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Performance of a multiplexed serological microarray for the detection of antibodies against central nervous system pathogens

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ABSTRACT

Central nervous system (CNS) infections have multiple potential causative agents for which simultaneous pathogen screening can provide a useful tool. This study evaluated a multiplexed microarray for the simultaneous detection of antibodies against CNS pathogens. The performance of selected microarray antigens for the detection of IgG antibodies against herpes simplex virus 1 and 2 (HSV-1 and HSV-2), varicella-zoster virus (VZV), adenovirus, *Mycoplasma pneumoniae* and *Borrelia burgdorferi* sensu lato, was evaluated using serum sample panels tested with reference assays used in a routine diagnostic laboratory. The microarray sensitivity for HSV-1, HSV-2, VZV, adenovirus and *M. pneumonia* ranged from 77% to 100%, and the specificity ranged from 74% to 97%. Very variable sensitivities and specificities were found for borrelial antigens of three different VlsE protein IR(6) peptide variants (IR6p1, IR6p2, IR6p4) and three recombinant decorin binding proteins A (DbpA; DbpAIa, DbpA91, DbpAG40). For single antigens, good specificity was shown for antigens of IR6p4 and DbpAIa (96%), while DbpA91, IR6p1 and IR6p2 were moderately specific (88–92%). The analytical sensitivity of the microarray was dependent on the borrelial IgG concentration of the specimen. The overall performance and technical features of the platform showed that the platform supports both recombinant proteins, whole viruses and peptides as antigens. This study showed diagnostic potential for all six CNS pathogens, including *Borrelia burgdorferi* sensu lato, using glutaraldehyde based microarray, and further highlighted the importance of careful antigen selection and the requirement for the use of multiple borrelial antigens in order to increase specificity without a major lack of sensitivity.

1. Introduction

One of the challenges in the laboratory diagnosis of central nervous system (CNS) infections is the relatively high number of potential causative pathogens, including e.g. herpes simplex virus 1 (HSV-1) and 2 (HSV-2), varicella zoster virus (VZV), *Borrelia burgdorferi* sensu lato, and *Mycoplasma pneumoniae*. Nucleic acid detection from cerebrospinal fluid (CSF) is usually the method of choice for the diagnosis of neuroinvasive virus infections, especially for different herpes viruses. However, virus serology from serum and CSF provides complementary information, and plays an important role particularly in prolonged infections, and in the investigation of long-term sequelae. For neuroinvasive *M. pneumoniae* infections, both nucleic acid detection and serology are generally used, while serology is most sensitive for neuroborreliosis. However, the serodiagnosis of *Borrelia burgdorferi* sensu lato is generally complicated by the very variable antibody responses observed between individuals and those at the different stages of the disease. Furthermore, the borrelial antibody response may not reflect an infection, but merely a contact with a tick carrying the pathogen. Due to the complex nature of borreliosis, several different diagnostic methods, e.g. enzyme immunoassays (EIA) and immunoblotting, are often used in parallel to obtain more specific and reliable results for diagnosis Lyme borreliosis. In addition, for the serodiagnosis of borrelial infection, it is generally suggested that two specific antigens should react with patient's serum to establish the diagnosis.

EIA is the most commonly used platform for the serodiagnosis of neuroinvasive infections, sometimes complemented by e.g. immunoblot. Simultaneous screening of antibodies against relevant viral and bacterial targets can provide an advantageous tool for the diagnosis of

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CNS infections. Serological platforms with a multiplexed format offer a rapid, cost-effective and clinically comprehensive approach. Such platforms include multiplex bead-based immunoassays and multiple antigen microarrays, which allow for parallel antibody detection. Different multiplex bead-based immunoassays have been developed for e.g. detection of antibodies against influenza virus, *Streptococcus pneumoniae*, Lyme borreliosis, or pneumococcal, meningococcal and haemophilus polysaccharides, and tetanus and diphtheria toxoids (Yan et al., 2005; Yu et al., 2011; Whitelegg et al., 2012; Gerritzen and Brandt, 2012).

Multiplexed serological microarrays have been developed for e.g. simultaneous detection of HIV and its coinfections (Lochhead et al., 2011), as well as for the diagnostics of atypical pneumonia (Gouriet et al., 2008). As the detection of CNS pathogens, previous microarray designs have focused on the viral nucleic acid detection (Jääskeläinen et al., 2006, 2008; Leveque et al., 2011; Mannonen et al., 2012) or detection of herpesvirus, rubella virus and *Toxoplasma gondii* antibodies using nitrocellulose based or amino-silane activated glass microarrays and proteins as antigens (Bacarese-Hamilton et al., 2004; Jääskeläinen et al., 2009). Proteome microarray has been set up to profile immune response to *Borrelia burgdorferi* sensu lato (Xu et al., 2008) but no serological or nucleic acid based microarrays have been described earlier for diagnosis of Lyme borreliosis.

