

HISTO SPOT AB Kits

Technical Report

Comparison of the HISTO SPOT[®] HLA AB with two other single antigen tests

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Introduction

The introduction of solid-phase immunoassays using recombinant single antigens (SA) for the detection and characterization of human leukocyte antigen (HLA) antibodies in transplantation has resulted in a new paradigm with respect to the interpretation of donor-specific antibodies (DSA). This is due to the fact that the SA tests provide a much higher sensitivity than the complement dependent lymphocytotoxicity (CDC) assays. Although the SA assays have permitted the detection of antibodies not detectable by CDC, the clinical significance of these antibodies is incompletely understood. Nevertheless, the detection of these antibodies has led to changes in the clinical management of sensitized patients. In addition, SA testing raises technical issues that require resolution and careful consideration when interpreting antibody results.

When the first SA assays were established on the Luminex instrument there was no gold standard to compare the results to and still there is no method that is reproducible enough and sufficiently validated to clearly decide which reactions are truly positive. This is illustrated as well by the huge variability in reported HLA antibodies in the different quality assessment schemes (e.g. INSTAND, EPT Eurotransplant), where a consensus of between 85% and 95% of the participating labs defines a correct positive reaction and 25% of discrepancies are accepted according to EFI standards.

In this study, the concordance of the newly developed HISTO SPOT[®] HLA AB test with the two other SA tests established in the market (LABScreen[®] Single Antigen, One Lambda and LIFECODES LSA Kit, Immucor) is analysed.

The HISTO SPOT[®] HLA AB test uses mostly recombinant single antigen proteins that are spotted on the bottom of a microtiter plate well to define HLA antibodies, whereas the other two tests use microsphere beads coated with recombinant single antigens.

The micro ELISA assay for the HISTO SPOT[®] HLA AB test runs fully automated on the MR.SPOT[®] processor.

Material and methods

44 patient sera from the Transfusion Center of the University Medical Center in Mainz, Germany, were tested with the HISTO SPOT[®] HLA AB test, the LABScreen[®] Single Antigen (One Lambda) and the LIFECODES LSA (Immucor) according to the instructions for use.

The HLA antibody detection process in the HISTO SPOT[®] HLA AB test is based on the interaction between the antibodies present in the sample and the antigen immobilized on the microarray. The antibodies specifically bind to their target antigen and are then recognized by an horse radish peroxidase conjugated anti-IgG. The presence of the antigen/antibody/anti-IgG product is detected by a coloured spot formed by tetramethylbenzidine (TMB).

The resulting antibody signals (coloured dots in the bottom of each test well) are photographed by the MR.SPOT[®] processor (Fusion 1.4.3.1) and the image is transferred into the HISTO MATCH interpretation software (Version 3.0.41). The image analysis software determines the mean colour intensity (MCI value) and the background (local background value) of each spot in the array. Based on the ratio of the MCI and the background the positivity of the spots is determined automatically by the software. Values that are very close to the cut off ($\pm 2\%$ = grey area) were regarded as questionable.

The results obtained with, the LABScreen[®] Single Antigen (One Lambda) and the LIFECODES LSA (Immucor) test were provided by the Transfusion Center of the University Medical Center in Mainz (Germany) as Excel files. The cut offs for the Luminex tests were the following:

LABScreen[®] Single Antigen: > 1000 MFI = weak positive, >2000 MFI = positive

LIFECODES LSA:

Class I: BCM > 1500, BCR=3, AD-BCR=4

Class II: BCM > 1500, BCR=4, AD-BCR=5

The concordance of the reported positive specificities (including weak positives) was analysed.

Results were classified as concordant if all three tests are negative (-/-/- = category 1) or all three tests are positive (+/+/+ = category 2). The result was regarded as discordant positive if the HISTO SPOT[®] HLA AB test is positive and the other two tests negative (+/-/- = category 3) and as discordant negative the other way round (-/+/+ = category 4). If the HISTO SPOT[®] HLA AB test was concordant with one of the two tests but not with the other the results was considered unclear (+/+/-, +/-/+, -/+/-, -/-/+ = categories 5, 6, 7 and 8). If the HISTO SPOT[®] HLA AB test result was in the grey area the result was regarded as questionable (category 0).

