Diagnostics alternatifs de la borréliose de Lyme (actualités)

A. Grillon

Laboratoire de bactériologie

UR 3073, Pathogens-Hosts-Arthropods-Vectors Interactions

Diagnostics alternatifs

Diagnostic indirect

- IFN-γ/ IL-1β/TTL
- Biomarqueurs (CXCL-13)
- Immuno-PCR
- IFN-α
- CD57
- Tests immunochromatographiques
- Spectroscopie Raman

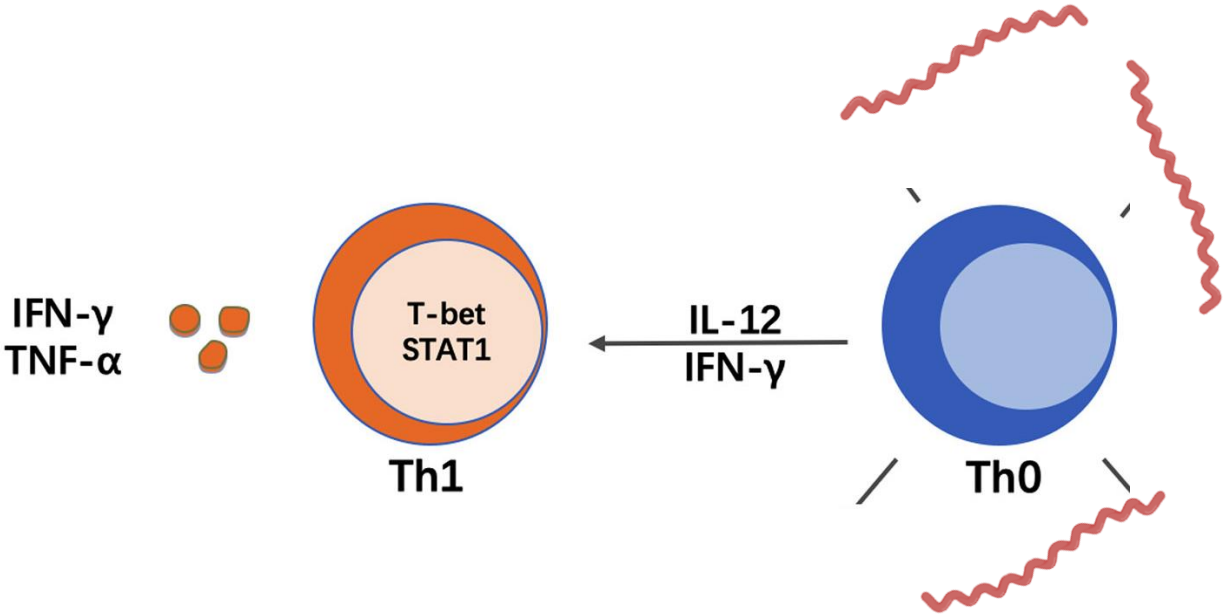
Diagnostic direct

- Microscopie
- Phelix-Phage
- Xenodiagnostic
- OspA

Réponse Immunitaire cellulaire

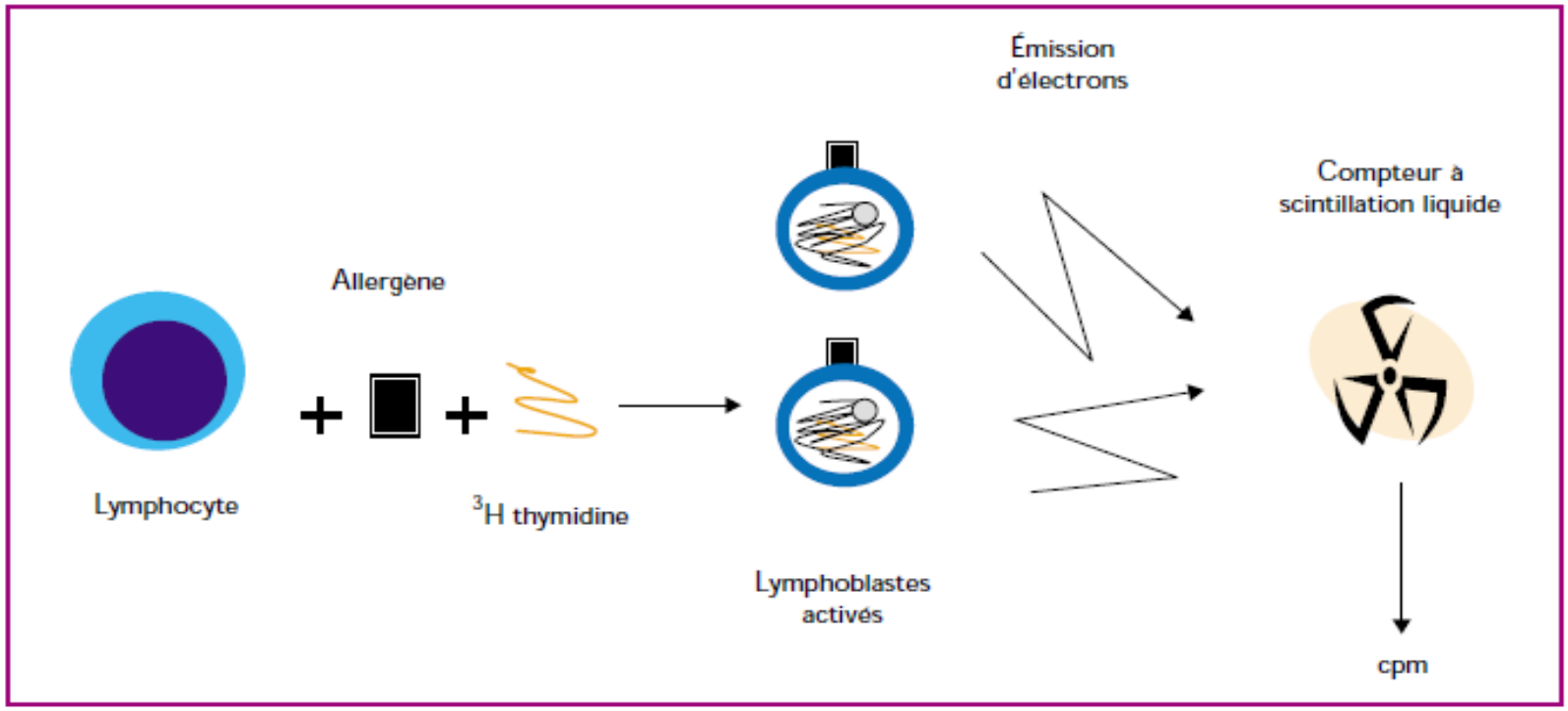
Interferon-γ

ELISpot



Gergel *et al.* Infect Immun 2004

Tests de transformation lymphocytaire



Résultats exprimés en « stimulation index » (+ si SI > 10)

