

# Ziplex System & Proteomic TipChip for High Speed Serodiagnosis of Infectious Disease

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## Introduction

**Infectious Disease Serology:** This type of assay is commonly performed in ELISA plates in Red Cross blood donation testing laboratories, clinical microbiology, and the communicable disease divisions of public health laboratories. Serum IgG and IgM antibody levels against such transmissible disease as HIV, Salmonella, hepatitis B, and C, Rubella and Chlamydia are performed by the thousands each day. 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 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, IgG class 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.

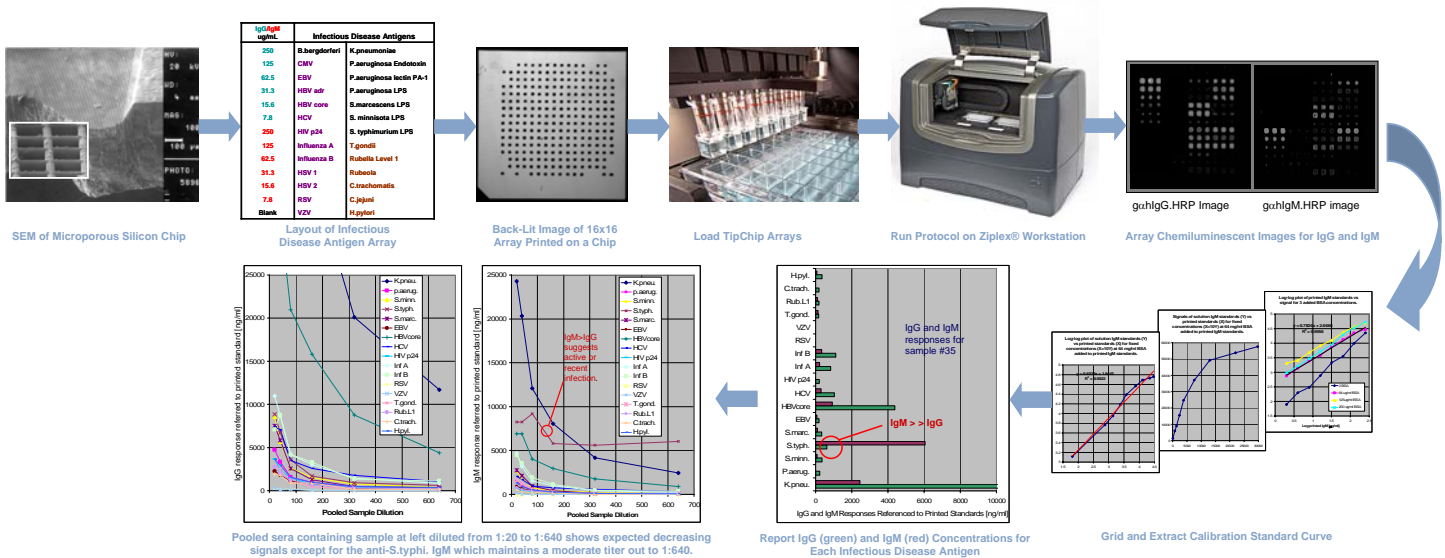
**On-Chip Calibration:** The microarray or microspot-based assay permits internal calibration. That is, subarrays of known standards (in this case pure human IgG and IgM) are assayed concurrently with, and within the sample. This "on-chip calibration" provides both internal quality control and quantitative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

## Methods

**Flow-Thru Silicon Substrates and Arrays:** Epoxysilane-coated microporous silicon chips 6 mm<sup>2</sup> were contact printed with Telechem SMP3 pins. Arrays of infectious disease antigens with subarrays 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 chips were assembled into (TipChips™) for automatic processing in the Ziplex® robotic 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 degrees 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 SuperSignal™ Femto), all volumes 150 μL. 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 microarray concurrently for many pathogens. The plates were loaded into the workstations and the walk-away assay protocol executed in 45 minutes.

## Proteomic TipChip Automation on the Ziplex® Platform



## 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 but may also be operated in manual mode. 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 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. With sample sizes less than 10 mL, this performance addresses the pediatric, STAT and emergency response, small animal veterinary, and high volume reference lab requirements for high speed immunoassay with one platform.

## References

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