P13016

APTarray: An Acquired Pathogen Titre Array for the Determination of Serum Antibody Levels

Rob Shipman, Sophia Fung, Ada Tang, Stuart Carmichael and Tom Ewart, NoAb BioDiscoveries Inc., CANADA

NoAb BioDiscoveries

ABSTRACT

Pathogenic bacterial and viral antigens were arrayed on activated, covalent binding glass slides together with a serial dilution of a human IgG standard (APTarray). Anonymous human sera were incubated with the APTarray and the bound IgG detected by a second incubation with a flourescent dye-labelled anti-IgG conjugate. Fluorescence images of the bound serum IgG were captured using a microarray slide scanner. Fluorescence intensity was compared to the IgG standard and the amount of IgG bound to each antigen determined. APTarray images revealed, at a glance, significant differences between individual serum IgG titres to the arrayed antigens. Absence of pathogen-specific IgG titres was indicated by the absence of fluorescence at specific antigen locations on the APTarray.
Blood donation screening for pathogen exposure or infection, pre-admission screening for IgG titres against common hospital-acquired pathogens, confirmation of the vaccinal response to community-acquired pathogens and bio-warfare agents are applications envisioned for the APTarray.

INTRODUCTION

NoAb activated slide surfaces covalently bind biomolecules to the slide surface via accessible primary amino, hydroxyl or thiol groups on the biomolecule. Although all six NoAb activated slide surfaces were used for the initial APTarray experiments, the epoxy slide surface was subsequently chosen for the APTarray due to the higher signal-to-noise ratio obtained with the APTarray protocol.
In this study we reformatted the method for determining a patient's antibody titre to an acquired pathogen, usually performed in microtitre plates, for the planar microarray on a glass slide. The objectives of this study were to (a) demonstrate the utility and convenience of a microarray in revealing, in one image or "at-a-glance", a patient's serum antibody titre against a library of bacterial and viral pathogens, and (b) emphasize how the compact microarray format could be exploited by incorporating a complete set of calibration standards in a subarray. The latter addition potentially contributing to the acceptance of an antigen microarray, including the APTarray, as a quantitative tool in immunodiagnostic protocols.
Numerous immunodiagnostic applications can be derived from this relatively straight-forward microarray method. Routine blood donor screening for transmissible infectious diseases by blood collection agencies is one application. Elective surgery pre-admission screening for protective antibody titres against prevalent hospital-acquired or ward pathogens is a second major application. This screening method is also applicable to the resurgent world-wide public health threats (TB, HIV, Cholera, Yersinia, Hepatitis B) and emerging infectious diseases (Cryptosporidium, Giardia, SARS, E. coli O:157, West Nile virus). Screening and vaccination of susceptible individuals could markedly reduce the host population through which these diseases propagate.
Screening for the development of a protective vaccinal response to an active vaccine or confirmation of protective antibody levels in response to passive immunotherapy is also important for both public health and military (biowarfare) operations.
Although this iteration of the APTarray falls under the aegis of human immunodiagnostic applications, this methodology could as equally be extended to the pathogens encountered in veterinary practice and associated epizootic diseases (equine encephalitis, brucellosis, rabies, foot and mouth disease, swine flu)

SUMMARY

1. The preliminary APTarray results highlight the potential of this array in providing an "at-a-glance" comprehensive view of the immunological status of a patient. The absence of pathogen-specific immunity from patient to patient is clearly evident even at this preliminary stage.

2. The library of viral and bacterial pathogens should be expanded. This might be accomplished with "killed" whole bacterial or viral preparations from culture. Clinical isolates from hospitals and community outbreaks, including the virulent and antibiotic-resistant serotypes, would be valuable in hospital infection monitoring and control.

3. The APTarray standards require a wider dynamic range of fluorescence proportionality as well as better spot uniformity. Improving the quantitative range and precision of the APTarray might also be addressed by labelling the printed material with a second fluorophor.

4. The APTarray test can be completed in 3 hours at ambient temperature with less than 100ul of serum. The APTarray method could be automated, customised and expanded to encompass all drug-resistant strains of a specific pathogen(s). The total cost of this test could be reduced to less than $10.00.

