Detection of granulocyte antibodies: Novel technologies

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Detection of granulocyte antibodies: standard techniques

- GIFT: Granulocyte immunofluorescence test
- LIFT: Lymphocyte immunofluorescence test
- GAT: Granulocyte agglutination test
- MAIGA: Monoclonal antibody-specific immobilization of granulocyte antigens

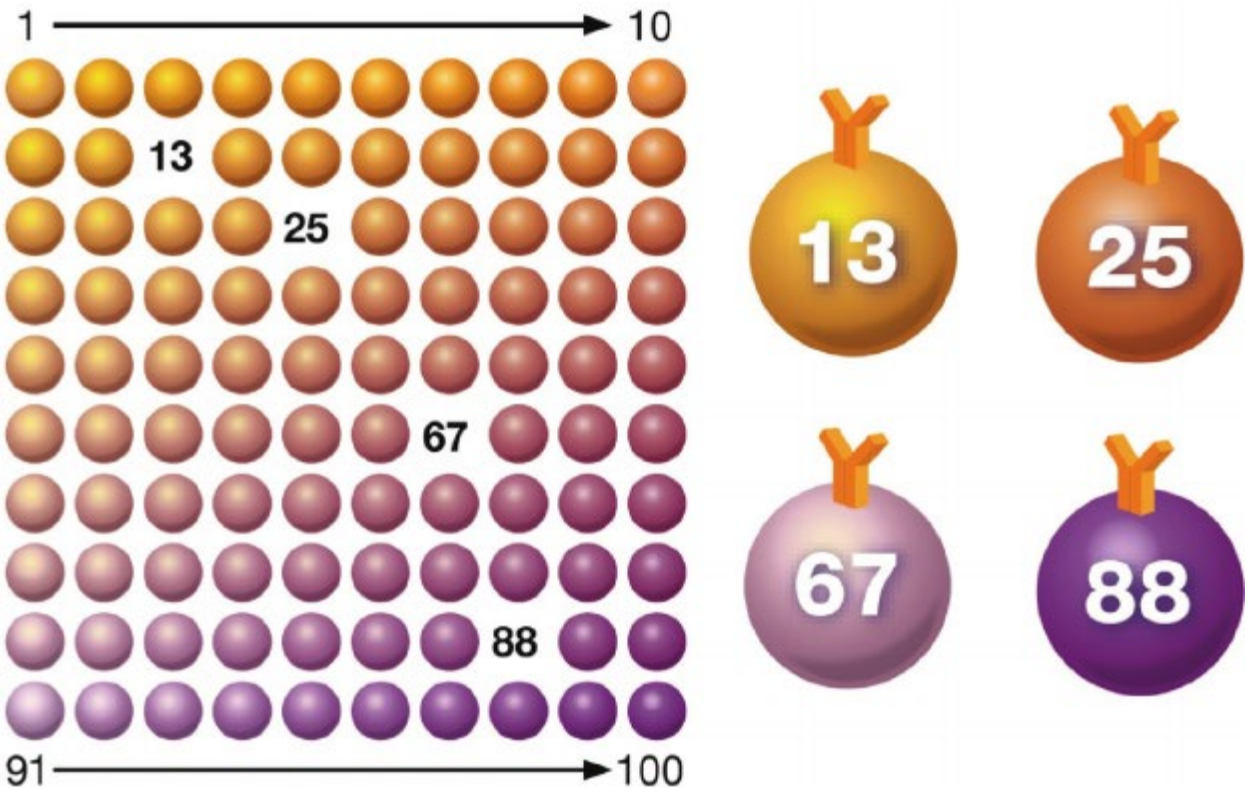
- *Most efficient on live donor-derived granulocytes (or lymphocytes) typed for HNA-alleles*
- *Tests with full antigen-coverage cause pressure on donors*
- *Only MAIGA eliminates the effect of HLA-antibodies*
- *Complicated tests requiring good health of the granulocytes and specially trained staff*

On the wish-list

- Off-the-shelf method
- Bulk analysis on stored samples at any time
- Relable antibody identification without allelic crossreactivities
- Eliminate the need for blood donors
- Eliminate the need for specially trained staff
- High throughput

