Summary of the Study Protocol

DIABOLYC (Diagnostic Test for Cutaneous Lyme Disease)

Research Title	**Lyme disease and early skin diagnosis**
Short Title	**DIABOLYC (Diagnostic Test for Cutaneous Lyme Disease)**
Advisor	**Strasbourg University Hospitals**
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HUS No.	**6084**
IDRCB No.	**2015-A00425-44**
Research Rationale	To develop a method for diagnosing Lyme disease based on skin tests that detect bacterial proteins rather than DNA. Epidemiology: 60,000 cases per year of Lyme borreliosis in France (Réseau Sentinelles data), 300,000 in the United States (CDC-Atlanta-USA) in 2013.
Main Objective	To compare the effectiveness of a microbiological skin test based on the detection of specific bacterial proteins from B. burgdorferi sensu lato (OspC and flagellin) using SRM-MS, compared with PCR and Borrelia culture.
Secondary objectives	(1) Search for other markers of Borrelia infection (decorin-binding protein or enolase, GroEl, lipoproteins found in mice) (2) Isolate Borrelia strains (3) Estimate the specificity of the method by evaluating control patients with negative serology for Borrelia.
Primary endpoint	Compare the sensitivity of SRM-MS with PCR and Borrelia culture techniques: percentage of positive tests using each of the three techniques.
Secondary endpoints	(1) In situ detection by SRM-MS of bacterial proteins other than OspC and flagellin, which have been well documented in the literature in skin biopsies of erythema migrans. Enolase and GroeL, for example, have been identified in Borrelia virulence vesicles (Toledo et al., 2012). Enolase, in particular, binds to plasminogen and facilitates the diffusion/migration of the bacterium into host tissues. (2) If the culture is positive, the bacterial strain will be added to the CNR collection. (3) Calculation of the specificity of SRM-MS based on the population of control patients with negative Lyme serology.

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Study design	A multicenter, prospective, and comparative study
Eligibility criteria	**Inclusion criteria:** - Men or women over the age of 18 - Patients who have dated and signed an informed consent form - Patients enrolled in a health insurance plan - Patients presenting with: - either typical erythema migrans according to the EUCALB diagnostic criteria - or a lesion suspected of being erythema migrans but not clinically typical, associated with a history of a tick bite. **For control patients:** - Men or women over 18 years of age - Patients who have dated and signed an informed consent form - Patients enrolled in a social health insurance plan - Patients hospitalized in the digestive surgery department of the HUS for a scheduled abdominal procedure who have given their consent to a skin biopsy of approximately 1 cm^2 at the scar site. - And who have tested negative for Lyme disease in serological testing performed in the preoperative phase (no more than one month before the procedure). **Exclusion criteria:** - Prior treatment of erythema migrans with antibiotics - Coagulation disorders identified during the patient interview - Contraindications to any product or device that may be used before, during, or after the biopsy - Systemic treatment likely to interfere with the healing process - Subjects who have undergone physical treatment (radiotherapy, etc.) on the area to be biopsied within the last 6 months - Known hypersensitivity to lidocaine hydrochloride, amide-type local anesthetics, or any of the excipients (sulfites) - Inability to provide the subject with informed consent (subject in an emergency situation, difficulty in understanding, etc.) - Subject under legal guardianship - Subject under conservatorship or curatorship - Subject currently excluded (as determined by a previous or ongoing study) For patients for whom local anesthesia with lidocaine/epinephrine is being considered: - Subject with recurrent porphyrias - Subject with coronary artery disease - Subject with ventricular arrhythmias

	- Subject with severe hypertension - Subject with obstructive cardiomyopathy - Patients with hyperthyroidism <u>For control patients:</u> - Surgical contraindication - History of Lyme disease or erythema migrans
Practical aspects of the trial	- Information and informed consent - Clinical interview and physical examination - Photograph of the erythema migrans - Two biopsies will be taken in close proximity to each other at the site of the erythema migrans. The following tests will be performed on these biopsies: ✓ Biopsy 1: PCR to detect Borrelia DNA, and culture to isolate the bacteria ✓ Biopsy 2: SRM-MS to detect Borrelia proteins (OspC and flagellin) **<u>For control patients:</u>** Patient recruitment for the control group will take place in the Department of Digestive and General Surgery at HUS. This department is located on the same site as the Department of Infectious Diseases and in close proximity to the bacteriology laboratory, which will facilitate communication and the collection of intraoperative samples.
Establishment of a biological sample collection	NO
Required number of subjects	74 +20 volunteer patients
Statistical method	In the main analysis, we will assess the difference in disease detection between the various techniques using two McNemar's chi-square tests: SRM-MS versus culture on the one hand, and SRM-MS versus PCR on the other. The significance level for each of the two tests will be set at 2.5%.
Preliminary timeline	Duration of the enrollment period: 6 years Maximum duration of participation for each subject: 10 days Total duration of the study: 6 years