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Evaluation of Surface Chemistries for Antibody Microarrays

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Abstract

Antibody microarrays are an emerging technology that promises to be a powerful tool for the detection of disease biomarkers. The current technology for protein microarrays has been primarily derived from DNA microarrays and is not fully characterized for use with proteins. For example, there are a myriad of surface chemistries that are commercially available for antibody microarrays, but no rigorous studies that compare these different surfaces. Therefore, we have used a sandwich enzyme-linked immunosorbent assay (ELISA) microarray platform to analyze 16 different commercially available slide types. Full standard curves were generated for 23 different assays. We found that this approach provides a rigorous and quantitative system for comparing the different slide types based on spot size and morphology, slide noise, spot background, lower limit of detection, and reproducibility. These studies demonstrate that the properties of the slide surface affect the activity of immobilized antibodies and the quality of data produced. Although many slide types produce useful data, glass slides coated with aldehyde silane, poly-L-lysine, or aminosilane, with or without activation with a crosslinker, consistently produce superior results in the sandwich ELISA microarray analyses we performed.

Keywords

ELISA; Antibody; Microarray; Solid Support

Introduction

Sandwich enzyme-linked immunosorbent assay (ELISA) microarrays have the potential to be a powerful tool for the screening and validation of biomarkers for cancer and other complex diseases, mainly due to the great promise of this technology for rapidly analyzing many antigens in very small sample volumes [1–6]. Even though the standard 96-well plate ELISA is routinely used for high-throughput detection of proteins, this platform has limited capacity to screen large numbers of proteins in an efficient manner, particularly when sample volumes are limited. In contrast, sandwich ELISA microarrays can simultaneously screen up to 50 proteins in small sample volumes (e.g., 15 μ L of diluted sample).

Originally, experimental protocols for ELISA microarray were based on a combination of the protocols for 96-well plate ELISA and DNA microarrays [6]. More recently, many studies

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have been directed towards optimizing conditions specifically for multiplexed protein assays including determination of the necessary time for sample incubation [7–9], reduction of cross-reactivity between assays and non-specific protein binding [10], and optimization of surface chemistries for immobilizing antibodies [11–14]. Despite these efforts, ELISA microarrays have yet to meet theoretical predictions for assay sensitivity [8], suggesting that assay conditions still have considerable room for improvement. That is, to achieve the full potential of the ELISA microarray technology, it will be necessary to develop an integrated platform that has been optimized at all processing steps.

Slide surface chemistry is an important aspect of a robust ELISA microarray platform, a point which is highlighted by the large variety of surface modifications and chemistries that are commercially available. A number of characteristics that are associated with good performance of ELISA microarrays must be evaluated when choosing the ideal surface for antibody immobilization, including spot size and morphology, total antibody binding, background signal (both surrounding and within the printed spot), lower limit of detection, and reproducibility across chips and experiments. Therefore, a good surface chemistry for antibody immobilization must have (i) high binding capacity, (ii) an ability to retain antibody activity, (iii) low variability between slides, and (iv) high signal-to-noise ratios. We have recently reviewed the literature on surface chemistries and found that there is a general failure to evaluate different surface chemistries in either a global or systematic manner [15]. As such, it has not been possible to critically determine which of the available slide chemistries are truly useful for antibody microarrays.

In this paper, we evaluate 16 commercially available slide types based on the criteria listed above. The slides were evaluated by comparing standard curves for 23 different assays, all previously determined to have good assay sensitivity and specificity (Gonzalez and Zangar, unpublished data). We find that three-dimensional slide surfaces tend to have higher background than two-dimensional surfaces, likely due to an inability to efficiently wash and/or block the surface. We also observe that non-covalent chemistries for capturing the antibody can work nearly as well as covalent ones. Overall, these experiments identify superior slide chemistries for antibody capture and define some basic principles that contribute to these superior chemistries.

