HISTO SPOT AB Kits

Technical Report

Influence of serum heat treatment on the performance of the HISTO SPOT® HLA AB test

Ing. Murielle Verboom, Hanover Medical School, Hanover, Germany
Dr. Michael Hallensleben, Hanover Medical School, Hanover, Germany
Dr. Kristin Launhardt, BAG Health Care GmbH, Lich, Germany

Introduction

Different sera treatment methods are commonly used before performing a test to detect HLA antibodies, e.g. centrifugation, filtration, heat or EDTA treatment. In this study the influence of heat inactivation on the results of the HISTO SPOT® HLA AB test has been examined.

The HISTO SPOT® HLA AB test uses 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

22 sera from patients on the kidney transplant waiting list at Hannover Medical School were tested with the HISTO SPOT® HLA AB class I and class II test 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 a horse radish peroxidase conjugated anti-IgG. The presence of the antigen/antibody/anti-IgG product is detected by a coloured spot formed by substrate TMB.

The resulting antibody signals (coloured 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. For interpretation the image analysis software determines the mean colour intensity (MCI-signal) and the background (local background value) of each spot in the array. Based on the ratio of the signal and the background the positivity of the spots is determined automatically by the software. Values that are very close to the cut off (± 2% = grey area) were regarded as questionable. For high precision and reproducibility the HISTO MATCH software provides an alternative algorithm that determines which of the reactions are significantly stronger than the others which helps to exclude potentially unspecific results. In addition, results can be reviewed and edited manually, if necessary, by critical inspection of the images.

For this study the images were also inspected visually and it was determined if there were unspecific background reactions with all or most of the antigens on the chip. All 22 sera were tested untreated and after heat inactivation.

Results

The majority of the heat inactivated sera showed an increased background compared to the untreated sera (Table 1). For the other sera there was no discernible difference between untreated and heat inactivated serum.

Table 1: Comparison of background for untreated and heat inactivated sera

	Class 1	**Class II**
Background higher with heat inactivated sera	16 sera	15 sera
No difference	6 sera	7 sera

Figure 1 shows the class I results for a typical serum showing increased background after heat inactivation. The positive reactions are much clearer for the untreated serum and interpretation is easier:

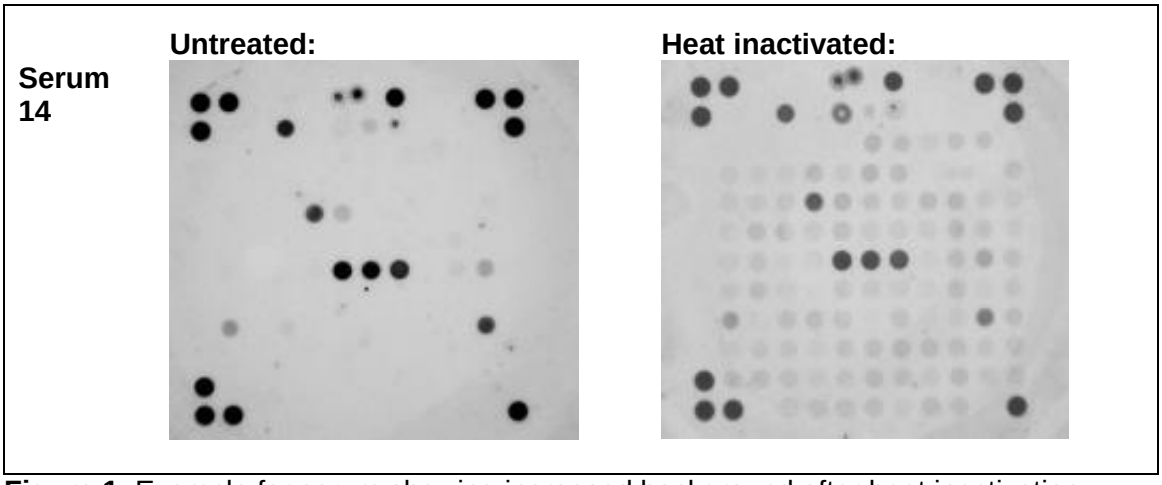


Figure 1: Example for serum showing increased background after heat inactivation

There were differences in the interpreted results as well which are summarized in Table 2. With one exception the differences in the interpretation were related to weak positive reactions.

