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Research Article

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# Lyme ELISA seronegative patients with borrelia antibody coated borrelia microbes in their blood

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## Abstract

*ELISA testing for detection of possible borrelia related antibodies in serum is an "Indirect" and antibody dependent technology because it seeks to identify the host antibody-dependent immune responses (free antibodies in serum) to past borrelia infections. Microscopic visualization of the actual borrelia spirochetes in blood or in tissue biopsies is a "direct" and antibody independent technology which is superior to "indirect" antibody dependent ELISA testing methods because it proves cases in which borrelia infection is ongoing, current, and unambiguous. Herein is a research study from thirty patients with ELISA negative serologies and whose physician-diagnosed symptom complexes were compatible with a status of "at risk for persistent/chronic Seronegative Lyme borreliosis". Monoclonal antibody confirmed borrelia individual spirochetes and borrelia biofilms in whole blood in the 30 ELISA seronegative patients in the study group of volunteers. All patient smears were subsequently analyzed with a Coombs reagent (rabbit species antiglobulins to human IgG) optimized for diagnostic use in hospital blood banks to detect naturally produced human immunoglobulins in the bloodstream. Duplicate examination of whole blood smears with the two reagents above confirmed a group of Lyme ELISA seronegative patients whose whole blood smears contained antibody coated borrelia spirochetes of the Bbss and BBsl Burgdorferi groups. Spirochete-bound antibodies from whole blood examination identify "solid phase on the bodies of spirochetes "antibody deposits which are absent in dilute serum separated from clotted whole blood. "Antibody cloaked spirochetes in whole blood" represent patient Lyme borreliosis immune responses to infection which are overlooked in current blood serum only ELISA Lyme tests.*

**Keywords:** ELISA testing, BBsl Burgdorferi, Clinical evaluation, Borrelia spirochetes, Whole blood smears, Lyme infections, facial nerve palsy.

## Introduction

Seronegative *Lyme borreliosis* is a legitimate clinical entity in which the diagnosis of Lyme disease is secured only by clinical signs and symptoms of the illness and by the physician's clinical evaluation of the patient's illness. The presence or the absence of free antibodies in the serum of the "patient is non-contributory because of any of the following scenarios. 1. Early Lyme infections with "a single "diagnostic lesion on the skin (Erythema migrans) are stand- alone proof of infection. 2. Earlystage patients with "paired concurrent" symptoms of facial nerve palsy and simultaneous arthritis or "paired and concurrent" symptoms of heart block and meningitis are standalone criteria for Lyme infections. 3. Later stage patients with fatigue, pain syndromes, cognitive issues, psychiatric symptoms may fail to produce detectable antibodies in their serum due to immune system suppressions due to HIV illness, corticosteroid medications, inherited immune system illnesses, Immune complexes in the serum "Immune complex disease", antigen excess "prozone "immune situations, or to immaturity of the immune system in pediatric years. *Lyme borrelia spirochetes* which bind to the Lyme related antibodies in whole blood can "permanently and incrementally remove" *Lyme borreliosis* disease infection specific "free and unbound "antibodies in serum fractions derived whole blood. If enough live spirochetes are present in the bloodstream (or in tissue sites) the total Lyme specific antibody in ***soluble phase*** in residual serum fraction after blood clotting will decline with each incremental increase in the actual number of solid spirochetes in the host's body. When total "antibody binding sites" on the total number of spirochetes in the body, is sufficient, a point is reached whereby all bloodstream soluble phase serum Lyme antibodies might be permanently "taken out of solution in the serum" because all diagnostic Lyme infection related antibodies are bound to the solid surfaces of whole borrelia spirochetes. Biofilm communities can also demonstrate "solid phase binding of Antibodies" in whole blood. The serum which remains after blood clotting will therefore fail to contain any residual "free antibodies" after the removal of whole blood antibodies in the solid phase

bound antibody fraction by clotting of the whole blood.

## Materials and Methods

### *Patient Selection*

An international group of Volunteer patients recorded their geographical residences as follows: England N=5, Denmark N=5, Norway N=2, California N=4. Wisconsin N=2, Michigan N=4, New York N=4, New Jersey N=2, Connecticut N=1, Massachusetts N=3.

Whole blood thin smears were prepared from EDTA anticoagulated phlebotomy vacutainer tubes and the thin smears were air dried at 24°C. A numerical code representing the date of birth and the patient first name initial and last name initials was written on each slide. The slides were then transported by USPS mail to the laboratory of the principal investigator where they were stored at room temperature until examination.