Our aim was to find a suitable microarray platform that supports different kinds of antigens, not just proteins, for the detection of viral and bacterial antibodies, and to set up a microarray for the detection of antibodies against *Borrelia burgdorferi* sensu lato for the diagnosis of Lyme borreliosis. In addition, our aim was to evaluate the feasibility of a multiplexed serological glutaraldehyde based microarray as a platform for comprehensive diagnostics of other CNS infections, especially HSV-1, HSV-2, VZV, adenovirus and *M. pneumoniae*, using serum samples. Glutaraldehyde coated microtiter plate based microarray (Viitala et al., 2013) was set up using different types of antigens, i.e. recombinant proteins, inactivated viruses and peptides, and evaluated for the simultaneous detection of antibodies against HSV-1, HSV-2, VZV, adenovirus, *M. pneumoniae* and *Borrelia burgdorferi* sensu lato from clinical serum specimens, by comparing them to commercial reference assays.

2. Materials and methods

2.1. Reference methods

HerpeSelect® HSV-1 IgG and HSV-2 IgG ELISA kits (Focus Diagnostics Inc, Cypress, CA, USA) were used for HSV antibody detection. *Mycoplasma pneumoniae* IgG EIA (Ani Labsystems Ltd, Vantaa, Finland) was used for *M. pneumoniae* antibody detection. *Borrelia afzelii* and VlsE IgG ELISA kit (Sekisui Virotech GmbH), Liaison® Borrelia IgG (DiaSorin), and Borrelia Europe Plus TpN17 Line IgG Line Immunoblot (Sekisui Virotech GmbH, Rüsselsheim, Germany) were used for the detection of borrelial IgG. The linear measurement ranges were 9–40 AU/ml for the Sekisui Virotech test, and 5–240 VE/ml for the DiaSorin test. A positive reaction in the immunoblot required distinct reactions against at least two of the antigens included in the assay (OspC, VlsE-mix, p39, DbpA, p58 or p83). All tests were performed according to the manufacturers' instructions. A validated and accredited in-house sandwich-EIA (HUSLAB, Finland) with VZV glycoprotein EIA antigen (Institute Virion Ltd, Rüschlikon/Zürich, Switzerland) and in-house adenovirus EIA (HUSLAB, Finland) with inactivated whole adenovirus antigen were used for VZV and adenovirus IgG detection, respectively. In short, the in-house adenovirus IgG EIA was carried out using 96-well microtiter plates coated with attenuated adenovirus 1 (strain 1A891) antigen (HUSLAB, Finland) followed by blocking with 1% bovine serum albumin (BSA; Jackson ImmunoResearch Europe Ltd, Suffolk, UK). Serum samples were diluted 1:100 in phosphate buffered saline (PBS) containing 0.05% Tween 20 (PBST) and incubated for 1 h at +37 °C followed by three washes with PBST. Rabbit anti-human IgG conjugated with horse radish peroxidase (HRP; Dako, Helsinki, Finland) was diluted 1:10,000 and used for a 1 h conjugation at +37 °C. 3,3′,5,5′-tetramethylbenzidine liquid substrate (Sigma-Aldrich) was used for colour reaction after washing three times with PBST. After 30 min incubation at RT, the colour reaction was stopped with 100 μL of 0.5 M H_2SO_4. The absorbance was read at 450 nm (Multiscan RC, Labsystems, Finland).

2.2. Clinical specimens

The study material included serum samples from individual patients tested for IgG antibodies against the following antigens at the routine diagnostics of the Helsinki University Central Hospital Laboratory (HUSLAB) in Finland in 2009–2012: HSV-1 ($n = 81$), HSV-2 ($n = 82$), VZV ($n = 77$), adenovirus ($n = 77$), *M. pneumoniae* ($n = 70$), and *Borrelia burgdorferi* sensu lato ($n = 53$). The sera tested for HSV-1, HSV-2, VZV, adenovirus and *M. pneumoniae* IgG antibodies were deemed either positive or negative by using the respective reference methods. The sera deemed positive for *Borrelia* IgG ($n = 35$) were reactive both in two different EIA tests (Sekisui Virotech and DiaSorin) and in IgG immunoblot. The sera deemed negative for *Borrelia* IgG ($n = 18$) were non-reactive in one EIA test (Sekisui Virotech). In order to better evaluate the analytical sensitivity of the microarray, the reactive borrelial specimens were further divided into three subcategories according to their IgG antibody concentrations as follows: low IgG concentration ($n = 9$), intermediate IgG concentration ($n = 13$), and high IgG concentration ($n = 13$). The sum of numeric values from the two EIA tests (Sekisui Virotech and DiaSorin) was used for this categorization (low IgG concentration 25–59; intermediate 60–179; high >180).