Duché *et al.*, *DM*T 2005

THE LANCET
Infectious Diseases

Diagnostic parameters of cellular tests for Lyme borreliosis in Europe (VICTORY study): a case-control study

M E Baarsma, Freek R van de Schoor*, Stefanie A Gauw, Hedwig D Vrijmoeth, Jeanine Ursinus, Nienke Goudriaan, Calin D Popa, Hadewych JM ter Hofstede, Mariska MG Leeflang, Kristin Kremer, Cees C van den Wijngaard†, Bart-Jan Kullberg†, Leo AB Joosten†, Joppe W Hovius†*

Etude prospective cas-témoin

- Spirofind (IL1-β)
- iSpot Lyme (INF-**γ**)
- LTT-MELISA (TTL)
- STTT (comparateur)

2022

	Sensitivity at baseline (all patients with Lyme borreliosis)		Specificity at baseline (participants without Lyme borreliosis [healthy controls])	
	True positive, n; N	Sensitivity (95% CI), %	True negative, n; N	Specificity (95% CI), %
Spirofind (per-protocol)	88; 204	43·1% (36·4–50·4)	140; 171	81·9% (76·1–87·2)
Spirofind (all samples)	116; 260	44·6% (38·7–50·6)	174; 216	80·6% (75·3–85·5)
iSpot Lyme (primary)	51; 94	54·3% (44·5–63·7)	32; 103	31·1% (21·5–40·3)
iSpot Lyme (alternate)	11; 94	11·7% (5·5–18·6)	79; 103	76·7% (67·3–84·5)
LTT-MELISA (primary)	66; 218	30·3% (23·8–36·7)	100; 190	52·6% (44·9–60·3)
LTT-MELISA (alternate)	42; 218	19·3% (14·1–25·0)	130; 190	68·4% (61·2–75·0)
C6-ELISA (primary)	135; 270	50·0% (44·5–55·5)	212; 228	93·0% (89·2–96·4)
C6-ELISA (alternate)	126; 270	46·7% (41·1–52·3)	214; 228	93·9% (90·5–97·1)
Standard two-tier testing (STTT C6-ELISA and immunoblot)	76; 270	28·1% (23·0–33·6)	216; 228	94·7% (91·5–97·7)

Limites

Prévalence : 1%

Sensibilité : 50%

Spécificité : 80%

Test	Test +	Test -	Total
Malades			100
Sains			9900
			10 000

Limites

Prévalence : 1%

Sensibilité : 50%

Spécificité : 80%

Test	Test +	Test -	Total
Malades	50 Vrai positifs	50 Faux négatifs	100
Sains			9900
			10 000

Limites

Prévalence : 1%

Sensibilité : 50%

Spécificité : 80%

Test	Test +	Test -	Total
Malades	50 Vrai positifs	50 Faux négatifs	100
Sains	1980 Faux positifs	7920 Vrai négatifs	9900
	2030	7970	10 000

Limites

Prévalence : 1%

Sensibilité : 50%

Spécificité : 80%

Test	Test +	Test -	Total
Malades	50 Vrai positifs	50 Faux négatifs	VPP : 2,5 %
Sains	1980 Faux positifs	7920 Vrai négatifs	VPN : 99,4 %
	2030	7970	

Clinical Infectious Diseases

CORRESPONDENCE

The Initial QuantiFERON-Lyme Prototype is Unsuitable for European Patients

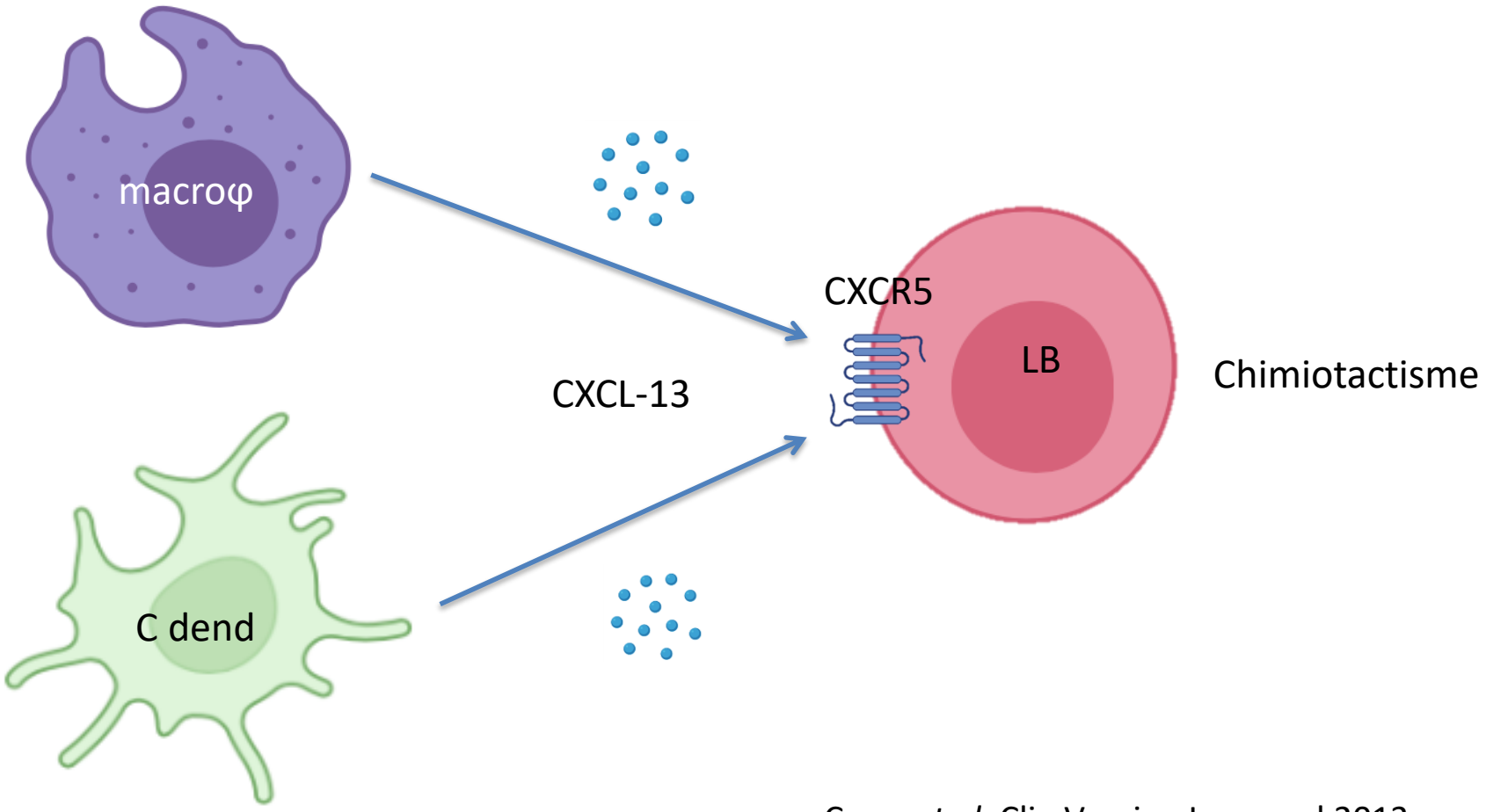
Our findings strongly suggest that the initial QuantiFERON-Lyme prototype is not suitable for use in Europe. These findings may be explained by less cross-reactivity than was presupposed by Callister and colleagues between the B31 strain of *Borrelia burgdorferi* sensu stricto, from which the antigens were derived, and genospecies generally causative of LD in Europe, such as *Borrelia afzelii* and *Borrelia garinii* [1, 4]. Alternatively, these

