The HLA Protein Chip

A High Performance Microarray Platform for Anti-HLA Antibody Detection Utilizing soluble HLA

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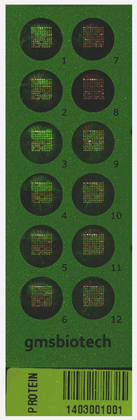
Abstract

Successful pairing of a transplant recipient with a donor organ depends on accurate prediction of risk factors for immunologic rejection. As such, there is much interest in accurate clinical measurement of HLA Ab specificities in recipient sera. Here we offer a new solution that is based on recent advancements made in the application of microarray technology in clinical diagnostics, to increase the precision and sensitivity of HLA Ab serology. HLA protein printed in this array system yielded up to 65,000 MFI with essentially no background signal. Accuracy and reproducibility required for a low cost HLA antibody-screening array was validated, resulting in a high degree of concordance with known monoclonal Ab patterns and established clinical reference sera. Conclusively, the combination of microarray and soluble HLA technology has resulted in a simple, accurate, and low cost method for high-performance multiplexed HLA Ab detection, which with additional development in progress will be ready to accommodate very high resolution HLA Ab screening, for pre-transplant evaluation and as a routine follow-up test to detect/monitor early stages of graft rejection.

The Protein Microarray

Multiple 12x12 microarrays were fabricated by microfluidic spotting of highly purified soluble HLA class I molecules under a range of conditions chosen to optimize protein stabilization on the microarray surface. Of particular interest was the evaluation of maximal signal and background intensities, signal uniformity between different HLA alleles and the structural integrity of the HLA proteins subsequent to microfluidic attachment to the microarray surface.
Our current prototype consists of:

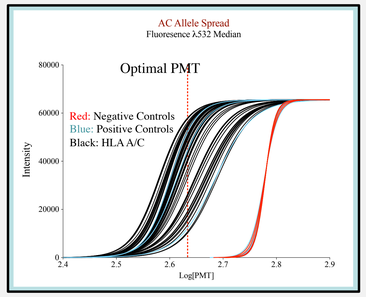
HLA1	121
Negative Controls	6
HLA related protein probes	5
Positive Control Human (Ig)	9
Positive Control Mouse (Ig)	3
Total	**144**



Color Legend:
Ratio images shown represent the comparison of HLA protein to a reference sample that is mixed with the arrayed HLA.
Red Laser: Cy5 reference sample (λ635 nm);
Green Laser: HLA/Ab interaction (PE) (λ532 nm).

Maximizing Dynamic Range

The complete dynamic range of the Chip scanner is being used when you see a range of intensities on the image from 1 to 65535. A pixel with an intensity of 65535 is saturated. Saturated pixels represent a condition in which there are more photons detected than the photo multiplier tube (PMT) can process. A saturated pixel is not an accurate measurement of the signal from the pixel, so it is imperative to set the PMT gain to avoid saturation. Auto PMT calculations delivering the optimum PMT value assure that the results will always be in the linear range and all signals will behave proportional to each other; an advantage the Luminex equipment is unable to address.

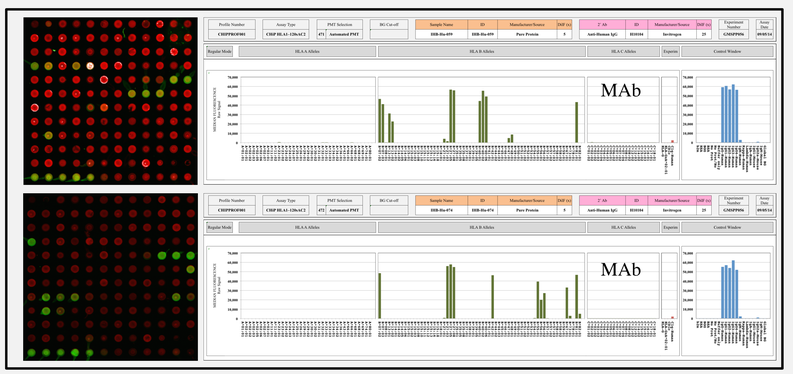


Conclusion

The combination of microarray and soluble HLA technology has resulted in a simple, accurate, and low cost method for high-performance multiplexed HLA Ab detection. Tests show all characteristics required to confirm the suitability of the assay platform for sera testing such as accuracy, specificity, and sensitivity. We believe that increased screening and the usage of additional evaluation tools such as the neutralizer technology with the Chip will greatly enhance our knowledge and contribute to a better risk management in Ab-related graft rejection.

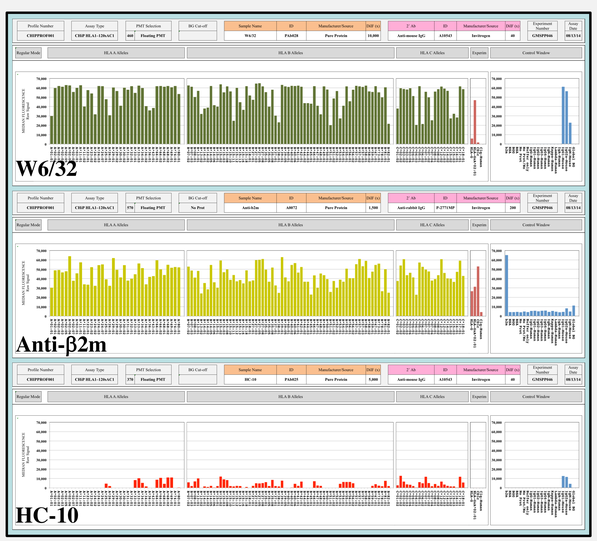
Epitope Validation

In order to validate epitope recognition, we investigated the performance of the Chip using test samples containing Bw4 recognizing Abs. Here we demonstrate that a clinical reference sera shows a very high degree of concordance with a known human monoclonal Ab profile (IHB-Hu-040; OUW4F11). Only alleles known to carry the Bw4 sequence were found to be positive. Visual inspection of the 12x12 sera grid showed no indication of high background compared to the "clean" monoclonal Ab system. Overall, testing many other epitope-specific human monoclonal Abs, which we could not all show here, confirm the structural integrity of specific epitopes on the printed sHLA molecule.



Quality Control

The key step in the development of a Chip platform is the selection and testing of suitable strategies to present the target antigen without loss of structural integrity. Conditions leading to degraded or denatured HLA proteins can contribute to false positive, false negative, and inconsistent signals and are therefore unacceptable. In order to distinguish between degrading and conserving conditions forced upon the trimeric structure of the HLA molecule during attachment/printing of the Chip, allele specific monoclonal/polyclonal antibodies were used. These antibodies were selected because of their unique characteristics to visualize sHLA molecules at different structural forms. W6/32 is known to recognize all native HLA molecules but only when structurally fully intact. HC-10 is generally used as indicator of structurally impaired HLA molecules as it recognizes free HLA heavy chains not associated with B2m. HC-10 is not recognizing all HLA molecules and lacks binding to several A and C type as well as a few B alleles. Anti-B2m antibody confirms the presence of the B2m subunit. Our assessment showed that structural integrity was greatly maintained demonstrating high W6/32 and anti-b2m signal and very low HC-10 activity.



Profile Validation

In order to demonstrate the capability of the HLA Chip to create clinically relevant reaction patterns, we tested multiple clinical reference sera of which only a few are presented here. We found that the obtained profiles were all in accord with their known recognition pattern obtained by previous tests conducted with the Luminex system. Most advantageous observation was that the chip showed extremely high signal-to-background ratios as one can imagine by visual inspection of the grid scan.