2.3. Microarray design

Single antigens for HSV-1, HSV-2, VZV, adenovirus and *M. pneumoniae* were used for simultaneous screening of specific IgG antibodies. The antigens used for the microarray were as follows: recombinant HSV-1 glycoprotein G (gG, amino acids 84–175; Acris Antibodies GmbH, Herford, Germany), recombinant HSV-2 glycoprotein G [gG, amino acids Leu343-Asp649 fused with human superoxide dismutase (SOD); Acris Antibodies GmbH, Herford, Germany], purified whole virus antigen of attenuated adenovirus 1 (strain 1A891) (HUSLAB, Finland), *M. pneumoniae* 1 AG (4MP67; Ani Biotech Oy, Vantaa, Finland), and recombinant VZV gE (University of Gothenburg, Gothenburg, Sweden; 11). This recombinant VZV gE antigen is produced in mammalian cells in large scale and is known to be specific for VZV showing no cross-reaction with HSV (Thomsson et al., 2011; Grahn et al., 2011).

The complex nature of the immune response to *Borrelia burgdorferi* sensu lato infection requires the use of multiple serological assays to increase diagnostic sensitivity and specificity (Bacon et al., 2003; Porwancher, 2003; Porwancher et al., 2011). With this in mind, six antigens for *Borrelia* were selected for the microarray: three different VlsE protein IR6 peptide variants (Core Facility of Protein Chemistry, Haartman Institute, University of Helsinki, Helsinki, Finland; Sillanpää et al., 2007) of *Borrelia burgdoferi sensu stricto* B31 (IR6p1), *B. garinii* IP90 (IR6p2), and *B. afzelii* ACAI (IR6p4), and three recombinant decorin binding proteins A (DbpA) (University of Helsinki, Helsinki, Finland; Panelius et al., 2007) derived from *B. burgdorferi sensu stricto* IA (DbpAIa), *B. garinii* 40 (DbpAG40), and *B. afzelii* A91 (DbpA91).

Prior to spotting, HSV-1, HSV-2, *M. pneumoniae*, and all six borrelial antigens were diluted in spotting buffer [10 mmol/L $NaHCO_3$ (pH 9.5)] in a final concentration of 0.1 mg/ml. VZV and adenovirus antigens were diluted in a final concentration of 1:10.

The 96-well polystyrene plates were activated with pre-polymerized glutaraldehyde, and each well was spotted with the 11 viral and bacterial antigens in triplicates by using the BioRobotics MicroGrid II microarray printer (BioRobotics, Cambridge, UK) as previously described (Viitala et al., 2013). Human IgG (~95% HPLC purified; Sigma-Aldrich, Helsinki, Finland) was spotted in triplicates and used as a positive control on the microarray. The spotting buffer and rabbit myosin (Sigma–Aldrich)

were included as negative controls. In summary, there were 42 spots on each polystyrene plate well. One well was used per serum sample for simultaneous and parallel screening of different viral and bacterial antibodies.

2.4. Microarray reactions

Once the antigens were spotted, the microarrays were blocked with PBS containing 2% BSA (Jackson ImmunoResearch Europe Ltd) for 30 min, followed by three washes with PBS containing 0.1% Tween 20 (PBST2). Blocked microarrays were dried, and stored at −70 °C prior to use.

Serum samples (volume 2 μl) were diluted 1:100 in 0.5% BSA (Jackson ImmunoResearch Europe Ltd) in PBS, incubated for 15 min at +37 °C on the microarrays, and washed three times with PBST2. Peroxidase conjugated Affinipure donkey anti-human IgG (H + L) (Jackson ImmunoResearch Europe Ltd; diluted 1:5000 in PBS) was inclubated on the microarrays for 15 min at +37 °C. The microarrays were washed again (3 × PBST2), subjected to 3,3′,5,5′-tetramethylbenzidine chromogen of ep(HS)TMB-mA (SDT Stereospecific Detection Technologies, Baesweiler, Germany) for 5 min, and dried followed by imaging.

