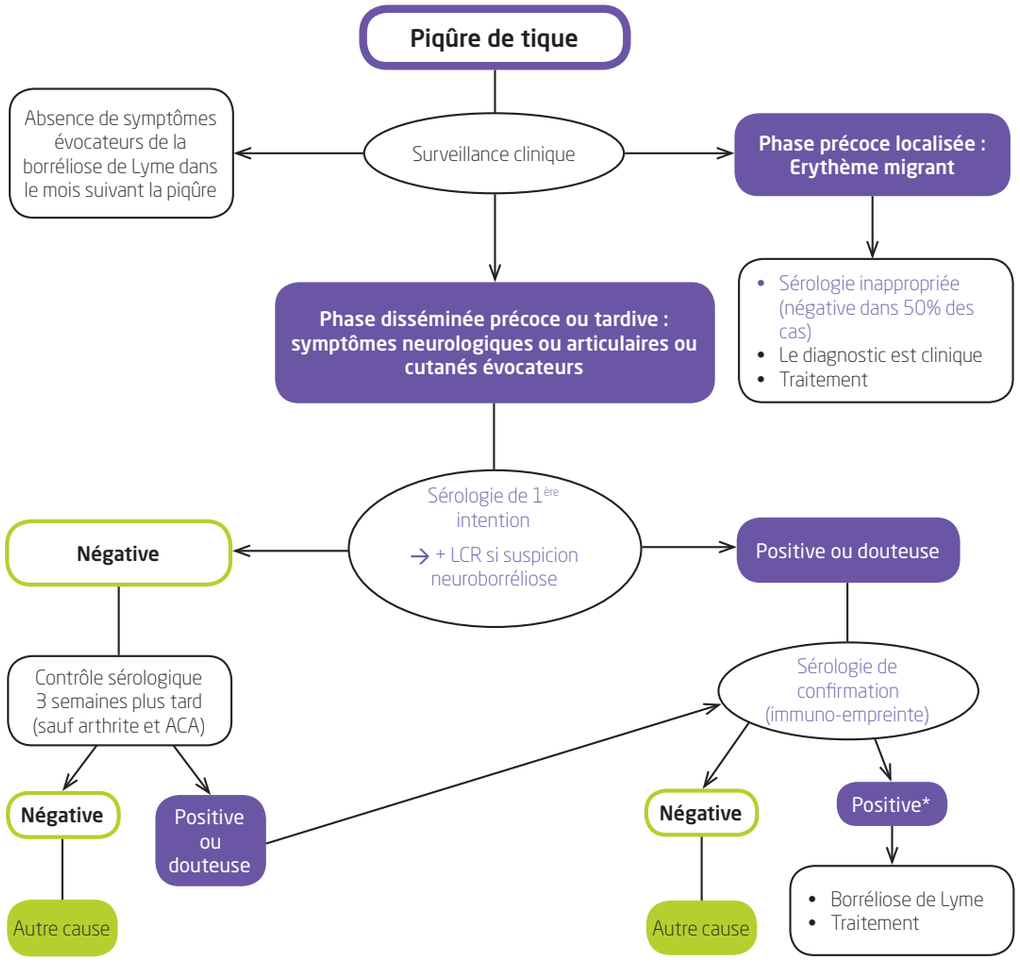
### → Limites de la sérologie

*A l'exception de l'érythème migrant typique, la positivité d'un test biologique est requise pour confirmer le diagnostic de borréliose de Lyme.*