Materials and Methods

Materials

Disuccinimidyl suberate (DSS) and bis[sulfosuccinimidyl] suberate (BS³) were purchased from Pierce (Rockford, IL). Purified antibodies and antigens were purchased from R&D Systems (Minneapolis, MN), Lab Vision Corporation (Fremont, CA), Fitzgerald (Concord, MA), MBL International (Woburn, MA), and BiosPacific (Emeryville, CA). Blocking solution containing 10 mg/mL casein in phosphate buffered saline (PBS) was purchased from Bio-Rad Laboratories (Hercules, CA), the TSA biotin system including streptavidin-conjugated horse radish peroxidase, amplification diluent, and biotinyltyramide was purchased from Perkin Elmer (Wellesley, MA), and streptavidin-conjugated Cy3 was obtained from Amersham Biosciences (Piscataway, NJ).

Erie Scientific (Portsmouth, NH) kindly provided aminosilane-, epoxysilane-, poly-L-lysine-, and aldehyde silane-coated slides, as well as HD and ES surface modifications for epoxysilane-coated slides. Full Moon protein slides were provided by Full Moon BioSystems (Sunnyvale, CA), slides H, AL, and E were provided by Schott (Elmsford, NY), and dendron slides were provided by NSB Postech (Pohang, Korea).

Microarray Preparation

A GeSiM NanoPlotter 2.1 (Quantum Analytics; Foster City, CA) non-contact printer with humidity control was used to print the antibodies. All slides were used as provided by the manufacturer with the exception of aminosilane- and dendron-coated slides. In some cases, these slides were treated with a 0.2 mg/mL solution of the homobifunctional cross-linker DSS in ethanol to create a covalent binding site for amines. Alternatively, aminosilane-coated slides were treated with 0.3 mg/mL of BS³ in PBS to compare the two cross-linkers. After incubation with the cross-linker for five minutes, the slides were rinsed with 100% ethanol and dried under argon.

Capture antibodies were suspended in PBS to a concentration of 0.5 to 0.8 mg/mL. The spots were printed 0.5 mm apart, in quadruplicate, on each array. Each slide contained 16 arrays in an 8 × 2 pattern, with 9 mm spacing between arrays. Following printing, the slides were incubated in the printer for 1 or 12 hours, as suggested by the manufacturer (see Table 1 for details). The arrays were outlined with a Pap pen to create a hydrophobic barrier for processing arrays individually. The successful printing of the spots was confirmed by scanning the slides using the Red Reflect setting on a Perkin Elmer ScanArray Express HT microarray scanner. The slides were blocked at room temperature for one hour in 10 mg/mL casein in PBS. Slides were then washed with PBS-T (0.05% Tween 20 in PBS) and processed immediately.

Slide Processing

Slides were processed as previously published (Gonzalez, et al., in press). Briefly, all incubation steps were performed in a humid chamber, at room temperature, in the dark, with gentle mixing. The slides were thoroughly washed between each processing step by soaking in PBS-T for 3 to 10 minutes. The slides were incubated with a mixture of antigens in 1 mg/mL casein in PBS for 4 hours. Standard curves were created utilizing a three-fold dilution series of the antigen mix along with an antigen-free blank, for eight total dilutions that were run in duplicate on each slide type. The slides were then incubated with biotinylated detection antibody at 25 ng/mL in 1 mg/mL casein in PBS for 1 hour. The signal was amplified using the TSA amplification system according to manufacturer's instructions, and then the slides were incubated with a solution of 1 µg/mL streptavidin-conjugated Cy3 in PBS-T for 30 minutes. A Perkin Elmer ScanArray Express HT laser scanner was used to image the Cy3 fluorescence signal on the slides.

Data Analysis

The slide images were quantified using ScanArray Express software (Perkin Elmer). Standard curves were created using ProMAT version 2.0, a custom software program we developed specifically for the analysis of ELISA microarray data [16;17]. This program is freely available at <http://www.pnl.gov/statistics/ProMAT>. The standard curves were fit to four-parameter logistic curves, except in the cases when the data would not converge to this model. In such cases, the standard data were automatically fit by ProMAT using a spline model (Daly, Anderson, White, Gonzalez, and Zangar, unpublished data).

In most cases, three replicate experiments were performed using all slide types, with each experiment being conducted using slides from the same manufacturer lot that were printed and processed on different days. The only exception to this was for the dendron 27 slides and the aminosilane-coated slides activated with BS³, both of which were only analyzed once. The data are presented as the mean and the standard deviation from the three replicate experiments, unless noted otherwise (*i.e.* Figures 1 and 6). "Slide noise" was defined as the background fluorescent signal in the immediate vicinity of a spot, a value which was automatically determined by the ScanArray Express software. The median value from the 1472 spots on each slide was used as the slide noise value for each experiment and slide type. "Spot background"

was defined as the median fluorescent intensity within a spot that was in the antigen-free blank. There are two antigen-free blank arrays per slide, 23 assays in quadruplicate spots on each array, resulting in 192 spots that were used to define spot background on each slide. However, since spot background was dependent upon the capture antibody, spot background values were first normalized as a ratio of the median value for each assay across all the slides. The median value of these normalized spot backgrounds from the 23 assays was used as the value for that experiment and slide type. The lower limit of detection (LOD) was calculated by ProMAT, which defines this assay parameter as the concentration point on the standard curve that corresponds to the mean of the log-transformed fluorescent values for the antigen-free blank plus three standard deviations of the fluorescent values. For this study, the fluorescent values used in this calculation are from eight spots per assay (four spots on each of two arrays) for each slide. LOD values were normalized and compared in the same manner as described for the spot background.

Results

We have evaluated sixteen commercially available slide types with a variety of surface modifications and reactive chemistries. An overview of these slide types and their surface chemistries is shown in Table 1. The slide types were chosen for their potential to immobilize active antibody and because they do not require special processing of the antibody prior to printing, such as commonly used for attachment through protein thiol or carbohydrate groups. Slides were processed according to manufacturer instructions, unless specified in Table 1. For example, some slides were treated with a homobifunctional crosslinker prior to printing to allow for covalent attachment of the antibodies. Slide performance was determined by using data from full standard curves and was assessed by comparing spot size and morphology, slide noise, spot background, assay lower limit of detection, and reproducibility.

Evaluation of Multiple Assays

It has been suggested in the literature that it is necessary to compare multiple assays in order to properly evaluate various slide chemistries [13]. Therefore, we investigated the various slide types by evaluating standard curves for 23 antibody assays. We observe clear and reproducible differences between the different slide types. When the comparisons are based on full standard curves rather than a single concentration point the rank order of the slide types is relatively constant for all assays, although some differences are apparent. A typical example of this observation is shown in Figure 1 (standard curves for the remaining assays are shown in Figure S1 in the supplemental data). With the exception of the Full Moon slide, the standard curves for E-Selectin are very similar across the various slide types (Figure 1A). However, greater differences in the shape of the standard curves across the slide types are observed for intracellular adhesion molecule 1 (Figure 1B), prostate specific antigen (Figure 1C), and RANTES (Figure 1D). For all assays, though, the general ranking of the slide types is maintained. Therefore, it does appear important to test several antibody assays when evaluating slide chemistry, but since most assays behave similarly it is possible to draw conclusions from these analyses that are generally applicable to all or almost all antibody assays. In the current study, we commonly use the median value of 23 different assays to compare slide characteristics, thereby ensuring that our comparisons are representative of a “typical” assay.

Spot Size and Morphology

Certain slide surfaces are designed to limit spot spreading and thereby decrease spot diameter, improve spot morphology, and allow for more densely printed arrays. Erie Scientific has designed two surface modifications for these purposes: a hydrophobic surface (“HD”) and an enhanced, or roughened, surface (“ES”). Erie’s HD slides are intended to decrease the spot size through hydrophobic interactions of the antibody and the slide surface. ES slides utilize a

surface with microscopic canyons and valleys to limit spot spreading, while maintaining the chemistry and hydrophilicity of the desired surface coating (e.g., aminosilane or epoxysilane). In theory, the ES slide surface allows for spotting of antibodies with decreased spot size, but with less chance of protein denaturation that may be associated with hydrophobic surfaces.

For the slides we have selected, there is very little difference observed for spot size (data not shown). Interestingly, this is true even for the ES and HD surface modifications. While the manufacturer has shown that these slides result in decreased spot size for DNA printing, we do not see the same effect with antibodies. This difference may reflect the composition of the print buffers, contact versus non-contact printing, other common differences in print conditions between antibody and DNA microarrays, as well as basic biochemical differences between antibodies and DNA.

