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Exploring novel sero-epidemiological tools—Effect of different storage conditions on longitudinal stability of microarray slides comprising influenza A-, measles- and *Streptococcus pneumoniae* antigens

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A B S T R A C T

In this study we evaluated the long-term stability of a microarray-based serological screening platform, containing antigens of influenza A, measles and Streptococcus pneumoniae, as part of a preparedness research program aiming to develop assays for syndromic disease detection. Spotted microarray slides were kept at four different storage regimes with varying temperature and humidity conditions. We showed that under the standard storage condition in a temperature-controlled (21 °C) and desiccated environment (0% relative humidity), microarray slides remained stable for at least 22 months without loss of antigen quality, whereas the other three conditions (37 °C, desiccated; Room temperature, non-desiccated; Frozen, desiccated) produced acceptable results for some antigens (influenza A, *S.pneumoniae*), but not for others (measles). We conclude that these arrays for multiplex antibody testing can be prepared and stored for prolonged periods of time, which aids laboratory-preparedness and facilitates sero-epidemiological studies.

1. Introduction

Infectious diseases can be diagnosed using two types of laboratory tests: [a] pathogen detection, for instance, polymerase chain reaction (PCR), immunoassays or culture, or [b] detection of antibodies against infectious agents, such as, the hemagglutination inhibition assay, microneutralization assay, enzyme-linked immunosorbent assay or Western Blotting. Molecular methods, like PCR, are fast and useful tools, given correct types of samples are collected within an appropriate time from onset of illness. However, with some acute infections e.g. caused by Dengue- (WHO, 2009) or Chikungunya virus (Taubitz et al., 2007), the relatively short period of viremia limits the applicability of virus detection methods as they are usually rapidly cleared in an immunocompetent host. Serological methods, on the other hand, detect antibody responses triggered by infection, and can thereby provide information on exposure when the agent may no longer be present. Antibody detecting techniques are not only useful to retrospectively confirm infections in individuals when paired sera are available; they also provide important information during outbreak settings. The ability to detect mild or asymptomatic infections allows estimation of attack rates, transmissibility and geographic distribution of a pathogen on a population level, as well as unbiased case fatality rates. In combination with epidemiological and clinical data, these measures are important to guide effective control strategies to contain infectious disease outbreaks (Cauchemez et al., 2012; Kumar and Henrickson 2012; Laurie et al., 2013).

For example, patients with influenza-like illness or acute respiratory infection are identified when referred by physicians. This forms the basis for the global sentinel surveillance systems (Beauté et al., 2012). Whereas such a symptom-dependent system is useful for virological surveillance, it tends to predominantly capture the most severe cases, as patients with mild- or asymptomatic infection are less likely to seek health care (Gibbons et al., 2014). Hence, during infectious disease outbreaks there is a risk that morbidity and

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mortality rates can be biased when only severe cases are included (Ejima et al., 2012; Gibbons et al., 2014).

With the recent influenza pandemic caused by a novel H1N1 subtype in 2009 [A(H1N1)pdm09], the importance of including serological studies into pandemic preparedness planning and the use of standardized serological assays for improved comparability between studies became apparent (Laurie et al., 2013). Serological methods, such as the hemagglutination inhibition- and microneutralization assay were widely used during different stages of the pandemic. However, despite the availability of an international antibody standard, limited awareness thereof precluded its wide use. Another challenge was that laboratory capacity and storage for conducting extensive and high-volume serological studies was insufficient (Laurie et al., 2013).

We previously reported on the development and use of a standardized serological assay termed protein microarray, which is a platform that is able to simultaneously screen for antibodies against multiple influenza hemagglutinin types in humans (Koopmans et al., 2012; Boni et al., 2013; Huijskens et al., 2013; de Bruin et al., 2014), chickens (Freidl et al., 2014) and bats (Freidl et al., 2015). This antibody detection assay was developed as part of an emerging disease preparedness program, and was piloted to monitor the evolution of the A(H1N1)pdm09 in13 countries (de Bruin et al., 2014). In another study, this technique was used in real-time to assess pre-existing antibody levels to H7 subtypes during the emergence of a novel zoonotic A(H7N9) avian influenza virus subtype in rural and urban locations of Vietnam (Boni et al., 2013). For such large-scale seroepidemiological studies, a high number of spotted microarray slides are required and are ideally stockpiled within the framework of laboratory preparedness for rapid deployment during outbreak situations. The potential use of this technology is conditional on validation of storage conditions. In the current study, we evaluated the long-term stability of viral proteins and bacterial polysaccharides printed onto microarray slides and investigated the influence of four different storage conditions on antigen quality over a period of 22 months.