Baarsma et al. 2021 CID

Biomarqueurs (CXCL-13)

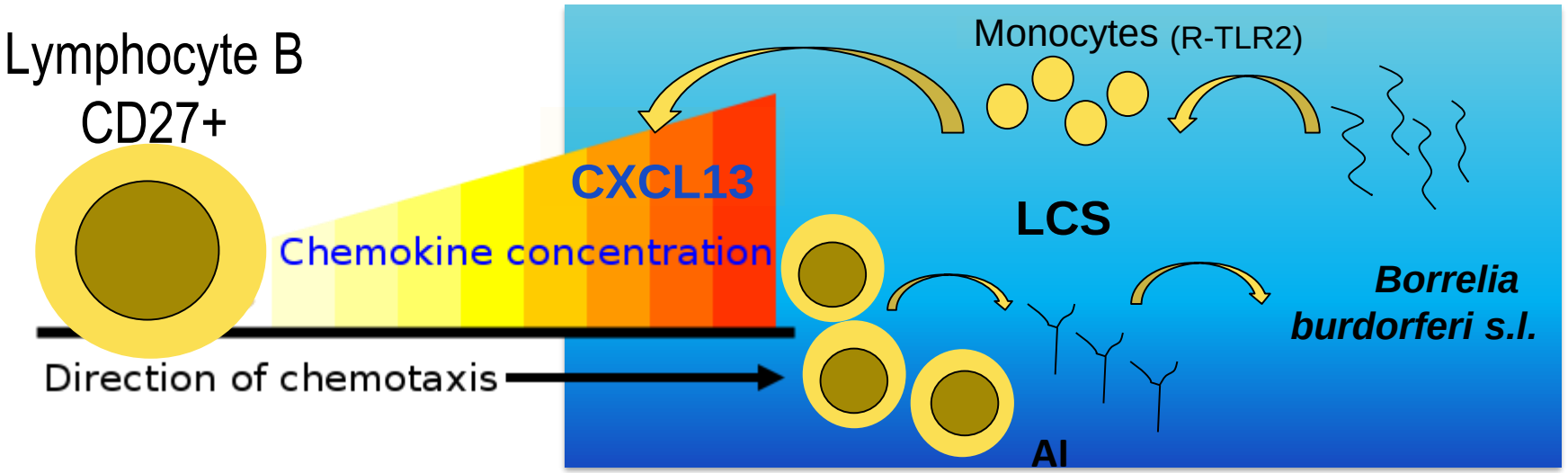
CXCL-13

C-X-C motif chemokine ligand 13



Cerar *et al*. Clin Vaccine Immunol 2013
Wagner et al. J Neurol 2017

CXCL-13 intrathécal



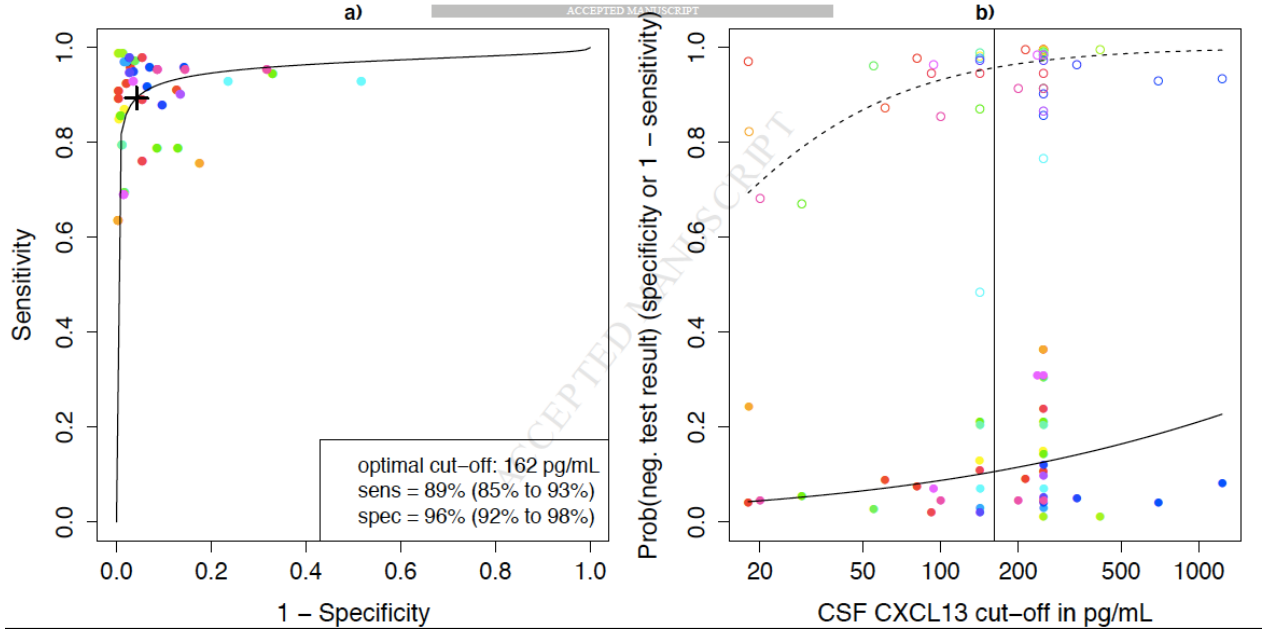
Lymphopléiocytose

Rupprecht *et al*. Neurology 2005

CXCL-13 intrathécal

Bonne performance diagnostique

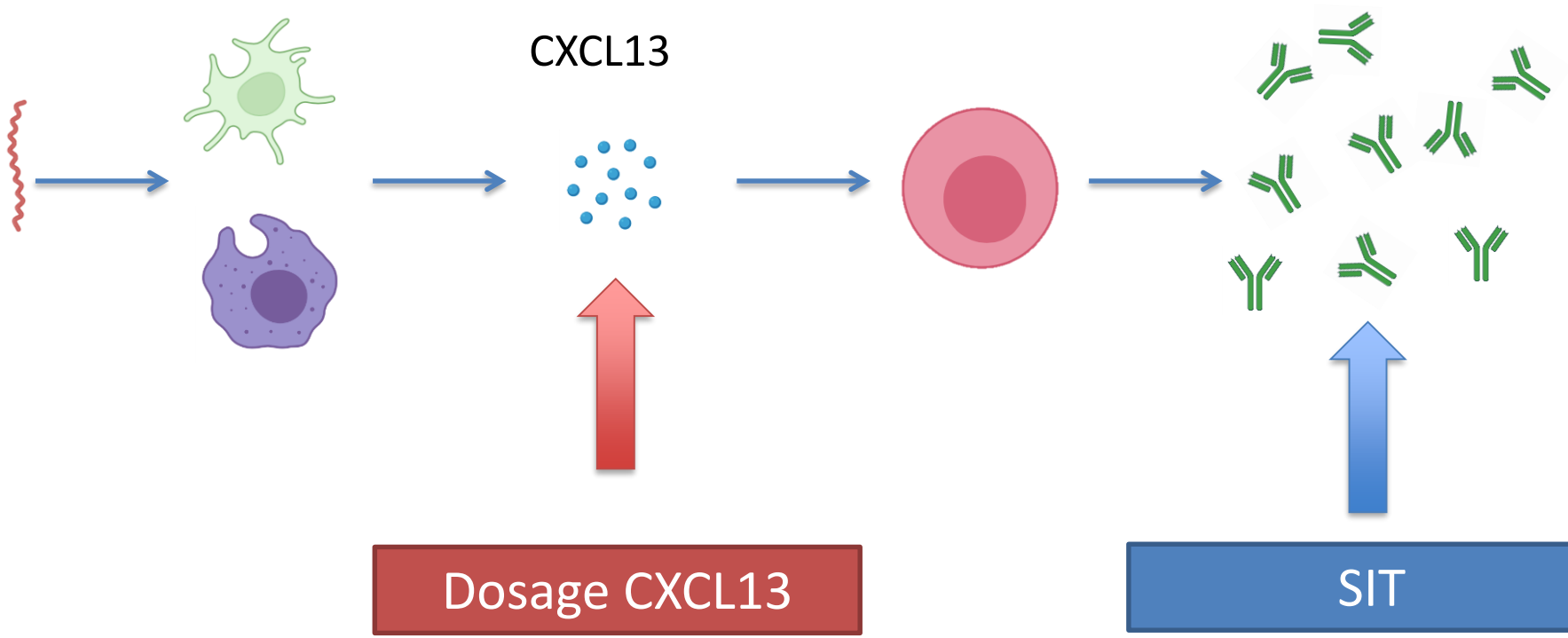
- Se : 85-93%
- Sp : 92-98%



Rupprecht *et al*. Clin Microbiol Infect 2018

CXCL-13 intrathécal

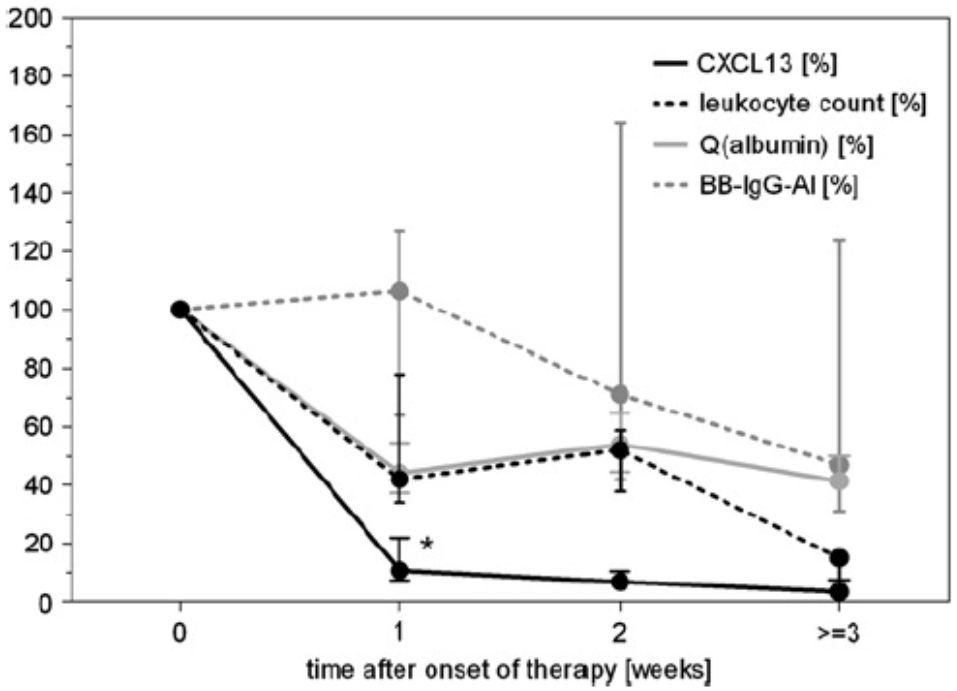
Diagnostic précoce, avant positivité de la SIT



Ljostad *et a*l. J Neurol 2008;Rupprecht *et al*. Nervenartz 2006;Rupprecht *et al*. Nervenartz 2014; Eckman et al. CID 2018

CXCL-13 intrathécal

Marqueur de l'efficacité du traitement



28 patients NB

Senel *et al*. J Neurol Neurosurg Psychiatry 2010

CXCL-13 intrathécal

Utilisable chez l'enfant

Remy *et al*. J Neuroinflam 2017
Skogman *et al*. Eur J Clin Microbiol Infect Dis 2017
Van Burgel *et a*l. J Clin Microbio 2011
Knudtzen *et al*. Eur J Clin Microbiol Infect Dis 2020
Sillanpää *et al.* Scand J Infect Dis 2013

Autres étiologies d'élévation du CXCL13		Exemples
Sprirochétose	Neurosyphilis	
Autres bactéries	Méningites, méningoencéphalites ou encéphalites bactériennes	*S. aureus*, neurotuberculose
VIH	Séropositivité VIH avec ou sans encéphalites	
Autres virus	Méningites, méningoencéphalites ou encéphalites virales	HSV, VZV, enterovirus, TBE
parasites et champignons	Méningites, méningoencéphalites ou encéphalites mycologiques	C. neoformans, encéphalites T. gondii, Trypanosomiase
Pathologies inflammatoires	Maladies inflammatoires SNC	SEP, Neurosarcoidose, vascularites, Guillain Barré, Encéphalomyélite disséminée
cancers	Cancers du SNC	Lymphomes cérébraux, cancers hématologiques avec atteintes méningées, méningites carcinomateuses