Viral Antigen	Bacterial Antigen	LPS Antigen	Human IgG
Rabella virus (1mg/ml in PBS pH7.2)	Toxoplasma gondi (1mg/ml in PBS pH7.2)	P. aeruginosa LPS serotype 10 (1mg/ml in NPB pH8.5)	500ng/ml
		P. aeruginosa Endotoxin A (0.5mg/ml in PBS pH7.2)	500ng/ml
Hepatitis B virus subtype Ad (1mg/ml in PBS pH7.2)	Chlamydia trachomatis (1mg/ml in PBS pH7.2)	P. aeruginosa Lectin PA-1 (1mg/ml in NPB pH8.5)	250ng/ml
			250ng/ml
Hepatitis B virus subtype Ay (0.5mg/ml in PBS pH7.2)	Helicobacter pylori (1mg/ml in PBS pH7.2)	Klebsiella pneumoniae (1mg/ml in NPB pH8.5)	125ng/ml
			125ng/ml
Hepatitis A virus (1mg/ml in PBS pH7.2)	Campylobacter jejuni (1mg/ml in PBS pH7.2)	Serratia marcescens (1mg/ml in NPB pH8.5)	63ng/ml
			63ng/ml
Respiratory Syncytial virus (1mg/ml in PBS pH7.2)	Borrelia burgdorferi (1mg/ml in PBS pH7.2)	Shigella flexneri (1mg/ml in NPB pH8.5)	
Cytomegalovirus (1mg/ml in PBS pH7.2)	Vibrio cholerae Inaba 5698 LPS (1mg/ml in NPB pH8.5)	Salmonella typhimurium (1mg/ml in NPB pH8.5)	

Table 1. APTarray antigens.
The antigen concentrations and buffers used for the antigens prior to printing the APTarray on NoAb Epoxy activated slides.

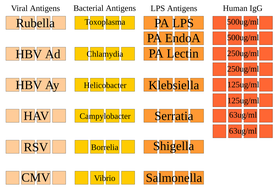

Figure 1. APTarray antigen schematic.
The positions of each viral or bacterial antigen or human IgG standard in the APTarray are shown as they were printed on NoAb Epoxy activated slides.