- Pour le réactif de 1ère intention, la spécificité est ≥ 90% ;
- Pour le réactif immuno-empreinte, la spécificité est ≥ 95% ;
- L'immuno-empreinte n'étant globalement pas plus sensible que l'ELISA, il n'y a donc pas d'indication à la faire en première intention ;
- Une sérologie positive ne permet pas de distinguer une infection active d'une infection ancienne (traitée ou non) ou asymptomatique ;
- La présence d'IgG isolées (sans IgM) ne signifie pas obligatoirement une « cicatrice sérologique » (par ex. absence d'IgM fréquente dans l'arthrite et l'ACA) ;
- La présence isolée d'IgM ne signifie pas obligatoirement une infection récente active ;
- La sérologie de 1ère intention peut être faussement positive (surtout en IgM) et non confirmée en immuno-empreinte : réactions croisées avec d'autres pathologies infectieuses (EBV, HSV, CMV, syphilis) ou des pathologies auto-immunes ;
- Une sérologie positive ne signifie pas que les symptômes soient en relation avec une maladie de Lyme ;
- La sérologie peut rester positive longtemps après un traitement efficace => la surveillance post thérapeutique est clinique ;
- Les anticorps spécifiques ne protègent pas contre une nouvelle infection à *B. burgdorferi* sensu lato.

# Diagnostic biologique Borréliose de Lyme

## Démarche bioclinique




* index élevé nécessaire pour le diagnostic dans la zone d'endémie.

En cas de difficulté, possibilité de contacter le Centre National de Référence (CNR) des Borrelia : cnr.borrelia@unistra.fr

Pour en savoir plus :


- Borrelia burgdorferi sensu lato, Société Française de Microbiologie ED., REMIC, 5e édition, 2015. P 465-470.
- Maladie de Lyme, ministère chargé de la santé : www.sante.gouv.fr/maladie-de-lyme.html
- Centre National de Référence (CNR) des Borrelia : www.chru-strasbourg.fr/Les-centres-de-reference/Borrelia
- SPILF : www.infectiologie.com/site/medias/_documents/consensus/Lyme-06/2006-lyme-depliant.pdf



Document réalisé par :

CNR Borrelia, InVS, ANSM, DYOMEDEA, Institut de Microbiologie CHU de Lille, APHP, DGS, selon les recommandations de la Société Française de Microbiologie.

Direction générale de la santé - 12/15

# Diagnostic biologique Borréliose de Lyme

**Piqûre de tique**

Surveillance clinique

Absence de symptômes évocateurs de la borréliose de Lyme dans le mois suivant la piqûre

**Phase précoce localisée : Erythème migrant**

- Sérologie inappropriée (négative dans 50% des cas)
- Le diagnostic est clinique
- Traitement

**Phase disséminée précoce ou tardive : symptômes neurologiques ou articulaires ou cutanés évocateurs**

Sérologie de 1ère intention

→ + LCR si suspicion neuroborréliose

**Négative**

Contrôle sérologique 3 semaines plus tard (sauf arthrite et ACA)

**Négative**

Autre cause

Positive ou douteuse

Positive ou douteuse

Sérologie de confirmation (immuno-empreinte)

**Négative**

Autre cause

**Positive***

- Borréliose de Lyme
- Traitement

# En cas de difficulté diagnostique


- Envoi d'échantillons au CNR
  - confirmation de tests sérologiques
  - recherche directe par culture et/ou PCR
  - analyses non facturées (fiche renseignement)
- Fiche de renseignements

# En cas de difficulté diagnostique

**Centre National de Référence des *Borrelia* - Tel : 03 69 55 14 27**
PTM – HUS 1 rue Koeberlé 67085 Strasbourg

**FICHE DE RENSEIGNEMENTS BORRELIOSE DE LYME**

Médecin prescripteur : ……
Hôpital et service : ……...

Laboratoire : ……
Biologiste : ……...