2.5. Microarray image analysis

Images of microarrays were taken by an inverted microscope (Olympus CKX41; Olympus Finland Oy, Espoo, Finland) using Olympus DP12 digital microscope camera (Olympus Finland Oy). One microarray image consisted of the 42 spots. Spot intensities for each antigen and local background signals on the microarrays were calculated using ImageJ software (http://rsbweb.nih.gov/ij/). Cut-off values were determined for each antigen separately using five to nine negative serum samples. Briefly, negative serum samples were screened in triplicate and analysed. The spot intensities were corrected for background signal, and cut-off values were determined for each antigen by multiplying the mean value of intensities by two. The equivocal range was set at +/− 10% from the cut-off intensity value.

3. Results

The serum samples panels were tested in parallel with the serological microarray and the reference methods. Equivocal intensity values in the microarray were interpreted as negative throughout the calculations. Table 1 summarizes the performance of the microarray for HSV-1, HSV-2, VZV, adenovirus, and *M. pneumoniae*. The sensitivity of the microarray for these targets ranged from 77% to 100%, and the specificity ranged from 74% to 97%. The overall sensitivity of the microarray for all these five targets was 91%, while the overall specificity was 85%. The best concordance as compared to reference tests was observed for HSV-1 and VZV (Table 1).

Table 2 summarizes the performance of the microarray for Lyme borreliosis. Due to the nature of microarray technique, the seroresponse detected using multiple antigens can be analysed using different rule sets to see whether some individual antigens or antigen combinations are superior over others for detecting borrelial antibodies. First, specificity and sensitivity were calculated separately for each borrelial antigen to assess their individual performance. Thereafter, the performance values were calculated for selected antigen combinations, with the requirement of simultaneous reactivity (sensitivity) and non-reactivity (specificity) against two different antigens. Finally, the performance was analysed for the three *Borrelia* species separately, with the requirement of reactivity against either of the two antigens specific for the species.

Good specificity was shown for IR6p4 and DbpAIa (96%), while DbpA91, IR6p1 and IR6p2 were moderately specific (88–92%). The specificity of DbpG40 was poor (63%), but showed the highest sensitivity (89%). The sensitivities of DbpAIa and IR6p2 were 77% and 83%, respectively, and these two antigens demonstrated the best overall performance. For *B. burgdorferi sensu stricto*, a moderate specificity (88%) and sensitivity (86%) were observed. Sensitivity for *B. afzelii* was moderate (88%), but with a poor sensitivity (57%). For *B. garinii*, in contrast, sensitivity (97%) was superior of the specificity (58%). When samples positive for borrelial IgG antibodies, were divided into three subcategories (low, intermediate and high), the sensitivities were more diverse between different antigens. Highest sensitivity values, ranging from 77% (DbpA91) to 100% (DbpAG40) were found among the samples showing high concentration of borrelial IgG, while the sensitivity of samples with intermediate IgG concentration varied from 23% (DbpA91) to 85% (IR6p2). Among the low IgG concentration *B. burgdorferi* sensu lato samples, sensitivity levels were weak for IR6p4 (11%), DbpA91 (11%), and IR6p1 (11%), but higher sensitivity values were found for DbpAIa (67%), IR6p2 (67%) and DBpAG40 (89%) antigens.

4. Discussion

The microarray technique enables simultaneous evaluation of multiple antigens, and the performance of the selected antigens can be validated using sample panels tested with selected reference assays. This study evaluated antigens for the detection of IgG antibodies against important CNS pathogens, namely HSV-1, HSV-2, VZV, adenovirus, *M. pneumoniae* and species of *B. burgdorferi sensu stricto*, which is the predominant species in North America, as well as *B. garinii* and *B. afzelii*, both predominant in Eurasia (Steere, 2001; Qiu et al., 2002; Hengge et al., 2003; Bunikis et al., 2004), causing Lyme borreliosis. Microarrays have been used for screening purposes (Bacarese-Hamilton et al., 2004; Xu et al., 2008; Jääskeläinen et al., 2009; Lochhead et al., 2011; Ardizzoni et al., 2011; Viitala et al., 2013), but they can be applied also for settings requiring enhanced specificity. Recent investigations exploited the microarray platform for profiling of immune responses to *Borrelia burgdorferi* sensu lato (Xu et al., 2008), and another to influenza viruses, suggesting that some level of humoral immunity may not

Table 1
Microarray performance for HSV-1, HSV-2, VZV, adenovirus and *M. pneumoniae* in comparison to reference methods. Equivocal microarray test results were interpreted in this analysis as negative. Equivocal microarray results were observed as follows: HSV-2/Ref- ($n = 1$), VZV/Ref + ($n = 1$), and *M. pneumoniae*/Ref + ($n = 2$).