### *Immunohistochemistry Technique*

Fluorescent labeled Monoclonal Antibody CB10 [1] to borrelia burgdorferi group major protein OSP A was Biotium Inc Mix-n-Stain CF660R (red chromogen) antibody labelling Kit Catalog 92243 according to the manufacturer recommendations. Red fluorochrome labeled CB10 anti OSPA was layered over one thin smear glass slide from each patient and incubated at 24°C. for 30 minutes. The slide was then washed in PBS, pH 7.4. The slides were examined for detection of areas of red color fluorescent antibody attached to any area of the thin blood smear. Fluorescent labeled Antiglobulin reagent was prepared from a validated Coombs reagent [2] (Ortho Clinical Diagnostics, Anti-Human globulin Anti IgG (Rabbit) heavy chain specific, Catalog 716360). (This Coombs antiglobulin reagent is used in many USA hospital blood banks to evaluate potential blood transfusion units and to select safe recipient-compatible red blood cell transfusion units for patients with immune hemolytic anemias). A fluorescent label Biotium Inc Mix n Stain 405 S (a blue color fluorochrome) was attached to the commercial Coombs reagent. The blue color fluorochrome labeled Coombs reagent was layered over unstained blood smears for 30 minutes at 24°C. the slides were then washed with PBS pH 7.4. Fluorescent microscopy detected any Coombs reagent positive bound areas in the blood smears.

## Results

Unstained blood smears which were individually immunostained and examined in this study of thirty international European and USA residents whose physicians determined that each patient volunteer in this study presented with "at risk for chronic borrelia burgdorferi infection" produced unanimous discoveries of borrelia spirochetes in their bloodstreams. Furthermore, it was demonstrated that microscopic evidence for existence of borrelia spirochetes and borrelia biofilms in their peripheral whole blood smears was robust, and not difficult to identify. It is inferred that all European volunteers were chronically infected with European strains of Borrelia burgdorferi group BBsl including afzelii, garinii, and other European Burgdorferi strains). It is also inferred that all USA patients were infected with USA strains borrelia including possible cases of Borrelia americanum or possible borrelia mayonii in two Wisconsin patients and other known strains of USA borrelia in the BBsl group.

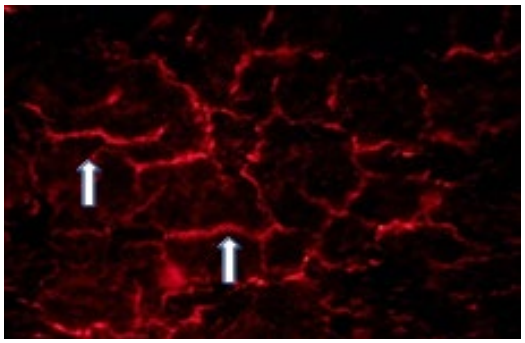

**Figure 1A:** Blood smears with positive for binding of Monoclonal Antibody CB10 to OSP A of borrelia spirochetes demonstrate live borrelia in whole blood.

*Borrelia burgdorferi* group BBss and Group BBsl all contain a major protein OspA on their surfaces which binds Monoclonal Antibody CB10 (red color fluorescence). Magnification 1000x of original.

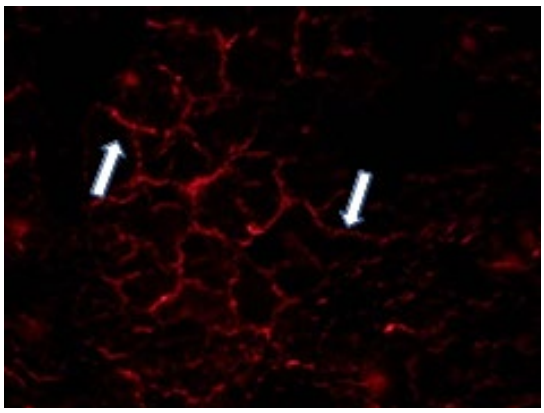

**Figure 1B:** Blood smears with positive for binding of Monoclonal Antibody CB10 to OSP A of borrelia spirochetes demonstrate live borrelia in whole blood.

*Borrelia burgdorferi* group BBss and Group BBsl all contain a major protein OspA on their surfaces which binds Monoclonal Antibody CB10 (red color fluorescence). Magnification 1000x of original.