Nature du prélèvement : …….. Date : /_ _/_ _/ _ _/
Examen demandé : Sérodiagnostic : ❒ PCR ❒ Culture : ❒

**PATIENT: Nom :**…… **Prénom :**…… Sexe : ❒ F ❒ H
Date de naissance : /_ _/_ _/ _ _/ Code postal du domicile : /_ _ _ _ _/
Profession : ……

**FACTEURS DE RISQUE** :

- Activités de loisirs : ❒ Non ❒ Oui Si oui, nature : ……
- Contacts avec des animaux **?** ❒ Non ❒ Oui Si oui, lesquels ? : ……..
- Exposition aux tiques (fréquentation de milieux forestiers…) : ❒ Non ❒ Oui
- Antécédents de piqûre de tique ? ❒ Non ❒ Oui Si oui, ❒ unique ou ❒ multiple?
- **Antécédent** d'érythème migrant : ❒ Non ❒ Oui Si oui, constaté par un médecin ❒ Oui ❒ Non
- Notion de piqûre de tique précédant **l'épisode actuel :** ❒ Non ❒ Oui
  Si oui, date de cette piqûre : /_ _/_ _/ _ _/ Durée de l'attachement : ….. ❒ heures ou ❒ jours
  Sur quelle partie du corps : ……
  Lieu de la piqûre (commune, forêt, vallée) : …….. Département : /_ _/

**SYMPTOMATOLOGIE AU MOMENT DU DIAGNOSTIC :** Date des premiers symptômes : /_ _/_ _/ _ _/
Date du diagnostic : /_ _/_ _/ _ _/

❒ ***Manifestations générales***
❒ Syndrome algique ❒ Syndrome fébrile : ….…. °C ❒ Asthénie

❒ ***Manifestations cutanées***
❒ Erythème migrant (> 5 cm) ❒ Lymphocytome ❒ Acrodermatite ❒ Autre (à préciser) :
Localisation : ……

❒ ***Manifestations neurologiques***
❒ Atteinte méningée : ❒ Non ❒ Oui Si oui, ❒ atteinte clinique ❒ uniquement biologique
❒ Atteinte périphérique :
Si oui, ❒ Paralysie faciale ❒ Radiculite Localisation : ……...
❒ Atteinte d'une autre paire crânienne, si oui, laquelle : ……..……..…
❒ Atteinte centrale : ❒ Non ❒ Oui Si oui, laquelle : ……..

**CYTOLOGIE DU LCR :** ❒ Non faite ❒ Si oui, date : /_ _/_ _/ _ _/ ❒ Lymphocytose : ……/ $mm^3$

❒ ***Manifestations articulaires*** Articulation(s) touchées(s) : ……...
❒ Arthralgies seules ❒ Arthrite aiguë ❒ Arthrite chronique
❒ Mono-arthrite ❒ Oligo-arthrite ❒ Polyarthrite

❒ ***Autres manifestations*** (à préciser) : ❒ Cardiaques ❒ Oculaires : …….

**Traitement antibiotique :** ❒ Non ❒ Oui
Nature et posologie : …….. du : /_ _/ _ _/ _ _/ au : /_ _/ _ _/ _ _/

**Fiche à transmettre CNR des *Borrelia***
**Plateau Technique de Microbiologie, Hôpitaux Universitaires de Strasbourg, 1 rue Koeberlé, 67085 Strasbourg**
**Fax: 03 69 55 16 98 –– E-mail : cnr.borrelia@unistra.fr**

## Envoi d'échantillons au CNR

- confirmation de tests sérologiques
- recherche directe par culture et/ou PCR
- analyses non facturées (fiche renseignement)

## Fiche de renseignements

# En cas de difficulté diagnostique

Centre National de Référence des *Borrelia*
Plateau technique de Microbiologie - HUS
1, place de l'Hôpital
BP 426
67091 Strasbourg Cedex

