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CHARACTERIZATION OF SECRETED SINGLE CLASS II HLA-DR & -DQ MOLECULES.
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**Aim:** HLA class II molecules are highly polymorphic cell surface antigens that are targeted by allogeneic immune responses during the transplantation of organs and bone marrow. The accurate detection of allogeneic antibodies directed towards specific class II molecules is an important determinant for successful organ transplantation. A limiting reagent for the implementation of class II HLA antibody screening assays is the availability of native individual class II molecules. Here we demonstrate the ability to secrete single class II HLA molecules from immortalized cell lines and we test the recognition of these class II molecules with defined clinical sera.

**Methods:** A cell line that does not express HLA class II molecules was transfected with leucine-zipper modified HLA $\alpha$- and $\beta$-chain cDNA, which resulted in the secretion of single class II HLA molecules. These soluble HLA class II molecules were then purified from the cell supernatant with a specific anti-HLA class II antibody (L243) coupled to CNBr-activated Sepharose 4B. The protein concentration was determined by a micro-BCA protein assay. To test for antigenicity, purified sHLA class II molecules were exposed to defined patient sera.

**Results:** Results demonstrate that transfected cell lines have the ability to secrete single class II HLA molecules. The sHLA class II titer of a typical production run was found to be in the area of 4-5 mg/liter of growth media. Antigenicity testing using defined antibodies showed accurate specificity. Comparison of the soluble HLA with full-length molecules showed no differences in antigenicity.

**Conclusions:** The usage of purified, single specificity soluble HLA class II-molecules in sera screening applications is highly feasible.

## 196-P

IMPROVED METHODOLOGY FOR TESTING "HIGH-BACKGROUND" SERA USING THE DynaChip® ANTIBODY ANALYSIS PLATFORM.
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**Aim:** The DynaChip® Antibody Analysis System is a microarray based platform that allows simultaneous detection of HLA Class I and Class II antibodies in patient serum. Although very reliable, occasional sera show high backgrounds, resulting in "void spots" which can not be interpreted. Factors that cause void spots can range from simple presence of dirt on the chip to nonspecific interaction of unknown serum components with the proteins on the microarray. More than 6 void spots for Class I and 4 for Class II invalidate an assay. Thus, it is critical to keep the number of void spots to minimum to avoid skewing the interpretation of the results.

**Methods:** To resolve the issue with these serum samples, each step in the protocol was examined. To lower background, we evaluated a panel of 16 blocking compounds. We also investigated the use of various detergents, and the effects of additional washing steps.

**Results:** We saw significant improvement in the number of void spots following optimization. We also noticed significant increase in the signal to noise ratio especially with our qualified negative serum samples. Following implementation of the new protocol, results generated were highly reproducible and produced consistent PRA and specificity data, even using sera that previously showed high background. Further, we compared the new protocol to the original protocol to ensure that the results were equivalent and that we observed true specificities.

**Conclusions:** Careful analysis of the different steps of DynaChip® protocol provides us with a good handle on the assay and ability to resolve background issues with difficult serum samples.