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A new platform for serological analysis based on porous 3-dimensional polyethylene sinter bodies

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ABSTRACT

A new sensitive and selective platform, three-dimensional immunosensor, has been developed for a rapid serological diagnosis; detection of a *Borrelia* infection was considered as a model assay. The immunosensor is based on a 3-dimensional (3D) porous solid surface (sinter body) with dimensions of 2 × 2.5 mm where a recombinant variable lipoprotein surface-exposed protein (VlsE; *Borrelia*-antigen) is immobilized by different techniques. The sinter body served as a robust and inexpensive carrier, which facilitated a successful hydrophobic adsorption as well as covalent immobilization of the antigen with sufficient amounts of on the surface. Because of sinter body's porosity, the detection could be performed in an immune affinity flow system based on a little disposable plastic column. The flow of reagents through the column is advantageous in terms of reducing the non-specific interaction and shortening the test time. Furthermore, three labels were tested for a colorimetric detection: i) a horseradish peroxidase (HRP) labeled secondary antibody, ii) nanoparticles based on Sudan IV, and iii) gold nanoparticles modified with protein A. HRP secondary labeled antibody provides the most sensitive test, 1000 fold dilution of serum sample can be clearly detected in only 20 min. Gold nanoparticles modified with protein A were used as a direct label or as a catalyst for reduction of silver ions. Direct detection with gold nanoparticles provides short time of analysis (5 min) while detection of metallic silver required longer time (12 min) but with improved sensitivity. Nanoparticles based on Sudan IV showed high background and were less favorable. The assay is distinctive because of the rapid analysis time with all used labels, longest 20 min. Compared to classical serological methods for *Borrelia* diagnosis, the developed method offers a simple, rapid and reliable tool of analysis with minimal cost and can be easily transferred to other infectious diseases.

1. Introduction

At time, there are many infectious diseases worldwide on progress. An antibiotic therapy is usually successful only if it is started in the early stage of the infection. Therefore, there is an urgent need for rapid, simple and inexpensive detection methods, mainly based on immunological reactions, allow point of care (POC) diagnostics. One of the most common complicated infectious diseases of North America and Europe is the 'lyme borreliosis', which is caused by tick-borne spirochaete bacteria known as *Borrelia burgdorferi* in a broad sense [1]. At least five different *Borrelia burgdorferi* genospecies are pathogenic in human, *Borrelia afzelii*, *Borrelia garinii*, *Borrelia burgdorferi*, *Borrelia spielmanii*, and *Borrelia bavariensis* [2]. *Borrelia* is transmitted to human by specific *Ixodes* spp ticks; the transmission takes place during the tick feeding, which occurs through injection of tick saliva to the host. Usually 36 h as feeding period are needed for the transmission. Three clinical stages of lyme borreliosis could appear and may overlap at the patient: [3] i) Erythema migrans, a rash increase centrifugally, appears after days to few weeks of the tick bite [4], ii) *Borrelia* spreads into bloodstream causing clinical signs of the early dissemination; many organs may be affected at this stage including nervous system, the joints, and the heart. This stage appears after few weeks to months from infection [5]; iii) if lyme borreliosis is not treated, the third stage that appears as chronic organ involvement occurs, persistent joint inflammation and joint swelling. In addition, direct involvement of the eye could take place at this stage [6].

Directly after infection there is good chance that the disease can be successfully treated with antibiotics (mainly with tetracyclines). Therefore, a rapid and early POC diagnostics is required. Detection of lyme borreliosis can be directly performed when the

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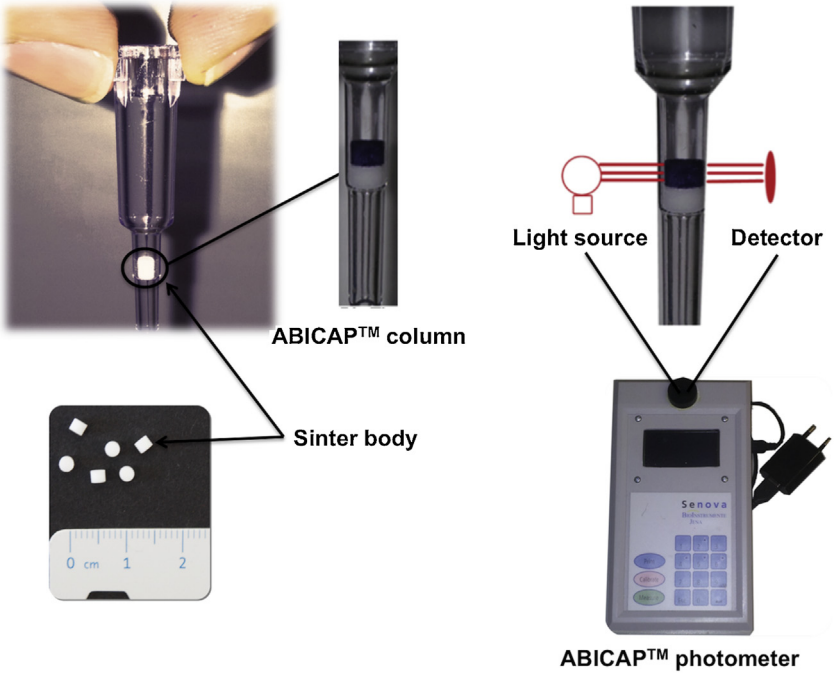