2. Materials and methods

2.1. Antigen selection and production of microarray slides

We evaluated the stability of recombinant proteins of the HA1 of different influenza virus hemagglutinins, whole inactivated measles virus and capsular polysaccharides of *Streptococcus pneumoniae* spotted onto microarray slides (Table 1). The stability of antigens was evaluated at four different temperature- and humidity conditions as further specified below. Recombinant HA1 proteins were produced in HEK293 cells and purified using HIS-tag purification as described by the manufacturers (Table 1). Antigen H1.09 was validated extensively in serosurveillance studies in humans during the H1N1 influenza virus pandemic of 2009 (Koopmans et al., 2012; Huijskens et al., 2013; de Bruin et al., 2014). Antigens H5.05 and H5.07 were validated for antibody screening of chicken serum samples (Freidl et al., 2014). Measles virus and *Streptococcus pneumoniae* (used as a surrogate for *Streptococcus suis*) antigens are currently being validated for the use in diagnostics and were included to evaluate antigen stability in the microarray platform for future purposes.

Optimal concentration per antigen was determined by checkerboard titration using antisera as shown in Table 1. Influenza and measles viral antigens were diluted in working strength protein arraying buffer (Maine Manufacturing, ME, USA) containing proteinase inhibitor cocktail (BioVision, Mountain View, CA, USA). Bacterial polysaccharides were printed in working strength protein arraying buffer only. All antigens (Table 1) were spotted onto nitrocellulose-coated glass slides of the same lot number (16-pad, Oncyte Avid, Grace Biolabs, Bend, OR, USA) using a non-contact spotter (Piezorray, Perkin Elmer, Mass., USA). Two microarray batches were produced on the same day using the same reagents and antigens. Each batch consisted of 25 slides, which constituted the maximum capacity per spot run. Immediately after spotting, slides were transferred to a dark plastic box and were stored in a drying chamber to allow optimal protein linkage to the nitrocellulose. All slides were kept in the drying chamber with an average temperature of 21 °C under dark conditions until further use (~3 weeks later). Based on previous experience with short-term storage of spotted slides it is known that antigen quality does not change within three weeks. For quality control prior to the study, we tested one slide per batch at the onset of the study and demonstrated that results with slides from different batches were comparable as overall antibody titers did not differ significantly at baseline (Time point 0, Fig. 2; Wilcoxon rank sum test, p-value = 0.69). Similarly, batches 1 and 2 did not differ over the entire study period (Wilcoxon rank sum test, p-value = 0.92). Calculation of geometric coefficients of variation (GCV) showed comparable variations in titers for both batches (GCV batch 1: 126%, GCV batch 2: 130%).

2.2. Microarray protocol

Microarray slides were essentially tested as described before (Koopmans et al., 2012). Briefly, we first incubated microarray slides with Blotto blocking buffer containing 0.1% Surfact-Amps (both Thermo Fisher Scientific, Rockford, MA, USA), followed by incubation of serum pools (see explanation hereafter) and finally used specific conjugates to visualize bound antibodies. All incubation steps were one hour in duration. Conjugates used were AlexaFluor647 AffiniPure labeled goat-anti-rabbit IgG, and Alexa647 AffiniPure labeled goat-anti-human IgG (both Fc-fragment specific and polyclonal, Jackson Immuno Research, West Grove, USA), at dilutions 1: 1300 as determined using checkerboard titration. Following the manufacturer's instructions, we updated both conjugates once during the study period. Before replacing the conjugates (same product from same manufacturer), we tested and verified that old- and new conjugates yielded comparable fluorescence signals at the same dilution (data not shown). After slide analysis, fluorescent signals were quantified using a ScanArray Gx Plus microarray scanner (Perkin Elmer) and sigmoidal fluorescence curves were converted into titers as described previously (Koopmans et al., 2012).

Antisera used for checkerboard titration were used to prepare specific antiserum pools, one containing rabbit antisera (i.e. anti-influenza and anti-S. *pneumoniae*) and another one consisting of human antisera (i.e. anti-measles) (Table 1). After pooling, we prepared twelve aliquots per serum pool which were subsequently stored at −80 °C to keep them stable until further use. At every 2-months interval, we used one aliquot per the serum pool to test the stability slides. From each aliquot we prepared two-fold dilution series in Blotto blocking buffer containing 0.1% Surfact-Amps, starting at a dilution of 1:80 for the rabbit- (anti-influenza A and anti-*S.pneumoniae*), and 1:320 for the human serum pool (anti-measles). For periodic testing, four slides – one per storage condition – were tested simultaneously.

2.3. Storage conditions

All slides were stored under dark conditions. The stability of spotted microarray slides was evaluated under the following four storage conditions: