



Monoclonal antibody immobilization of granulocyte specific antigens (MAIGA) assay

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Analysis of granulocyte-reactive antibodies using an immunoassay based upon monoclonal-antibody-specific immobilization of granulocyte antigens

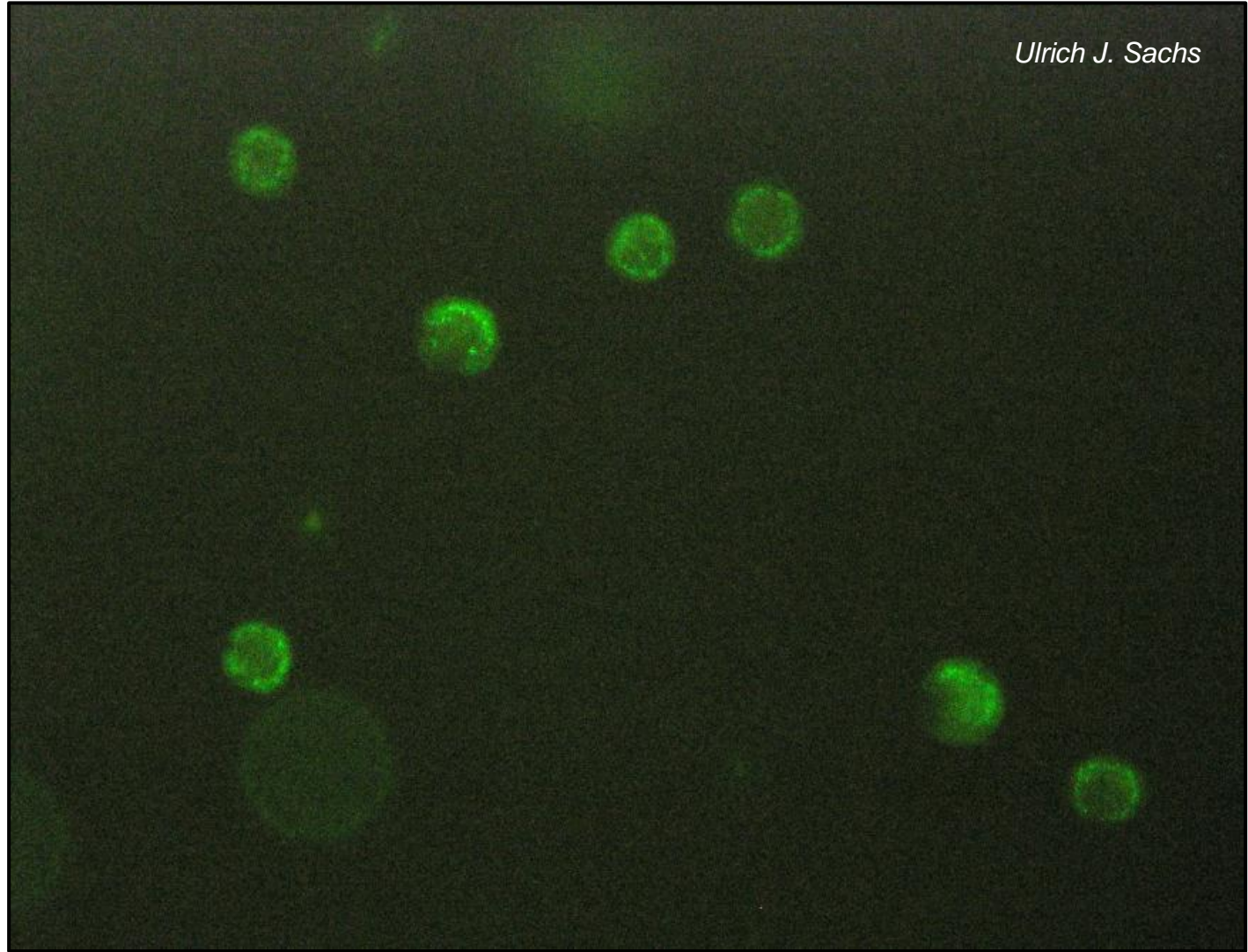
J. Bux, B. Kober, V. Kiefel and C. Mueller-Eckhardt *Institute for Clinical Immunology and Transfusion Medicine, Justus-Liebig-University Giessen, Germany*

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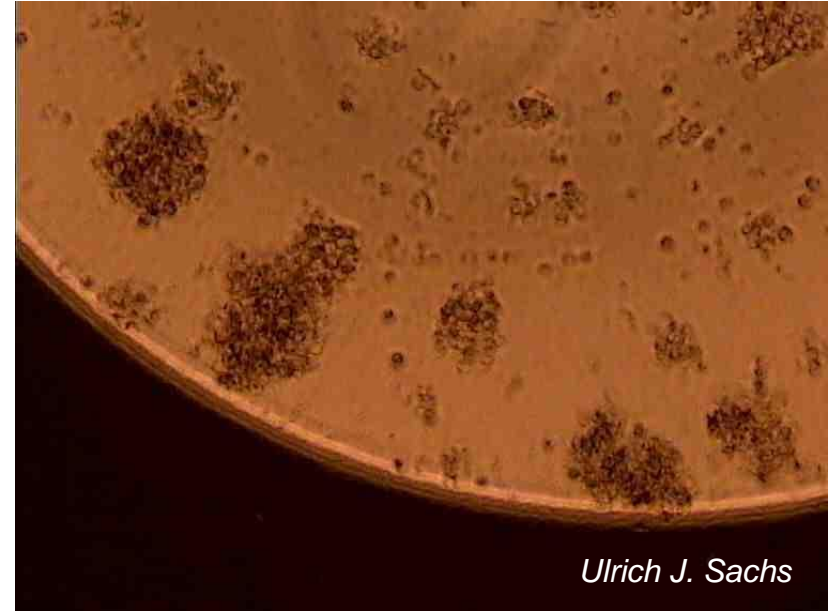
Genetics of human neutrophil antigens (HNA)

| HNA | GP | Antigen | | Allele | Allele defining base exchanges | | | | | |
|-----|--------------------|------------------|------|-----------------|--------------------------------|------------|------|------|------|------|
| | | | | | | | | | | |
| 1 | FcγRIIb (CD16b) | HNA-1a | NA1 | FCGR3B*01 | 108G | 114C | 194A | 233C | 244G | 316G |
| | | HNA-1b | NA2 | FCGR3B*02 | 108C | 114T | 194G | 233C | 244A | 316A |
| | | HNA-1c | SH | FCGR3B*03 | 108C | 114T | 194G | 233A | 244A | 316A |
| | | HNA-1d | - | FCGR3B*02 | 108C | 114T | 194G | 233C | 244A | 316A |
| | | HNA-1null | - | FCRG3B*null | <i>gene depletion</i> | | | | | |
| 2 | CD177 | HNA-2 | NB1 | CD177*01 | 843A | 1011G | | | | |
| | | <i>HNA-2null</i> | - | <i>CD177*02</i> | 843T | 1011G | | | | |
| | | <i>HNA-2null</i> | - | <i>CD177*03</i> | 843A | 1011Δ G | | | | |
| 3 | CTL2 | HNA-3a | 5b | SLC44A2*01 | 451C | 455G | | | | |
| | | HNA-3a | 5b | SLC44A2*03 | 451T | 455G | | | | |
| | | HNA-3b* | - | SLC44A2*02 | 451C | 455A | | | | |
| 4 | Mac-1 (CD11b) | HNA-4a | Mart | ITGAM*01 | 230G | | | | | |
| | | HNA-4b | - | ITGAM*02 | 230A | | | | | |
| 5 | LFA-1 (CD11a) | HNA-5a | Ond | ITGAL*01 | 2372G | | | | | |
| | | - | - | ITGAL*02 | 2372C | | | | | |

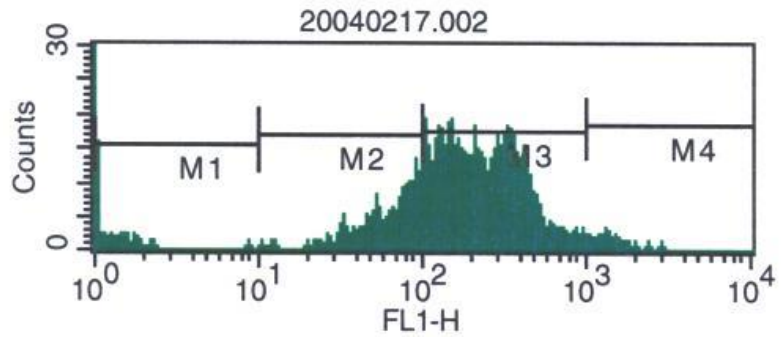
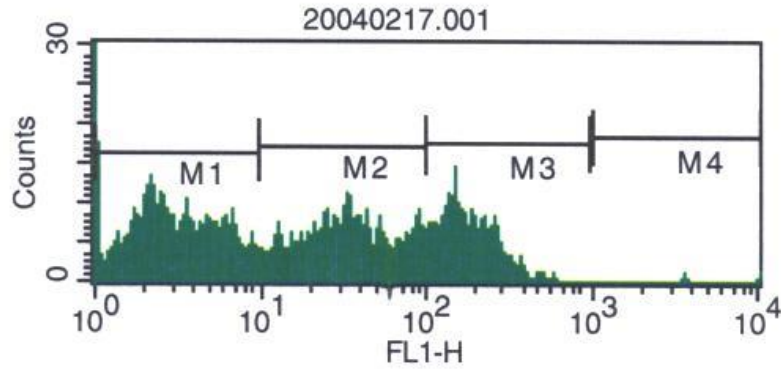
GIFT (an indirect immunofluorescence test)



GAT (an indirect agglutination assay)



LIFT (an indirect lymphocyte immunofluorescence test)



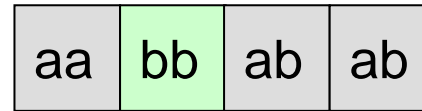
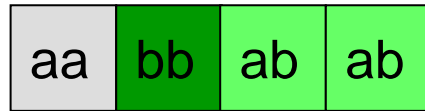
Test cell set-up (example)

GIFT

GAT

LIFT

HNA-1



HNA-2

>80%, 30-50%, 0%

HNA-3

aa, bb

aa, bb

HNA-4

ab

HNA-5

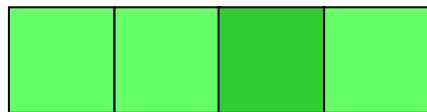
ab

Serological pattern in GIFT, GAT, and LIFT

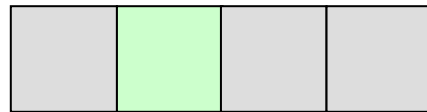
GIFT

GAT

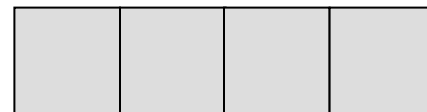
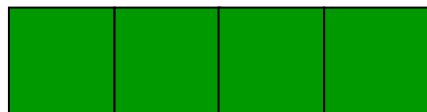
LIFT



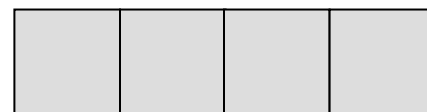
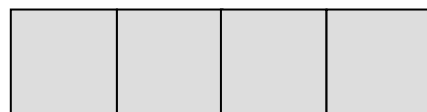
HLA?



anti-HNA-1b

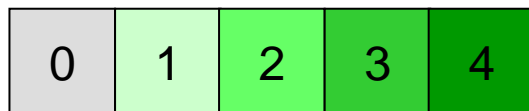


Auto-Ab



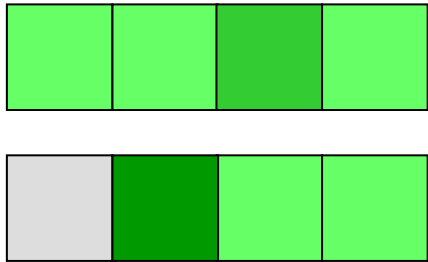
negative

Scoring

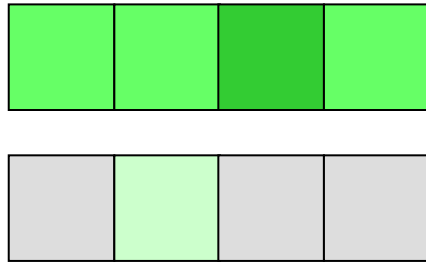


One major problem: HLA immunization

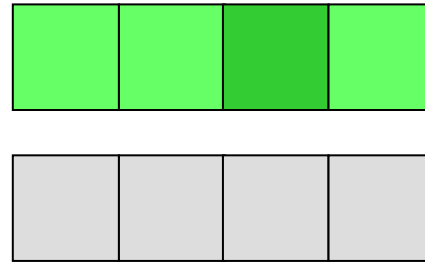
GIFT



GAT



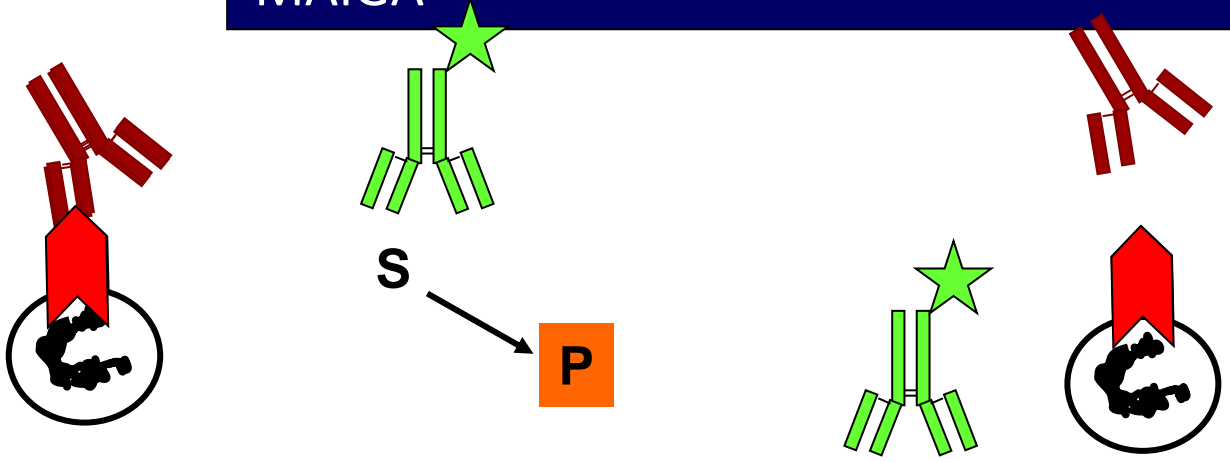
LIFT

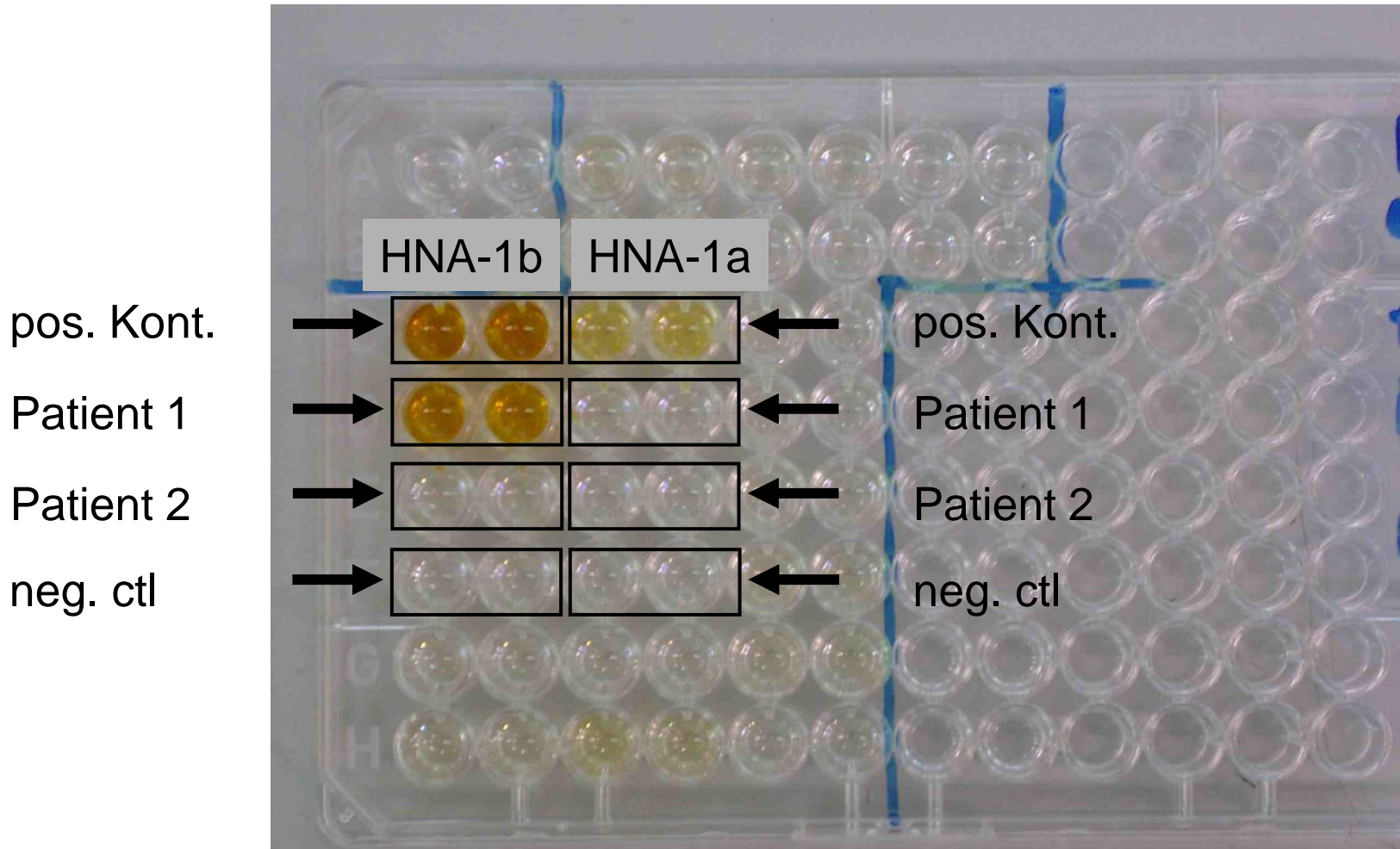


HLA

? anti-HNA-1b

MAIGA





patient 1: anti-HNA-1b; patient 2: negative

Prepare a plate with goat-anti-mouse.

Select your cell panel (based on suspicion).

Co-incubate fixed neutrophils with serum and moab (mouse-anti-human). A trimolecular complex will form.

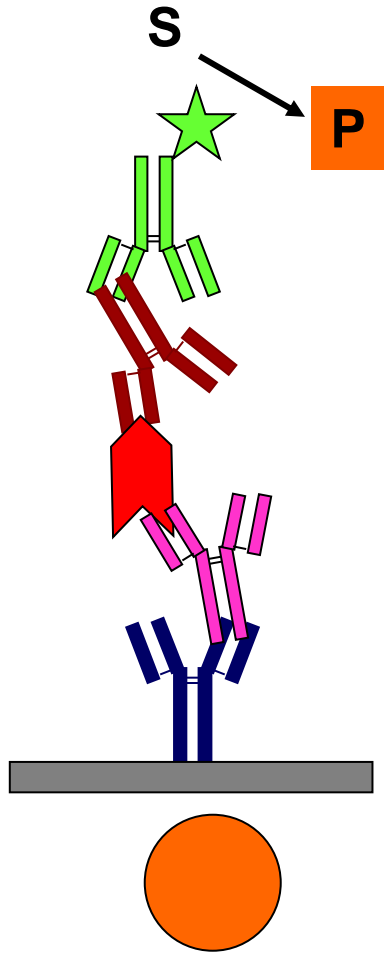
Wash and lyse.

Introduce the lysate to the plate. A 4-molecular complex will form.

Wash and add rabbit-anti-human, enzyme-labeled. A 5-molecular complex will form.

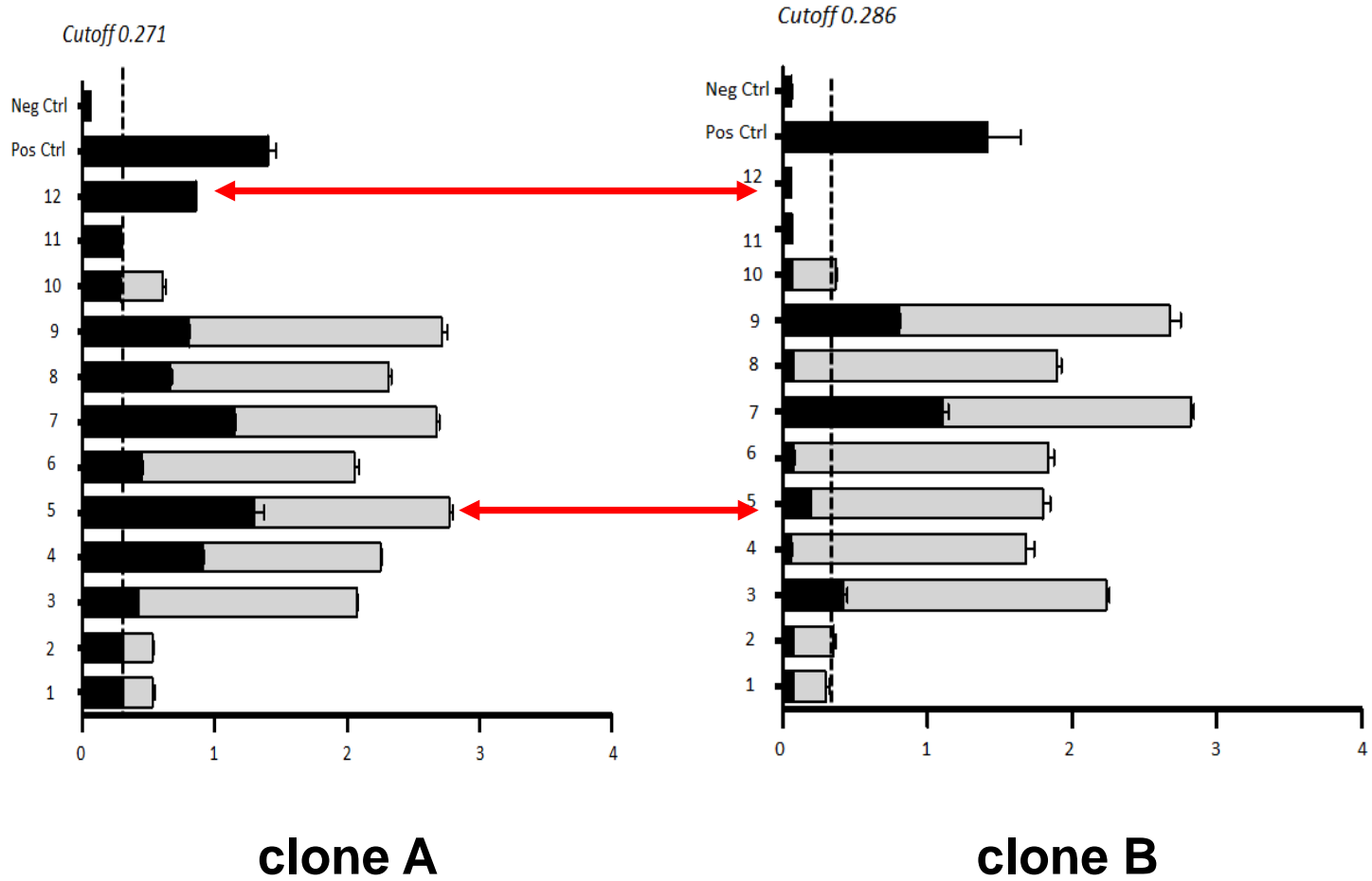
Wash and add substrate. Read OD and compare to negative and positive controls.

Monoclonals make the difference



| Antigen | HNA | monoclonal antibody (clone) |
|---------|-----|-----------------------------|
| CD16b | 1 | 3G8, DJ130c, BW209 |
| CD177 | 2 | 7D8, MEM166 |
| CTL2 | 3 | -* |
| CD11b | 4 | e.g. BEAR-1 |
| CD11a | 5 | e.g. MEM-25 |

Auto- and iso-antibodies against CD177



Do autoantibodies have specificities?

| auto-anti- | | | | |
|-----------------------------|-----------------------|------------|-----------|----------|
| GIFT | MAIGA (3G8, BW209) | | | |
| | HNA- 1a | HNA- 1b | both | none |
| 1a (68/77) | 28 | 19 | 16 | 5 |
| 1b (9/77) | 3 | 5 | 0 | 1 |
| pan (0/77) | 0 | 0 | 0 | 0 |

High specificity, low sensitivity. A test for **antibody differentiation**, not a screening test.

Well-established to identify the specificity of alloantibodies. Advantage: native antigens! Relevance of differentiating autoantibodies is unclear. Autoantibodies are more often missed in the MAIGA assay (a problem of avidity?).

Very **laborious** test. Requires **experienced** staff.

No international consensus on immobilizing antibodies. No commercial controls. No HNA-2 detection.