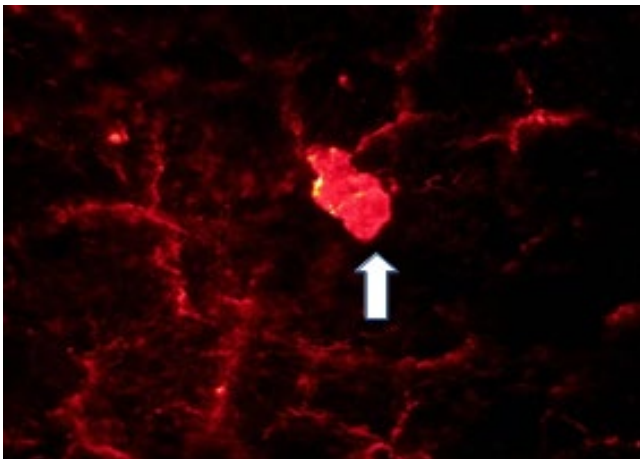

**Figure 1C:** Blood smears with positive for binding of Monoclonal Antibody CB10 to OSP A of *borrelia spirochetes* demonstrate live borrelia in whole blood.

*Borrelia burgdorferi* group BBss and Group BBsl contain a major protein OspA which binds Monoclonal Antibody CB10 (red color fluorescence). Magnification 1000x of original.

A single Biofilm community of *borrelia burgdorferi* spirochetes with a globular shape (white arrow) is demonstrated and additional red color single *borrelia spirochetes* are distributed across the blood smear.

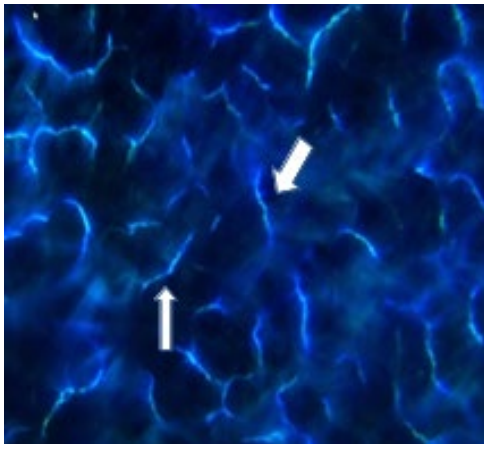

**Figure 2A:** Fluorescence positive blood smears with Coombs Test using human antibodies bound to individual Borrelia spirochetes in blood smear.

Single separate borrelia spirochetes (blue fluorescence) are stained with the Blue labeled Coombs reagent which binds to the human immunoglobulin IgG antibodies attached to the surface of each spirochete. (Blue bright signal fluorescence from the Coombs reagent).

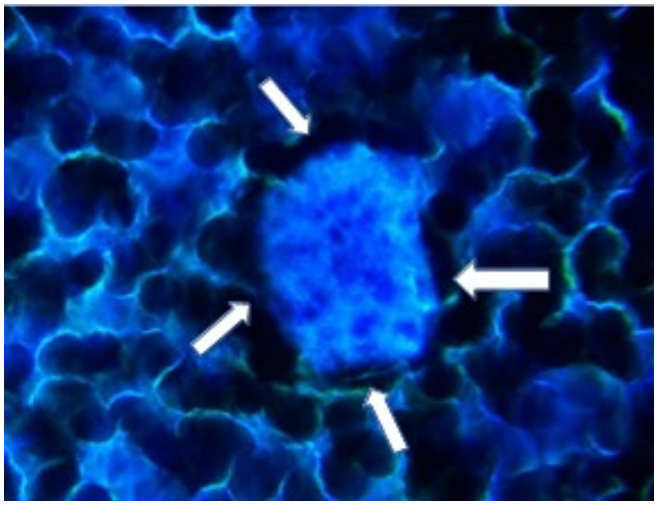

**Figure 2B:** Fluorescence positive blood smears with Coombs Test using human antibodies bound to Biofilm ***communities of Borrelia spirochetes in blood smear.***

Biofilm community of *borrelia burgdorferi* (White arrow) demonstrates aglobular pattern high signal blue color fluorescence and is remarkable for "Swiss cheese pattern "empty spaces of water channel spaces which are typical for biofilm communities. Individual single *borrelia spirochetes* bind blue color Coombs reagent antibodies to attached patient produced antibodies to the *Lyme borreliosis* infection which have previously bound to Proteins on the surface of free *borrelia spirochetes* in the whole blood smear. Magnification 1000x original.

