# The HISTO SPOT AB assay does not detect antibodies to denatured HLA antigens in patient serum

**J.Smith et al., 2018, BSHI, prize for the best abstract presentation un the UK meeting**

- **Heart Science Centre at Harefield Hospital: exclusively cardiothoracic transplantation**
- **all transplants proceed based on the virtual crossmatch**
- **4000 samples per year using the Luminex based assays**
- **extremely sensitive (probably too sensitive)**
- **Many of the beads have a proportion of the protein on the bead in a denatured format which causes issues as they often have common antibody profiles.**
- **The assays are prone to issues with 'over reactive' beads.**
- **Number of repeat or additional tests with other kits are often required to determine that the reactivity is irrelevant.**

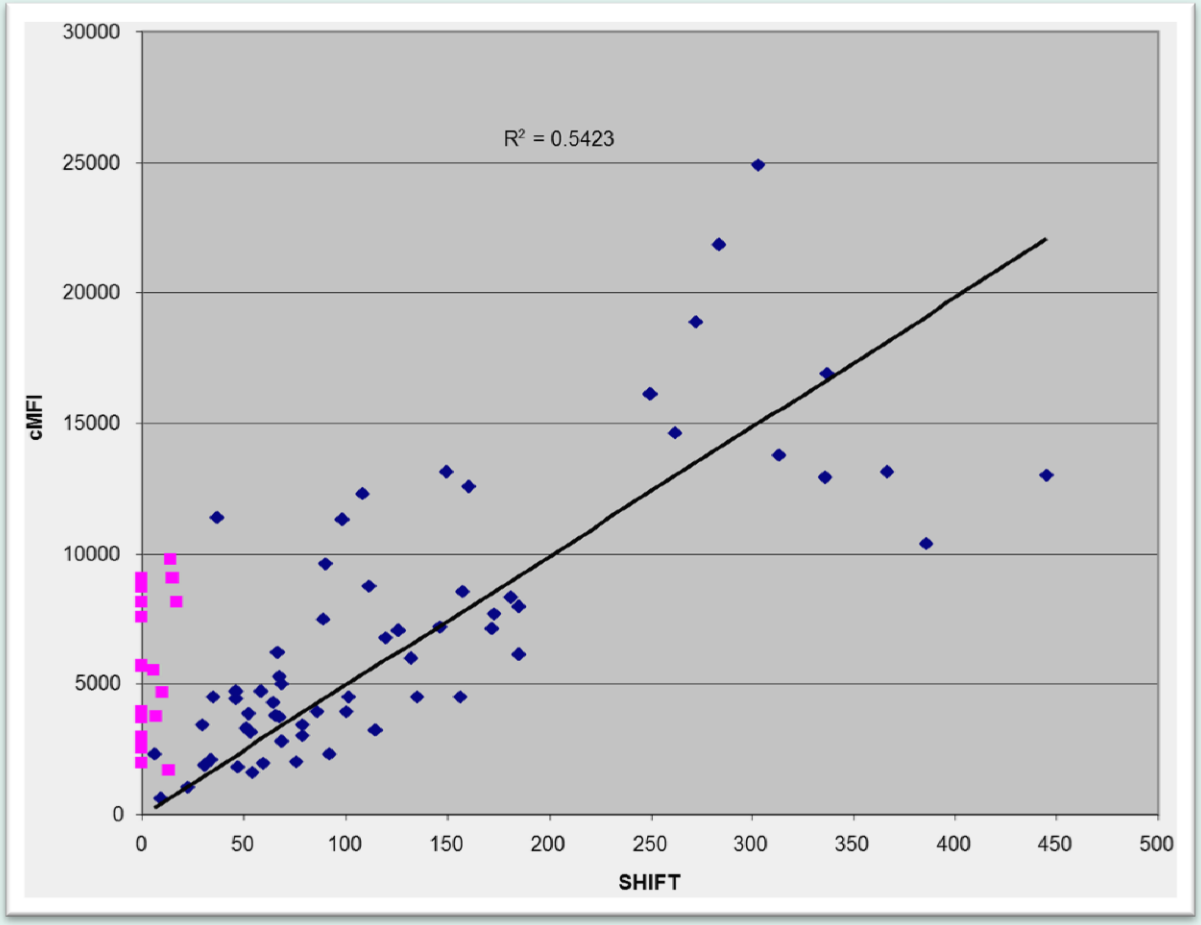
**An alternative to Luminex assays is desperately needed.**

# Denatured proteins

**Some of the proteins on the Labscreen (and probably Immucor) Luminex single antigen beads are in a denatured format (dnHLA) i.e not associated with β2-microglobulin.**

**They have previously shown that antibodies to dnHLA do not cause a positive flow crossmatch and if not confirmed can lead to patients being denied access to transplant.**

# Abs against denatured antigens do not cause a positive flow crossmatch

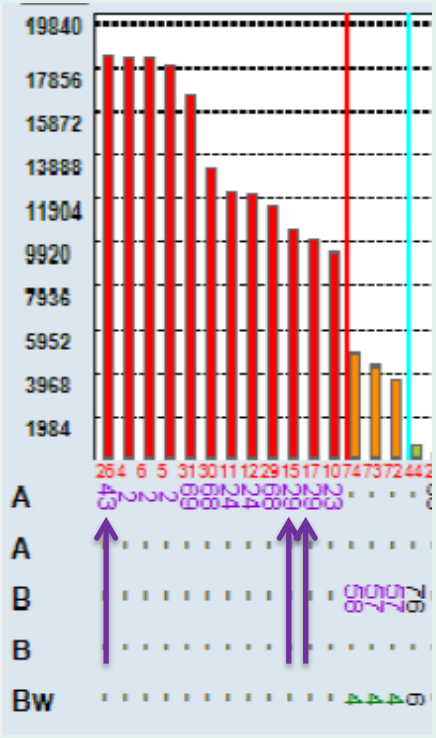



**Blue:**
**reactions with intact HLA antigens**

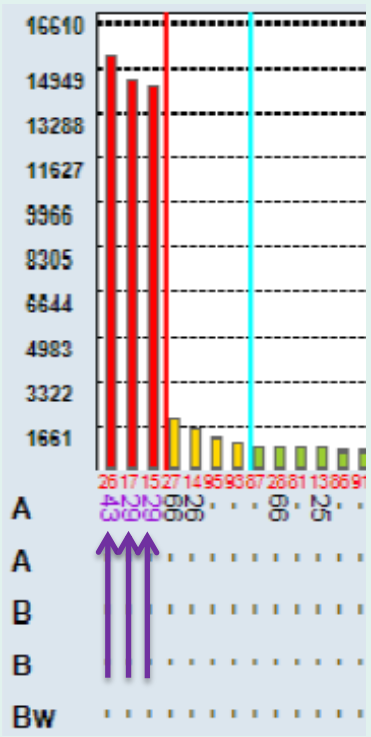
**Pink:**
**reactions with denatured antigens**

# Method to detect denatured antigens on the beads

**Normal IgG Assay**




**Denature beads with acid treatment before the assay (0.3M Glycine-HCL pH2.7 for 30 mins)**




- **Reactivity against denatured antigens will remain or increase**
- **Reactivity against intact antigens will be absent**

**In this case:**

- **A43 and A29 are detected by ABS against denatured antigens and are not clinically relevant**
- **Both have MFIs > 10.000**