For DQ and DP the results were matched according to the DQB1/DPB1 specificity. The DQA1/DPA1 the specificities are not always identical for the three tests. However, no obvious DQA1 or DPA1 antibodies were detected in this study.

Results

Comparison of the antigen panels

For the class I tests the antigen panels are different but the total number of antigens is similar and the coverage of allele groups and serological equivalents is comparable (Fig.1).

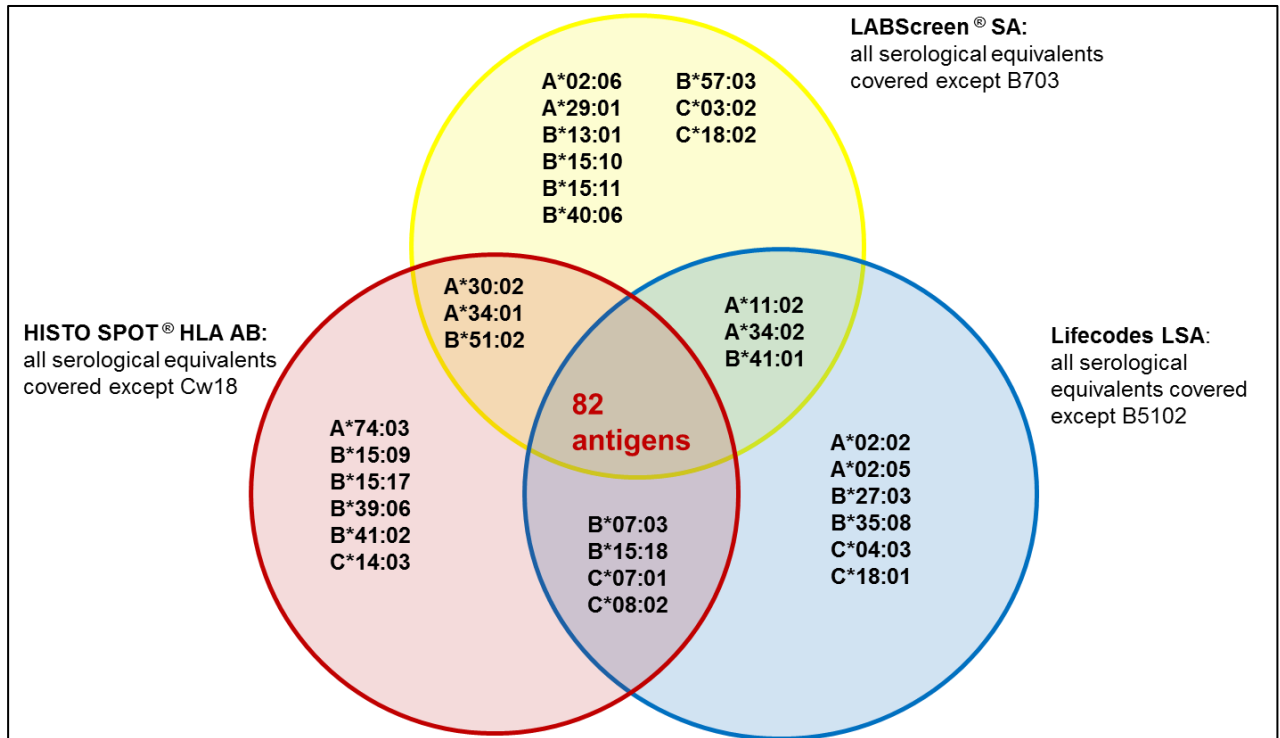


Figure 1: Comparison of antigen panels for class I

For the comparison of the antigen panels for class II the different loci DR, DQ and DP are analysed separately, because for DQ and DP the number of different combinations between the alpha and the beta chain of the protein has to be taken into account, too.

Figure 2 shows that all serological DR equivalents are covered with all three tests. The the HISTO SPOT® HLA AB test and the LIFECODES LSA test have a comparable number of antigens, the LABScreen® Single Antigen test contains some more DRB1 specificities than the other two tests.