Poor spot morphology can lead to inferior slide performance due to decreased antibody activity and difficulties with signal quantification. Spot morphology was visually evaluated based on variations of signal intensity within individual spots. Similar to spot size, there were very few differences in spot morphology between the different slide types. An image of a typical array with good spot morphology is shown in Figure 2A. One exception was slide H, which had an irregular spot morphology (Figures 2C and 2D). Large variations in signal intensity were observed between the outer circumference and the center of the spot, creating a “halo effect”. Figure 2 is discussed in more detail below.

Slide noise and spot background

We define slide noise as the fluorescent signal that appears on parts of the slide that have not been printed with antibody. Spot background refers to signal that appears on a printed protein spot when no antigen is included during slide processing procedure. An increase in either slide noise or spot background can reduce assay sensitivity by decreasing signal to noise ratios, particularly near the lower limit of detection.

In general, slide noise was very low for the slide types studied here (Figures 2A and 3). Some slides, such as slide E and dendron 9, showed slightly elevated levels of slide noise. This increase in slide noise could be caused by denaturation of the blocking protein on the slide surface and increased non-specific binding of signal-related proteins. Slide H and ES-epoxysilane also have relatively high slide noise levels. These latter two slide types both have an increased binding surface area, which potentially leads to increased non-specific protein binding most likely caused by the complex surface structure and/or inefficient blocking of the slide surface.

Spot background is primarily due to non-specific binding of proteins to the spots, a process that would be expected to be increased by surface chemistries that induce protein denaturation. While an increase in spot background can potentially affect the lower limit of detection, it does not affect assay specificity. We find that the absolute fluorescent intensity associated with spot background is nearly always greater than slide noise. As shown in Figure 4, slide E (panel B) has a higher amount of spot background for most assays than poly-L-lysine (panel A). Note that two assays (epidermal growth factor and basic fibroblast growth factor, indicated by arrows in Figures 4A and 4B) consistently have higher spot background regardless of slide type, demonstrating that some antibodies are inherently more prone to non-specific protein interactions. Our quantitative comparison of spot background (Figure 4C) uses the median value from the 23 assays and therefore is not influenced by these outliers. By comparing the average of these median values from 3 replicate experiments, we observe greater levels of spot background for slide H and, to a lesser extent, all epoxysilane-coated slides and all three types of Full Moon slides (Figure 4C).

Lower Limit of Detection

The lower limit of detection is defined as the lowest antigen concentration that can be detected with confidence, and is a direct assessment of assay sensitivity. We evaluated the various slide types by comparing the relative lower limits of detection for all assays across three replicate experiments. For a typical experiment, the lower limits of detection for all assays across all slide types range from 0.1 – 475 pg/mL, with a median value of 3 pg/mL. Figure 5A shows an example of a typical standard curve generated by ProMAT, with the useful range of the assay indicated by the gray shading. The vertical, red-dashed line on the left of this gray area approximately corresponds to the lower limit of detection. As depicted in Figure 5B, lower limits of detection were greater for dendron 27, slide AL, and slide H. Relatively poor lower limits of detection were also observed for epoxysilane-UV and dendron 9 slides. All other slide types resulted in similar lower limits of detection.

Reproducibility

It is essential that the chosen slide chemistry give results that are highly reproducible both between arrays and experiments. Here we have visually assessed reproducibility across experiments, as demonstrated in Figure 2 for poly-L-lysine slides (panels A and B) and slide H (panels C and D). While most of the slide types investigated here exhibit similar levels of reproducibility, slide H generally shows poorer reproducibility than the other slides. The images in Figures 2C and 2D clearly show the poor reproducibility observed for slide H. Poly-L-lysine slides from replicate experiments (Figures 2A and 2B) are representative of a slide type that had generally good reproducibility. While Full Moon slides show good reproducibility within this study, previous studies in our laboratory have revealed that there are significant variations between manufacturer lots, with some lots performing significantly better or worse than the results presented here (data not shown). For this reason, we consider Full Moon slides to have poor reproducibility but also to have considerable potential if the manufacturing process could be uniformly optimized.