# 14 patient sera known to contain antibodies to denatured HLA antigens were selected for study

## Previously determined with acid treated beads in the LABScreen assay

| Specificity of antibodies to denatured antigens | IgG |
|---|---|
| A1 | A1 |
| A1,25,26,33,34,66,68,69,80 | A11,A26,A33,A66,A69 WEAK A68 |
| A11,25,26,66,43, B8,65,59,64 | A11,A25,A26,A66,A43, B8,B65,B59,B64 |
| A25,26,33,34,66,68,69,80 | A25,A26,A33,A34,A66,A68,A69,A80 |
| A25,26,34,43,66 | A25,A26,A34,A43,A66 |
| A29,43 | A2,A23,A24,A29,A43,A68,A69, B57,58 |
| B7,37,54,55,56,42,67,81,82 | B7,37,54,55,56,42,67,81,82 |
| B7,42,54,55,56,67,75,81,82 | A*11:02,A23,A*34:01,A66 B7,42,54,55,57,58,67,75,81,82 |
| B7,42,54,55,56,67,81,82 | B7,42,54,55,67,81,82 WEAK B56 |
| Cw1,12,15 | A43 Cw1,12,15 |
| Cw1,12,15 | B7,13, Cw1,12,15 |
| Cw1,12,15 | Cw1,12,15, |
| Cw1,5,12,15 | Cw1,5,12,15 |
| Cw7 | Cw7 |

# 14 patient sera known to contain antibodies to denatured HLA antigens were selected for study

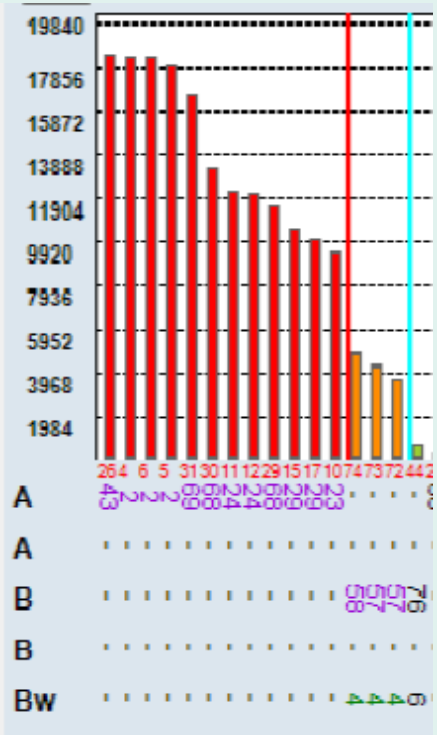
**Previously determined with acid treated beads in the LABScreen assay**

| Specificity of antibodies to denatured antigens | IgG |
|---|---|
| A1 | A1 |
| A1,2 | A11,A26,A33,A66,A69 WEAK A68 |
| A | A11,A25,A26,A66,A43, B8,B65,B59,B64 |
| | A25,A26,A33,A34,A66,A68,A69,A80 |
| | A25,A26,A34,A43,A66 |
| | A2,A23,A24,A29,A43,A68,A69, B57,58 |
| | B7,37,54,55,56,42,67,81,82 |
| L | A*11:02,A23,A*34:01,A66 B7,42,54,55,57,58,67,75,81,82 |
| B7, | B7,42,54,55,67,81,82 WEAK B56 |
| Cw1,12 | A43 Cw1,12,15 |
| Cw1,12,15 | B7,13, Cw1,12,15 |
| Cw1,12,15 | Cw1,12,15, |
| Cw1,5,12,15 | Cw1,5,12,15 |
| Cw7 | Cw7 |

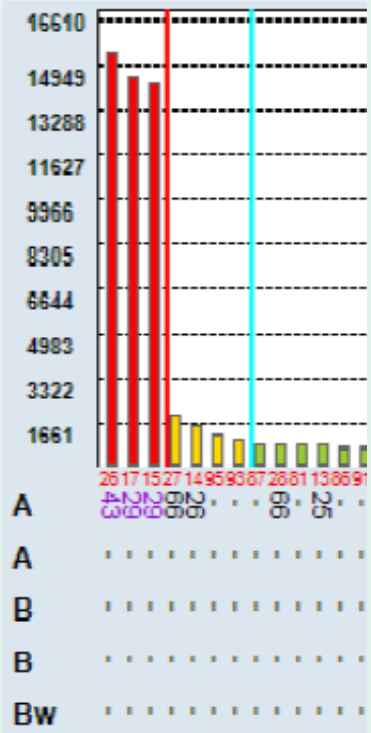
Only specificities in **black** are clinically relevant – the others are directed against denatured antigens