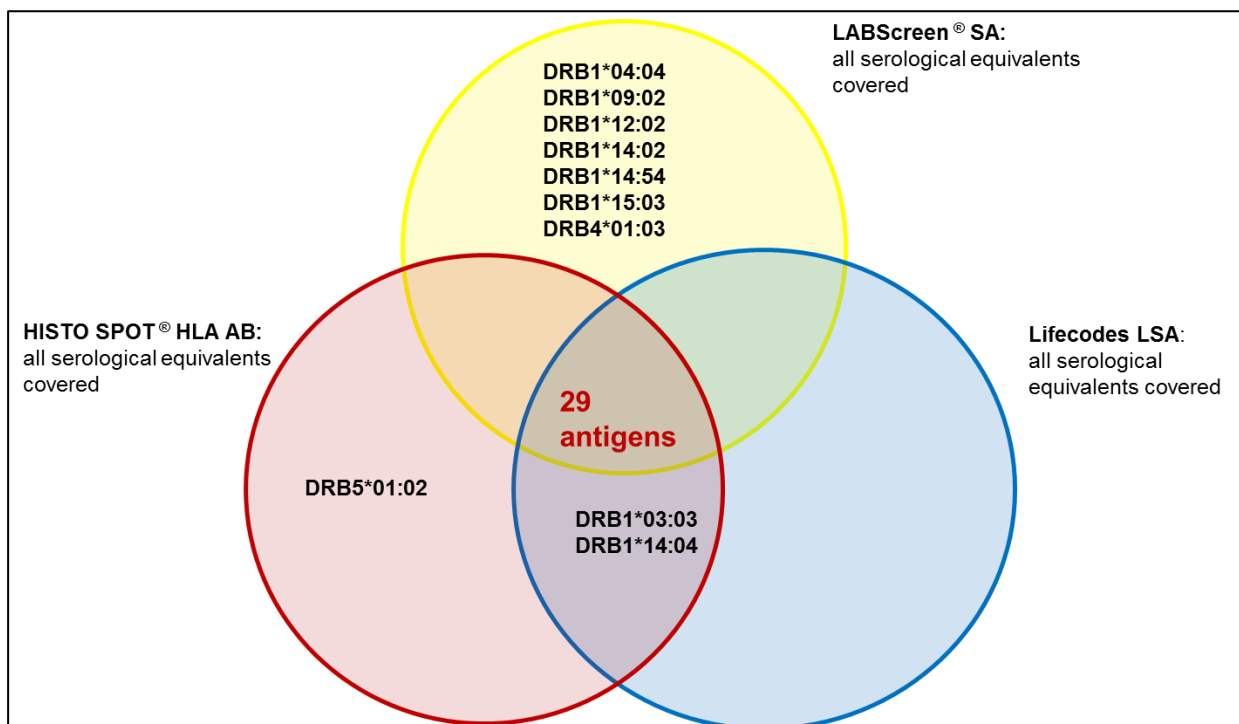


Figure 2: Comparison of antigen panels for the DR locus

For the DQ locus the HISTO SPOT® HLA AB test covers all serological equivalents but all specificities are present only once, e.g. combined with one DQA1 allele. The other two tests have the most common specificities in at least two different DQB1-DQA1 combinations (Fig. 3).