	Reference	Array + (N)	Array − (N)	Total (N)	Sensitivity % [95% CI]	Specificity % [95% CI]
HSV-1	Ref+	31	3	34	91 [77, 97]	89 [77,95]
	Ref−	5	42	47		
HSV-2	Ref+	22	3	25	88 [70, 96]	74 [61, 83]
	Ref−	15	42	57		
VZV	Ref+	59	5	64	92 [83, 97]	92 [67, 99]
	Ref−	1	12	13		
Adenovirus	Ref+	58	0	58	100 [94, 100]	84 [62, 94]
	Ref−	3	16	19		
M. pneumoniae	Ref+	33	10	43	77 [62, 87]	97 [82, 99]
	Ref−	1	26	27		

Ref+, positive result in reference test; Ref−, negative result in reference test; Array+, positive result in microarray; Array−, negative result in microarray. CI, confidence interval.

Table 2
Performance of the microarray for different borrelial antigens and antigen combinations using different rule sets. Rule sets for antigen combinations: with the word "and", both antigens have to react with serum; with the word "or", one of the antigens has to react with the serum. The analysis included 18 *B. burgdorferi* sensu lato IgG negative and 35 *B. burgdorferi* sensu lato IgG positivive serum samples. The positive samples were further divided into three subcategories according to their IgG antibody levels as follows: low IgG concentration ($n = 9$), intermediate IgG concentration ($n = 13$), and high IgG concentration ($n = 13$).

Antigen or antigen combinations	Specificity % [95% CI]	Sensitivity %			
		All samples [95% CI]	Low borrelial IgG concentration[1] [95% CI]	Intermediate borrelial IgG concentration[2] [95% CI]	High borrelial IgG concentration[3] [95% CI]
IR6p4	96 [80,99]	54 [38, 70]	11 [2, 44]	46 [23, 71]	92 [67, 99]
DbpAIa	96 [80,99]	77 [61, 88]	67 [35, 88]	69 [42, 87]	92 [67, 99]
DbpA91	92 [74, 98]	40 [26, 56]	11 [2, 44]	23 [8, 50]	77 [50, 92]
IR6p1	92 [74, 98]	51 [36, 67]	11 [2, 44]	38 [18, 65]	92 [67, 99]
IR6p2	88 [69,96]	83 [67, 92]	67 [35, 88]	85 [58, 96]	92 [67, 99]
DbpAG40	63 [43,79]	89 [74, 96]	89 [57, 98]	77 [50, 92]	100 [77, 100]
DbpAIa and IR6p2	83 [64, 93]	63 [46, 77]	33 [12, 65]	62 [36, 82]	85 [58, 96]
DbpAIa and DbpAG40	63 [43,79]	74 [58, 86]	67 [35, 88]	62 [36, 82]	92 [67, 99]
DbpAIa and DbpAG40 and IR6p2	58 [39, 76]	60 [44, 74]	33 [12, 65]	54 [29, 77]	85 [58, 96]
B. burgdorferi sensu stricto antigens: IR6p1 or DbpAIa	88 [69,96]	86 [71, 94]	78 [45, 94]	85 [58, 96]	100 [77, 100]
B. afzelii antigens: IR6p4 or DbpA91	88 [69,96]	57 [41, 72]	11 [2, 44]	54 [29, 77]	92 [67, 99]
B. garinii antigens: IR6p2 or DbpAG40	58 [39, 76]	97 [85, 99]	100 [70, 100]	92 [67, 99]	100 [77, 100]

CI, confidence interval. IR6p1, VlsE protein IR(6) peptide variant from *B. burgdoferi sensu stricto* B31. IR6p2, VlsE protein IR(6) peptide variant from *B. garinii* IP90. IR6p4, VlsE protein IR(6) peptide variant from *B. afzelii* ACAI. DbpAIa, decorin binding recombinant protein A from *B. burgdorferi sensu stricto* IA. DpbpAG40, decorin binding recombinant protein A from *B. garinii* 40. DbppA91 decorin binding recombinant protein A from *B. afzelii* A91.

be measured by the traditional hemagglutination inhibition assays (Koopmans et al., 2012). Recently, a similar approach was applied for the different coronaviruses (Reusken et al., 2013). We have earlier demonstrated that detection of viral antibodies and quantitation of C-reactive protein is feasible in a single microarray (Viitala et al., 2013). With the microarray technique, multiple antigens can be present in the same reaction and immunologic history can be examined simultaneously for several viral or bacterial targets. This provides an opportunity for more comprehensive and rapid identification of the causative pathogen.