# Same serum as shown before tested with the HISTO SPOT® HLA AB test

## Normal IgG assay




## Denatured




## MR.SPOT ®

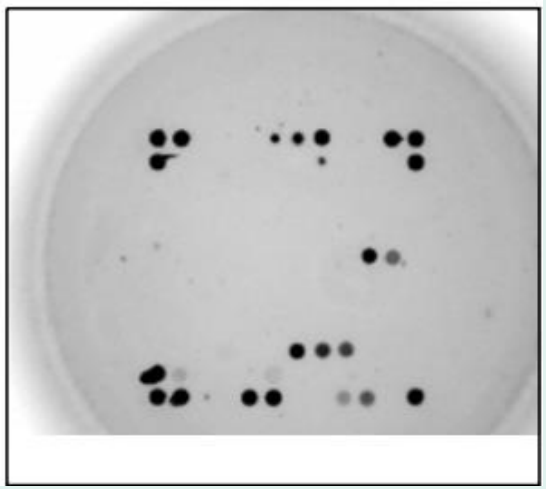
**Summary:** A2;A203;A23;A24;A68;A69;B57;B58

PRA 20.43 Algorithm Normal

Specificity A2;A203;A23;A24;A68;A69;B57;B58

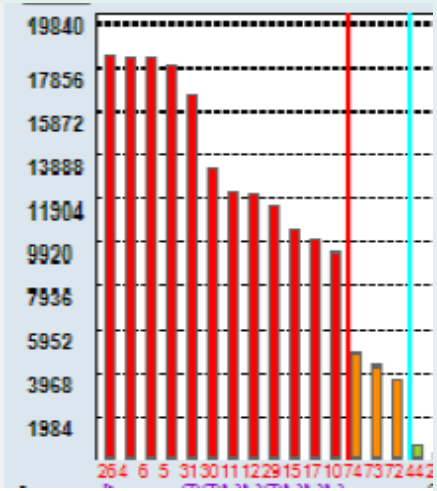
Excluded

| Name | Creg | Signal | Normalized Signal | CutOff | Value | Signal |
|---|---|---|---|---|---|---|
| A*68:01 | | 182.70 | 4.11 | 1.32 | + | |
| A*02:01 | 2C | 189.72 | 4.00 | 1.32 | + | |
| B*57:01 | | 179.01 | 3.96 | 1.32 | + | |
| A*02:03 | | 187.88 | 3.90 | 1.32 | + | |
| A*68:02 | 10C,2C | 141.17 | 3.40 | 1.32 | + | |
| A*69:01 | 10C,2C | 116.77 | 2.94 | 1.32 | + | |
| A*24:02 | 1C,2C,Bw4 | 95.11 | 2.49 | 1.32 | + | |
| B*58:01 | | 89.66 | 2.48 | 1.32 | + | |
| A*23:01 | | 46.39 | 1.75 | 1.32 | + | |
| A*24:03 | | 22.39 | 1.35 | 1.32 | ? | |
| A*29:02 | 1C | 13.25 | 1.23 | 1.32 | - | |