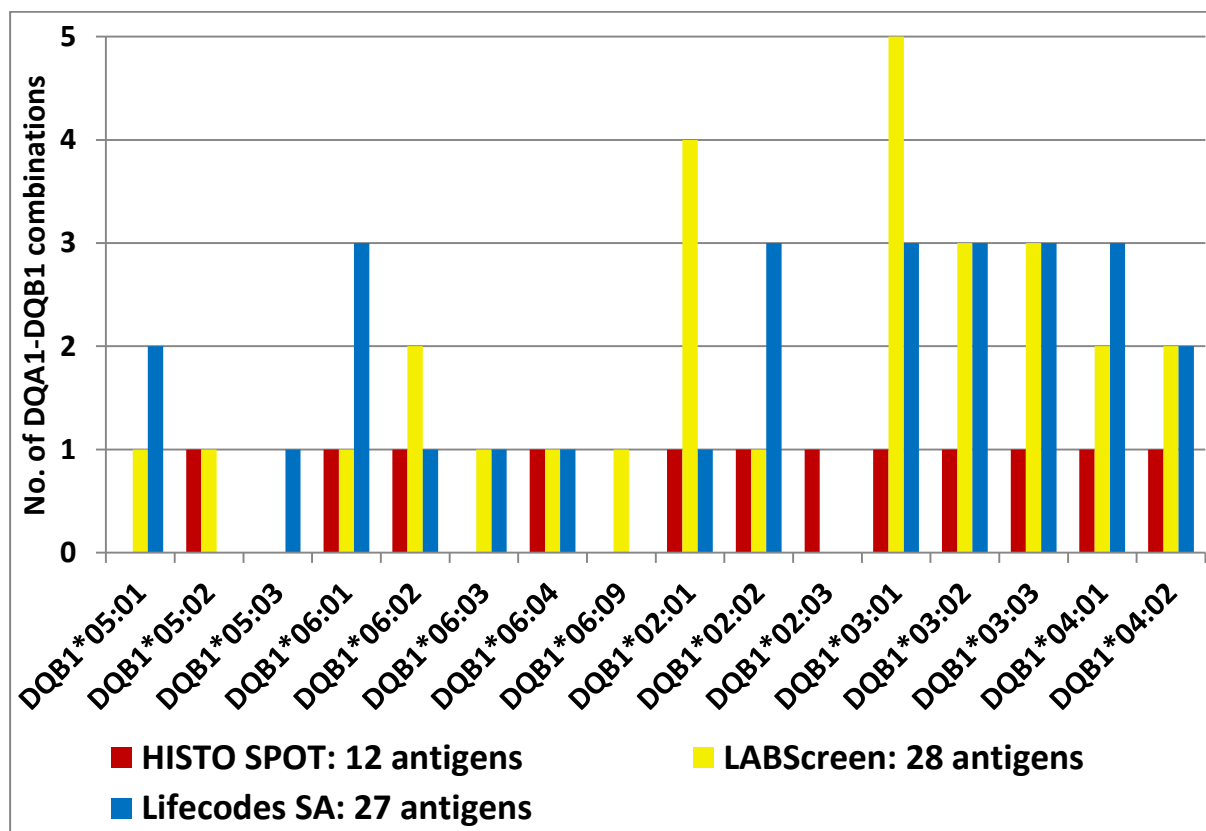


Figure 3: Comparison of antigen panels for the DQB1 locus

The coverage of DQA1 alleles is better for the LABScreen® Single Antigen test and the LIFECODES LSA test than for the HISTO SPOT® HLA AB test (Tab. 1).

Table 1: DQA1* antigens represented in the three SA tests

HISTO SPOT HLA AB	LABScreen SA	Lifecodes LSA
	DQA1*01:01	DQA1*01:01
DQA1*01:02	DQA1*01:02	DQA1*01:02
DQA1*01:03	DQA1*01:03	DQA1*01:03
DQA1*01:04		DQA1*01:04
DQA1*02:01	DQA1*02:01	DQA1*02:01
	DQA1*03:01	DQA1*03:01
	DQA1*03:02	DQA1*03:02
	DQA1*03:03	
DQA1*04:01	DQA1*04:01	DQA1*04:01
	DQA1*05:01	DQA1*05:01
	DQA1*05:03	
	DQA1*05:05	
	DQA1*06:01	DQA1*06:01

The coverage of DPB1 alleles is most complete for the LABScreen® Single Antigen test with 19 alleles. The HISTO SPOT® HLA AB test represents 16 alleles and the LIFECODES LSA test 12 alleles. Like for DQB1/DQA1 there is only one DPB1/DPA1 combination per allele in the HISTO SPOT® HLA AB test, whereas in the other two test there are up to five different combinations per allele (Fig. 4).

Table 2 shows that the number of DPA1 alleles represented in the tests is the same for the HISTO SPOT® HLA AB test and LIFECODES LSA test. The LABScreen® Single Antigen test has two more alleles.

