BAG
DIAGNOSTICS

HISTO SPOT®
HLA AB SYSTEM

EVALUATION OF THE DIAGNOSTIC
SENSITIVITY AND SPECIFICITY OF THE
HISTO SPOT® HLA AB SYSTEM – PRECISION
AND EXCELLENCE IN A NEW FORM

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INTRODUCTION

The success of an allotransplantation heavily depends on accurate donor–recipient HLA matching [1-3]. HLA profiling with the established Luminex single antigen bead (SAB) test is known to produce a certain amount of false results indicating that there is room for improvement regarding HLA typing technology [4-8].

Our HISTO SPOT® HLA AB System that utilizes microtiter plates coated with recombinant single antigen proteins to detect anti-HLA antibodies represents a good alternative to Luminex tests. Here, we evaluated the diagnostic sensitivity and specificity of the HISTO SPOT® HLA AB System by analyzing 60 sera for class I and 104 sera for class II from a round-robin test. Because of their high sensitivity SAB assays have been found to detect the presence of anti-HLA antibodies in sera of individuals, which have not been immunized before and which therefore should not produce these types of antibodies. In some cases these false positive results are due to a reaction of "natural antibodies" against denatured HLA antigens attached to SABs and do not justify the exclusion of potential donor-recipient matches for organ transplants. Here, we also compared the performance of the HISTO SPOT® HLA AB System with respect to the detection of these "natural antibodies" in comparison to the Luminex-based CE certified LABScreen™ SA Class II kit.

MATERIAL AND METHODS

DETERMINATION OF THE DIAGNOSTIC SENSITIVITY AND SPECIFICITY

60 sera provided by the Eurotransplant Reference Lab (ETRL) were examined with the HISTO SPOT® HLA AB class I test and 104 sera provided by the ETRL and the Société Francophone de Histocompatilibité et d'Immunogénétiques (SFHI) were analyzed with the HISTO SPOT® HLA AB class II test according to the instruction manual with the help of the fully automated MR.SPOT® processor. Antibodies in the sera selectively bound their target antigens, which are coated to the surface of the microtiter plates. Afterwards, antibody-antigen complexes were labelled with a horse radish peroxidase (HRP) conjugated anti-IgG followed by the addition of the chromogenic substrate tetramethylbenzidin (TMB). Because of the catalytic activity of HRP the TMB was oxidized, which turned it from colorless to blue. This color reaction was then captured with FUSION Software Version 1.6 and the images were assessed with HISTO MATCH HLA AB module version 4.2.1. The values measured by the HISTO MATCH software were not manually edited unless the gridding had to be corrected, the interpretation algorithm had to be changed or visible dust in a well affected the measurement. The results obtained with the HISTO SPOT® HLA AB class I and class II test were compared to the published consensus data for the quality exchange sera from the SFHI and the ETRL ascertained previously with Luminex-based single antigen tests by around 70 independent laboratories in a ring trial, which hereafter are referred to as the expected results. In order to interpret the data we adhered to the method of the Eurotransplant quality exchange that defines a specificity as positive if 95% or more of the participating laboratories report a positive result. On the other hand, a negative specificity was determined if less than 5% of the laboratories observed it. Everything between 5% and 95% was classified as "no consensus" (NC) and excluded from further analyses.

DETECTION OF SAMPLES WITH „NATURAL ANTIBODIES"

30 samples from healthy donors from a blood bank were tested for the presence of HLA-specific antigens in parallel with the HISTO SPOT® HLA AB System according to the instruction manual and the Luminex-based CE certified LABScreen™ SA Class I kit (OneLambda) according to the manufacturer's instructions and performances were compared.

RESULTS

HISTO SPOT® HLA AB CLASS I

Out of the 60 sera used in this test only one (EPT 2017) or 2% yielded highly unspecific results and was therefore not incorporated in the analysis of diagnostic sensitivity (the rate of truly positive test results) and specificity (the rate of truly negative test results). For the 59 remaining samples the published consensus data reported 1873 positive consensus specificities. The HISTO SPOT® HLA AB class I test succeeded in identifying 1800 of these consensus specificities with only 73 being falsely detected as negative. This equaled an outstanding diagnostic sensitivity of 96%. With regard to diagnostic specificity, 2377 negative consensus specificities were published and expected. Here, the HISTO SPOT® HLA AB class I test successfully identified 2179 negative specificities while only displaying 180 false positive reactions resulting in a very high diagnostic specificity of 92%.
In terms of detection of "natural antibodies" the HISTO SPOT® HLA AB class I test yielded 17 positive and 3 questionable results adding up to 67% of all samples tested. However, this is still significantly lower compared to the 80% (14 positive and 10 questionable results) obtained with the LABScreen™ SA Class I kit **(Table 1)**.

	OneLambda	HISTO SPOT®
Positive	14	17
Questionable	10	3
Negative	6	10

Table 1: Head-to-head performance comparison between the LABScreen™ SA Class I kit from OneLambda (left) and the HISTO SPOT® HLA AB class I test (right) with respect to the detection of "natural antibodies" in blood samples from healthy donors.