Overall Comparison of Slide Chemistries and Types

Figure 6 shows typical standard curves for platelet-derived growth factor AA, which are representative of the trends across all 23 antibody assays (see Figure S1). The standard curves in Figure 6A compare immobilization via physical adsorption onto untreated aminosilane-coated slides versus covalent binding on the same slide surface activated with either BS³ or DSS, which crosslink using NHS esters. Crosslinking using either agent leads to similar standard curves. The use of untreated aminosilane-coated slides results in consistently lower spot signal intensities than the slides treated with cross-linker when antigen is present. Regardless of the difference in absolute signal intensity, the lower limit of detection (~2 pg/mL) and other assay parameters for all three standard curves are very similar, indicating that the aminosilane-coated slides perform similarly regardless of immobilization technique.

The plot in Figure 6B compares epoxysilane-coated slides with two different immobilization protocols, (i) incubation at high humidity for 12 hours (epoxysilane, ES-epoxysilane) and (ii) incubation at high humidity for 1 hour followed by 350 mJ UV cross-linking (epoxysilane-UV), as well as two different surface types, (i) plain glass (epoxysilane, epoxysilane-UV) and (ii) enhanced surface (ES-epoxysilane). There are few differences observed between these slides with the different immobilization techniques.

Three different protein slides from Full Moon BioSystems were evaluated: Full Moon, Full Moon B, and Full Moon C. Full Moon slides are coated with a thin-film proprietary polymer that contains reactive sites, including amines, epoxides, aldehydes, NHS esters, and others. Full Moon B and C slides differ from Full Moon slides by the inclusion of a nano-layer of cellulose between the glass slide and the polymer film. Full Moon B and C slides contain

different numbers of reactive sites in the thin-film polymer from one another. There were only minor differences observed between these slide types (Figure 6C).

Figure 6D compares standard curves representative of the five major types of slide chemistry we have investigated: hydrogel (Full Moon), aminosilane treated with DSS, epoxysilane, aldehyde silane, and poly-L-lysine. Some general observations include that the spot background is higher for the hydrogel, consistent with a previous report [13].

Discussion

The rank performance of each slide type based on spot size, spot morphology, slide noise, spot background, lower limit of detection, and reproducibility is summarized in Table 2. The slide types are divided into three general groups based on overall performance. The slide types that performed well in all categories are shown in the upper grouping, while the poorer performing slide types are at the bottom. It is clear from these rankings that some broad generalizations can be made about slide surface characteristics and antibody performance.

One slide surface characteristic we examined relates to immobilization strategy: physical adsorption and covalent attachment. Physical adsorption refers to the association of antibodies with the slide surface via hydrophobic, hydrogen bonding, or ionic interactions. While this technique is simple, the lack of covalent bonds means that there is potential for exchange of the capture antibodies or the blocking agent with assay reagents such as antigens or detection antibodies [18;19]. The exchange of non-covalently attached reagents would be expected to increase slide noise and spot background, resulting in poor assay sensitivity. In our study, both aminosilane and poly-L-lysine surface chemistries utilize physical adsorption. In general, these slide chemistries result in lower signal intensities across the standard curve, possibly due to a lower density of immobilized antibody. However, since the lower limits of detection did not appear to be adversely affected (Figure 5), we conclude that this general reduction in signal intensity did not adversely affect assay performance. We also did not observe an increase in either slide noise or spot background for these slide types, suggesting that reagent exchange is not a significant factor. As such, we conclude that non-covalent binding chemistries performed comparably to the best of the covalent chemistries in all categories (Table 2).

