APPLICATION NOTE

Ziplex System & Proteomic TipChip for High Speed Serodiagnostics of Infectious Disease*

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Infectious Disease Serology. This type of assay is commonly performed in ELISA plates for analyzing serum IgG and IgM antibody levels against such transmissible diseases as HIV, Salmonella, Hepatitis B and C, Rubella and Chlamydia. Despite the availability of species-specific RNA/DNA PCR methods for direct detection of pathogens, recovery of viruses and bacteria from samples is not highly reliable, and the assays are both time-consuming and expensive. Serological antibody tests therefore continue to be the mainstay of infectious disease surveillance.

When implemented on a microarray, the format of the assay consists of printing an array of infectious disease antigens on the microarray substrate. These may range in complexity from whole killed bacteria and viruses to pure recombinant proteins. The patient's serum is incubated on the array and the antibodies that bind to the individual pathogenic antigens are "reported" by labeled secondary antibodies specific for the IgG, IgM, and IgA classes. IgM class antibodies form the dominant first phase (acute) response, while IgG class antibodies form the mature or post-recovery response. When attempting to classify the onset of a disease such as HIV or hepatitis C it is particularly important to determine the relative levels of IgM and IgG antibodies. Dominant IgM would signify a recent exposure.

On-Chip Calibration. The microarray, or microspot-based assay, permits internal calibration. Subarrays of known standards (in this case pure human IgG and IgM) are assayed concurrently with, and within, the sample. This "on-chip calibration" provides both internal quality control and quantitative correction for many factors such as sample quality (lipemia, hemolysis), reagent, and instrumentation variation.

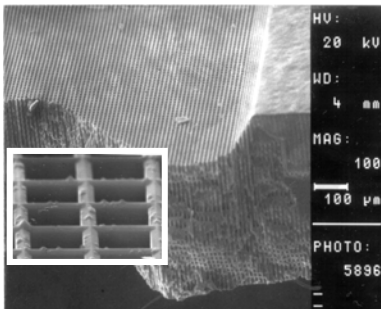
Methods

Flow-Thru Silicon Substrates and Arrays:
Epoxysilane-coated microporous silicon chips 6 mm^2 were contact printed with Telechem SMP3 pins. Arrays of infectious disease antigens with sub-arrays of IgG and IgM dilution series calibrators were printed in triplicate. Antigen concentrations were individually optimized in print buffer (10 mM Phosphate buffer pH 7.2 in 690 mM (5X) NaCl) and ranged between 500 and 2000 mg/ml. Printed arrays were assembled into TipChips for automatic processing in the Ziplex® Automated Workstation. The arrays were pre-blocked for 3 hours in 1% BSA-TBS pH 8 (assay buffer), then air dried and stored in desiccated packets at 4°C until use.

***Assays*:**
Whatman (#7701-1651) 96 well microwell plates were filled with 8 TipChips, 8 samples diluted in assays buffer, wash buffer (10 mM PBS pH 7.2), and chemiluminescence substrate (Pierce Super Signal™ Femto), all volumes 150 mL. Samples were diluted 1:20, 1:40, 1:80, 1:160, 1:320 and 1:640 to show that classical titer may be determined by microarrays concurrently for many pathogens. The plates were loaded into the Ziplex Automated Workstations and the walk-away assay protocol executed in 45 minutes.

* For Research Use Only. Not for Use in Diagnostic Procedures.

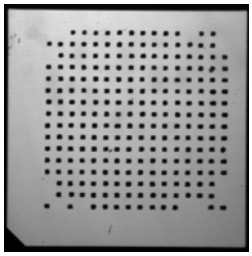
Process Flow



SEM of Microporous Silicon Chip

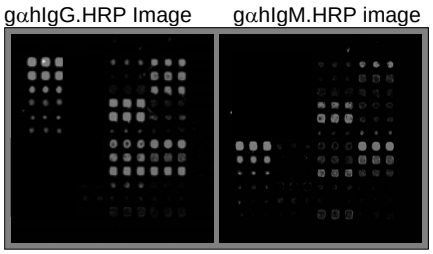
IgG/IgM ug/mL	Infectious Disease Antigens	
250	B.bergdorferi	K.pneumoniae
125	CMV	P.aeruginosa Endotoxin
62.5	EBV	P.aeruginosa lectin PA-1
31.3	HBV adr	P.aeruginosa LPS
15.6	HBV core	S.marcescens LPS
7.8	HCV	S. minnisota LPS
250	HIV p24	S. typhimurium LPS
125	Influenza A	T.gondii
62.5	Influenza B	Rubella Level 1
31.3	HSV 1	Rubeola
15.6	HSV 2	C.trachomatis
7.8	RSV	C.jejuni
Blank	VZV	H.pylori