Table 2: DPA1* antigens represented in the three SA tests

HISTO SPOT HLA AB	LABScreen SA	Lifecodes LSA
DPA1*01:03	DPA1*01:03	DPA1*01:03
	DPA1*01:04	
	DPA1*01:05	
DPA1*02:01	DPA1*02:01	DPA1*02:01
DPA1*02:02	DPA1*02:02	DPA1*02:02
DPA1*03:01	DPA1*03:01	DPA1*03:01
DPA1*04:01	DPA1*04:01	DPA1*04:01

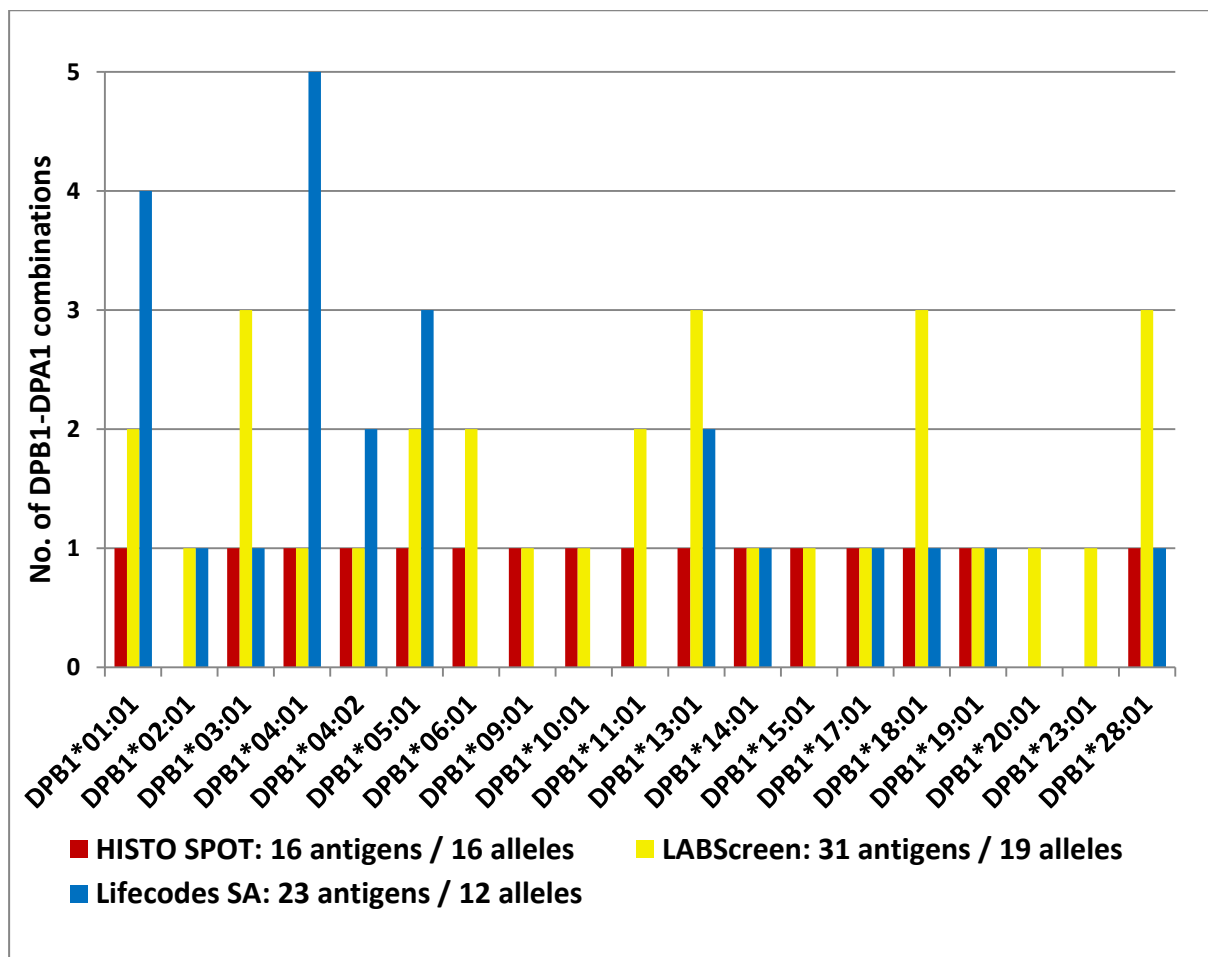


Figure 4: Comparison of antigen panels for the DPB1 locus

Concordance of the results

The table in Annex 1 shows the concordance of the results for class I antibodies for the antigens that were present in all three assays and for all the 44 sera tested. 87% of the results were concordant, 6% were discordant positive or discordant negative (Figure 5). There were more discordant positive (4%) than discordant negative (2%) reactions. Some of the discordant positivity can be attributed to the antigens C*04:01 and C*16:01 which tend to react unspecifically positive. If these two antigens are excluded from the analysis the percentage of discordant positive reactions is reduced to 3%.