Antibodies immobilized through covalent bonds are not susceptible to exchange, as in physical adsorption, but are more likely to lose activity due the effects of covalent interactions [15]. Specifically, the potential for multiple covalent attachments per antibody increases the likelihood that the structure of the antigen-binding regions will be altered and thus lose activity. We have investigated a variety of covalent chemistries, including epoxides, NHS-esters, aldehydes, and these three chemistries in combination with other surface modifications (i.e., Full Moon, dendron, and slide H slides). All of these slide chemistries allow for attachment via primary amines on the antibody surface. We found that covalent binding can result in superior slide performance, such as observed with the aminosilane-coated slides activated with a homobifunctional NHS-ester crosslinker (i.e., DSS or BS³), but that not all covalent binding slide types work well (Table 2). In addition, the introduction of active sites apparently did not lead to an increase in performance for aminosilane slides (Figure 6A). While the higher signal intensity at high antigen concentrations for activated aminosilane slides implies that there is more active antibody bound to the slide, there was not an increase in slide performance compared to the untreated slides by any of the criteria used here.

The ideal slide chemistry for antibody microarrays would optimize immobilization efficiency as well as antibody function. Antibodies are relatively stable proteins, and the potential for denaturation is much less than most other proteins. However, there is still a possibility for problems to occur and even small levels of protein denaturation will expose hydrophobic

regions and thereby increase non-specific protein binding. Researchers have used slides with a three-dimensional coating, or hydrogel based on the theory that this surface will maintain protein activity by creating an environment that is partially hydrated, but still supports the protein structure [13]. Additionally, the three-dimensional structure allows for a higher antibody binding capacity due to increased surface area [13]. However, the complex surface structure of hydrogels decreases diffusion rates, thereby slowing assay kinetics and decreasing assay sensitivity [13]. The hydrogel slides that we have studied here include three types of Full Moon slides and slide H, a glass slide coated with a hydrogel that contains reactive NHS esters.

These hydrogel slides performed poorly due to high spot background levels and, in some cases, high slide noise and poor spot morphology. While slide H has been used successfully by other research groups [20;21], in our hands the results were poor. The differences between groups may relate to the protocol followed, and more specifically the spotting buffer used. We felt it was necessary to consecutively print all slides in a single run in order to prevent potential print run variation. Therefore all antibodies were printed from an aqueous PBS solution, which maintains antibody stability in solution and we have found to work well with most slide types. The manufacturer of slide H recommends a much more complex buffer containing glycerol, surfactant, and albumin. We have previously found that inclusion of surfactant or albumin in the spotting buffer results in poor spot morphology or lower antibody binding, respectively, for most slide surfaces. The higher slide noise we observe with Slide H, however, can not be attributed to the printing buffer. It seems likely that this higher noise is inherently a property of the complex, three-dimensional surface structure that increases the surface area available for non-specific protein binding, limits protein mobility and prevents efficient washing. We have observed that for proteins other than antibodies, hydrogels can increase protein activity (unpublished observations). As such, hydrogels may be useful for proteins that are prone to denaturation but appear to be poorly suited for antibody microarrays.

Another three-dimensional slide surface that we have investigated is dendron-coated slides. Dendrons are essentially half of a dendrimer, and the synthesis can be controlled to regulate the size of the dendron and to include only one reactive group per molecule. This combination allows for regular spacing of the reactive groups across the glass surface and can restrict the number of covalent attachments to one or two per antibody [22]. These slides have been shown to work well for DNA microarray assays [23–26], but we know of no reports on the utility of dendrons for antibody microarrays. Here we have investigated two dendron slides with varying spacing between the reactive amine groups, which were activated with a homobifunctional linker to form an active NHS ester. Overall, we found the dendron-coated slides to have relatively poor performance, with suboptimal lower limits of detection.

Based on our investigations, we can make several generalizations regarding optimal slide chemistries for ELISA microarrays. First, we find that hydrogels have high slide noise and spot background, presumably due to slow diffusion rates [13]. In addition, we find that hydrogel slides have poor reproducibility, and in some cases high slide noise and suboptimal lower limits of detection. We also found that dendron-coated slides and some aldehyde slides show poor results for antibody microarrays, with unacceptable lower limits of detection. Overall, our results suggest that total background signal is directly proportional to surface area, at least for a similar binding chemistry. In addition to the results with the hydrogel and dendron slides, this conclusion is supported by the fact that the ES-epoxysilane slides, which have an irregular surface, also have greater levels of slide noise than other epoxysilane slides (Figure 3). Therefore, our results suggest that simple, flat slide surfaces for ELISA microarrays produce superior background signals relative to more complex surface structures. We found that the various epoxysilane-coated slides have average performance. Specifically, they showed poor performance based on slide noise, spot background, and lower limit of detection, as

summarized in detail in Table 2. It is possible that there is a partial denaturation of the antibody on the surface that adversely affects overall assay performance.