Table 2: Summary of differences in the interpretation for untreated and heat inactivated sera

Serum	**affected antigens**	**untreated**	**Heat inactivated**	**Remarks**
7	C*08:01, C*15:02	negative	weak positive	with heat inactivation interpretation questionable (Figure 2)
13	C*12:03, C*15:02, C*16:01	negative	weak positive	with heat inactivation interpretation questionable
4	B*53:01	weak positive	negative	
5	B*48:01, B*82:01	weak positive	negative	weak reaction disappears after heat inactivation because background reactions have the same strength (Figure 2)
17	A*80:01	weak positive	negative	
20	B*59:01	weak positive	negative	
19	C*07:01, C*07:02	strong positive	negative	strong reactions untreated

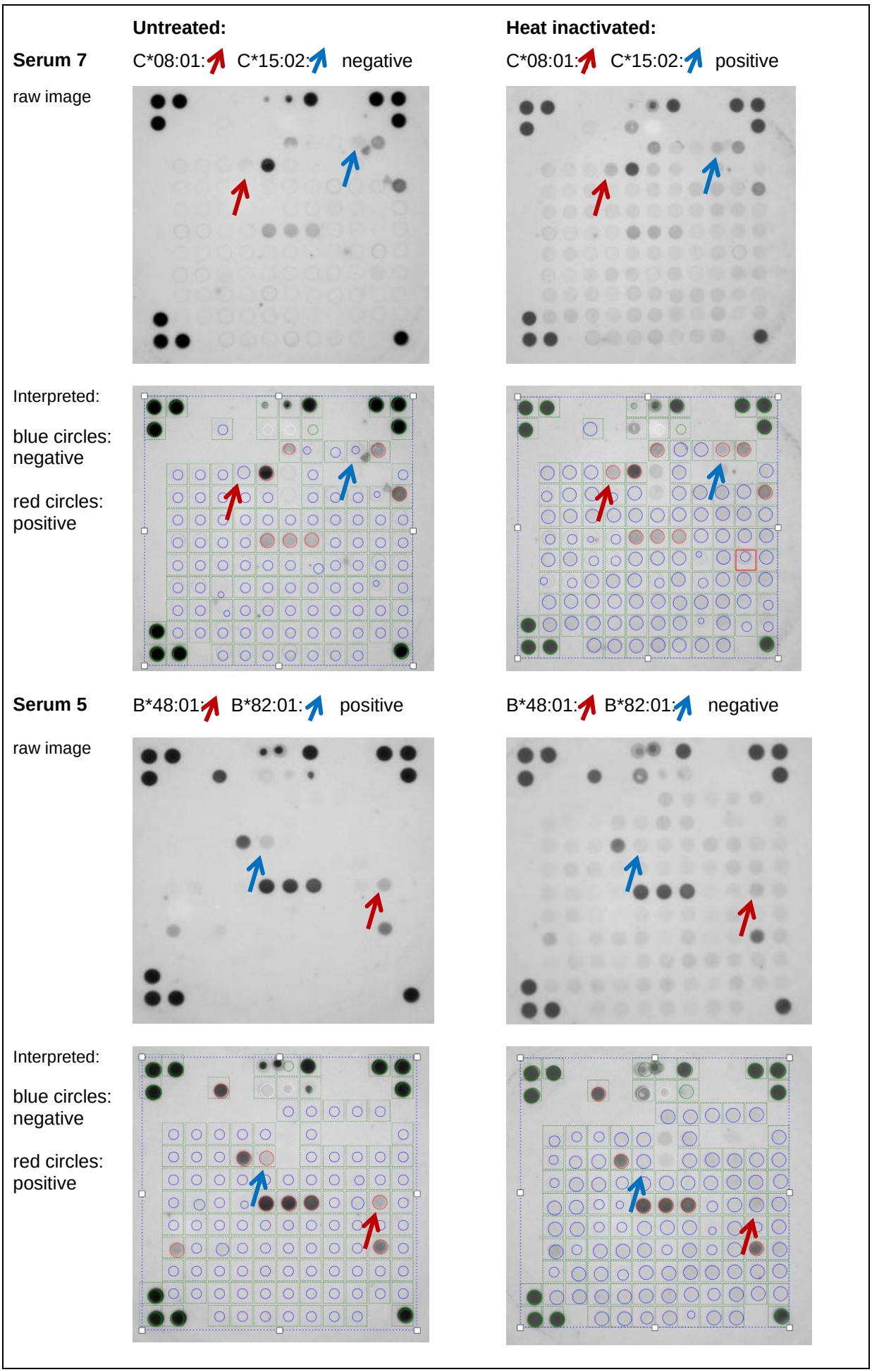


Figure 2: Examples for different interpretations for untreated and heat inactivated sera

In Figure 2 two examples are shown to illustrate the differences that were observed in the interpretation of the results. For serum 7 two antigens were classified as negative with the untreated serum, but assigned as positive by the algorithm for unspecific results in the HISTO MATCH Software because they were slightly stronger than the other unspecific reactions. Considering the overall background obtained with the heat treated serum the result from the untreated serum looks more reliable.

For serum 5 there were two antigens clearly positive (though weak) with the untreated serum that disappeared after heat inactivation because the unspecific reactions with all the negative antigens became equally strong as these positive reactions.

In serum 19 there were strong positive reactions for C*07:01 and C*07:02 which disappeared completely after heat inactivation. Further tests are under evaluation to confirm this finding and rule out any experimental errors.