Autres étiologies d'élévation du CXCL13		Exemples
Sprirochétose	Neurosyphilis	
Autres bactéries	Méningites, méningoencéphalites ou encéphalites bactériennes	S. aureus, neurotuberculose
VIH	Séropositivité VIH avec ou sans encéphalites	
Autres virus	Méningites, méningoencéphalites ou encéphalites virales	HSV, VZV, enterovirus, TBE
parasites et champignons	Méningites, méningoencéphalites ou encéphalites mycologiques	C. neoformans, encéphalites T. gondii, Trypanosomiase
Pathologies inflammatoires	Maladies inflammatoires SNC	SEP, Neurosarcoidose, vascularites, Guillain Barré, Encéphalomyélite disséminée
cancers	Cancers du SNC	Lymphomes cérébraux, cancers hématologiques avec atteintes méningées, méningites carcinomateuses

Autres étiologies d'élévation du CXCL13		Exemples
Sprirochétose	Neurosyphilis	
Autres bactéries	Méningites, méningoencéphalites ou encéphalites bactériennes	S. aureus, neurotuberculose
VIH	Séropositivité VIH avec ou sans encéphalites	
Autres virus	Méningites, méningoencéphalites ou encéphalites virales	HSV, VZV, enterovirus, TBE
parasites et champignons	Méningites, méningoencéphalites ou encéphalites mycologiques	C. neoformans, encéphalites T. gondii, Trypanosomiase
Pathologies inflammatoires	Maladies inflammatoires SNC	SEP, Neurosarcoidose, vascularites, Guillain Barré, Encéphalomyélite disséminée
cancers	Cancers du SNC	Lymphomes cérébraux, cancers hématologiques avec atteintes méningées, méningites carcinomateuses

Autres étiologies d'élévation du CXCL13		Exemples
Sprirochétose	Neurosyphilis	
Autres bactéries	Méningites, méningoencéphalites ou encéphalites bactériennes	S. aureus, neurotuberculose
VIH	Séropositivité VIH avec ou sans encéphalites	
Autres virus	Méningites, méningoencéphalites ou encéphalites virales	HSV, VZV, enterovirus, TBE
parasites et champignons	Méningites, méningoencéphalites ou encéphalites mycologiques	C. neoformans, encéphalites T. gondii, Trypanosomiase
Pathologies inflammatoires	Maladies inflammatoires SNC	SEP, Neurosarcoidose, vascularites, Guillain Barré, Encéphalomyélite disséminée
cancers	Cancers du SNC	Lymphomes cérébraux, cancers hématologiques avec atteintes méningées, méningites carcinomateuses

CXCL-13 intrathécal

absence de seuil universel

Seuil des études dépend :

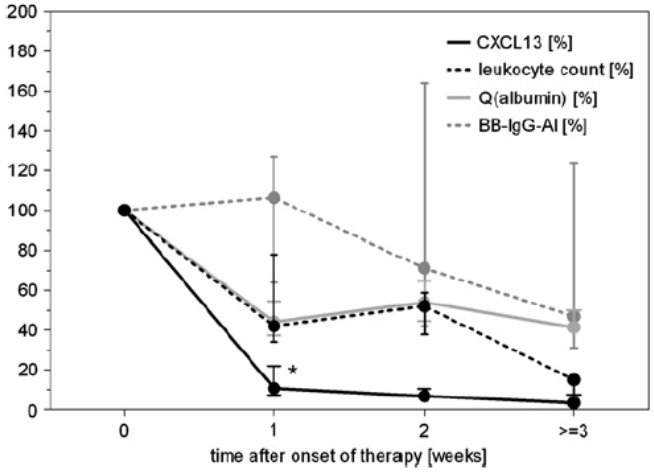
- Kit utilisé
- Population (enfant/adulte)
- groupes de patients utilisés comme témoins pos et neg

Seuil utilisable actuellement : 162 pg/mL (Se 89%; Sp 96%) ou 91 pg/mL (Se 96%; Sp 94%)

Rupprecht *et al.* Clin Microbiol Infect 2018

CXCL-13 intrathécal

Utilisable uniquement en absence d'antibiothérapie préalable



Chute de sensibilité **de plus de 20%** si antibiothérapie préalable

Knudtzen et al. Eur J Clin Microbiol Infect Dis 2020

CXCL-13 intrathécal

Et la NBL tardive ?

Les taux de CXCL13 auraient tendance à se normaliser avec le temps

=> Place inconnue

=> Intérêt pour différencier infections actives/passées ?

Strle et al. 2020 CID

Microscopie

Microscopie

Biological and Biomedical Reports, 2013, 3(1), 15-28

Research Article

A simple method for the detection of live *Borrelia* spirochaetes in human blood using classical microscopy techniques

Morten M. Laane[1], Ivar Mysterud[2,*]

[1]Department of Molecular Biosciences, University of Oslo, P.O. Box 1041 Blindern, 0316 Oslo, Norway

[2]Department of Biology, University of Oslo, P.O. Box 1066 Blindern, 0316 Oslo, Norway