Finally, of the slide types tested, aminosilane-coated slides, either untreated or activated with homobifunctional cross-linker, aldehyde silane-coated slides, or poly-L-lysine slides appear to be superior for use in an ELISA microarray. These slide types all exhibited superior spot size and morphology, low slide noise and spot background, high assay sensitivity, and good reproducibility. We found that these characteristics held true for essentially all of the 23 antibody assays we evaluated.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Differences in standard curves on multiple slide types for representative antibody assays. Standard curves comparing the major slide chemistries investigated (Full Moon, epoxysilane, aminosilane treated with DSS, aldehyde silane, and poly-L-lysine) for four different assays: (A) E-selectin; (B) intracellular adhesion molecule 1; (C) prostate specific antigen; and (D) RANTES. All slides were printed and processed at the same time. Data points and cross bars represent the mean and standard deviation, respectively, of 8 spots from a single experiment. These data are representative of three replicate experiments conducted on separate days.

Figure 2. Representative images for superior and inferior slide performance for spot morphology, slide noise and reproducibility. Typical images for poly-L-lysine (A and B) and slide H (C and D) for two replicate experiments conducted on different days. Panels A and B show an example of a slide type with superior performance while panels C and D show an example of a slide type with poor performance. Panel E shows the array layout used for all experiments. Cy3 – Cyanine3-conjugated IgG (positive control); AmR – amphiregulin; CD14 –CD14; EGF – epidermal growth factor; EGFR – epidermal growth factor receptor; Esel – E-selectin; bFGF – basic fibroblast growth factor; HBEGF – heparin-binding epidermal growth factor; Her2 – c-erbB-2 extracellular domain; HGF – hepatocyte growth factor; ICAM –intracellular adhesion molecule 1; IGF1 – insulin-like growth factor 1; IL1 α – interleukin 1-alpha; IL18 – interleukin 18; MMP1 – matrix metalloprotease 1; MMP2 – matrix metalloprotease 2; MMP9 – matrix metalloprotease 9; PDGF – platelet-derived growth factor AA; PSA – prostate specific antigen; RANTES – RANTES (CCL5); TGF α – transforming growth factor alpha; TNF α – tumor

necrosis factor alpha; uPAR – urokinase-type plasminogen activator receptor; VEGF – vascular endothelial growth factor-A.

Figure 3.

Evaluation of slide noise for the various slide types. Columns and cross bars represent the mean and standard deviation, respectively, of the median value for each slide type. The data are from three replicate experiments, each of which was performed with slides that were printed and processed on different days. Superior performance is defined as slide noise less than 200, normal performance is between 200 and 600, and poor performance is greater than 600, as summarized in Table 2.

Figure 4.

Comparison of spot background across multiple slide types. The images in panels A and B represent low relative background (poly-L-lysine) and high relative background (slide E), respectively. Arrows indicate two assays (4 replicate spots per assay) that have high background regardless of slide type. The plot in panel C shows a comparison of the relative spot background for all slide types. To calculate these values, the spot background signal for each slide type was normalized based on the median value for that assay across all slide types. Columns and cross bars represent the mean and standard deviation, respectively, of the median spot background for each slide type across three replicate experiments. Each experiment was performed with slides that were printed and processed on different days. Superior performance is defined as relative spot background less than 1, normal performance is between 1 and 4, and poor performance is greater than 4, as summarized in Table 2.

Figure 5.

Comparison of the relative lower limits of detection for all slide types. Panel A shows a representative curve with the gray regions defining the upper and lower limits of detection. Panel B shows the relative lower limits of detection across the three replicate experiments. To calculate these values, the lower limit of detection was normalized using the median value from all slide types for a given assay. Columns and cross bars represent the mean and standard deviation across the three replicate experiments, respectively, of the median of the normalized lower limit of detection across all assays. Each experiment was performed with slides from separate prints and processed on different days. Superior performance is defined as relative lower limit of detection less than 2, normal performance is between 2 and 4, and poor performance is greater than 4, as summarized in Table 2.