HISTO SPOT® HLA AB CLASS II

After having evaluated the performance of the HISTO SPOT® HLA AB class I test we proceeded to do the same with the HISTO SPOT® HLA AB class II test. For this purpose, we analyzed 104 sera provided by the ETRL and the SFHI. For these sera, published consensus data reported 1109 positive consensus specificities. With the HISTO SPOT® HLA AB class II test we could identify 1013 of these positive consensus specificities, while 96 were reported as false negative. The 1013 true positive results added up to a diagnostic sensitivity of 91%. Manual correction even increased the number of true positive results to 1044, resulting in a final diagnostic sensitivity of 94%. Looking at each serological specificity separately our test detected not less than one antigen per specificity with a diagnostic sensitivity of ≥ 75%. Concerning the diagnostic specificity, we were expecting 4550 negative consensus specificities that had been published previously. The HISTO SPOT® HLA AB class II test detected 4449 true negative specificities and showed 101 false positive results. From these results, we could calculate a diagnostic specificity of 98%. Manual correction resulted in a reduction of the amount of true negative results to 4418, which equaled a diagnostic specificity of 97%.
The analysis of blood donor samples with the HISTO SPOT® HLA AB class II test for the presence of "natural antibodies" yielded 18 negative results adding up to 60% of all samples tested. The same analysis with the LABScreen™ SA Class I kit amounted to only 5 negative (17%) and 10 questionable results, which represent one third of all samples **(Table 2)**. Finally, we would like to point out that only for one (EFS-NDF 287) out of the 133 sera that were analyzed with the HISTO SPOT® HLA AB class II test analyzed we obtained a result that could not be interpreted, which equals very low 0.75%.

	HISTO SPOT®	LUMINEX
Positive	11	14
Questionable	0	10
Negative	18	5

Table 2: Evaluation of 30 sera from blood bank donors (no reactivities = negative (NEG), at least one positive reaction = positive (POS), at least one questionable reaction, no positive reactions = questionable (Q)). * EFS NDF 287 reacts generally unspecific and would have been rated as uninterpretable.

CONCLUSION

In order to maximize the prospects of success of a solid organ transplant, it is crucial to match the HLA patterns of the donor and the recipient as precise as possible. In this study, we compared the performances of established Luminex-based SAB systems and our newly developed HISTO SPOT® HLA AB class I and class II test in terms of specifically and reliably detecting antibodies directed against HLA antigens in the sera of patients. Our examination clearly showed that the HISTO SPOT® HLA AB class I test with a diagnostic sensitivity of 96% and a diagnostic specificity of 92% as well as the HISTO SPOT® HLA AB class II test with a diagnostic sensitivity of 92% and a diagnostic specificity of 97% exhibited outstanding performances that definitely allow the conclusion that the HISTO SPOT® HLA AB System can be utilized instead of Luminex-based technologies without compromising the quality of the analysis. This conclusion is further supported by the very low percentage of serum samples that did not yield interpretable results.

We could additionally demonstrate that the HISTO SPOT® HLA AB System is less prone to identify the so-called "natural antibodies", which account for a significant amount of false positive results during HLA matching, in serum samples of individuals that have not been sensitized to HLA antigens. A comparative analysis with the Luminex-based LABScreen™ SA Class I and Class II kit revealed a markedly higher number of potentially positive or questionable results demonstrating that the HISTO SPOT® HLA AB System is indeed a good alternative to established HLA typing technologies.

In summary, we have shown here that the HISTO SPOT® HLA AB System is an accurate and reliable method for the detection of anti-HLA antibodies that will undoubtedly pass external quality assessments and provide the user with the information to take decisions for the benefit of their patients.

REFERENCES

[1] Gerhard Opelz, Bernd Döhler. Effect of human leukocyte antigen compatibility on kidney graft survival: comparative analysis of two decades. Transplantation. 2007 Jul 27; 84(2):137-43.

[2] Andrea A Zachary, Mary S Leffell. HLA Mismatching Strategies for Solid Organ Transplantation - A Balancing Act. Front Immunol. 2016 Dec 7:7:575.

[3] Roberto Crocchiolo, Gianni Rombolà. Human Leucocyte Antigen System and Selection of Unrelated Hematopoietic Stem Cell Donors: Impact of Patient-Donor (Mis)matching and New Challenges with the Current Technologies. J Clin Med. 2023 Jan 13; 12(2):646.

[4] Luis E Morales-Buenrostro, Paul I Terasaki, Lluvia A Marino-Vázquez, Jar-How Lee, Nadim El-Awar, Josefina Alberú. „Natural" human leukocyte antigen antibodies found in nonalloimmunized healthy males. Transplantation. 2008 Oct 27; 86(8):1111-5.

[5] Ji Won In, Eun Youn Rho, Sue Shin, Kyoung Un Park , Eun Young Song. False-positive reactions against HLA class II molecules detected in Luminex single-antigen bead assays. Ann Lab Med. 2014 Sep; 34(5):408-10.

[6] Christina L Dean, Scott M Krummey, Howard M Gebel, Robert A Bray, Harold C Sullivan. Identification of a recurrent pattern of false-positivity by Luminex HLA MHC class I single antigen bead testing. Hum Immunol. 2020 Feb-Mar; 81 (2-3):73-78.

[7] Borae G Park, Younhee Park, Beom Seok Kim, Yu-Seun Kim, Hyon-Suk Kim. False Positive Class II HLA Antibody Reaction Due to Antibodies Against Denatured HLA Might Differ Between Assays: One Lambda vs. Immucor. Ann Lab Med. 2020 Sep; 40(5):424-427.

[8] Boyeon Kim, Sinyoung Kim, Younhee Park, Hyon-Suk Kim. False-positive reactivity of anti-human leukocyte antigen antibodies detected using the single-antigen bead assay. Hum Immunol. 2021 Jun; 82(6):409-413.