Fig. 1. ABICAP™ system, 2 × 2.5 mm 3-DPESB were placed inside a small column or a pipette tip. Optical density after employing the label can be measured at 650 nm or 520 nm using ABICAP™ photometer.

patient shows the typical borreliosis symptom, erythema migrans with fever following a tick bite in an endemic lyme disease area. Diagnosis of lyme borreliosis in laboratory are mainly based on four different techniques: i) the direct detection of intact *Borrelia* spirochetes using microscope based observation [7], ii) cultivating of *Borrelia* in Barboure-Stoennere-Kelly (BSK) [8] medium followed by microscopic detection of spirochetes [9], iii) detection of *Borrelia* specific DNA sequence using the polymerase chain reaction (PCR) [10], and iv) the indirect detection based on measurement of *Borrelia* specific IgG and IgM antibodies using serological immunoassays [11]. Serological diagnosis as an indirect detection method mainly comes in laboratory as a confirmatory test due to the high specificity of the antibody-antigen interaction, besides the high sensitivity of the test arising from the detecting labels. After 2 – 6 weeks of Erythema migrans, serological diagnosis is mainly positive with one tier test. In early stage, the main disadvantage of serology represented in the possibility of false-positive results arising from the cross reaction with other non pathogenic organisms; therefore two-tier test [12] was recommended by US Centers for Disease Control and Prevention (CDC) [13]. However the method is still time consuming and intensive. As an alternative, choosing a highly specific antigen, which induces a strong antibody response that can be detected from early to late phase during the disease, shows low cross reaction with the other non pathogens, and gives the same results as two-tier test could be an advantage to overcome the long analysis time of the two-tier test.

As the assay presented here has been designed as serological test for detection of antibodies directed against *Borrelia*, highly specific VlsE has been chosen as antigen. This protein induces a strong and specific antibody response that can be detected from early to late phase during the disease in humans infected with *Borrelia burgdorferi* or with European genospecies including *Borrelia afzelli* and *Borrelia garlnii*. Simultaneous it shows low cross-reaction with the other non-pathogenic *Borrelia* strains [14]; this was utilized to improve the validity of the test. In this work a new assay platform based on three-dimensional polyethylene sinter bodies (3-DPESB) has been developed for a rapid serological diagnosis. The 3-DPESB can be either placed in a small column (Fig. 1, ABICAP™ system) or alternatively directly in a pipette tip. 3-DPESB with dimension of 2 × 2.5 mm were used as robust and inexpensive carriers for the immunoassay. Three different labels incorporated with the 3-DPESB have been tested in a noncompetitive assay (Fig. 2), namely horseradish peroxidase (HRP) secondary labeled antibodies, nanoparticles based on Sudan IV and gold nanoparticles modified with protein A.

2. Experimental

2.1. Materials and buffers

If not otherwise mentioned, chemicals were obtained from Fluka, Sigma-Aldrich or Merck, Germany, in analytical grade quality. Benzophenone and 4-(dimethylamino)-pyridine were obtained from Merck-Schuchardt, Germany. N,Ní-disuccinimidyl carbonate ≥ 95% (NMR) and anti-human IgG (γ-chain specific)-biotinylated antibody were from Sigma-Aldrich, Germany. Allyl alcohol 99.5% (GC) and ethanolamine 99% were purchased from Fluka, Germany. Acetone 99.5% and methanol were obtained from Fisher scientific, UK. Ethanol and bovine serum albumin (BSA) (albumin fraction V, from bovine serum for biochemistry) were from Merck (Darmstadt, Germany).

Full-length 56 kDa VlsE *Borrelia* recombinant antigen in PBS (pH 7.2–7.4; 0.15 M NaCl) (*Borrelia afzelii,* expressed in *E.coli* and purified with affinity chromatographic techniques), positive human-sera anti-VlsE (IgG) that derived from patients with assumed lyme borreliosis, negative human-sera are from normal blood donors (sera samples pre-tested by Seramun luminescence immunoassay LIA-006 for IgG, anti-human IgG-HRP, and 3,3′, 5,5′-tetramethylbenzidine (TMB/substrate solution) were from Seramun Diagnostica, Heidesee, Germany.

Casein buffer concentrate (CBC) (5.5%) and conjugate buffer [antibody/antigen (poly HRP) conjugate stabilizer (AA1)] were from Stereospecific Detection Technologies, Baesweiler, Germany. ABICAP™ column, sinter bodies (3-DPESB) and photometer with