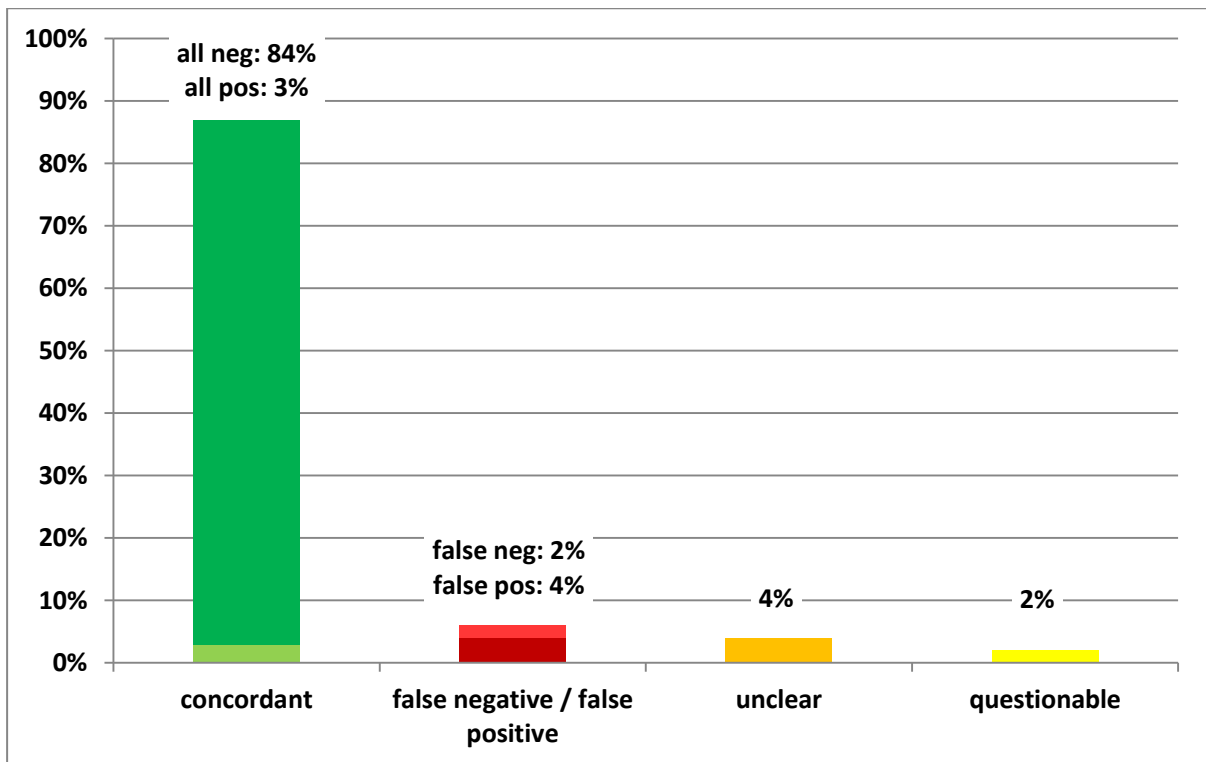


Figure 5: Percentage of concordant and discordant results for class I antibodies

The analysis of discordant positive and discordant negative results from the perspective of the other two tests shows that the percentage of discrepancies is quite similar to the HISTO SPOT® HLA AB test (Table 1).

Table 3: Comparison of the percentage of discordant positive / discordant negative results for the three tests (Class I)

	HISTO SPOT® HLA AB (BAG Health Care)	LABScreen® SA (One Lambda)	LIFECODES LSA (Immucor)
Presumably false positive	3%* (+/-/-)	3% (-/+)	1% (-/-/+)
Presumably false negative	2% (-/+)	0% (+/-/+)	1% (+/+/-)

* C*04:01, C*16:01 excluded

Some of the sera show more problematic results than others, e.g. for the serum in position A6 51 out of 83 antigens give unclear results.