The sensitivity and specificity values of the developed microarray were based on a comparison with existing reference assays. All of the reference tests in this study are being used in a large scale routine diagnostic laboratory (HUSLAB, Finland), and are considered as reliable test platforms. Some of the serum samples were reactive in the microarray test without reaction in the reference test; e.g. for HSV-1 there were 5/47, and for HSV-2 15/57 such samples. It must be noted that the specimen panels did not represent an unbiased general population, but rather individuals suspected of a particular infection. Therefore, some of these discordant samples may be truly positive for the respective targets. This is highlighted particularly in the case of adenovirus for which the microarray was reactive for all positive panel samples (100% sensitivity), and for 3/19 of the negative panel specimens. The same whole adenovirus antigen was used both in the reference assay and in the microarray, and thus the superior sensitivity of the microarray platform is plausible.

For the serodiagnosis of Lyme borreliosis, specificity is a critical factor, and reactions to several antigens are required to determine a history of *Borrelia* contact. In the microarray developed in this study, six borrelial antigens were used, and results from single antigens, as well as antigen combinations were evaluated. As demonstrated in Table 2, the requirement of simultaneous reactivity (sensitivity) and non-reactivity (specificity) against several antigens considerably decreased the performance values. Sensitivity values can be artificially increased by using less strict rules, e.g. with the requirement of reactivity for only one out of two antigens representing the same *Borrelia* species (Table 2). However, this will subsequently result in less optimal specificity values, and does not reflect the requirements of a diagnostic laboratory, which cannot rely on a single antigen. Our study highlighted the importance of a careful selection of diagnostic criteria and antigens when such an approach is adapted for clinical use. If the diagnostic criteria are chosen without proper validation, very variable results may be obtained when using multiple antigens.

In this study, only two antigen sites of VlsE and DbpA were used. We tested also the commercial *B. burgdorferi* recombinant protein p41 (ProSpec-Tany TechnoGene Ltd, Rehovot, Israel) and *B. afzelii* lysate (Institut Virion\Serion GmbH, Würzburg, Germany) (data not shown), but these antigens did not prove useful for the microarray platform. The DbpA sequence site has high inter- and intraspecies heterogeneity and it is considered to be a sensitive antigen in the late phase of Lyme borreliosis, and particularly in neuroborreliosis (Roberts et al., 1998; Heikkilä et al., 2002a,b, 2003; Schulte-Spechtel et al., 2006; Panelius et al., 2007). The use of several DbpA antigens from different species may increase specificity and sensitivity of the microarray assay. However, as DbpA is known to be more sensitive in the late phase of Lyme borreliosis, other antigens from different antigenic sites are needed to maintain a good performance of the microarray assay throughout the course of illness.

In this study we demonstrate the diagnostic potential of a multiplexed serological microarray platform for the detection CNS pathogens. In the case of *B. burgdorferi* sensu lato infection, specificity is the major challenge of laboratory diagnostics. This study further highlighted the requirement for the use of multiple borrelial antigens in order to increase specificity without a major lack of sensitivity. The overall performance of the platform proved promising in terms of test performance and technical features, and while it only requires a small samples volume, it is potentially adaptable also for CSF specimens.

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References

Ardizzoni, A., Baschieri, M.C., Manca, L., Orsi, C.F., Venturelli, C., Meacci, M., Peppoloni, S., Farina, C., Blasi, E., 2011. The protein "mycoarray": a novel serological assay for the laboratory diagnosis of primitive endemic mycoses. New Microbiol. 34, 307–316.

Bacarese-Hamilton, T., Mezzasoma, L., Ardizzoni, A., Bistoni, F., Crisanti, A., 2004. Serodiagnosis of infectious diseases with antigen microarrays. J. Appl. Microbiol. 96, 10–17.

Bacon, R.M., Biggerstaff, B.J., Schriefer, M.E., Gilmore Jr., R.D., Philipp, M.T., Steere, A.C., Wormser, G.P., Marques, A.R., Johnson, B.J., 2003. Serodiagnosis of Lyme disease by kinetic enzyme-linked immunosorbent assay using recombinant VlsE1 or peptide antigens of *Borrelia burgdorferi* compared with 2-tiered testing using whole-cell lysates. J. Infect. Dis. 187, 1187–1199.

Bunikis, J., Garpmo, U., Tsao, J., Berglund, J., Fish, D., Barbour, A.G., 2004. Sequence typing reveals extensive strain diversity of the Lyme borreliosis agents *Borrelia burgdorferi* in North America and *Borrelia afzelii* in Europe. Microbiology 150, 1741–1755.

