Voortgangsrapportage Nieuwe diagnostiek voor de ziekte van Lyme
30 augustus 2018

Project Title:	Development of a Novel Diagnostic Test for Lyme Borreliosis
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Post-Doctoral Researcher:	Dr. Abhijeet Nayak
Involved PhD Student:	Jeanine Ursinus

Aim of the Project

The project aims to implement a protein microarray approach for identification of new antigens during different phases of *B. afzelii* infection. The whole proteome of *B. afzelii* will be printed on a microarray slide and will be probed with human patient and mouse serum samples to identify unique antigens at different stages (early, late and post-treatment) of Lyme disease. This will result in identification of novel biomarkers that can differentiate between an active versus a past *B. afzelii* infection, ultimately resulting in a more sensitive diagnostic test and appropriate treatment methods. We are now approx. three months into the project.

Overall plan

The project has been divided into four different stages and work flow is depicted as a flow chart below

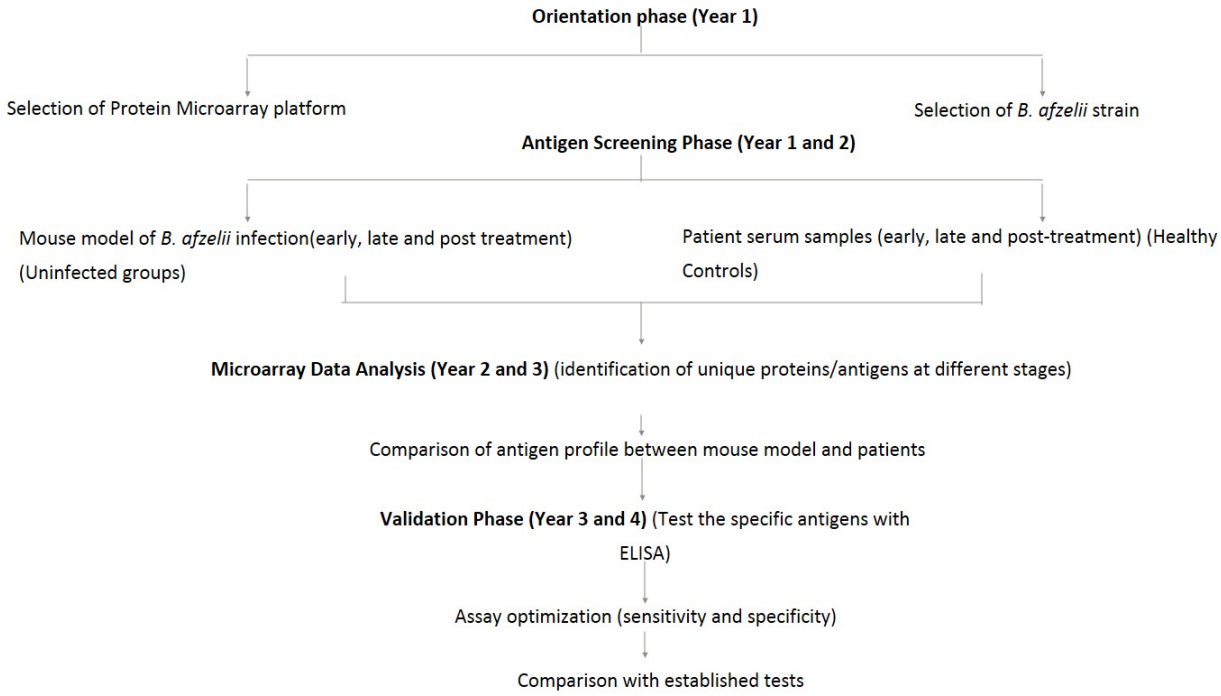

Figure 1: Work flow representing four different phases and the associated plan at each stage of the project.

Progress

Phase I - Selection of protein microarray platform

Multiple commercial companies were contacted in order to choose the most appropriate microarray platform, primarily companies producing whole protein microarray and/or peptide microarrays.

Whole Protein Microarray – Approach 1

Based on the search, Antigen Discovery Inc. (in the U.S.A) was chosen as a potential company since the company has already established a proprietary microarray chip comprising of whole proteins of *Borrelia afzelii* (the primary *Borrelia* genospecies that is most clinically prevalent in Europe). After initial discussion through tele-conference meetings legal procedure to establish a mutual research agreement has already been initiated and is envisioned to be finished within September/October 2018. Through the AMC foundation this has been discussed in more detail with Horsting-Stuit Foundation.

Peptide Microarray – Approach 2

As a back-up plan, Pepper Print (in Germany) has been contacted as a potential company. The company is specialized in producing peptide microarrays for several different pathogens (bacteria and viruses – most notably a peptide microarray chip for tick borne encaphalitis virus). We will try to perform a small pilot study by the end of the year.

