Novel monovalent and multivalent recombinant proteins of *Borrelia burgdorferi* sensu lato as potential antigens for the detection of specific antibodies in the sera of Lyme patients

Lyme borreliosis (LB) is a multisystem disease caused by a group of related spirochetes, *Borrelia burgdorferi* sensu lato (sl), that are transmitted to the skin during the blood sucking process by ticks belonging to some species of the genus *Ixodes*. Despite substantial efforts to improve surveillance and control of LB in recent decades, it is still the most prevalent arthropod-borne disease in the temperate regions of the northern hemisphere. The disease can be found mainly in Europe, North America and temperate Asia. The risk of LB is related to tick abundance and exposure and so it has a higher incidence among subjects living in rural areas, forestry and farm workers, hunters, mushroom gatherers, and berry pickers. The symptoms of this disease can vary, though most of them are not specific. The most common symptom of Lyme borreliosis is the typical ring-shaped redness (EM, *Erythema migrans*) surrounding the location of the tick bite. Usually, it may be observed in the first few days following a tick bite, but could also take several weeks to develop. In addition, EM also can be absent in up to 20 to 50% of patients. Other than the rash, there are also more general symptoms observed in the early stages of borreliosis, such as fatigue, fever and headaches. Furthermore, the infecting pathogen can spread to other tissues and organs, causing more severe manifestations that can involve nervous system, joints, or heart. The *B. burgdorferi* sl complex currently comprises at least 20 genospecies. In Europe, several of these are pathogenic to humans: *B. afzelii, B. garinii, B. burgdorferi* sensu stricto (ss), *B. bavariensis* (previously *B. garinii* OspA serotype 4) and *B. spielmanii*, while the pathogenicity of others such as *B. lusitaniae, B. valaisiana*, and *B. bissettiae* is still uncertain. Although all pathogenic genotypes may cause erythema migrans, the late stage of Lyme disease is highly dependent on the genospecies of *B. burgdorferi* sl that caused the disease. *B. garinii* and *B. bavariensis* infections often cause neurological symptoms (NB, neuroborreliosis), and *B. burgdorferi* ss. causes mainly Lyme arthritis (LA). *B. spielmanii* has so far been isolated only from skin lesions.

Current diagnosis of Lyme disease is based on clinical symptoms and the two-tier detection of anti-*Borrelia* antibodies. First, an ELISA is performed, followed by a Western blot to confirm the results from the first serology, as well as to identify the infecting *Borrelia* species. Unfortunately, commercially available diagnostic tests differ in their specificity and sensitivity. These tests are based on whole-cell lysate preparations of native antigens, purified spirochete antigens (such as, flagellar components) or whole-cell antigens combined with recombinant proteins. The complexity of the antigenic composition among the *Borrelia* genospecies and differential expression of proteins in host and vector (temporal and spatial antigenic variability) has posed challenges for the serodiagnosis of borreliosis. Recent achievements in genetic engineering and biotechnology could rise these challenges by the use of newly constructed monovalent and multivalent/chimeric recombinant proteins synthesized in microorganism transformed with genes encoding conserved fragments of *B. burgdorferi* sl complex antigens. A new trend in generation of recombinant antigens relies on the development of chimeric recombinant antigens (multivalent), composed of fragments derived from two, three or more antigens. For this reason, the aim this project is generation of a new recombinant proteins of *B. burgdorferi* sl in prokaryotic expression system, and then determination of their antigenic properties. In our opinion, employment of B-cell epitopes from the strains specific for defined geographic areas or highly conserved fragments of proteins has the potential to improve the performance of serological diagnosis. Thus, new chimeric antigens, composed of selected epitopes/fragments of proteins, give hope to solve the current problem of diagnosis of Lyme disease caused by different genospecies. Adequate selection of regions from *Borrelia* antigens, construction of chimeric proteins followed by a confirmation of their antigenic properties is the initial step of new immunoassay development.