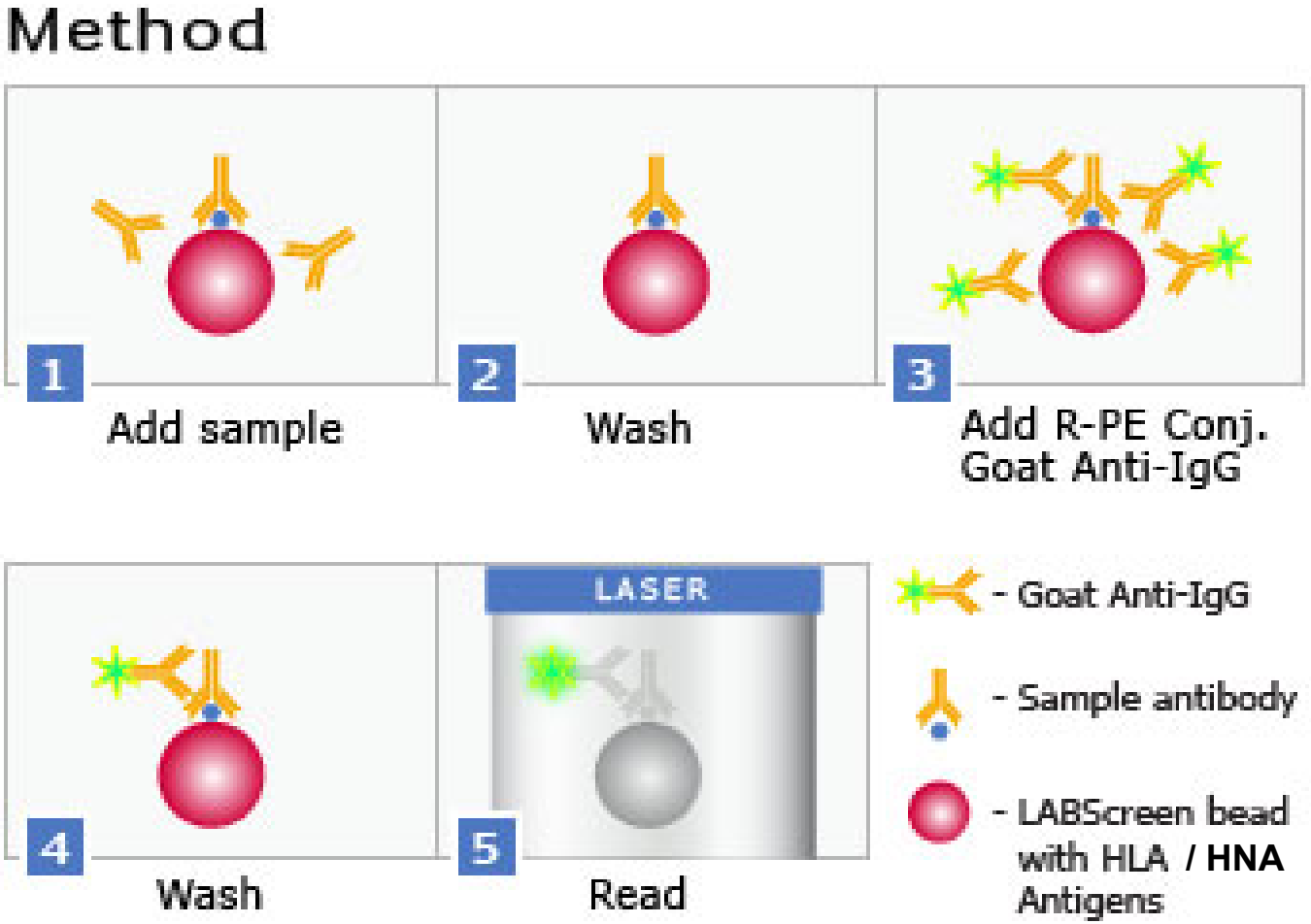
- *Luminex-based technologies are promising ”novel” tools*

Principle: bead-technology



Each bead is individually detectable by a distinct fluorescence sgnal

LabScreen Multi (LSM – One Lambda)



LSM setup

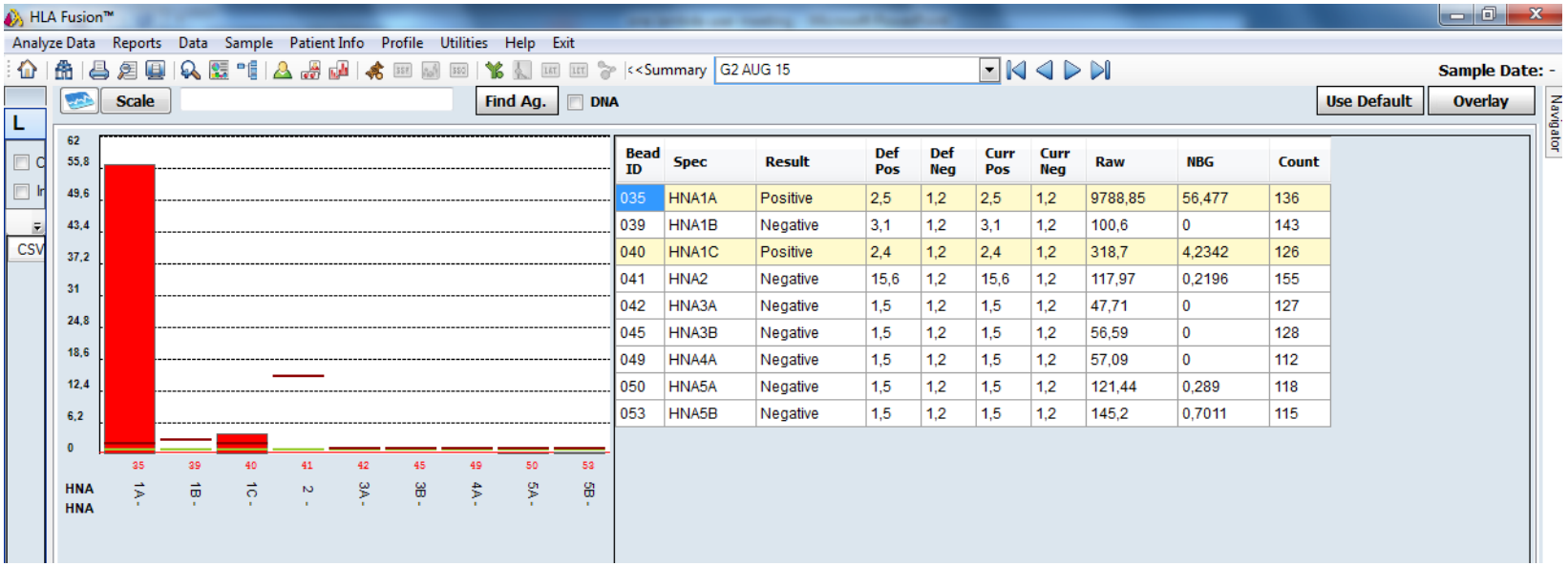
HLA-cass I:	**12 beads** with different combinations of specificities
HLA-class II:	**5 beads** with different combinations of specificities
HNA:	**9 beads** each expressing the following pecific antigens: HNA-1a, HNA-1b, HNA-1c, HNA-2, HNA-3a, HNA-3b, HNA-4a, HNA-5a, HNA-5b
Performance:	Incubation 1: 20 µL sample incubated with beads Incubation 2: anti-IgG- PE
Results:	$\text{NBG ratio} = \frac{\text{S\#N - SNC bead}}{\text{BG\#N - BGNC bead}}$

S#N	Sample-specific fluorescent value for bead #N
SNC bead	Sample-specific fluorescent value for Negative Control bead
BG#N	Background NC Serum fluorescent value for bead #N
BGNC bead	Background NC Serum fluorescent value for Negative Control bead

Alloantibodies against HNA (and HLA)

- Alloimmunization against HNA-antigens can occur during pregancy or blood transfusion.
- Alloantibodies against HNA-antigens (in particular anti-HNA-3 antibodies) can cause transfusion-reated acute lung injury (TRALI).
- Antibodies to foregin HLA can also cause TRALI.
- Plasma from female donors is generally not used clinically.
- Screening of blood donors for HLA/HNA-antibodies reduce TRALI incidence.
- Important aspect of granulocyte antibody laboratories to identify such antibodies.