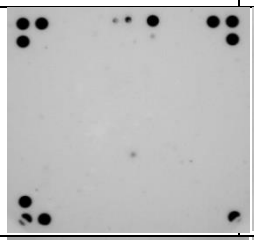
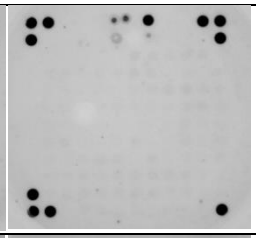
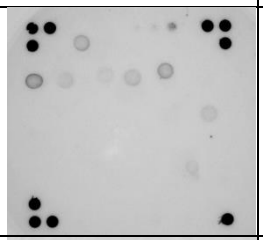
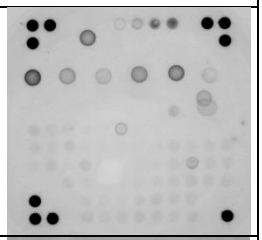
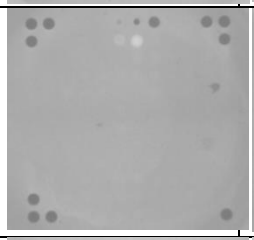
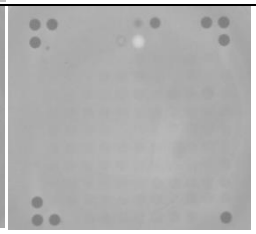
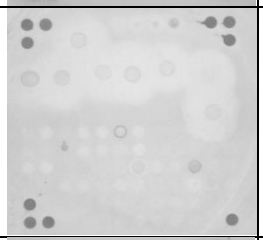
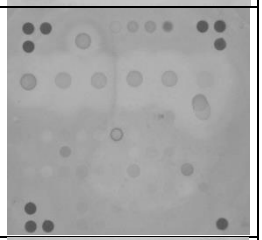
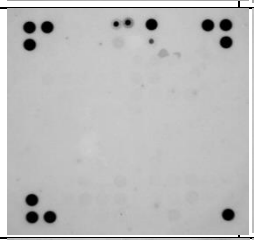
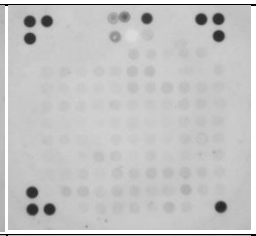
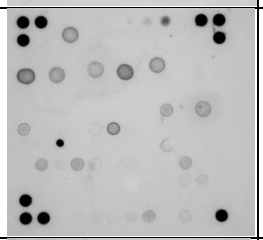
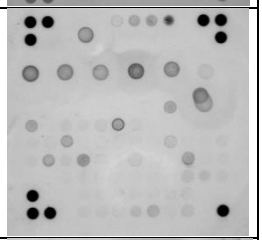
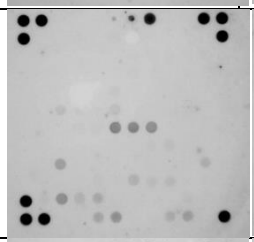
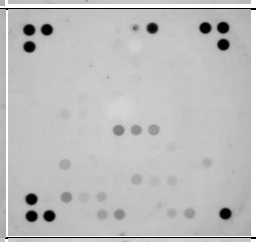
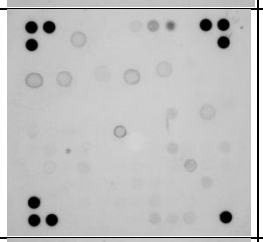
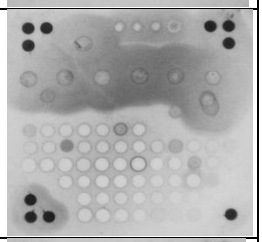
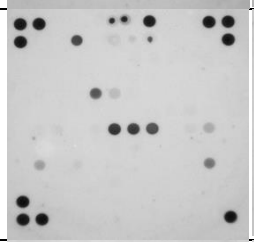
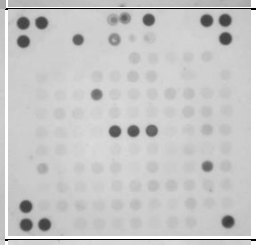
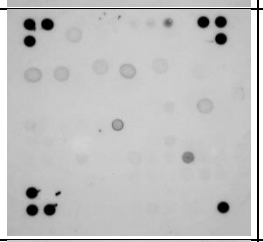
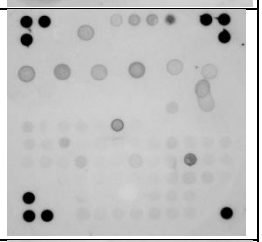
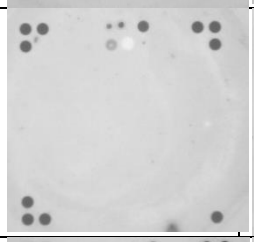
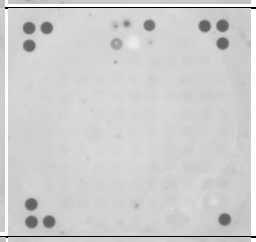
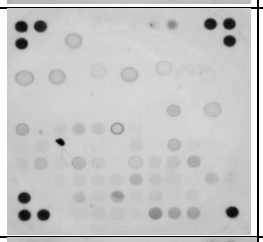
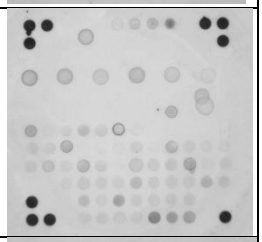
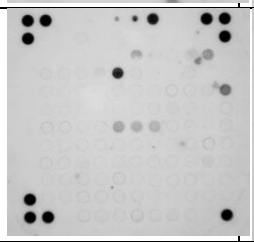
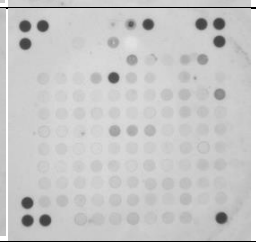
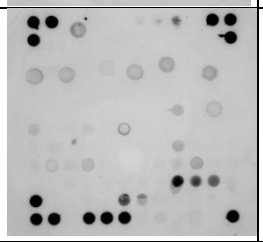
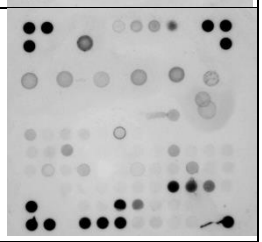
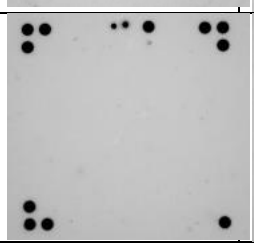
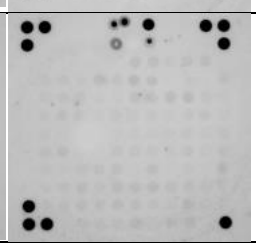
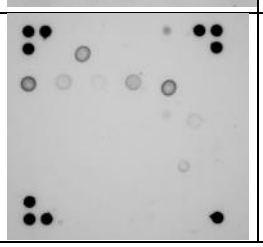
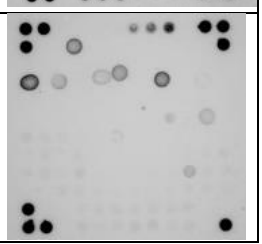
Conclusion:

The results of this study clearly show that heat inactivation of the sera increases unspecific background reactions with the HISTO SPOT HLA AB test and makes interpretation difficult or sometimes impossible. Therefore, sera should not be heat inactivated for this test.

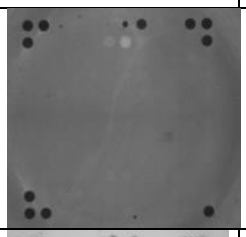
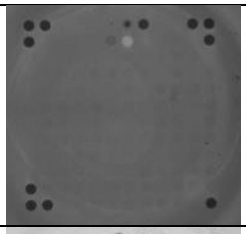
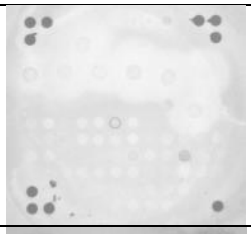
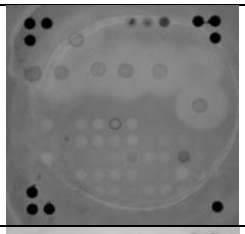
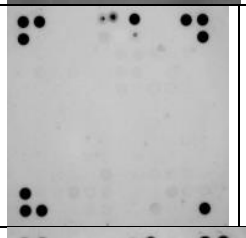
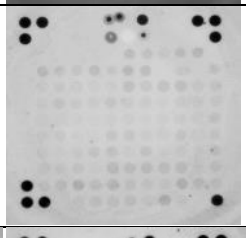
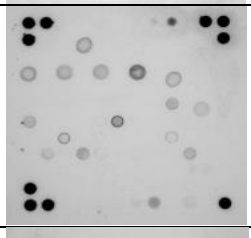
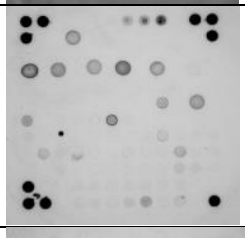
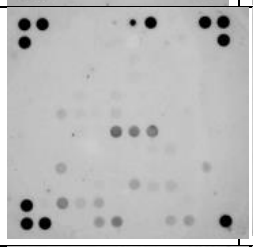
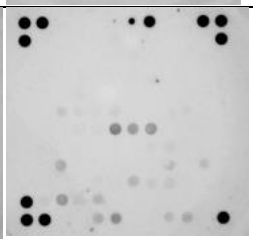
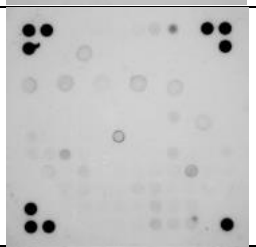
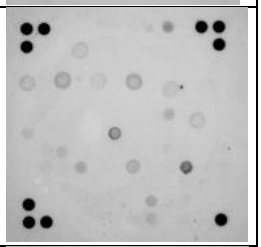
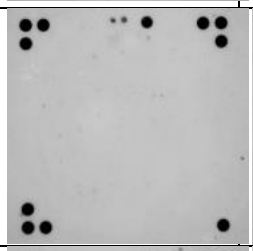
