HISTO SPOT® AB Kits – Technical Report

Repeatability study of the HISTO SPOT® HLA AB Screen/ID test

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Introduction

This study has the aim to show repeatability when testing sera for HLA Antibodies with the HISTO SPOT® HLA AB Screen/ID Kit. The repeatability is an essential parameter for an accurate and stable test. The HISTO SPOT® HLA AB Screen/ID test is a microtiter plate-based test that runs on an automated bench top system, the MR.SPOT® Processor. This assay is capable of detecting class I or class II antibodies via immobilized native as well as recombinant antigens.

Material and Methods

The HISTO SPOT® HLA AB Screen/ID Test is a microtiter plate based test. In the individual test vessels, the HLA class I or class II antigens are immobilized to which the specific anti-HLA antibodies bind during the automatic assay processed on the MR.SPOT®. Antibodies bound to the antigens are detected by an enzymatic colour reaction. The coloured spots are photographed at the end of the assay and can be evaluated in the HISTO MATCH interpretation software. The software calculates the mean colour intensity (MCI) of each spot which can be used for a semi quantitative analysis.

The samples used for repeatability testing are listed in Table 1. One serum was tested with a single antigen test on the Luminex instrument for comparison. The repeatability was evaluated with three different methods. First, the pictures were compared visually (examples see Figure 1 and 2). Second, the variability of the intensity of the reactions i.e. the coefficient of variance (CV = Standard deviation / Mean) for the MCIs of each antigen was calculated for the 7, 8 or 9 repeat tests.

Table 1: Samples used for the study

Sample	No. of tests
Positive Controls	
Class I (monoclonal Ab reacting with all class I antigens, 25 ng/µl)	9x
Class II (monoclonal Ab reacting with public epitopes on HLA-DR, DP, DQ2, 25 ng/µl)	8x
Class I positive	
BAG 108H0290	7x
Leiden 584961	7x
Leiden 547826	8x
EPT 2015 B	8x
EPT 2015 C	8x
EPT 2015 K	8x
Class II positive	
BAG 93H808	7x
BAG 107H0850	7x
Leiden 1054499	8x
EPT 2015 J	8x
EPT 2016 E	8x
EPT 2016 J	8x
Single Antigen Test on the Luminex® instrument	
BAG 105H0030	7x

BAG: human sera from BAG Health Care lab
Leiden: human sera from Leiden University
EPT: sera from Eurotransplant quality exchange

The global CV was then calculated as the mean from the CVs of the 96 antigens for class I and 59 antigens for class II. As an additional parameter the percentage of antigens with a CV higher than 15% was determined. For the serum tested on the Luminex instrument the same calculations were done using the MFI (Mean fluorescence intensity) values. In a third step the results were interpreted with the HISTO MATCH software. The positive, negative or questionable results of the individual antigens are automatically suggested by the HISTO MATCH software based on the MCIs compared to the background. The concordance of assigned results in repeated tests was analysed.

Results

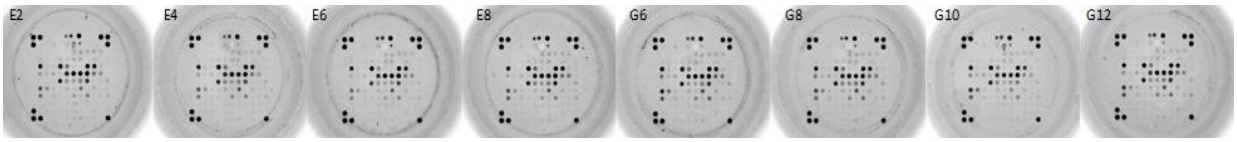


Figure 1: Class I test with the serum Leiden 547826

Figure 2: Class II test with the serum Leiden 1054499

Figure 1 and 2 show the photos of the repeat tests of the serum Leiden 547826 for class I and of the serum Leiden 1054499 for class II. The photos of the other serums tested are shown in the appendix. In the various pictures of the sera, it is noticeable that there are hardly any visible differences in the intensities of the reactions. The global CV was used as a quantitative measure of the repeatability. The global CV was between 6% and 9% for serums tested for class I and between 4% and 8% for class II. The percentage of antigens with a CV of 15% or higher ranged between 0% and 5% for class I and between 0% and 12% for class II (Figure 3 and 4). The results also show that the variability of the reactions strongly depend on the serum that is tested. The more questionable reactions there are present in the test the higher is the variability. The global CV for the positive control was lower than for the serums with 1% for class I and 2% for class II. The global CV of the single serum tested on the Luminex was 13% and the percentage of antigens with CV higher than 15% was 24%. The analysis of the interpreted results shows that the variability of the MCIs sometimes leads to discrepancies in the automatic interpretation of the results if the signals are weak and close to the cut off. The same is true for the serum tested on the Luminex® instrument. Most of the discrepancies will be removed after reviewing and editing of the results by the user.

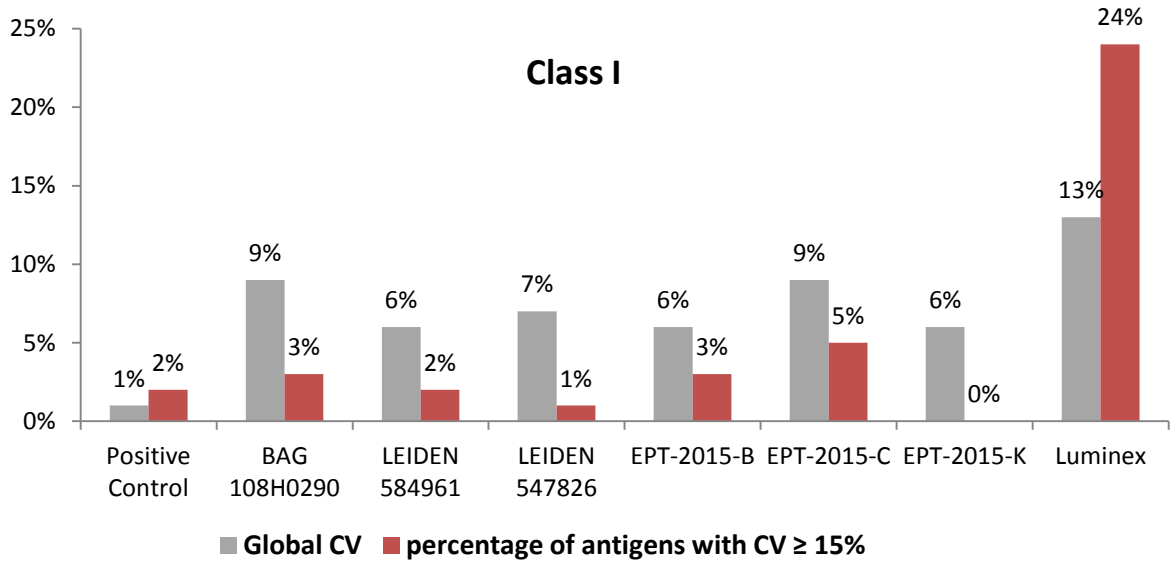


Figure 3: Global CV and percentage of antigens with a CV higher than 15% for the class I test

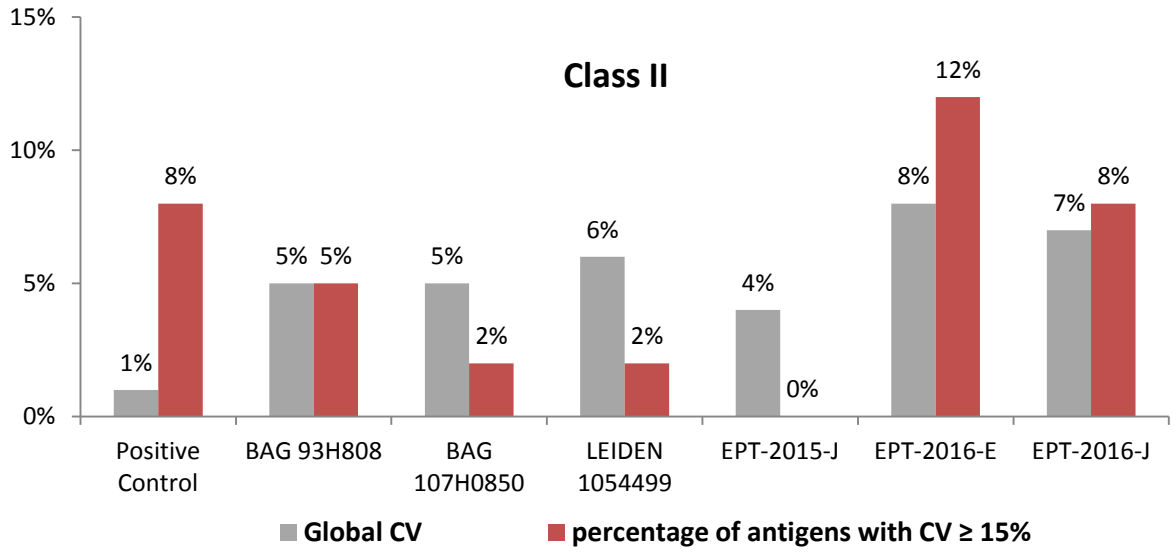


Figure 4: Global CV and percentage of antigens with a CV higher than 15% for the class II test

Discussion

The repeatability study for the HISTO SPOT® HLA AB Screen/ID Kit for class I and class II shows a very low variability. The CVs are below 10% for the class I and class II tests. In a similar experimental setting for a single antigen assay on the Luminex® instrument Reed et al. (2013) found a much higher CV of around 25% for sera. For the single serum tested with a Class I single antigen assay on the Luminex® instrument in this study the variability in signal was found to be higher as well.

For monoclonal antibodies used as a positive control in the single antigen assay on the Luminex® instrument CVs below 10% have been reported (Congy-Jolivet, 2013). The CVs of 1% found in this study are far below this value.

Therefore, it seems that a better repeatability can be reached with the HISTO SPOT® HLA AB Screen/ID Kit than with the single antigen assay on the Luminex® instrument. A higher number of sera should be tested on the Luminex® instrument to confirm this finding.