Gerritzen, A., Brandt, S., 2012. Serodiagnosis of Lyme borreliosis with bead based immunoassays using multiplex technology. Methods 56, 477–483.

Gouriet, F., Levy, P.Y., Samson, L., Drancourt, M., Raoult, D., 2008. Comparison of the new InoDiag automated fluorescence multiplexed antigen microarray to the reference technique in the serodiagnosis of atypical bacterial pneumonia. Clin. Microbiol. Infect. 14, 1119–1127.

Grahn, A., Studahl, M., Nilsson, S., Thomsson, E., Bäckström, M., Bergström, T., 2011. Varicella-zoster virus (VZV) glycoprotein E is a serological antigen for detection of intrathecal antibodies to VZV in the central nervous system, without cross-reaction to herpes simplex virus type-1. Clin. Vaccine Immunol. 18, 1336–1342.

Heikkilä, T., Seppälä, I., Saxen, H., Panelius, J., Yrjänäinen, H., Lahdenne, P., 2002a. Species-specific serodiagnosis of Lyme arthritis and neuroborreliosis due to *Borrelia burgdorferi sensu stricto*, *B. afzelii*, and *B. garinii* by using decorin binding protein A. J. Clin. Microbiol. 40, 453–460.

Heikkilä, T., Seppälä, I., Saxen, H., Panelius, J., Peltomaa, M., Huppertz, H.I., Lahdenne, P., 2002b. Cloning of the gene encoding the decorin-binding protein B (DbpB) in *Borrelia burgdorferi* sensu lato and characterisation of the antibody responses to DbpB in Lyme borreliosis. J. Med. Microbiol. 51, 641–648.

Heikkilä, T., Huppertz, H.I., Seppälä, I., Sillanpää, H., Saxen, H., Lahdenne, P., 2003. Recombinant or peptide antigens in the serology of Lyme arthritis in children. J. Infect. Dis. 187, 1888–1894.

Hengge, U.R., Tannapfel, A., Tyring, S.K., Erbel, R., Arendt, G., Ruzicka, T., 2003. Lyme borreliosis. Lancet Infect. Dis. 3, 489–500.

Jääskeläinen, A.J., Piiparinen, H., Lappalainen, M., Koskiniemi, M., Vaheri, A., 2006. Multiplex-PCR and oligonucleotide microarray for detection of eight different herpesviruses from clinical specimens. J. Clin. Virol. 37, 83–90.

Jääskeläinen, A.J., Piiparinen, H., Lappalainen, M., Vaheri, A., 2008. Improved multiplex-PCR and microarray for herpesvirus detection from CSF. J. Clin. Virol. 42, 172–175.

Jääskeläinen, A.J., Moilanen, K., Bühler, S., Lappalainen, M., Vapalahti, O., Vaheri, A., Piiparinen, H., 2009. Serological microarray for detection of HSV-1, HSV-2, VZV, and CMV antibodies. J. Virol. Methods 160, 167–171.

Koopmans, M., de Bruin, E., Godeke, G.J., Friesema, I., van Gageldonk, R., Schipper, M., Meijer, A., van Binnendijk, R., Rimmelzwaan, G.F., de Jong, M.D., Buisman, A., van Beek, J., van de Vijver, D., Reimerink, J., 2012. Profiling of humoral immune responses to influenza viruses by using protein microarray. Clin. Microbiol. Infect. 18, 787–807.

Leveque, N., Van Haecke, A., Renois, F., Boutolleau, D., Talmud, D., Andreoletti, L., 2011. Rapid virological diagnosis of central nervous system infections by use of a multiplex reverse transcription-PCR DNA microarray. J. Clin. Microbiol. 49, 3874–3879.

Lochhead, M.J., Todorof, K., Delaney, M., Ives, J.T., Greef, C., Moll, K., Rowley, K., Vogel, K., Myatt, C., Zhang, X.Q., Logan, C., Benson, C., Reed, S., Schooley, R.T., 2011. Rapid multiplexed immunoassay for simultaneous serodiagnosis of HIV-1 and coinfections. J. Clin. Microbiol. 49, 3584–3590.

Mannonen, L., Vainionpää, R., Kauppinen, J., Lienhard, R., Tritten, M.L., Cannon, G., Hall, W.W., Moilanen, K., Häkkinen, M., Jääskeläinen, A.J., Piiparinen, H., Mäki, M., Järvinen, A.K., Lappalainen, M., 2012. Evaluation of multiplex polymerase chain reaction and microarray-based assay for rapid herpesvirus diagnostics. Diagn. Microbiol. Infect. Dis. 73, 74–79.