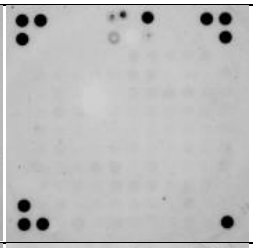
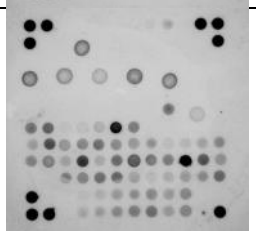
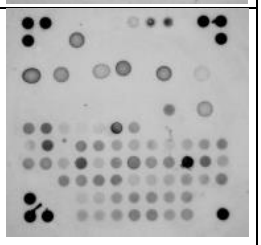
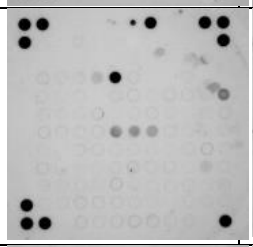
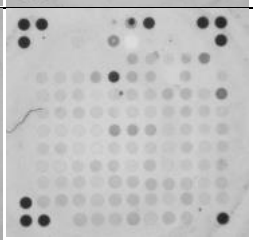
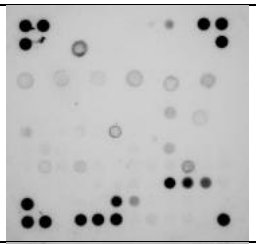
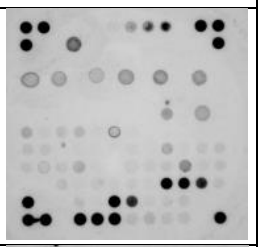
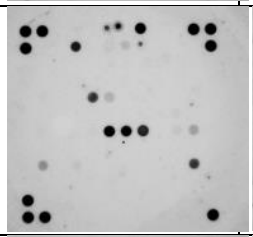
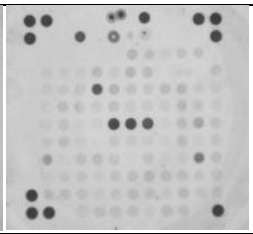
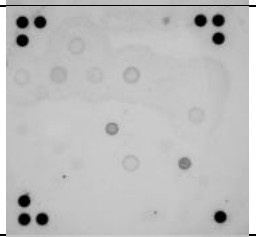
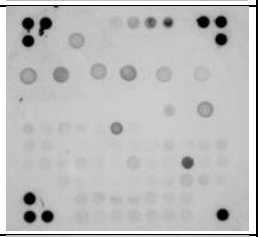
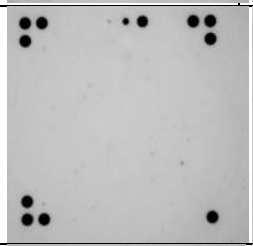
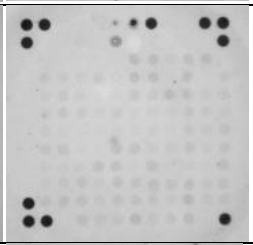
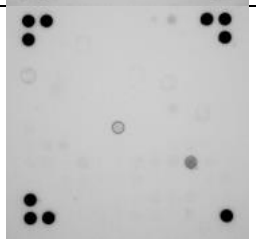
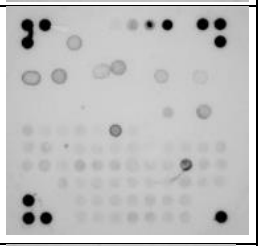
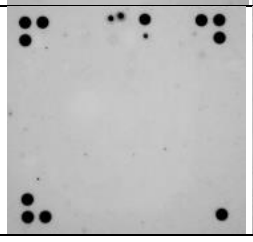
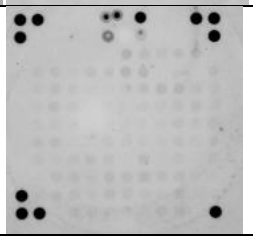
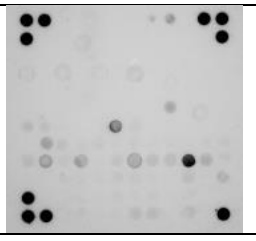
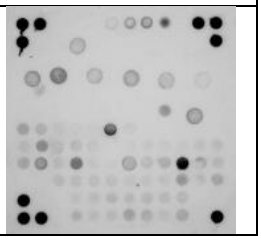
April 2018