Overall, the variability for the class II chip is higher than for the class I chip. This might be due to the fact that the class II chip contains some native antigens in addition to the recombinant ones and that the native antigens show a higher variability.

Literature

Reed, E.F., et al.; Comprehensive Assessment and Standardization of Solid Phase Multiplex-Bead Arrays for the Detection of Antibodies to HLA, Am. J. Transplant. (2013), 13(7)

Congy-Jolivet, N. et al., Production and characterization of chimeric anti-HLA monoclonal antibodies targeting public epitopes as tools for standardizations of the anti-HLA antibody detection, J. Immunol. Methods (2013)

Appendix

Table 1: Images of the six tested sera of class I

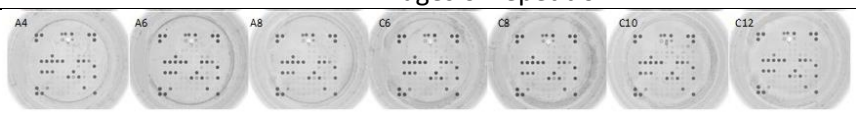
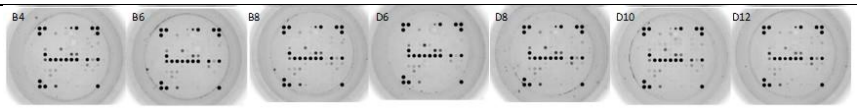
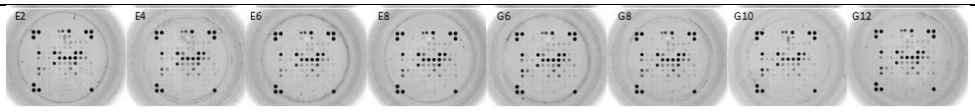
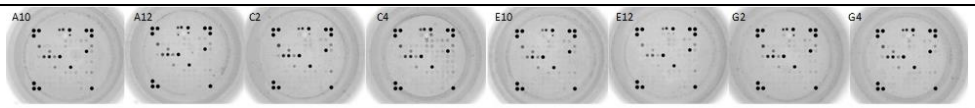
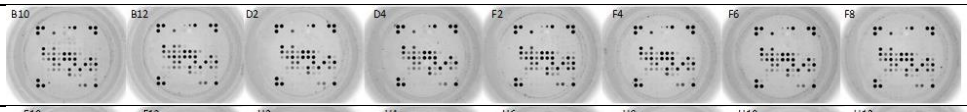
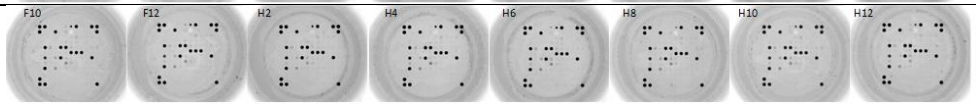
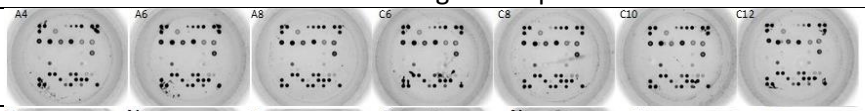
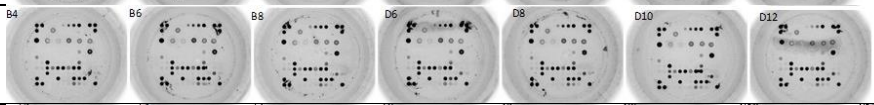
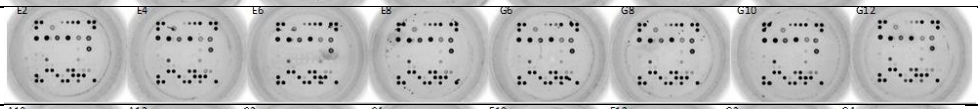
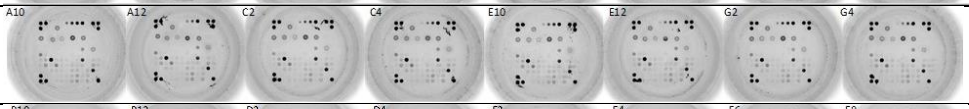
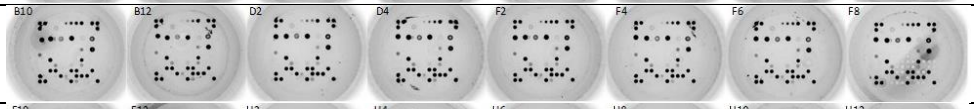
Serum	Images of repetition
BAG108H0290	
LEIDEN 584961	
LEIDEN 547826	
EPT – 2015 – B	
EPT – 2015 – C	
EPT – 2015 – K	

Table 2: Images of the six tested sera of class II

Serum	Images of repetition
BAG 93H808	
BAG 107H0850	
LEIDEN 1054499	
LEIDEN EPT 2015 - J	
LEIDEN EPT 2016 - E	
LEIDEN EPT 2016 - J