Panelius, J., Sillanpää, H., Seppälä, I., Sarvas, H., Lahdenne, P., 2007. Antibodies to recombinant decorin-binding proteins A and B in the cerebrospinal fluid of patients with Lyme neuroborreliosis. Scand. J. Infect. Dis. 39, 775–780.

Porwancher, R., 2003. Improving the specificity of recombinant immunoassays for Lyme disease. J. Clin. Microbiol. 41, 2791.

Porwancher, R.B., Hagerty, C.G., Fan, J., Landsberg, L., Johnson, B.J., Kopnitsky, M., Steere, A.C., Kulas, K., Wong, S.J., 2011. Multiplex immunoassay for Lyme disease using VlsE1-IgG and pepC10-IgM antibodies: improving test performance through bioinformatics. Clin. Vaccine Immunol. 18, 851–859.

Qiu, W.G., Dykhuizen, D.E., Acosta, M.S., Luft, B.J., 2002. Geographic uniformity of the Lyme disease spirochete (*Borrelia burgdorferi*) and its shared history with tick vector (*Ixodes scapularis*) in the northeastern United States. Genetics 160, 833–849.

Reusken, C., Mou, H., Godeke, G.J., van der Hoek, L., Meyer, B., Müller, M.A., Haagmans, B., de Sousa, R., Schuurman, N., Dittmer, U., Rottier, P., Osterhaus, A., Drosten, C., Bosch, B.J., Koopmans, M., 2013. Specific serology for emerging human coronaviruses by protein microarray. Eur. Surveill. 18, 20441.

Roberts, W.C., Mullikin, B.A., Lathigra, R., Hanson, M.S., 1998. Molecular analysis of sequence heterogeneity among genes encoding decorin binding proteins A and B of *Borrelia burgdorferi* sensu lato. Infect. Immun. 66, 5275–5285.

Schulte-Spechtel, U., Fingerle, V., Goettner, G., Rogge, S., Wilske, B., 2006. Molecular analysis of decorin-binding proteinA (DbpA) reveals 5 major groups among European *Borrelia burgdorferi* sensu lato strains with impact for the development of serological assays and indicates lateral genetransfer of the DbpA gene. Int. J. Med. Microbiol. 296, 250–266.

Sillanpää, H., Lahdenne, P., Sarvas, H., Arnez, M., Steere, A., Peltomaa, M., Seppälä, I., 2007. Immune responses to borrelial VlsE IR6 peptide variants. Int. J. Med. Microbiol. 297, 45–52.

Steere, A.C., 2001. Lyme disease. N. Engl. J. Med. 345, 115–125.

Thomsson, E., Persson, L., Grahn, A., Snäll, J., Ekblad, M., Brunhage, E., Svensson, F., Jern, C., Hansson, G.C., Bäckström, M., Bergström, T., 2011. Recombinant glycoprotein E produced in mammalian cells in large-scale as an antigen for varicella-zoster-virus serology. J. Virol. Methods 175, 53–59.

Viitala, S.M., Jääskeläinen, A.J., Kelo, E., Sirola, H., Moilanen, K., Suni, J., Vaheri, A., Vapalahti, O., Närvänen, A., 2013. Surface-activated microtiter-plate microarray for simultaneous CRP quantification and viral antibody detection. Diagn. Microbiol. Infect. Dis. 75, 174–179.

Whitelegg, A.M., Birtwistle, J., Richter, A., Campbell, J.P., Turner, J.E., Ahmed, T.M., Giles, L.J., Fellows, M., Plant, T., Ferraro, A.J., Cobbold, M., Drayson, M.T., MacLennan, C.A., 2012. Measurement of antibodies to pneumococcal, meningococcal and haemophilus polysaccharides, and tetanus and diphtheria toxoids using a 19-plexed assay. J. Immunol. Methods 377, 37–46.

Xu, Y., Bruno, J.F., Luft, B.J., 2008. Profiling the humoral immune response to Borrelia burgdorferi infection with protein microarrays. Microb. Pathog. 45, 403–407.

Yan, X., Zhong, W., Tang, A., Schielke, E.G., Hang, W., Nolan, J.P., 2005. Multiplexed flow cytometric immunoassay for influenza virus detection and differentiation. Anal. Chem. 77, 7673–7678.

Yu, J., Lin, J., Kim, K.H., Benjamin Jr., W.H., Nahm, M.H., 2011. Development of an automated and multiplexed serotyping assay for Streptococcus pneumoniae. Clin. Vaccine Immunol. 18, 1900–1907.