Figure 6.

Standard curves for platelet-derived growth factor AA printed on various slide chemistries and surface modifications. (A) aminosilane-coated slides; (B) epoxysilane-coated slides; (C) Full Moon slides; (D) comparison of the major slide surfaces studied here. Data points and cross bars represent the mean and standard deviation, respectively, of 8 spots from a single experiment. All slides were processed at the same time and are representative both of a majority of the antibody assays and of the three replicate studies.

Table 1

Summary of commercially available slide types investigated. Slide treatment refers to processing of slides required prior to printing. Immobilization refers the amount of time allowed for antibody binding to the slide surface immediately after printing but before the blocking step was initiated.

Slide Type	Manufacturer	Slide Treatment	Reactive Site (Slide/ Antibody)	Immobilization
Aminosilane	Erie	None	NA	1 h incubation
Aminosilane-DSS [*]	Erie	0.2 mg/mL DSS	NHS Ester/Primary amine	1 h incubation
Aminosilane-BS ³ *	Erie	0.3 mg/mL BS ³	NHS Ester/Primary amine	1 h incubation
Epoxyasilane	Erie	None	Epoxide/Primary amine	12 h incubation
Epoxyasilane-UV	Erie	None	Epoxide/Primary amine	1 h incubation + 350 mJ UV crosslink
ES-epoxyasilane	Erie	None	Epoxide/Primary amine	12 h incubation
HD-epoxyasilane	Erie	None	Epoxide/Primary amine	12 h incubation
Aldehyde Silane	Erie	None	Aldehyde/Primary amine	1 h incubation
Poly-L-lysine	Erie	None	NA	12 h incubation
Protein (proprietary polymer)	Full Moon	None	Various sites/ Primary amine	12 h incubation
Protein MC Type B (cellulose + polymer)	Full Moon	None	Various sites/ Primary amine	12 h incubation
Protein MC Type C (cellulose + polymer)	Full Moon	None	Various sites/ Primary amine	12 h incubation
Slide AL	Schott	None	Aldehyde/Primary amine	1 h incubation
Slide E	Schott	None	Epoxide/Primary amine	1 h incubation
Slide H [*] (hydrogel with NHS esters)	Schott	None	NHS Ester/Primary amine	1 h incubation
Dendron 9 [*]	NSB	0.2 mg/mL DSS	NHS Ester/Primary amine	1 h incubation
Dendron 27 [*]	NSB	0.2 mg/mL DSS	NHS Ester/Primary amine	1 h incubation

* Slide was not processed according to manufacturer instructions.

Table 2

Ranking of slide types tested based on spot size, spot morphology, slide noise, spot background, lower limit of detection, and reproducibility (defined in Materials and Methods). Slides are grouped into three blocks, which are listed in order of overall ranking.

Overall Slide Performance	Slide Type	Size	Morphology	Slide Noise	Spot Background	Lower Limit of Detection	Reproducibility
Good	Aminosilane	**	**	***	***	***	**
	Aminosilane-DSS	**	**	***	***	***	**
	Aminosilane-BS ³	**	**	***	***	***	ND
	Poly-L-lysine	**	**	***	***	***	**
Average	Aldehyde Silane	**	**	***	***	***	**
	Epoxysilane	**	**	***	**	***	**
	Epoxysilane-UV	**	**	***	**	***	**
	ES-epoxysilane	**	**	**	***	***	**
	HD-epoxysilane	**	**	***	**	***	**
	Slide E	**	**	**	**	***	**
Poor	Full Moon	**	**	***	**	***	*
	Full Moon B	**	**	***	**	***	*
	Full Moon C	**	**	***	**	***	*
	Dendron 9	**	**	**	***	***	**
	Dendron 27	**	**	***	***	*	ND
	Slide AL	**	**	***	***	*	**
Slide H		ND	*	*	*	*	*

ND - not determined

*

poor performance

**

normal performance

superior performance