Corresponding author*: ivar.mysterud@bio.uio.no

ABSTRACT

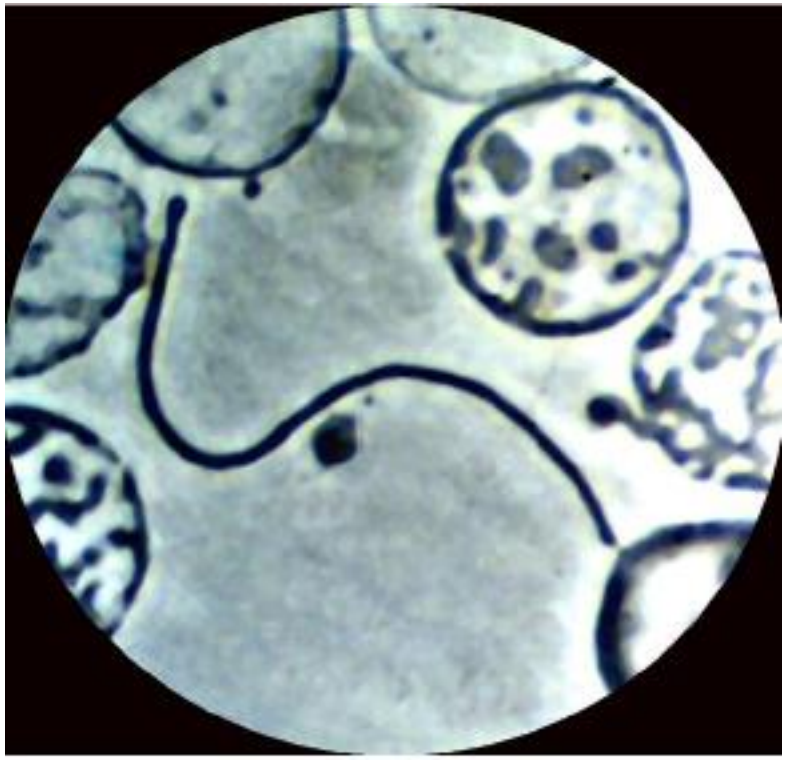
We have developed a simple method for the detection of live spirochaete stages in blood of patients where chronic borreliosis is suspected. Classic techniques involving phase-contrast and fluorescence microscopy are used. The method is also quite sensitive for detecting other bacteria, protists, fungi and other organisms present in blood samples. It is also useful for monitoring the effects of various antibiotics during treatment. We also present a simple hypothesis for explaining the confusion generated through the interpretation of possible stages of *Borrelia* seen in human blood. We hypothesize that these various stages in the blood stream are derived from secondarily infected tissues and biofilms in the body with low oxygen concentrations. Motile stages transform rapidly into cysts or sometimes penetrate other blood cells including red blood cells (RBCs). The latter are ideal hiding places for less motile stages that take advantage of the host's RBCs blebbing-system. Less motile, morphologically different stages may be passively ejected in the blood plasma from the blebbing RBCs, more or less coated with the host's membrane proteins which prevent detection by immunological methods.

Keywords: spirochaetes; Lyme disease; Borrelia; borreliosis; microscopical detection method; human blood

Introduction

Since the discovery of *Borrelia burgdorferi*, the Lyme disease spirochaete, borreliology has evolved from a microbiological curiosity to a major branch of bacterial pathogenesis research. Lyme disease is a global health problem; indeed few infectious diseases have garnered more sustained attention from the scientific and, notably, the lay media [1]. *Borrelia*-related diseases are a major challenge in medical research in many countries of the world, and we refer to comprehensive sources for the history and characteristics of this dangerous disease [2]. The disease is of great relevance today, and will be even more important tomorrow [3].

The most serious challenges are connected to diagnosis. The relatively large *Borrelia*, i.e. *B. burgdorferi*, is in general not readily detectable in blood smears of varying thickness from Lyme disease patients and suspected host animals. Yet blood engorgement from infected host result in up to 100% infected ticks [4].



Borrelia spirochaete attached to RBC
Structure inside the inflated RBC are probably infection sites

Microscopie

INFECTIOUS DISEASES, 2016
VOL. 48, NO. 6, 411–419
http://dx.doi.org/10.3109/23744235.2016.1144931

Taylor & Francis

ORIGINAL ARTICLE

Validate or falsify: Lessons learned from a microscopy method claimed to be useful for detecting *Borrelia* and *Babesia* organisms in human blood

Audun Aase[a], Ondrej Hajdusek[b], Øivind Øines[c], Hanne Quarsten[d], Peter Wilhelmsson[e], Tove K. Herstad[a], Vivian Kjelland[f,g], Radek Sima[b], Marie Jalovecka[b], Per-Eric Lindgren[e,h] and Ingeborg S. Aaberge[a]

[a]Department of Bacteriology and Immunology, Norwegian Institute of Public Health, Oslo, Norway; [b]Institute of Parasitology, Biology Centre, Czech Academy of Sciences, Ceske Budejovice, Czech Republic; [c]Section for Virology, Norwegian Veterinary Institute, Oslo, Norway; [d]Department of Medical Microbiology, Sørlandet Hospital Health Enterprise, Kristiansand, Norway; [e]Department of Clinical and Experimental Medicine, Division of Medical Microbiology, Linköping University, Linköping, Sweden; [f]Department of Engineering and Science, University of Agder, Kristiansand, Norway; [g]Research Unit, Sørlandet Hospital Health Enterprise, Kristiansand, Norway; [h]Medical Services, County Hospital Ryhov, Jönköping, Sweden

ABSTRACT

Background: A modified microscopy protocol (the LM-method) was used to demonstrate what was interpreted as *Borrelia* spirochetes and later also *Babesia* sp., in peripheral blood from patients. The method gained much publicity, but was not validated prior to publication, which became the purpose of this study using appropriate scientific methodology, including a control group. **Methods** Blood from 21 patients previously interpreted as positive for *Borrelia* and/or *Babesia* infection by the LM-method and 41 healthy controls without known history of tick bite were collected, blinded and analysed for these pathogens by microscopy in two laboratories by the LM-method and conventional method, respectively, by PCR methods in five laboratories and by serology in one laboratory. **Results** Microscopy by the LM-method identified structures claimed to be *Borrelia*- and/or *Babesia* in 66% of the blood samples of the patient group and in 15% in the healthy control group. Microscopy by the conventional method for *Babesia* only did not identify *Babesia* in any samples. PCR analysis detected *Borrelia* DNA in one sample of the patient group and in eight samples of the control group, whereas *Babesia* DNA was not detected in any of the blood samples using molecular methods. **Conclusions** The structures interpreted as *Borrelia* and *Babesia* by the LM-method could not be verified by PCR. The method was, thus, falsified. This study underlines the importance of doing proper test validation before new or modified assays are introduced.

ARTICLE HISTORY
Received 7 August 2015
Revised 5 January 2016
Accepted 18 January 2016
Published online 15 February 2016

KEYWORDS
Lyme disease; Lyme borreliosis; babesiosis; *Borrelia burgdorferi* sensu lato; *Babesia* spp.; microscopy; PCR

Introduction

The diagnosis of various infections following tick bite may be challenging.[1–3] Lyme borreliosis (LB), also called Lyme disease, is caused by infection with the spirochete *Borrelia burgdorferi* sensu lato and remains the most prevalent tick-borne infection in Europe and Northern America. This bacterium has a very complex genome and may change phenotypical expression and biological function depending on natural environments (reviewed by Samuels and Radolf [4]) and may reveal different morphological variants, at least *in vitro*, although their role in LB could not be confirmed.[5]

Borrelia miyamotoi is another *Borrelia* sp. related to the relapsing fever *Borrelia* group and has recently been found in ticks in Norway.[6] Although fever seems to be a common clinical manifestation of *B. miyamotoi* infection, other non-specific symptoms may be present and, unlike *B. burgdorferi* s.l., *B. miyamotoi* is detected in blood of infected patients by PCR or by microscopy.[7,8]