Layout of Infectious Disease Antigen Array

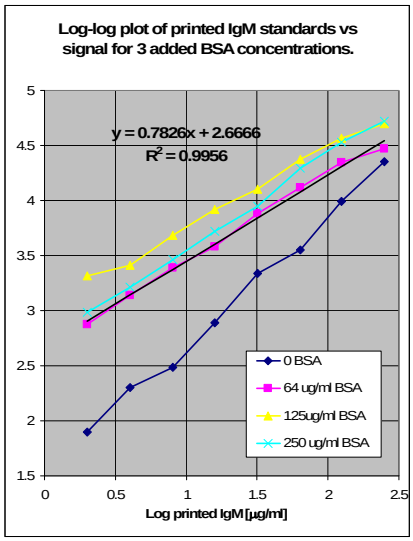


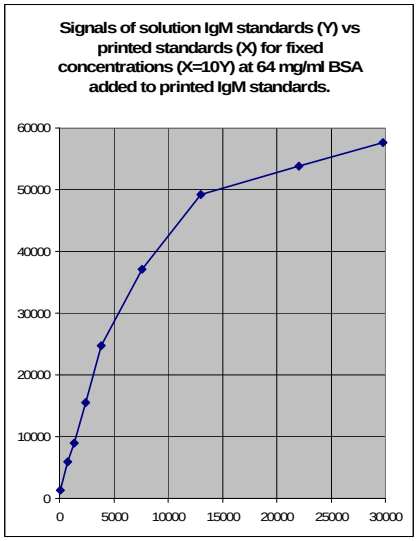
Back-Lit Image - Array Printed on a Chip

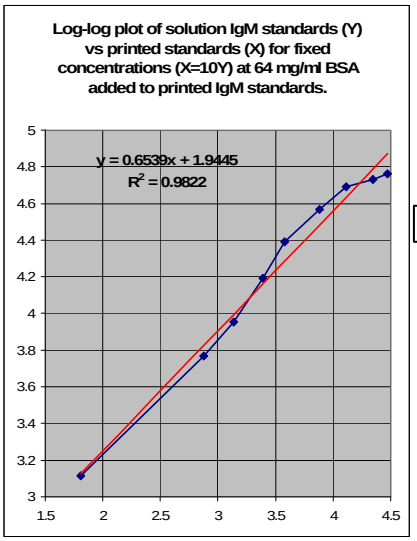
Assay Processing on Ziplex Automated Workstation