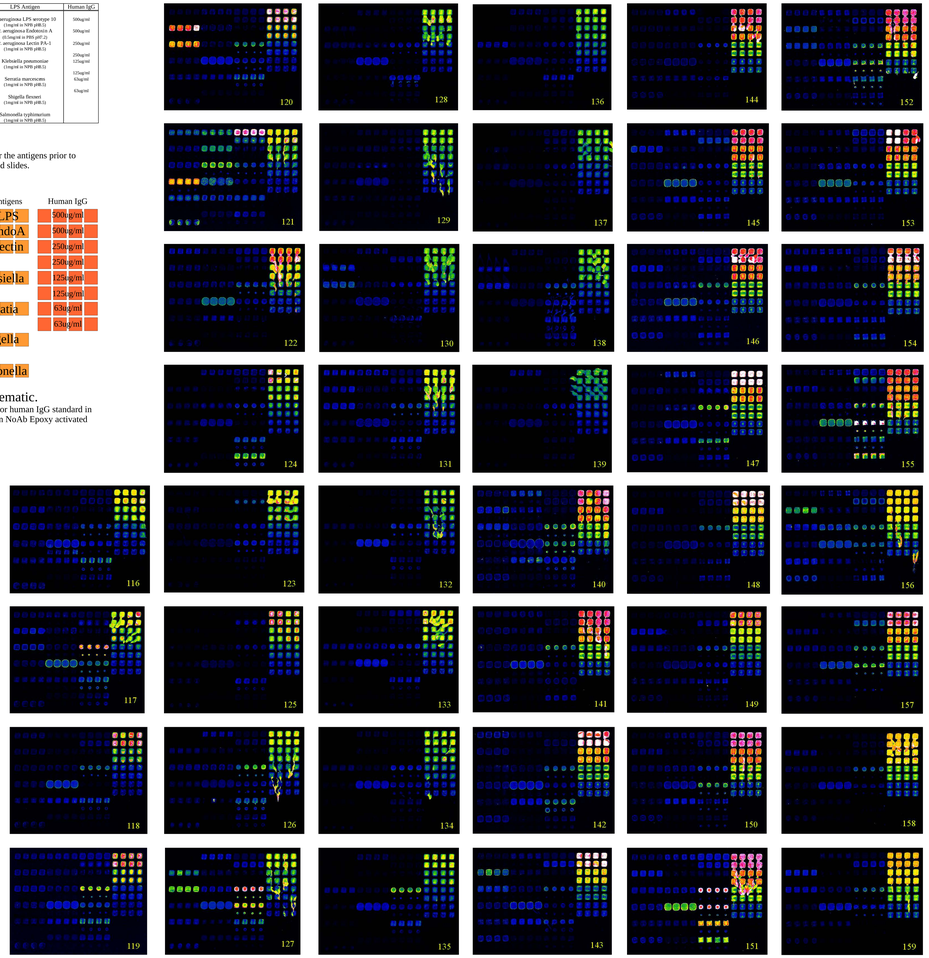

Figure 2. Fluorescence images of various anonymous serum samples on the APTarray
Bacterial and viral antigen preparations were resuspended in PBS pH7.4 and printed on NoAb BioDiscoveries Epoxy activated glass slide substrates using a Virtek SDDC-2 arrayer and Telechem SMP5 pins. APTarrays were dried overnight in the arrayer cabinet. APTarrays were blocked in PBS pH7.4; 1% BSA; 0.5% Tween 20; 0.5% sodium azide for 1 hour at RT. Sera were diluted 1:10 in PBS pH7.4; 1% BSA; 0.5% Tween 20; 0.5% sodium azide. 200ul of the diluted serum solution was applied directly to the APTarray grid on the slide and incubated for 1 hour at RT "array-side up" in a humidified chamber. APTarrays were washed by dipping the slides 5 times in PBS pH7.4; 0.5% Tween 20; 0.5% sodium azide. 200ul of Goat anti-Human IgG-Cy3 conjugate [diluted 1:100 in PBS pH7.4; 1% BSA; 0.5% Tween 20; 0.5% sodium azide] was applied directly to the APTarray grid on the slide and incubated for 1 hour at RT "array side up" in a humidified chamber. APTarrays were washed by dipping the slides 5 times in PBS pH7.4; 0.5% Tween 20; 0.5% sodium azide, once in deionized water and "spin dried" in a bench- top centrifuge at 1200rpm for 10 minutes. APTarrays were scanned at 10um resolution in a GSI Lumonics ScanArray Lite at a laser setting of 80 and PMT gain of 65. A Human IgG standard dilution series is located in the last four columns of each APTarray for quantitation purposes.

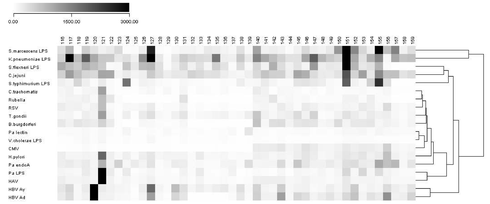

Figure 3. Serum sample reactivity to APTarray antigens.
The concentration of antibodies specific to each antigen on the APTarray was determined for each serum sample. These values were then used to rank antigens on the APTarray that were most commonly detected in the serum samples. Hierarchical clustering algorithms in GeneLinker Gold v.4.0 were used to cluster APTarray antigens with similar reactivity profiles and create the "heat map" above of the APTarray antigen immuno-reactivity profile of each serum sample.

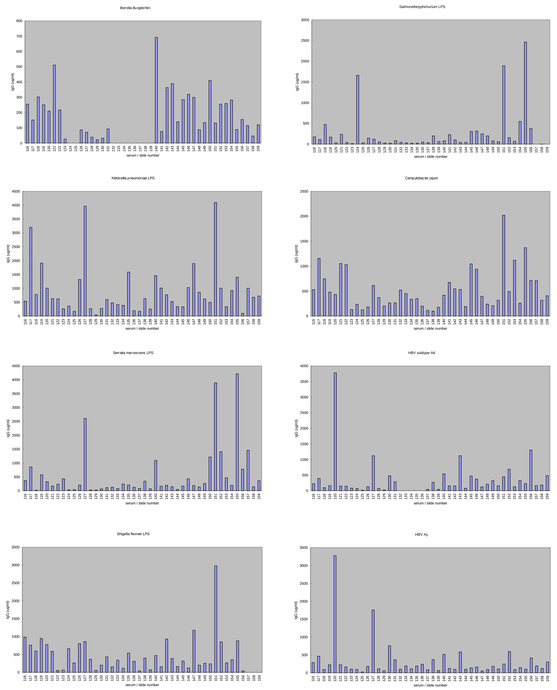

Figure 4. APTarray antigen profiles.
The concentration of antibodies specific to each antigen on the APTarray was determined for each serum sample. These values were then used to produce a histogram of the reactivity of each serum sample to specific antigens on the APTarray. Reactivity profiles for several of the APTarray antigens are shown above.

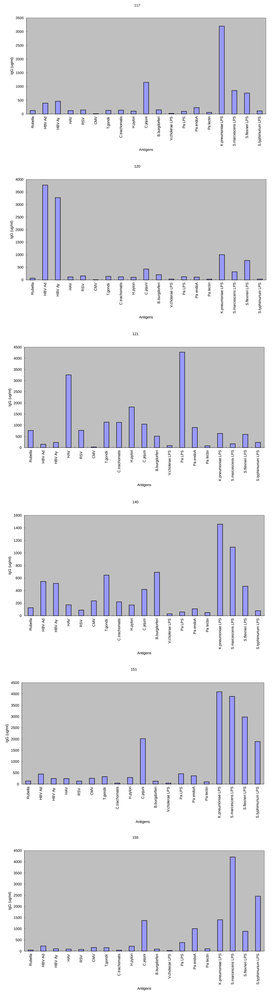

Figure 5. APTarray serum sample profiles.
The concentration of antibodies specific to each antigen on the APTarray was determined for each serum sample. These values were then used to produce a histogram of the reactivity of each serum sample to the antigens on the APTarray. Reactivity profiles for several of these serum samples are shown above.