Direct identification of the *B. burgdorferi* s.l. in blood by polymerase chain reaction (PCR) has relatively low diagnostic sensitivity, assumedly due to the low number of spirochetes in blood and temporary bacteremic phase,[2,9] whereas PCR on biopsy material or synovial fluid are more suitable for the detection of *Borrelia* spp. in specific disease manifestations. Accordingly, cultivation of *B. burgdorferi* s.l. from blood specimens has low sensitivity and, when present, they require special growth media and long incubation periods before they can be detected by microscopy. Hence, indirect detection methods such as identification of *Borrelia*-specific antibodies in serum or cerebrospinal fluid by ELISA or immune blot remain the most used methods in clinical diagnosis.[2] The sensitivity of the serological methods has been improved and the detection rate for disseminated early disease is 70–90% and for late disease (6–8 weeks after onset of symptoms) nearly 100%.[10–12] However, the interpretation of positive samples is hampered by a high seroprevalence of antibodies against *Borrelia* spp. in the general population, particularly in endemic areas.[13–15]

Babesiosis is another tick-borne disease that may affect humans, particular immunocompromised individuals.[16,17] Studies from Norway indicate, however, that human

CONTACT Audun Aase audun.aase@fhi.no Department of Bacteriology and Immunology, Norwegian Institute of Public Health, PO Box 4404, NO-0403 Oslo, Norway
This Original Article is linked to an Editorial Commentary also published in Infectious Diseases – DOI – 10.3109/23744035.2016.1144932 – Microscopy of human blood for *Borrelia burgdorferi* and *Babesia* without clinical or scientific rationale.

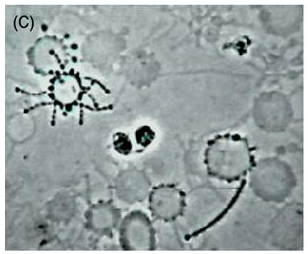
Table 2. Proportion (%) of subjects positive for *Borrelia* and *Babesia* like structures (95% CI) by microscopy; Lab. 1 using the LM-method and Lab. 2 using conventional protocol of the patient group ($n = 21$) and the control group ($n = 41$).

	Lab. 1 (LM-method)		Lab. 2 (Conventional method)	
	Patient group	Control group	Patient group	Control group
Borrelia	52 (30–74)	61 (45–76)	nd*	nd
Babesia	57 (34–78)	85 (71–94)	0	0
Double infection	43 (22–66)	59 (42–74)	na*	na

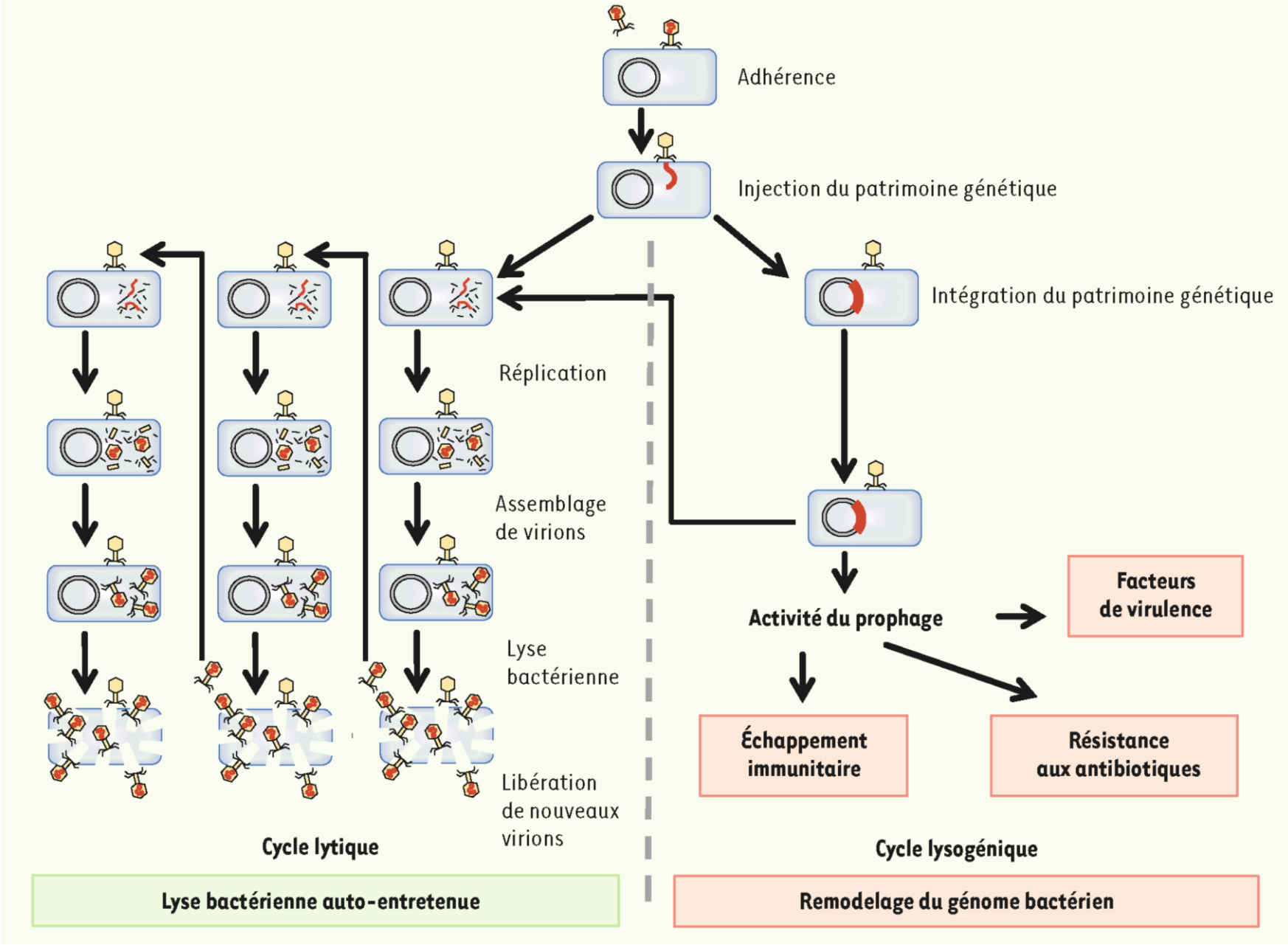
nd, not done at this Lab; na, not applicable.

Nombre de positifs :
groupe « contrôle » >> groupe « patient »

Aucun positif chez les témoins positifs !