## Discussion

The unanimous presence of live *borrelia spirochetes* in the bloodstreams of thirty international residents who were "ELISA Lyme seronegative" by CDC criteria but who were clinically positive for "at-risk Chronic, or" Long Haul" clinically symptomatic borrelia infections is unpredicted but is perhaps relevant for the greater world- wide community of Lyme borreliosis infected patients with long term unrelenting active symptoms and signs of " Lyme borreliosis-like' infections which fail to satisfy the Centers for Disease Control (CDC) definitions for declaration of "significant" serum antibody levels in their commercial laboratory blood test results. Further research with whole blood specimens from ELISA Lyme seronegative patients with active persistent symptoms and clinical signs of "at- risk chronic Lyme borreliosis infections" is needed. There is an urgent need to revise the CDC Lyme diagnostic "rules" for the interpretation of results of serum specimen-based Lyme ELISA antibody testing and recommended guidelines for the interpretation and clinical action recommendations for physicians who are responsible for the care of individual chronically ill Lyme borreliosis patients internationally. Conventional opinions in the Lyme disease medical community for the past 40 years discuss that in Lyme disease patient, the peripheral blood smears very rarely demonstrate circulating borrelia spirochetes after the initial tick bite exposure. These false opinions are not based on any published and peer reviewed actual microscopic study of individual patient

whole blood smears and therefore do not represent a product of peer reviewed manuscripts and "precision medicine." Biofilms of any class of microbe in any peripheral blood are a potential marker of chronic infection. Current opinions among academic observers in the Lyme disease medical community dispute the legitimacy of bona fide chronic infections with live spirochetes in late Lyme disease patients. The entity of so called "post-Lyme syndrome" has been entrenched in the literature. Recently the Johns Hopkins Lyme Center has coined the term "long Haul" Lyme disease to fill the void which separates "Early" from "Late" Lyme disease without engaging proof that live borrelia infections continue to live in the body of Chronic Lyme patients. A third academic group of physicians recognizes that in late disease, the spirochetes live on in biofilm communities which are resistant to antibiotic killing of spirochetes inside the Biofilm community. Microbiologists agree that biofilm lifestyles are a reality for more than 99% of all categories of disease-causing bacteria. Biofilms of infectious microbes in human blood or in human tissue sites are microscopic visually compelling evidence of proof of chronic infections at the site of biofilm deposits. Thirty international patients who volunteered to participate in this research study universally demonstrated that persistent borrelia genus spirochetes were present in their blood stream thin smears with Monoclonal antibody staining. Individual borrelia spirochetes in whole blood smears and Monoclonal antibody-stained biofilm communities of borrelia in whole blood smears were robustly present. Thirty international patients, all had ELISA negative serologies in their serum but demonstrated, in the judgment of their personal physicians, clinical signs and symptoms "at risk for chronic borrelia disease". Thirty international patients demonstrated that microscopically visible species of borrelia burgdorferi actually bound human antibodies attached to the surfaces of the bloodstream borrelia with a well-controlled fluorescent labeled Coombs antiglobulin reagent, for detection of bound human antibodies. This is the very first discovery of "spirochete bound human antibodies" in the blood streams of chronically infected borrelia species infected human patients with negative Lyme ELISA test results. In this patient group of thirty chronic Lyme disease patients, a negative ELISA Lyme test result was used as **"evidence in argument against their true biologically positive chronic infection status".** Borrelia infection was present in each patient's blood. Biologically misleading medical information transmitted in Lyme ELISA test reports was not a consequence of "failure" of the blood specimens to harbor human antibodies to Lyme borreliosis anywhere in their bloodstream or in their body organs, but rather a consequence of the laboratory failure to analyze the blood clots which actually contained the patient's antibodies to *Lyme borreliosis* spirochetes which were bound to the surfaces of the microbes in the blood clot portion of their venipuncture specimen. The true discrimination of persistent active infections in thirty patients was revealed by microscopic detection "direct microbe diagnosis by visual identification" and the triumph of the "direct detection microscopy" over indirect free antibody in serum testing in the ELISA test method. The analytical tools for parameters of "Sensitivity" and "Specificity" and "Predictive Value" of medical laboratory testing were elegantly defined in 1975 by Drs. Galen and Gambino in their classic monograph **"Beyond normality: The predictive value and efficiency of medical diagnoses."** [3]. The current versions of internationally utilized techniques in medical diagnostic blood serum ELISA Lyme technologies today receive a grade of "poor data results" when viewed through the lens of Dr. Galen and Dr. Gambino's public health guidelines. The much-sought caveats of **"positive results in disease and negative results in health."** define the absolute best practices that can be demanded of Lyme borreliosis blood testing in year 2022. Going forward, better medical diagnostic results in laboratory testing for Lyme borreliosis will be garnered only when the monograph medical diagnostic guidance principles in the book **"Beyond Normality**" [3] are heeded. Attention to a new "high quality "analytical product, namely microscopic evaluation of "Whole blood" received for analysis from at risk chronic *Lyme borreliosis* patients and which is subjected to Coombs reagent microscopic analysis provide an opportunity to discover heretofore "hidden" infectious microbes which are coated with host produced antibodies.

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