Phase I – Selection of *Borrelia afzelii* strain to be used for the protein microarray

As depicted in Figure 1, protein microarray will be examined with serum samples derived from humans at different stages of the disease. As a parallel approach, the protein microarray chip will also be interrogated with serum sample derived from experimentally infected mice. To mimic the natural route of *Borrelia* infection, our tick-*B. afzelii*-mouse model will be implemented. The protein microarray established by Antigen Discovery Inc. is based on *Borrelia afzelii* strain BO23 and PKo. However, the tick-*B. afzelii*-mouse model established in our laboratory is based on *Borrelia afzelii* strain CB43. Therefore, to test the cross-reactivity of CB43 and Pko strains a western blot was performed with whole cell lysates of the respective strains. *Borrelia afzelii* strain Pko clearly demonstrated cross-reactivity with serum derived from mice infected with CB43 strain (Figure 2). High reactivity was observed with CB43, which was to be expected since probing was performed with serum derived from the mice infected with the same strain.

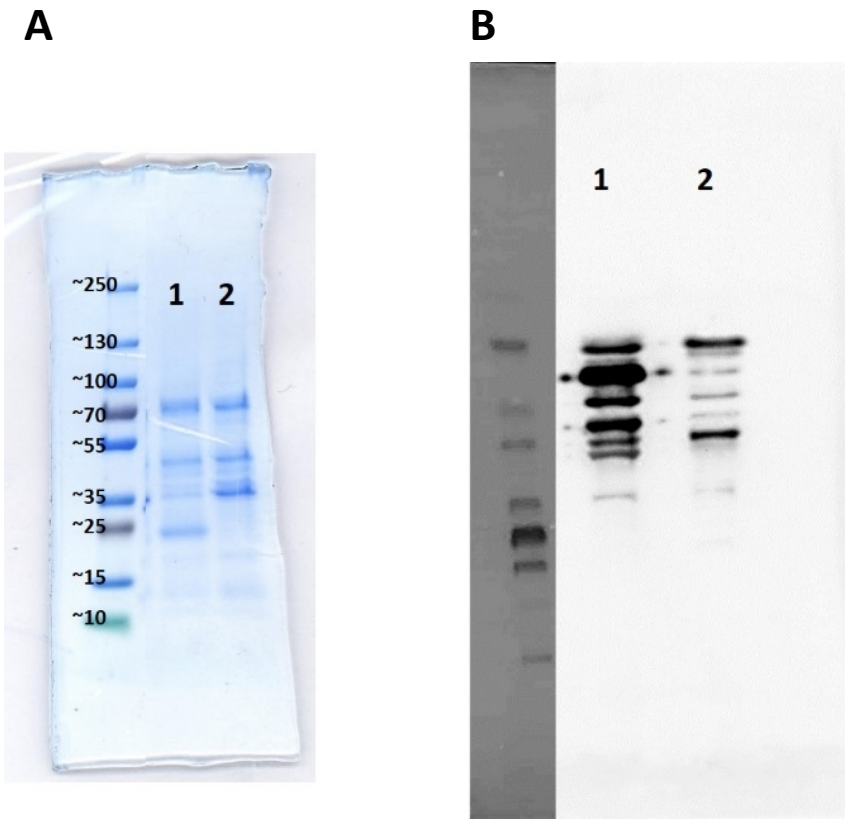

Figure 2: SDS-PAGE and Western Blot to examine serum cross-reactivity of *Borrelia afzelii* strains. **A.** SDS-PAGE was performed in order to account for protein loading concentration. Lane 1 – CB43 whole cell lysate and Lane 2 – Pko whole cell lysate. 2.1. µg of protein was loaded onto each well of 4-20% Tris-glycine gel. **B.** The western blot was probed with serum (1:200) derived from mice infected with ticks harboring *Borrelia afzelii* CB43 strain. Lane 1 – CB43 whole cell lysate and Lane 2 – Pko whole cell lysate.

In addition, *in silico* analyses showed that there is a high percentage of identity of several protein sequences available in public databases of the different *B. afzelii* isolates. Therefore, it was concluded that a CB43 tick-*B. afzelii*-mouse model can be implemented for obtaining serum samples that will be used to probe a whole protein microarray chip based on PKo strain.

Phase I – Selection of human patient groups to obtain well-characterized serum samples

The final selection of the human patient serum samples from our biobank was organized in collaboration with Jeanine Ursinus (PhD student in Professor Hovius' group). The patient groups and numbers are described in table 1. We envision to use the protein array from Antigen Discovery (see above), enabling us to use all of the selected sera to probe their protein chip.

Table 1: Patient selection.

Case definition (disease stage)	Estimated number of samples
Erythema migrans (early disease)	20-25
Acroedermatitis chronica atrophicans (late disease)	20-25
Post treatment lyme disease syndrome	20-25
Healthy volunteers / blood donors	20-25
Treated and cured LB patients	To be determined

* Case definition were largely based on Coumou et al. CMI 2016.

Additional points

a. I will undertake an Article 9 laboratory animal science course in September in order to obtain official permission so that I can initiate the mouse experiment thereafter
b. As a back-up plan for the tick-Borrelia-mouse model I have also ordered ticks infected with *B. afzelii* strain IS1, from Insect services GmbH