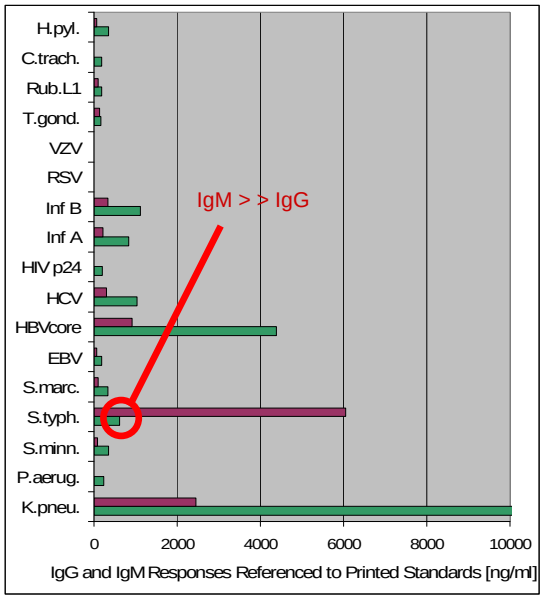
Chemiluminescent Images for IgG and IgM



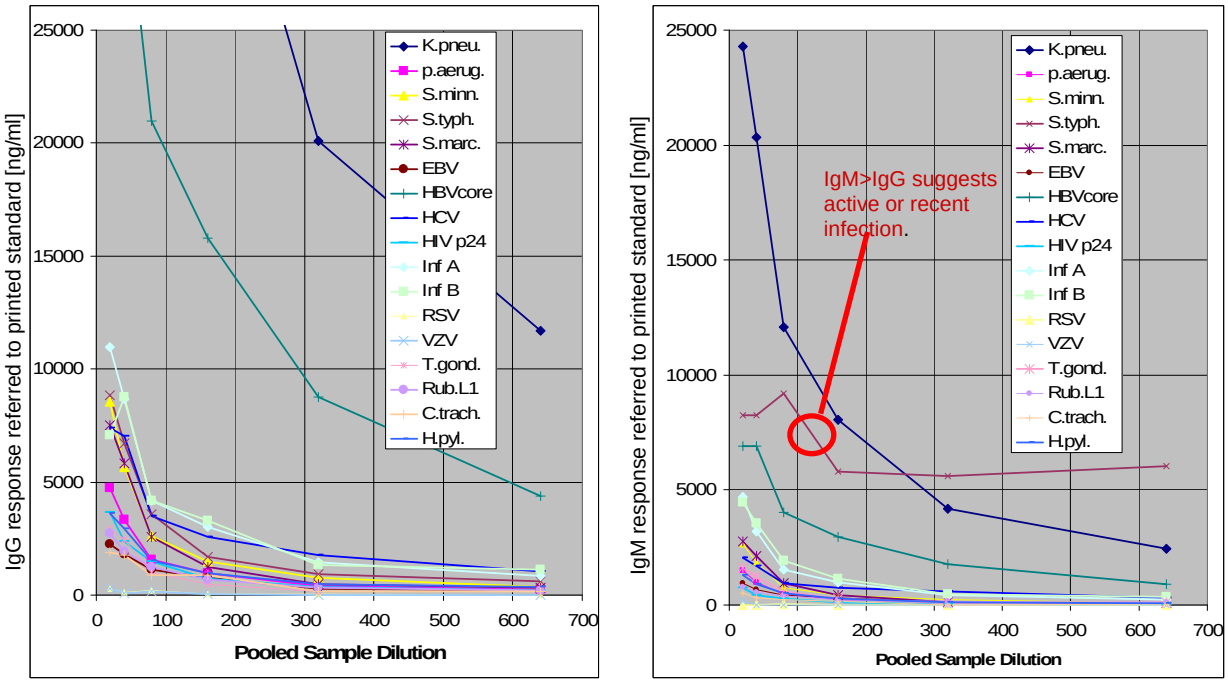




Grid and Extract Calibration Standard Curve



IgG (green) and IgM (red) Concentrations for Each Infectious Disease Antigen



Pooled sera containing sample (from above) diluted from 1:20 to 1:640 shows expected decreasing signals except for the anti-S.typhi. IgM which maintains a moderate titer out to 1:640.

Results

All 8 infectious disease arrays were processed in 45 minutes. Actual immunoassay chemistry was completed in 8 minutes; the remaining 37 minutes being required for chemiluminescence imaging with the present single CCD camera instrument. The Ziplex software automatically grids and quantifies arrays. CVs for triplicate standards and antigen spots were 7 - 8%. There was a good fit to the log-log response of the calibration standard data (linear regression coefficient > 0.99). The relationship between printed and solution calibration standards was determined using arrays with printed mouse immunoglobulin standards and solution standards (mouse monoclonal antibodies against selected antigens e.g., hIgM, EBV, CMV, Rubella, and *T. gondii*). This relationship was regular over 2 orders of magnitude and modeled with log (printed standard) – log (solution standard) fit (regression coefficient >0.98). This is equivalent to the working range of ELISA assays of this type.

IgG and IgM binding to the pathogen spots was quantitatively and qualitatively similar to results previously reported for manually performed assays [1, 2] with epoxysilane glass substrate microarrays using the same serum samples. Dynamic range and sensitivity were also equivalent. IgM responses higher than IgG against a given pathogen suggest current or recent infection (or vaccination) by that antigen. One example is shown for which anti-*Salmonella typhimurium* IgM was markedly high. Each data point is the mean of triplicates and each dilution represents one array (1440 results). Six dilutions of the sample demonstrated the predictable monotonically decreasing IgG and IgM signals, except for the anti-*S.typhimurium* IgM.

The apparent increase in the IgM signal with dilution between 20 and 80 times is consistent with steric hindrance (hook effects) in immunoassay as previously explained [1]. The fact that the IgM level is elevated out to 1:640 dilution is also consistent with the concept of antibody titer due to polyclonal, polyvalent B-lymphocyte responses.

Conclusions:

Even for this small array of 90 test spots, throughput (over 950 tests/ hour) was several times that of current high speed immuno-chemistry instruments. A 16x16 array is shown in the back-lit image. Presently, arrays are manufactured at densities up to 22x22 spots. At these densities, the Ziplex System could deliver over 5000 multiplexed immunoassay tests/hour.

References

1. Ewart T, Carmichael S, Lea P. High quality epoxysilane substrate for clinical multiplex serodiagnostic proteomic microarrays. In: Chan CW, Yu K, Krull UJ, Hornsey RI, Wilson BC, Weersink RA, eds. Photonics North 2005, Proceedings of SPIE Vol. 5969. Toronto, Canada, 2005.

2. Ewart T, Raha S, Kus D, Tarnopolsky M. Imaging combined autoimmune and infectious disease microarrays (Proceedings Paper). In: Mathieu P, ed. Photonics North 2006, Proceedings of SPIE, Vol. 6343. Quebec City, Canada, 2006.

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