BAG Health Care GmbH, Amtsgerichtsstr. 1-5, 35423 Lich / Germany

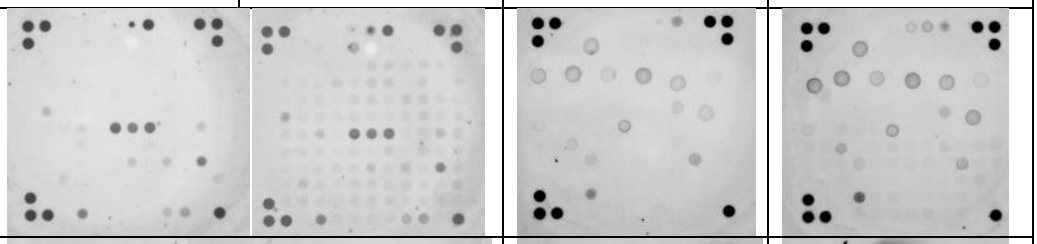
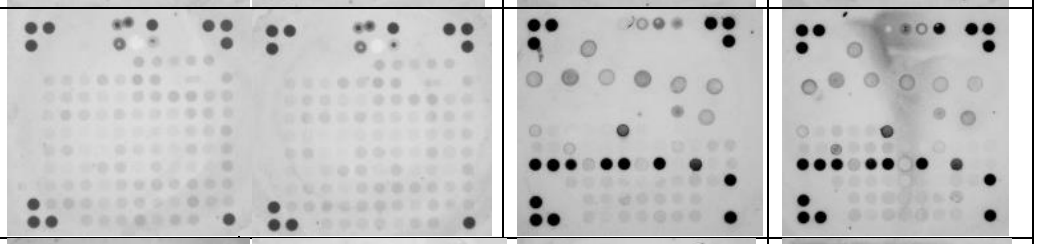
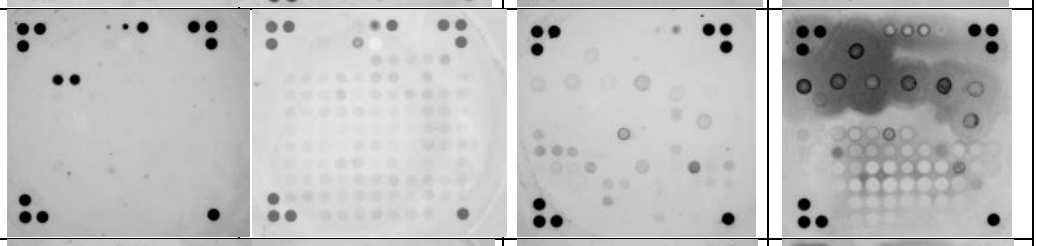
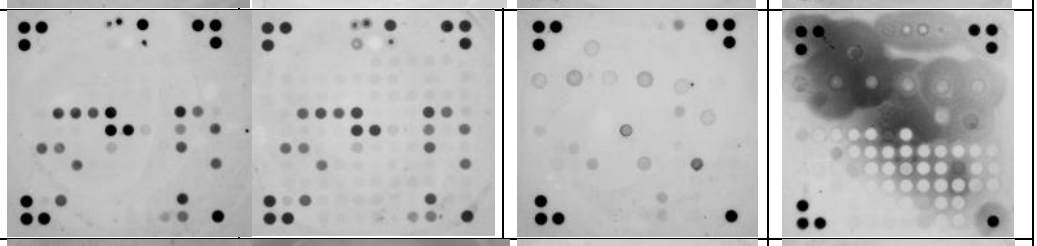
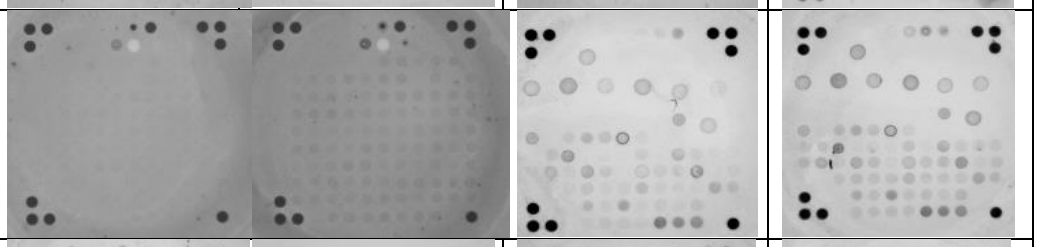
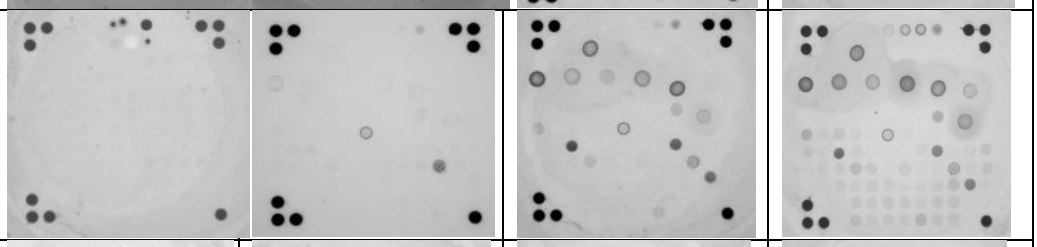
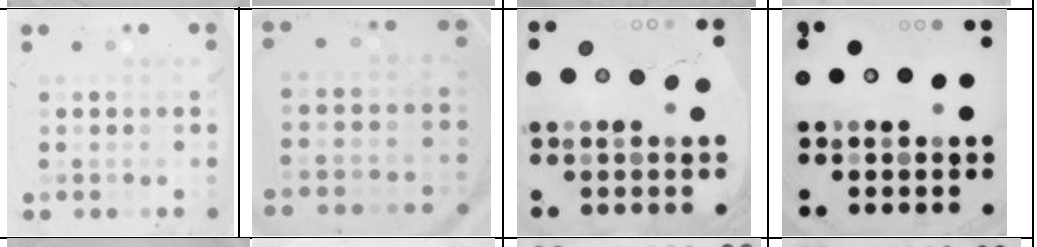
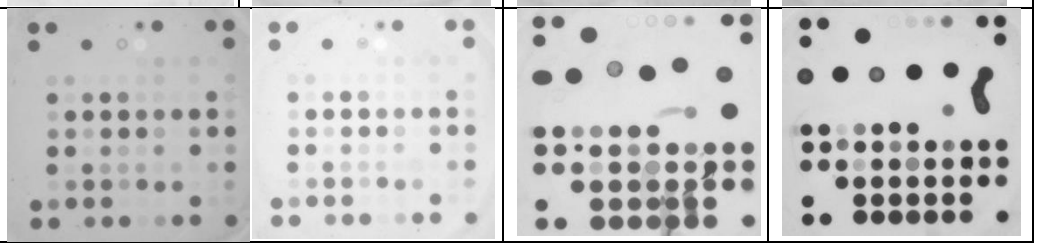
Annex 1: Images obtained with untreated und heat inactivated sera