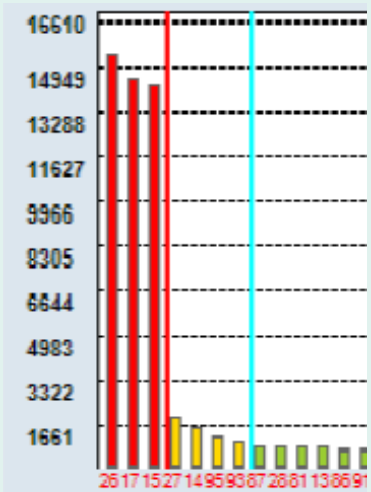
# Same serum as shown before tested with the HISTO SPOT® HLA AB test

## Normal IgG assay




A2, A23, A24, A29, A43, A68, A69
B57, B58

## Denatured



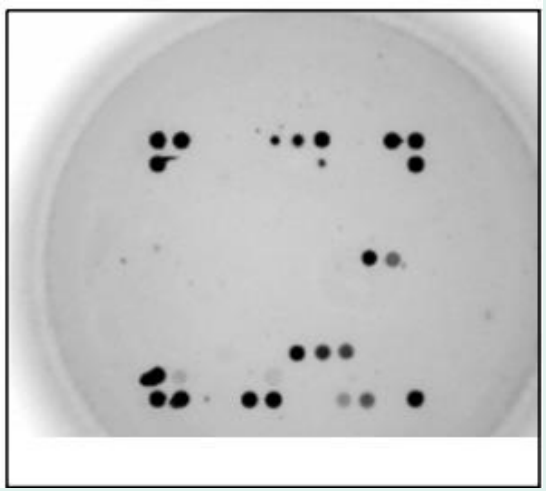

A29, A43

## MR.SPOT ®

A2, A23, A24, A68, A69
B57, B58

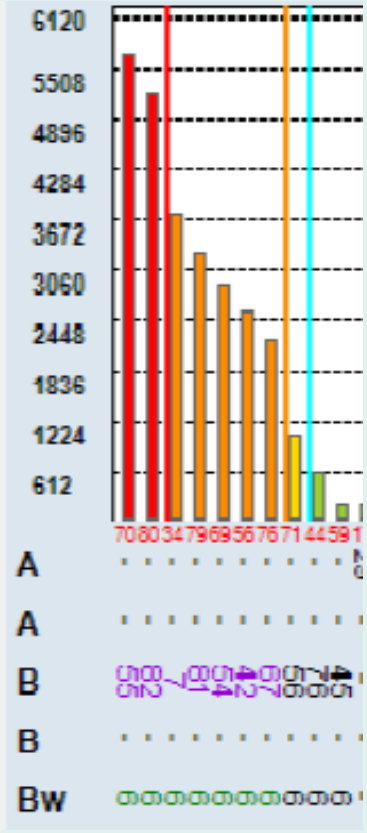
Not the denatured specificities!

| | | | | | |
|---|---|---|---|---|---|
| B*58:01 | | 89.66 | 2.48 | 1.32 | + |
| A*23:01 | | 46.39 | 1.75 | 1.32 | + |
| A*24:03 | | 22.39 | 1.35 | 1.32 | ? |
| A*29:02 | 1C | 13.25 | 1.23 | 1.32 | - |

