Clinical Infectious Diseases

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The Initial QuantiFERON-Lyme Prototype is Unsuitable for European Patients

To the Editor—The QuantiFERON-Lyme, a novel test for Lyme disease (LD), was discussed in *Clinical Infectious Diseases* by Callister and colleagues [1]. Based on the platform commonly used for tuberculosis [2], it functions by overnight in vitro stimulation of whole blood with synthetic *Borrelia* peptides, after which interferon-γ (IFN-γ) in the supernatant is quantified. Callister and colleagues found the initial QuantiFERON-Lyme prototype to have a sensitivity of approximately 70% in patients with an erythema migrans (n = 29), a considerable improvement over the 17% sensitivity of standard 2-tier serology in their study [1]. More importantly, the IFN-γ response significantly decreased in the majority of patients after treatment, implying its usefulness as test-of-cure [1].

We recently performed the Validation of Cellular Tests for Lyme borreliosis (VICTORY) study to assess the diagnostic parameters of their assay and 3 other cellular assays in a cohort of European patients with physician-confirmed LD and controls. VICTORY's study design, inclusion criteria, and test procedures have been published previously [3].

Unexpectedly, our study did not confirm the findings of Callister and colleagues in their sample of American patients [1]. Only a very few patients showed an IFN-γ response that exceeded the manufacturer-prescribed cutoff of ≥ 0.33 IU/mL (Table 1). Our study design made it challenging to start incubation < 16 hours after the blood was drawn, resulting in a substantial number of tests that were excluded from the primary analysis. However, predefined secondary analyses showed no appreciable differences between tests from LD patients that were processed strictly according to protocol and those that were not. Only

Table 1. Reactivity of the Initial QuantiFERON-Lyme Prototype

	LD Patients at t = 0 wk		LD Patients at t = 6 wk		LD Patients at t = 12 wk	
	No. Positive (%)	95% CI	No. Positive (%)	95% CI	No. Positive (%)	95% CI
QFN-Lyme: per-protocol samples only	1/58 (1.7)	0.0–9.2	3/52 (5.7)	1.2–15.6	1/53 (1.9)	0.1–10.1
C6 ELISA	39/58 (67.3)	53.7–79.0	24/52 (46.2)	32.2–60.5	27/53 (50.9)	36.8–64.9
Secondary analyses						
QFN-Lyme: samples from patients with disseminated LD, per-protocol	1/15 (6.7)[a]	0.2–32.0	1/10 (10.0)[a]	2.5–44.5	1/15 (6.7)[a]	0.2–32.0
C6 ELISA	15/15 (100)	78.2–100	10/10 (100)	69.2–100	15/15 (100)	78.2–100
QFN-Lyme: samples with protocol violations	2/201 (1.0)[a]	0.1–3.6	2/190 (1.1)[a]	0.1–3.8	2/157 (1.3)[a]	0.2–4.5
C6 ELISA	84/200 (42.0)	35.1–49.2	70/190 (36.8)	30.0–44.1	42/157 (26.8)	20.0–34.4
	Reactivity in Healthy Controls		Reactivity in Cross-reactive Controls (Infectious Diseases)		Reactivity in Cross-reactive Controls (Autoimmune Conditions)	
	No. Positive (%)	95% CI	No. Positive (%)	95% CI	No. Positive (%)	95% CI
QFN-Lyme: per-protocol samples only	1/40 (2.5)	0.1–13.2	1/8 (12.5)	3.2–52.7	0/22 (0)	0–15.4
C6 ELISA	1/40 (2.5)	0.1–13.2	0/8 (0)	0–36.9	0/22 (0)	0–15.4

C6-ELISA results are shown only for available QFN-Lyme samples. For the C6-ELISA, an equivocal result was interpreted as negative (strict interpretation). Tests may not have been performed at a given time point because there was insufficient usable material to perform the test, or because a participant neglected to send in a follow-up sample. The t = 12 time point was facultative. QFN-Lyme protocol violations may include time from phlebotomy to incubation > 16 hours, duration of incubation for < 20 or > 24 hours, or other technical error. Technically nonvalid tests were excluded.

Abbreviations: CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; LD, Lyme disease; QFN, QuantiFERON.

[a]Reactivity was comparable to EM group and per-protocol samples, respectively (Fisher's exact: $P > .05$).

a small number of samples were indeterminate due to insufficient reactivity in the positive control (10/744, 1.3% of total from LD patients). Because of study design, most samples from patients with disseminated LD were incubated within 2 hours after being obtained (all time points: 39/68, 61.5%), but these did not show any appreciable reactivity. Unsurprisingly, reactivity in controls was also largely absent. Receiver operating characteristic analysis did not yield any usable cutoff optimization strategies.

Importantly, no apparent technical difficulties were identified that could explain the observed low reactivity. The contents of the antigen-coated test tubes as provided by the manufacturer and used in the study were verified using high-performance liquid chromatography, and functional testing using the study tubes on known responders from the United States yielded reactivity (Supplementary File).

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 specific synthetic peptides might induce a more subtle IFN-γ response in European patients, which could be detected with a more sensitive immunoassay, or that necessitates more robust or different in vitro immunostimulants.

Whereas the QuantiFERON-Lyme's preliminary results from the United States show its promise as a diagnostic tool, more research into its applicability in Europe is warranted. Our findings highlight several important considerations for further development by the assay's manufacturers [5].

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Potential conflicts of interest. None of the authors have received any financial compensation from QIAGEN or any of the other companies participating in the study. M. E. B. and J. W. H. collaborate with Bio-Rad Laboratories and Pfizer on unrelated projects on Lyme disease (LD). J. W. H. reports funding from ZonMw as part of a VIDI grant and INTERREG as part of the European collaborative NorthTick project, outside the submitted work. F. v. d. S. and L. A. B. J. collaborate with Hycult Biotech on developing novel diagnostic tests for LD. B. J. K. and L. A. B. J. are co-inventors of the Spirofind, an experimental in-house assay for LD, which is owned by Radboudumc and was previously licensed for development to Boulder Diagnostics (Boulder, Colorado, USA) and subsequently Oxford Immunotec (Oxford, UK) until 2018. C. C. v. d. W. reports no potential conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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Safety and Tolerability of Fluoroquinolones for Periprosthetic Joint Infection

To the Editor—Periprosthetic joint infection (PJI) remains one of the most devastating and costly complications following total joint arthroplasty [1]. Perioperative administration of systemic and/or local antibiotics is an important