	Class I		Class II	
Serum	untreated	heat inactivated	untreated	heat inactivated
1				
2				
3				
4				
5				
6				
7				
8				

Annex 1: Images obtained with untreated und heat inactivated sera

	Class I		Class II	
Serum	untreated	heat inactivated	untreated	heat inactivated
9				
10				
11				
12				
13				
14				
15				
16				

Annex 1: Images obtained with untreated und heat inactivated sera

	Class I		Class II	
Serum	untreated	heat inactivated	untreated	heat inactivated
17				
18				
19				
20				
21				
22				
PC				
PC				

Annex 2: Detailed interpreted results with untreated (ut) and heat inactivated (hi) sera

Protein	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi
Serum	**1**		**2**		**3**		**4**		**5**		**6**		**7**		**8**		**9**		**10**		**11**		**12**		**13**		**14**		**15**		**16**		**17**		**18**		**19**		**20**		**21**		**22**	
A*01:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
A*02:01	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A*02:03	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A*03:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A*11:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A*23:01	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-	-	-
A*24:02	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-	-	-
A*24:03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-
A*25:01	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-
A*26:01	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A*26:02	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A*29:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A*30:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A*30:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A*31:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A*32:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-
A*33:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A*33:03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A*34:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A*36:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
A*43:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A*66:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A*66:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A*68:01	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A*68:02	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A*69:01	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A*74:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A*74:03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A*80:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
B*07:02	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B*07:03	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B*08:01	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B*13:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-
B*14:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B*14:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B*15:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
B*15:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B*15:03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
B*15:09	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B*15:12	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
B*15:13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-
B*15:16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-
B*15:17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-
B*15:18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B*18:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B*27:05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-
B*27:08	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B*35:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B*37:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B*38:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-

Annex 2: Detailed interpreted results with untreated (ut) and heat inactivated (hi) sera