The detailed results for class II are shown in the table in Annex 2. For class II the results are similar to the class I (Figure 2).

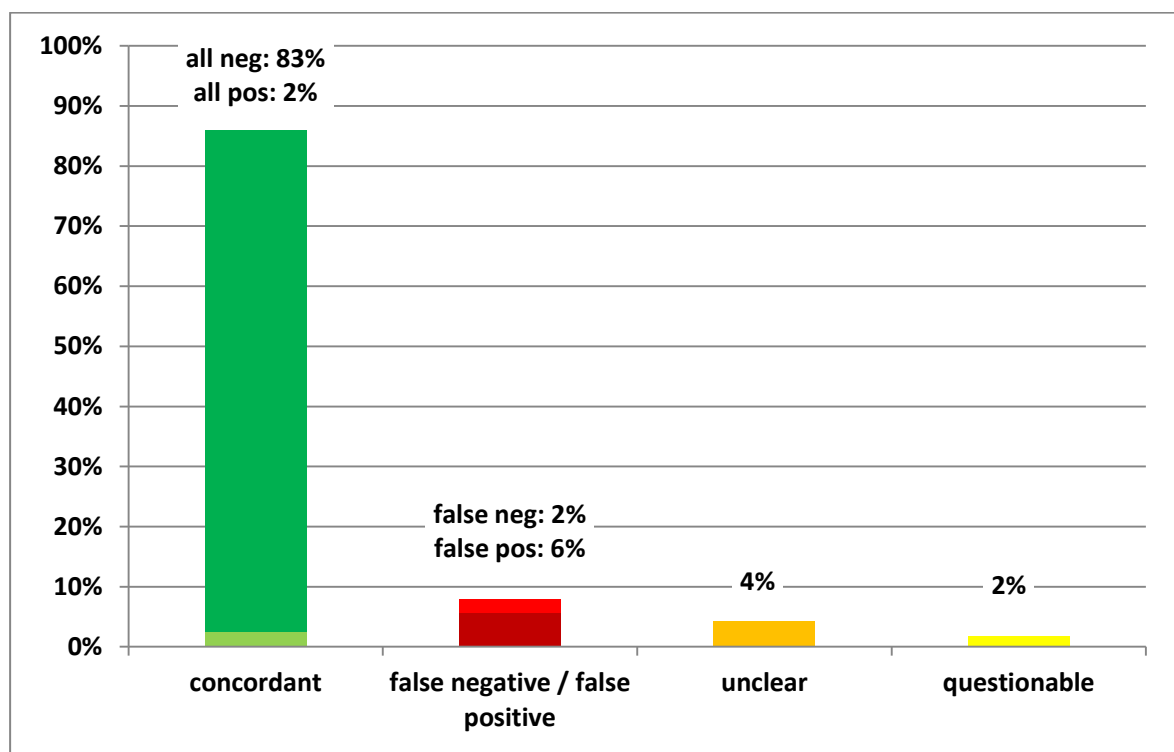


Figure 6: Percentage of concordant and discordant results for class II antibodies

The two native antigens DRB1*08:01 and DRB1*12:01 give an unspecific positive result in this batch and contributed to the discordant positivity for the HISTO SPOT[®] HLA AB class II test. If these two antigens are excluded from the analysis the percentage of discordant positive results goes down to 4%. The comparison of the percentage of discordant positive or discordant negative reactions from the perspective of the two other tests reveals that the concordance between the two Luminex tests is slightly higher than between the HISTO SPOT[®] HLA AB class II test and the Luminex tests.

Table 4: Comparison of the percentage of discordant positive / discordant negative results for the three tests (Class II)

	HISTO SPOT [®] HLA AB (BAG Health Care)	LABScreen [®] SA (One Lambda)	LIFECODES LSA (Immucor)
Presumably false positive	4%* (+/-/-)	2% (-/+)	2% (-/-/+)
Presumably false negative	2% (-/+/+)	0% (+/-/+)	0% (+/+/-)

* DRB1*08:01, DRB1*12:01 excluded

Conclusion:

The coverage of antigens for the HISTO SPOT[®] HLA AB test is equivalent to the two other SA tests for the class I test. The C*18 group should be included in the next batches.

