Contact Information:

Sarah Mo
373 280th St., Osceola, WI 54020
888-342-7272 x266
sarah.mo@neurorelief.com

Presenters:

Chenggang Jin, MD, PhD/Bradley Bush, ND
60 minutes
Development and Validation of a Novel Interferon-γ ELISPOT Assay for Sensitive and Specific Detection of Antigen-Specific T cell Response to *Borrelia burgdorferi*

The enzyme-linked immunospot (ELISPOT) technology has proven to be extremely sensitive in detecting antigen specific reactive T cells and has been applied in laboratory diagnostic for Tuberculosis approved by FDA. A novel T-cell based assay for diagnosis of Lyme disease -Lyme ELISPOT was successfully developed and validated.

Target Audience: Medical Practitioner, Researcher, Scientist, General Audience
No Pharmacy Discussion

ABSTRACT

Learning Objectives: After the presentation, an individual will be able to:

- Describe ELISPOT assay technology
- Contrast ELISPOT assays to conventional antibody detection methods utilized for the diagnosis of Lyme disease
- Evaluate the potential application of ELISPOT as a diagnostic tool for Lyme disease

Presentation Outline:

Background: Lyme disease, caused by infection with the spirochete *Borrelia burgdorferi*, is an emerging infectious disease in the United States that has become an important public health problem. Both B-cell immunity and T-cell immunity develop in natural infection with *Borrelia burgdorferi*. Detection of specific antibody response mediated by B cells against *Borrelia burgdorferi* is utilized conventionally in aiding the clinical diagnosis of Lyme disease. However, the limitation of these antibody-based immunoassays is that they have low sensitivity and specificity, causing significant false negative and false positive results. Furthermore, *Borrelia* specific antibodies cannot be detected at the early stage of infection and in a fraction of seronegative Lyme patients who lack *Borrelia* specific antibody responses. In contrast, *Borrelia* specific T-cell based immune assays have not yet been well developed. Thus, highly sensitive and specific T-cell based clinical laboratory assays are needed to help in diagnosing Lyme disease in conjunction with antibody-based immunoassays. The enzyme-linked immunospot (ELISPOT) technology has proven to be extremely sensitive in detecting antigen specific reactive T cells and has been

applied in laboratory diagnostic for Tuberculosis approved by FDA. We here explore the potential application of ELISPOT as diagnostic tool for Lyme disease.

Objective: The aim of this study is to develop and validate a novel T-cell based assay for diagnosis of Lyme disease using newly developed digitalized ELISPOT technology.

Methods: To develop the novel T-cell based diagnostic assay for Lyme disease, we detected the *Borrelia* antigen-specific memory T cells that were activated *ex vivo* by recombinant *Borrelia* specific antigens, using Th1 cytokine Interferon-γ ELISPOT at the single cell level. The human peripheral blood mononuclear cells (PBMC) were stimulated with single or a combination of recombinant *Borrelia* specific antigens, DbpA, OspC, p100 and VlsE. In addition, we added costimulatory cytokine IL-7 into the cell culture to increase the detection of T memory cells. The results of ELISPOT were analyzed using CTL S6 Ultimate-V Analyzer/BioSpot 5.0 Software and reported as IFN- γ Spot Forming Units (SFU). To validate the Lyme ELISPOT assay, a cohort of 21 clinically diagnosed Lyme patients and 45 healthy control subjects were further studied and compared with Western Blot test. The performance of the Lyme ELISPOT assay, including clinical sensitivity, clinical specificity, accuracy and precision, is also evaluated.

Results: The frequency of *Borrelia* specific T memory cells can be detected by Interferon- γ ELISPOT and therefore can be used as a biomarker for *Borrelia* infection. The detection of antigen specific T cells was significantly increased by a combination of recombinant *Borrelia* antigens and addition of constimulatory cytokine IL-7. The signal enhancing effect of IL-7 was observed even at saturating antigen concentration in terms of frequency, but IL-7 did not increase the amount of IFN- γ secreted by individual cells. A strong correlation was observed between ELISPOT and IFN- γ concentration measured by Bio-plex suspension system ($R=0.8$, $P<0.0001$). The Lyme ELISPOT assay cut-off value was determined using Receiver Operating Characteristic (ROC) curve analysis and it was found that a cut-off value of $\geq$25 PFU maximized assay sensitivity and specificity. It has a significantly higher specificity (96%) and sensitivity (76%) compared with Western blot (Sensitivity 24%). The results also demonstrated that there was dissociation between B cell response and T cell response during *Borrelia* infection, suggesting a comprehensive immunological diagnostic panel should include both B cell and T cell diagnostics. Further studies will include more Lyme patients, other related diseases and independent studies by other laboratories.

Conclusion: A novel T-cell based assay for diagnosis of Lyme disease -Lyme ELISPOT was successfully developed and validated. This newly developed Lyme ELISPOT assay may be a helpful laboratory diagnostic test for Lyme disease, especially for seronegative Lyme patients. A comprehensive evaluation of both antibody response and T cell response to *Borrelia* infection will provide new insights into the pathogenesis and diagnosis of Lyme disease.

Speaker Bios

Chenggang Jin, MD, PhD

Dr. Chenggang Jin holds a Ph.D in Immunology and is also an MD with 10 years of experience in medical research at Princeton University, The University of Iowa Hospitals and Clinics and Beth Israel Deaconess Medical Center/Harvard Medical School. Dr. Jin also holds certification from the Educational Commission for Foreign Medical Graduates in the field of medicine. His specialized training includes many years of technical and research experience in cellular immunology and molecular biology. He also has extensive experience with flow cytometry and PCR instrumentation. He is currently the Director of Laboratory Immunology at Pharmasan Labs and led the creation of new clinical laboratory assay, iSpot-Lyme, for Lyme disease.

Bradley Bush, ND

Dr. Bradley Bush received a N.D. degree from National College of Naturopathic Medicine in 2000 and is currently the Director of Clinician Affairs for NeuroScience, Inc. Dr. Bush specializes in neuro-endo-immune health, nutrition and infusion therapies. His focus is on addressing gastrointestinal and HPA axis disturbances in addition to nutritional deficiencies as a cornerstone of patient care. He is co-author of the ND: Notes Science Board Review and ND Notes: Clinical Board Review books. Dr. Bush has worked for years for manufacturers of nutritional supplements, is a founder and past-organizer of the annual Pharmaceutical Perspectives conference, and currently sits on the board of the Naturopathic Education and Research Consortium (NERC).