Phelix-Phage



Benech et al. Med Sci 2022

- Multiples plasmides
- Certains prophages
- Recherche par qPCR

frontiers in Microbiology

ORIGINAL RESEARCH
published: 15 March 2021
doi: 10.3389/fmicb.2021.651217

Targeting Multicopy Prophage Genes for the Increased Detection of *Borrelia burgdorferi* Sensu Lato (s.l.), the Causative Agents of Lyme Disease, in Blood

Jinyu Shan[1†], Ying Jia[1†], Louis Teulières[2], Faizal Patel[1] and Martha R. J. Clokie[1*]*

frontiers in Microbiology

OPINION
published: 13 December 2021
doi: 10.3389/fmicb.2021.802131

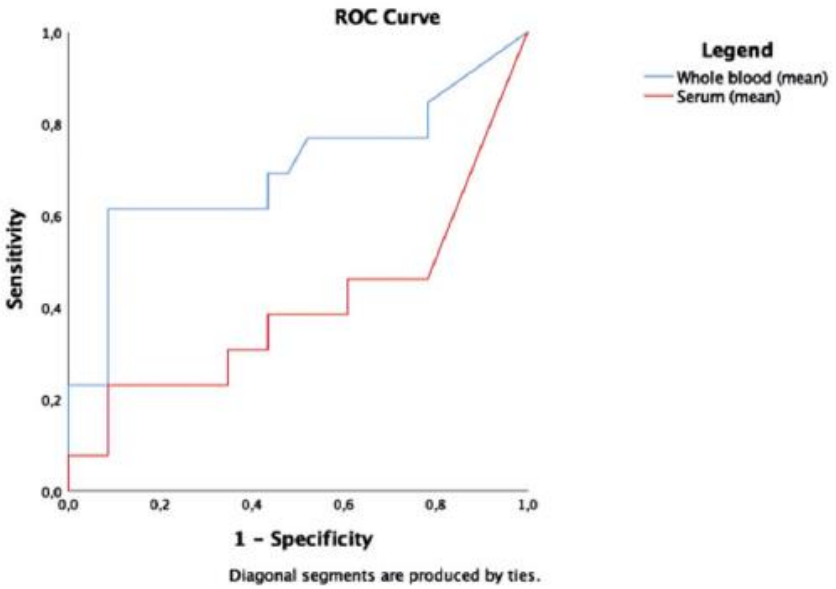
Opinion: Methodological Shortcomings in the Study on a Prophage-based PCR Test for Lyme Borreliosis

Freek R. van de Schoor[1†], M. E. Baarsma[2†], Mariska M. G. Leeflang[3], Volker Fingerle[4,5], Gabriele Margos[4,5], Joppe W. Hovius[2,5] and Alje P. van Dam[2,5]*

- Usage d’une souche (Bbss B31) qui possède 13 copies du gène recherché
- Patients diagnostiqués par un seul médecin (Critères ?)
- Sur les 23 VS, 21 ont un signal positif
- Soucis statistiques (gonflement artificiel de l’effectif)

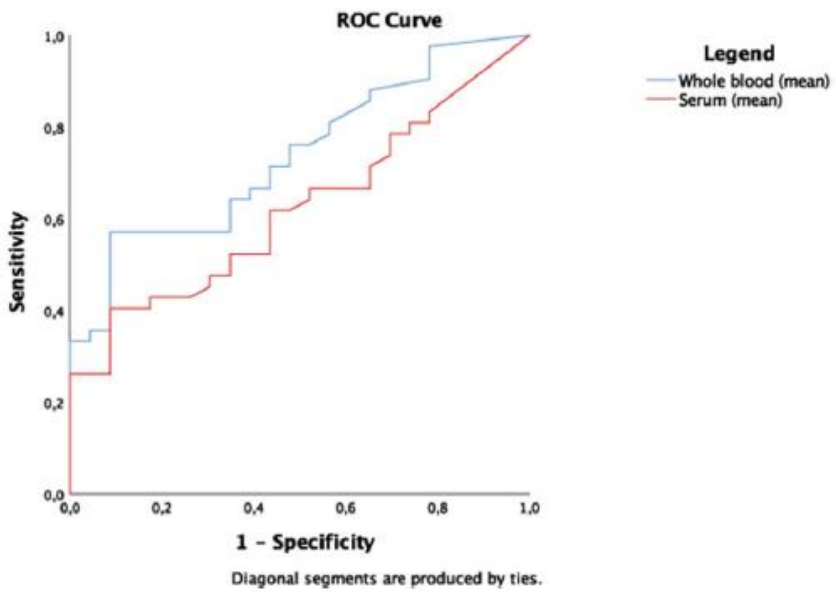
D

Healthy volunteers vs. early LB



E

Healthy volunteers vs. late LB



Au mieux, pour une spécificité de 90% :

- Se 62% early LB
- Se 57% late LB


That's all Folks

Spectroscopie Raman

Received: 2 December 2(
DOI: 10.1002/jbio.20200(

JOURNAL OF BIOPHOTONICS

Explorin copy for detectior

Charles Farb rouski[1]* |
Artem S. Rog

TABLE 1 Vibrational band assignments for Raman spectra of mouse blood

Band (cm^{-1})	Assignment
562	Fe-O_2 stretch (heme)[59]
676	Pyrrole symmetric bending (Heme)[59]
719	C–C–O related to glycosidic ring skeletal deformations[60]
752	Protein,[61] Heme ring breathing[59]
962	Associated with alpha CH of porphyrin ring[62]
1002	Phenylalanine ring breathing[39], CH_3 in-plane rocking of polyenes[39]
1126	C–C stretching[39]
1172	Trp, Phe[61]
1226	CH Bending (Heme)[59]
1249	*meso* CH of porphyrin ring[62]
1275	Lipids, Amide III[61]
1308	*meso* CH of porphyrin ring[62]
1340	Trp, Adenine, Lipids[61]
1376	Pyrrole ring[63]
1447	CH_2[39]
1462	CH_2, CH_3[64]
1516	C=C[39]

Received: 2 December 2020 | Revised: 18 January 2021 | Accepted: 19 January 2021

DOI: 10.1002/jbio.202000477

FULL ARTICLE

JOURNAL OF BIOPHOTONICS

Exploring a possibility of using Raman spectroscopy for detection of Lyme disease

Charles Farber[1] | Rohini Morey[1] | Mark Krimmer[1] | Dmitry Kurouski[1*] | Artem S. Rogovskyy[2*]

- Analyse en PLS-DA
- Aucune bande significative
- Bandes « discriminantes »: cycle de l'hème et alcène
- Calcul de probabilité de précision sans intervalle de confiance
- Quid des autres situations cliniques ?

Frontiers in Cellular and Infection Microbiology

TYPE Original Research
PUBLISHED 27 October 2022
DOI 10.3389/fcimb.2022.1006134

Testing Raman spectroscopy as a diagnostic approach for Lyme disease patients

Nicolas K. Goff[1], Tianyi Dou[1], Samantha Higgins[1], Elizabeth J. Horn[2], Rohini Morey[1], Kyle McClellan[1], Dmitry Kurouski[1*] and Artem S. Rogovskyy[3*]