REFERENTIEL DES ANALYSES DU
CENTRE NATIONAL DE REFERENCE DES BORRELIA

**Prélèvements pour recherche de *Borrelia* par biologie moléculaire :**

| Nature prélèvement | Conditionnement | Quantité minimale | Conservation/Transport | Délai d'acheminement |
|---|---|---|---|---|
| Biopsie cutanée | Tube stérile sans additif | Punch biopsie 3-4 mm | A congeler – Transport en carboglace | A envoyer du lundi au jeudi maximum |
| Biopsie tissulaire | Tube stérile sans additif | Punch biopsie 3-4 mm | A congeler – Transport en carboglace | A envoyer du lundi au jeudi maximum |
| Tissu synovial | 2 tubes stériles sans additif | 2 x 4 fragments | A congeler – Transport en carboglace | A envoyer du lundi au jeudi maximum |
| Liquide articulaire | Tubes stériles polypropylène à bouchon à vis | 2 mL (2 x 1 mL) | A congeler – Transport en carboglace | A envoyer du lundi au jeudi maximum |
| LCR | Tubes stériles polypropylène à bouchon à vis | 0,8 mL (2x 0,4 mL) | A congeler – Transport en carboglace | A envoyer du lundi au jeudi maximum |
| Humeur aqueuse | Tube stérile sans additif | 100 µL | A congeler – Transport en carboglace | A envoyer du lundi au jeudi maximum |
| Sang total : UNIQUEMENT pour recherche de fièvres récurrentes | Tube EDTA | 5 mL | Réfrigéré - +4°C | 48h |

Si le prélèvement ne peux être congelé, conserver à +4°C et acheminer le prélèvement dans les 48h.

## Envoi d'échantillons au CNR

- confirmation de tests sérologiques
- recherche directe par culture et/ou PCR
- analyses non facturées (fiche renseignement)

## Fiche de renseignements

## Modalités d'envoi

# En cas de difficulté diagnostique

**Prélèvements pour sérologie de Lyme et/ou demande de synthèse intrathécale spécifique des neuroborrélioses :**

| Nature prélèvement | Conditionnement | Quantité minimale | Conservation/Transport | Délai d'acheminement |
|---|---|---|---|---|
| Sang (sérum) | Tube sec | 2 mL | Réfrigéré - +4°C | 48h |
| LCR | Tube sec | 0,8 mL | Réfrigéré - +4°C | 48h |

**Prélèvements pour recherche de *Borrelia* par culture :**

Les tubes de milieu BSK stériles sont fournis sur demande au CNR : 03.69.55.14.27
Il est impératif de travailler dans des conditions stériles pour tout usage de BSK.

| Nature prélèvement | Conditionnement | Quantité minimale | Conservation/Transport | Délai d'acheminement |
|---|---|---|---|---|
| Biopsie cutanée | Tube de milieu BSK stérile | Punch 3-4 mm | A conserver et à transporter à T°C ambiante | A envoyer du lundi au jeudi maximum |
| Biopsie tissulaire | Tube de milieu BSK stérile | Punch 3-4 mm | A conserver et à transporter à T°C ambiante | A envoyer du lundi au jeudi maximum |
| Tissu synovial | Tube de milieu BSK stérile | 2 x 4 fragments | A conserver et à transporter à T°C ambiante | A envoyer du lundi au jeudi maximum |
| Liquide articulaire | Tube de milieu BSK stérile | 6 – 8 gouttes | A conserver et à transporter à T°C ambiante | A envoyer du lundi au jeudi maximum |
| LCR | Tube de milieu BSK stérile | 6 – 8 gouttes | A conserver et à transporter à T°C ambiante | A envoyer du lundi au jeudi maximum |

Après ensemencement du prélèvement dans le tube de BSK stérile, **bien** revisser le bouchon sur le tube et protéger le tube dans du papier absorbant.

## Envoi d'échantillons au CNR

- confirmation de tests sérologiques
- recherche directe par culture et/ou PCR
- analyses non facturées (fiche renseignement)

## Fiche de renseignements

## Modalités d'envoi

# Conclusion

- D'abord les données cliniques et anamnestiques
- La sérologie n'est pas le diagnostic, c'est une aide

⇒ sérologie + ≠ borréliose de Lyme active
IgM peu informatives (pas forcément aigu)

- Piqûre tique/érythème migrant : pas d'examen bio
- Neuroborréliose : sérologie (synthèse intra-thécale)
- Arthrite/ACA : 1. sérologie 2. +/- PCR, culture

⇒ utiliser les tests validés et recommandés