For the class II test the coverage of DRB alleles is the same as for the LIFECODES LSA test, the LABScreen[®] Single Antigen test has some more rare alleles. For the DQ and the DP loci there are not enough different combinations of α - and the β -chain to differentiate DQA1 or DPA1 antibodies from DQB1 or DPB1 antibodies in the HISTO SPOT[®] HLA AB test.

The antigens that are present in all three tests the HISTO SPOT[®] HLA AB test show a high concordance with the two other single antigen tests on the market. Some of the antigens (C*04:01, C*16:01 and DRB1*08:01, DRB1*16:01) show unspecific positive reactions and should be optimized for the next productions.

The overall performance of the HISTO SPOT[®] HLA AB class I test is comparable to the other established single antigen tests. The test is, therefore, suitable to be used in HLA antibody diagnostics.

For the HISTO SPOT[®] HLA AB class II test more antigens have to be included before the test is suitable for routine testing, though the performance of the antigens that are present is satisfying.

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Annex 2: Detailed results class II

	C7	D7	E7	F7	G7	H7	A8	B8	C8	D8	E8	F8	G8	H8	A9	B9	C9	D9	E9	F9	G9	H9	A10	B10	C10	D10	E10	F10	G10	H10	A11	B11	C11	D11	E11	F11	G11	H11	A12	B12	C12	D12	E12	F12								
DRB1*01:01	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1					
DRB1*01:02	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1			
DRB1*01:03	8	1	1	1	1	1	2	1	1	1	1	1	1	1	1	4	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1				
DRB1*03:01	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1					
DRB1*03:02	1	8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1					
DRB1*04:01	1	1	1	1	1	1	4	1	1	1	1	1	1	1	1	1	7	1	1	1	1	1	1	1	1	1	8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1				
DRB1*04:02	8	1	1	1	1	1	4	1	1	1	1	1	1	1	1	4	1	1	1	1	1	1	1	1	1	1	7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1				
DRB1*04:05	1	1	1	1	1	1	4	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1				
DRB1*07:01	1	1	1	3	3	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1				
DRB1*08:01n	8	3	0	1	1	1	2	1	3	3	3	3	3	3	1	4	1	3	1	1	3	1	1	3	1	1	3	3	3	3	3	3	3	3	3	1	1	2	3	3	3	3	3	1	1	3	3	3				
DRB1*09:01	8	1	1	1	1	1	2	1	1	1	4	1	8	1	8	1	2	1	1	1	1	1	1	1	1	1	1	4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1			
DRB1*10:01	1	1	1	1	1	1	4	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1				
DRB1*11:01	0	1	1	1	1	1	2	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1				
DRB1*12:01n	8	3	1	1	3	3	1	0	1	3	3	3	3	3	8	1	1	1	3	1	3	1	1	1	3	1	3	3	1	0	3	1	1	1	4	1	1	0	1	3	1	1	1	1	1	1	1	1	1			
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DRB4*01:01n	1	3	1	1	1	1	4	1	1	1	3	1	3	1	1	8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	7	1	1	1	1	1	1	1	1	1	1	1	1	1		
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DQA1*02:01-DQB1*02:02	1	1	7	1	1	1	1	3	1	1	1	1	1	1	1	1	1	8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	7	1	1	1	1	1	1	1	1	1	1	1	1	1		
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DQA1*02:01-DQB1*04:01	1	1	8	0	1	1	1	1	1	3	1	5	1	1	1	1	1	3	1	1	3	1	1	3	1	1	3	1	1	1	1	1	1	7	3	1	4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
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DQA1*01:03-DQB1*06:01	1	1	1	1	1	1	1	3	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
DQA1*01:01-DQB1*06:02	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
DQA1*01:03-DQB1*06:03	1	1	1	1	1	1	1	3	1	1	2	1	8	1	3	1	1	1	1	1	7	1	1	1	3	1	1	1	1	1	1	1	1	1	8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
DQA1*01:03-DQB1*06:04	1	1	1	3	1	1	1	3	1	1	6	0	8	1	1	1	1	0																																		