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Comparison of HLA antibodies identification methods for platelet products selection in HLA-mediated platelet refractory patients.

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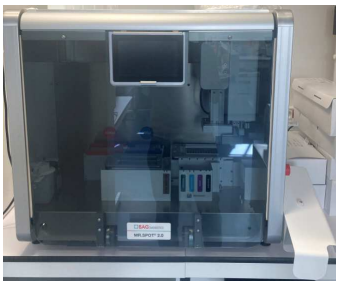
Background

The platelet refractory state still remains the major issue of platelet transfusions in patients with hematological diseases or aplasia. The first etiology of platelet transfusion ineffectiveness due to immunological conflict is HLA class I allo-immunization. The introduction of solid-phase immunoassays using recombinant single antigens (SA) (LIFECODES LSA Kit, Immucor® and LABScreen Single Antigen, One Lambda® (OL)) for detection and characterization of HLA antibodies in transfusion has resulted in a new paradigm with respect of HLA compatibility platelet products selection. However, the SA assay raises technical issues that require resolution and careful consideration when interpreting antibody identification results[1]. Some of the pitfalls in interpreting anti-HLA antibodies are linked to the co-identification of anti-HLA alloantibodies from "natural" type and antibodies directed against denatured HLA antigens[2,3,4,5]. A new HLA class I antibodies identification method, the HISTO SPOT® HLA AB test (BAG Health care GmbH, Lich, Germany) which is based on the interaction between the HLA antibodies and the recombinant single antigen immobilized on the microarray, has been recently commercialized.

The aim of this study is to determine the ability of HISTOSPOT for detecting anti-HLA class I antibodies in non-effective platelet transfusion compared to OL assay.

Firstly, the results of detection of HLA antibodies by HISTO SPOT® HLA AB test were compared to the results for the OL test. Then, the impact of the detection of HLA antibodies were evaluated in accordance to 1-hour corrected count increment (CCI) after the platelet transfusion.

Material & Methods

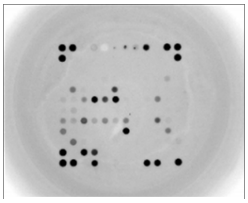


56 serum samples from 54 patients with platelet refractory were tested with the HISTO SPOT® HLA AB test and LABScreen® Single Antigen.

The HISTO SPOT® HLA AB Screen/ID test is a miniaturized and multiplexed solid phase enzyme linked immunosorbent assay. Each well of the microtiter plates is printed with various recombinant or native HLA antigens. Each antigen is printed on the bottom of the well to have an independent spot. The resulting antibody signals (colored dots in the bottom of each test well) are photographed by the MR.SPOT® processor and the image is transferred into the HISTO MATCH interpretation software. The image analysis software determines the color intensity and the background intensity of each spot in the array. Then a Mean Color Intensity (MCI) is calculated by subtracting the background value from the spot intensity. Based on spot intensity and the variability of the background the software gives a cut off value for each test, but results have to be reviewed and edited by the user.

To determine platelet transfusion efficiency, one-hour corrected count increments (CCIs) were calculated as follows: (post platelet count – pre platelet count in 10^9/L) × (body surface area in m^2) / (platelet dose transfused × 10^{11}).

To determine the impact of two assays on platelet transfusion efficiency, the sensitivity is calculated by TP/(TP+FN), the specificity by TN/(TN+FP), the positive predictive value by TP/(TP+FP) and the negative predictive value by TN/(TN+FN) with TP : true positive, TN : true negative, FP : false positive, FN : false negative.



Name	Creg	Signal	Normalized Signal	CutOff	Value	Signal
A*68:01	10C,2C	19845	4,73	140	+	
A*69:01	10C,2C	19529	4,37	140	+	
A*02:01	2C	19368	4,26	140	+	
A*02:03		19136	4,05	140	+	
A*68:02	10C,2C	17839	4,04	140	+	
B*57:01	2C,5C,Bw4	4326	1,86	140	+	
B*58:01	2C,5C,Bw4	4122	1,77	140	+	
B*51:02		987	1,19	140	-	
B*81:01	7C,Bw6	981	1,20	140	-	
B*41:02	7C,12C,Bw6	845	1,17	140	-	

Results

Single Antigen panel differences

ANTIGEN	A2	A11	A26	A29	A34	A74	B7	B13	B63	B70	B71	B71	B75	B39	B61	B41	B41	B57
OL	A*02:06	A*11:02		A*29:01	A*34:02			B*13:01			B*15:10		B*15:11		B*40:06	B*41:01		B*57:03
SPOT			A*26:02			A*74:03	B*07:03		B*15:17	B*15:09		B*15:18		B*39:06			B*41:02	

Table 1. differences between the OL and SPOT panel.

All antigens are present in the two kits. OL and HISTO SPOT® HLA AB Kits were composed of 81 and 79 class I SA, respectively. Ten SA and eight SA are unique in each assay.

Concordance between OL and HISTO SPOT results

MFI OL	OL	HISTO SPOT	CONCORDANCE	
> 10 000	570	549	96 %	**84%**
5 000 – 10 000	255	149	58 %	
2 000 – 5 000	317	140	44 %	
500 - 2 000	265	92	35 %	
< 500	7	5	71 %	
Total	1414	935	**66%**	

Table 2. concordance between OL and HISTOSPOT results

The global concordance is 66%. There is no difference between HLA-A and HLA-B
The concordance for HISTO SPOT® test for OL MFI > 5 000 were 84%.

Transfusion efficiency results

	CCI < 7 Non-effective	CCI > 7 Effective
OL + / HISTO SPOT – *(MFI)*	**9** *B35 (4 800)* *A24 (2 800)* *B7 (1 900)* *A2 (1 200)* *A2 (1 100)* *A24 (1 100)*	**7** *B7 (1 900)* *A32 (1 900)* *A19 (1 500)* *A30 (1 300)* *A2 (1 200)* *B50 (800)* *A24 (600)* *B55 (600)*
OL + / HISTO SPOT + *(MFI)*	**8** *B27 (8 000)* *B38 (7 500)* *B44 (7 300)* *A26 (6 800)* *A1 (6 300)* *B50 (5 900)* *A2 (4 200)*	**3** *A1 (6 300)* *A24 (4 500)* *A23 (2 700)*
OL - / HISTO SPOT -	**21**	**11**

Table 3. number of effective and non-effective transfusions according to DSA detection by OL and HISTO SPOT®

	OL	HISTOSPOT
Sensitivity	45%	21%
Specificity	52%	86%
Positive predictive value	63%	72%
Negative predictive value	34%	34%

Table 4. analytical performance of OL and HISTO SPOT®

The sensitivity is lower for HISTOSPOT. However, the specificity and the positive predictive value is better than OL.
The negative predictive value is identical for both methods.

Conclusion

The HISTO SPOT® HLA AB test is an easy method with friendly analysis software. Data of this study showed that this assay is less sensitive than the OL kit but more specific and with a better positive predictive value. However, these data were not sufficient to determine the level of relevance of the HISTO SPOT® HLA AB test compared to OL test on the selection of HLA compatible platelet products. A larger multicenter study should be performed to establish the performance of the HISTO SPOT® HLA AB test in the detection of HLA class I antibodies in platelet transfusion ineffectiveness context.

References

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