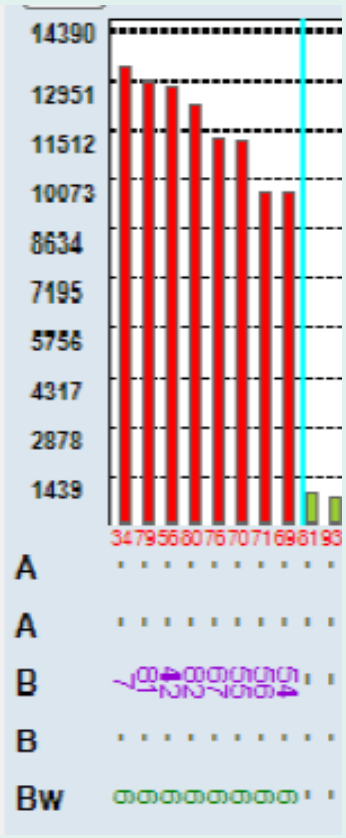


# Second example

## Normal IgG assay

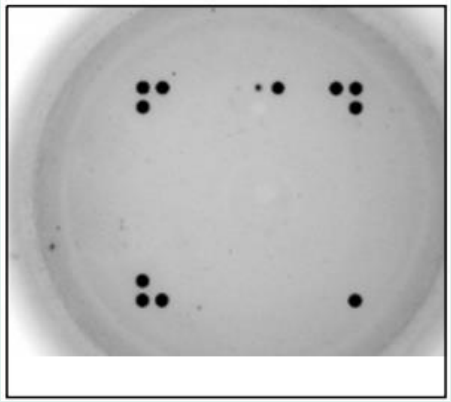



## Denatured



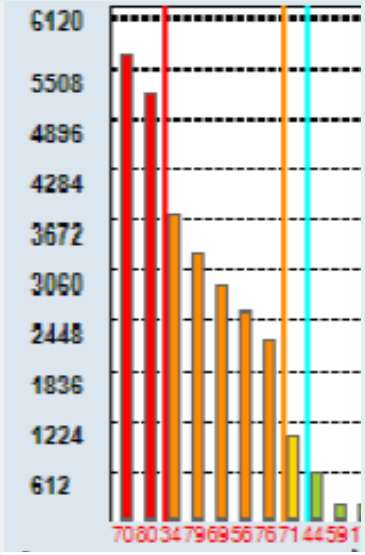

## MR.SPOT ®

| Name | Creg | Signal | Normalized Signal | CutOff | Value Signal |
|---|---|---|---|---|---|
| A*01:01 | | 21.57 | 1.32 | 1.31 | ? |
| B*27:05 | | 9.82 | 1.16 | 1.31 | - |
| B*59:01 | | 11.30 | 1.16 | 1.31 | - |
| B*50:01 | | 8.74 | 1.15 | 1.31 | - |
| B*14:01 | | 8.42 | 1.14 | 1.31 | - |
| B*45:01 | | 8.45 | 1.14 | 1.31 | - |
| C*08:02 | | 7.89 | 1.13 | 1.31 | - |
| B*56:01 | | 8.02 | 1.13 | 1.31 | - |
| C*06:02 | | 7.37 | 1.13 | 1.31 | - |
| C*07:02 | | 7.27 | 1.13 | 1.31 | - |
| B*51:01 | | 7.25 | 1.13 | 1.31 | - |
| A*30:02 | 1C | 8.33 | 1.13 | 1.31 | - |
| C*05:01 | | 8.84 | 1.13 | 1.31 | - |
| B*40:02 | 7C,12C,Bw6 | 7.15 | 1.12 | 1.31 | - |
| C*02:02 | | 7.31 | 1.12 | 1.31 | - |
| B*54:01 | 7C,Bw6 | 7.24 | 1.12 | 1.31 | - |
| B*39:01 | | 8.12 | 1.12 | 1.31 | - |
| C*04:01 | | 7.98 | 1.12 | 1.31 | - |
| B*53:01 | 5C,Bw4 | 7.02 | 1.12 | 1.31 | - |
| B*55:01 | 7C,Bw6 | 7.04 | 1.12 | 1.31 | - |
| B*44:02 | 12C,Bw4 | 6.98 | 1.12 | 1.31 | - |
| A*26:02 | 10C | 7.51 | 1.12 | 1.31 | - |
| B*58:01 | 2C,5C,Bw4 | 7.83 | 1.12 | 1.31 | - |
| C*15:02 | | 7.59 | 1.12 | 1.31 | - |
| C*07:01 | | 6.65 | 1.12 | 1.31 | - |
| A*26:01 | 10C | 7.55 | 1.12 | 1.31 | - |
| A*74:01 | 10C | 7.96 | 1.12 | 1.31 | - |
| B*81:01 | 7C,Bw6 | 6.68 | 1.12 | 1.31 | - |
| B*39:06 | 8C,Bw6 | 8.21 | 1.12 | 1.31 | - |
| A*11:01 | 1C,10C | 8.00 | 1.12 | 1.31 | - |