Protein	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi
Serum	**1**		**2**		**3**		**4**		**5**		**6**		**7**		**8**		**9**		**10**		**11**		**12**		**13**		**14**		**15**		**16**		**17**		**18**		**19**		**20**		**21**		**22**	
B*39:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B*39:06	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B*40:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B*40:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B*41:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
B*42:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B*44:02	-	-	-	-	-	-	+	+	+	+	-	-	+	+	-	-	-	-	-	-	+	+	-	-	+	+	+	+	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-
B*44:03	-	-	-	-	-	-	+	+	+	+	-	-	+	+	-	-	-	-	-	-	+	+	-	-	+	+	+	+	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-
B*45:01	-	-	-	-	-	-	+	+	+	+	-	-	+	+	-	-	-	-	-	-	+	+	-	-	+	+	+	+	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-
B*46:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B*47:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
B*48:01	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
B*49:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
B*50:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
B*51:01	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
B*51:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
B*52:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
B*53:01	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
B*54:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B*55:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B*56:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B*57:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
B*58:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
B*59:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
B*67:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B*73:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B*78:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B*81:01	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B*82:01	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C*01:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C*02:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C*03:03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C*03:04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C*04:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	?	-
C*05:01	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C*06:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C*07:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
C*07:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
C*08:01	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C*08:02	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C*12:03	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C*14:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C*14:03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C*15:02	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C*16:01	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C*17:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Annex 2: Detailed interpreted results with untreated (ut) and heat inactivated (hi) sera

sera treatment	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi	ut	hi
Serum No.	1		2		3		4		5		6		7		8		9		10		11		12		13		14		15		16		17		18		19		20		21		22					
DPA1*02:01-DPB1*01:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-				
DPA1*01:03-DPB1*03:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-				
DPA1*03:01-DPB1*04:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-				
DPA1*02:02-DPB1*04:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-				
DPA1*02:02-DPB1*05:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-				
DPA1*02:01-DPB1*09:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-				
DPA1*02:01-DPB1*10:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-				
DPA1*02:01-DPB1*11:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-				
DPA1*02:01-DPB1*14:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-				
DPA1*02:02-DPB1*15:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-				
DPA1*01:03-DPB1*17:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-				
DPA1*02:02-DPB1*18:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-				
DPA1*02:01-DPB1*19:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-				
DPA1*02:02-DPB1*28:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-				
DQA1*02:01-DQB1*02:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	+	+	-		-	-	-	-	-	-				
DQA1*02:01-DQB1*02:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	+	+	-		-	-	-	-	-	-				
DQA1*02:01-DQB1*02:03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	+	+	-		-	-	-	-	-	-				
DQA1*02:01-DQB1*03:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	+	+	-		-	-	-	-	-	-				
DQA1*02:01-DQB1*03:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	+	+	-		-	-	-	-	-	-				
DQA1*02:01-DQB1*03:03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	+	+	-		-	-	-	-	-	-				
DQA1*02:01-DQB1*04:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	+	+	-		-	-	-	-	-	-				
DQA1*04:01-DQB1*03:03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	-		?	-	-	-	-	-				
DQA1*04:01-DQB1*04:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-				
DQA1*01:04-DQB1*05:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	+	+	+		-	-	-	-	-	-				
DQA1*01:03-DQB1*06:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-				
DQA1*01:01-DQB1*06:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	+		-	-	-	-	-	-				
DQA1*01:02-DQB1*06:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	+		-	-	-	-	-	-				
DQA1*01:03-DQB1*06:03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	+		-	-	-	-	-	-				
DRB1*01:01	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			+	+	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-				
DRB1*01:02	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			+	+	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-				
DRB1*01:03	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			+	+	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-				
DRB1*03:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-				
DRB1*03:02-DRB1*08:02-DRB3*01:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-				
DRB1*04:01	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	?	-	-	-			-	-	-	-	-	-	-	-	-	-	-		-	-	+	+	-	-				
DRB1*04:02	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	-		-	-	+	+	-	-				
DRB1*04:05	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	-		-	-	+	+	-	-				
DRB1*07:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	+	+	-	-	-		-	-	-	-	-	-				
DRB1*08:01-DRB1*08:03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-				
DRB1*08:04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	+		-	-	-	-	-	-				
DRB1*09:01	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			+	+	-	-	-	-	-	-	-	-	-		-	-	+	+	-	-				
DRB1*10:01	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			+	+	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-				
DRB1*11:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-				
DRB1*11:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-				
DRB1*11:03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-				
DRB1*12:01-DRB3*02:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-				
DRB1*13:01-DRB3*02:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-				
DRB1*13:03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-				
DRB1*14:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-				
DRB1*14:04-DRB3*02:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-				
DRB1*15:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	+		-	-	-	-	-	-				
DRB1*15:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	+		-	-	-	-	-	-				
DRB1*16:01-DRB1*16:02-DRB5*02:02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-				
DRB3*01:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-				
DRB3*03:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-				
DRB3*02:02-DRB1*01:01-DRB1*11:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-				
DRB4*01:01-DRB1*01:01-DRB1*04:01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-				
DRB5*01:01	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			+	+	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-				
DRB5*01:02	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			+	+	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-				

unspecific - uninterpretable

unspecific - uninterpretable

unspecific - uninterpretable

unspecific - uninterpretable

black smear - uninterpretable