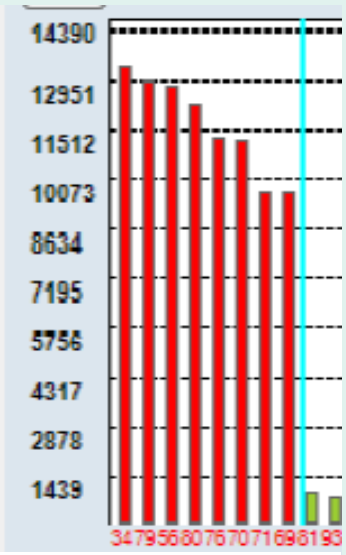


# Second example

## Normal IgG assay



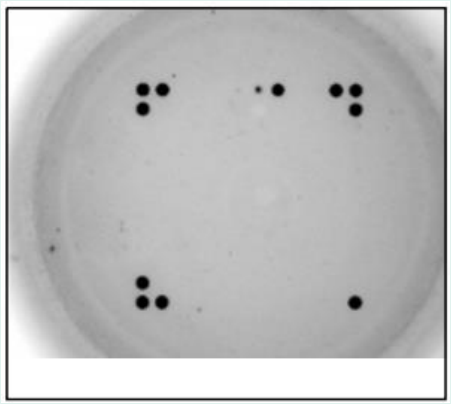

## Denatured




## MR.SPOT ®

**Negative.**

| Name | Creg | Signal | Normalized | CutOff | Value Signal |
|---|---|---|---|---|---|
| C*04:01 | | 7.98 | 1.12 | 1.31 | - |
| B*53:01 | 5C,Bw4 | 7.02 | 1.12 | 1.31 | - |
| B*55:01 | 7C,Bw6 | 7.04 | 1.12 | 1.31 | - |
| B*44:02 | 12C,Bw4 | 6.98 | 1.12 | 1.31 | - |
| A*26:02 | 10C | 7.51 | 1.12 | 1.31 | - |
| B*58:01 | 2C,5C,Bw4 | 7.83 | 1.12 | 1.31 | - |
| C*15:02 | | 7.59 | 1.12 | 1.31 | - |
| C*07:01 | | 6.65 | 1.12 | 1.31 | - |
| A*26:01 | 10C | 7.55 | 1.12 | 1.31 | - |
| A*74:01 | 10C | 7.96 | 1.12 | 1.31 | - |
| B*81:01 | 7C,Bw6 | 6.68 | 1.12 | 1.31 | - |
| B*39:06 | 8C,Bw6 | 8.21 | 1.12 | 1.31 | - |
| A*11:01 | 1C,10C | 8.00 | 1.12 | 1.31 | - |



**All specificities found are against denatured antigens!**

# Comparison for all 14 sera

| Specificity of antibodies to denatured antigens | MR SPOT finding | IgG |
|---|---|---|
| A1 | Negative | A1 |
| A1,25,26,33,34,66,68,69,80 | Negative | A11,A26,A33,A66,A69 WEAK A68 |
| A11,25,26,66,43, B8,65,59,64 | Negative | A11,A25,A26,A66,A43, B8,B65,B59,B64 |
| A25,26,33,34,66,68,69,80 | Negative | A25,A26,A33,A34,A66,A68,A69,A80 |
| A25,26,34,43,66 | Negative | A25,A26,A34,A43,A66 |
| A29,43 | A2,A23,A24,A68,A69,B57,B58 | A2,A23,A24,A29,A43,A68,A69, B57,58 |
| B7,37,54,55,56,42,67,81,82 | Negative | B7,37,54,55,56,42,67,81,82 |
| B7,42,54,55,56,67,75,81,82 | Negative | A*11:02,A23,A*34:01,A66 B7,42,54,55,57,58,67,75,81,82 |
| B7,42,54,55,56,67,81,82 | Negative | B7,42,54,55,67,81,82 WEAK B56 |
| Cw1,12,15 | Negative | A43 Cw1,12,15 |
| Cw1,12,15 | Negative | B7,13, Cw1,12,15 |
| Cw1,12,15 | Negative | Cw1,12,15, |
| Cw1,5,12,15 | Negative | Cw1,5,12,15 |
| Cw7 | Negative | Cw7 |

- **10 sera: only ABS against denatured antigens in Luminex – completely negative with on MR.SPOT®**

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|---|---|---|
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| A11,25,26,66,43, B8,65,59,64 | Negative | A11,A25,A26,A66,A43, B8,B65,B59,B64 |
| A25,26,33,34,66,68,69,80 | Negative | A25,A26,A33,A34,A66,A68,A69,A80 |
| A25,26,34,43,66 | Negative | A25,A26,A34,A43,A66 |
| A29,43 | A2,A23,A24,A68,A69,B57,B58 | A2,A23,A24,A29,A43,A68,A69, B57,58 |
| B7,37,54,55,56,42,67,81,82 | Negative | B7,37,54,55,56,42,67,81,82 |
| B7,42,54,55,56,67,75,81,82 | Negative | A*11:02,A23,A*34:01,A66 B7,42,54,55,57,58,67,75,81,82 |
| B7,42,54,55,56,67,81,82 | Negative | B7,42,54,55,67,81,82 WEAK B56 |
| Cw1,12,15 | Negative | A43 Cw1,12,15 |
| Cw1,12,15 | Negative | B7,13, Cw1,12,15 |
| Cw1,12,15 | Negative | Cw1,12,15, |
| Cw1,5,12,15 | Negative | Cw1,5,12,15 |
| Cw7 | Negative | Cw7 |

- **10 sera: only ABS against denatured antigens in Luminex – completely negative with on MR.SPOT®**
- **1 serum: all specificities against intact antigen found on MR.SPOT®, but not the two for denatured antigens**

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| Specificity of antibodies to denatured antigens | MR SPOT finding | IgG |
|---|---|---|
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| A11,25,26,66,43, B8,65,59,64 | Negative | A11,A25,A26,A66,A43, B8,B65,B59,B64 |
| A25,26,33,34,66,68,69,80 | Negative | A25,A26,A33,A34,A66,A68,A69,A80 |
| A25,26,34,43,66 | Negative | A25,A26,A34,A43,A66 |
| A29,43 | A2,A23,A24,A68,A69,B57,B58 | A2,A23,A24,A29,A43,A68,A69, B57,58 |
| B7,37,54,55,56,42,67,81,82 | Negative | B7,37,54,55,56,42,67,81,82 |
| B7,42,54,55,56,67,75,81,82 | Negative | A*11:02,A23,A*34:01,A66 B7,42,54,55,57,58,67,75,81,82 |
| B7,42,54,55,56,67,81,82 | Negative | B7,42,54,55,67,81,82 WEAK B56 |
| Cw1,12,15 | Negative | A43 Cw1,12,15 |
| Cw1,12,15 | Negative | B7,13, Cw1,12,15 |
| Cw1,12,15 | Negative | Cw1,12,15, |
| Cw1,5,12,15 | Negative | Cw1,5,12,15 |
| Cw7 | Negative | Cw7 |

- **10 sera: only ABS against denatured antigens in Luminex – completely negative with on MR.SPOT®**
- **1 serum: all specificities against intact antigen found on MR.SPOT®, but not the two for denatured antigens**
- **3 sera: no specifities agains denatured antigens on MR.SPOT® , but some discrepancies with the Luminex remain, that need to be resolved**