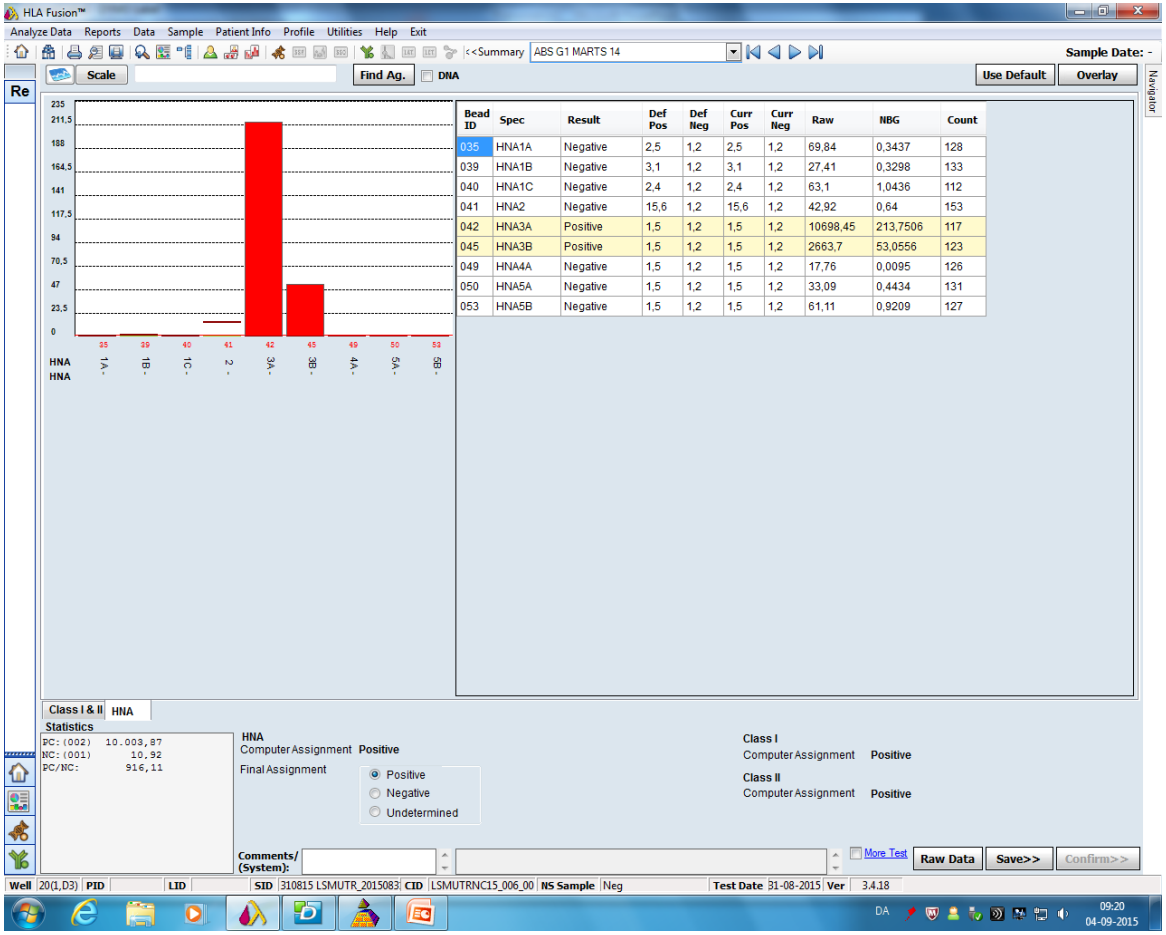
Example: detection of anti-HNA-1a alloantibody using LSM



Bead ID	Spec	Result	Def Pos	Def Neg	Curr Pos	Curr Neg	Raw	NBG	Count
035	HNA1A	Positive	2,5	1,2	2,5	1,2	9788,85	56,477	136
039	HNA1B	Negative	3,1	1,2	3,1	1,2	100,6	0	143
040	HNA1C	Positive	2,4	1,2	2,4	1,2	318,7	4,2342	126
041	HNA2	Negative	15,6	1,2	15,6	1,2	117,97	0,2196	155
042	HNA3A	Negative	1,5	1,2	1,5	1,2	47,71	0	127
045	HNA3B	Negative	1,5	1,2	1,5	1,2	56,59	0	128
049	HNA4A	Negative	1,5	1,2	1,5	1,2	57,09	0	112
050	HNA5A	Negative	1,5	1,2	1,5	1,2	121,44	0,289	118
053	HNA5B	Negative	1,5	1,2	1,5	1,2	145,2	0,7011	115

Courtesy of Kaspar René Nielsen, Aalborg University, Denmark

Example: detection of anti-HNA-3a alloantibody using LSM

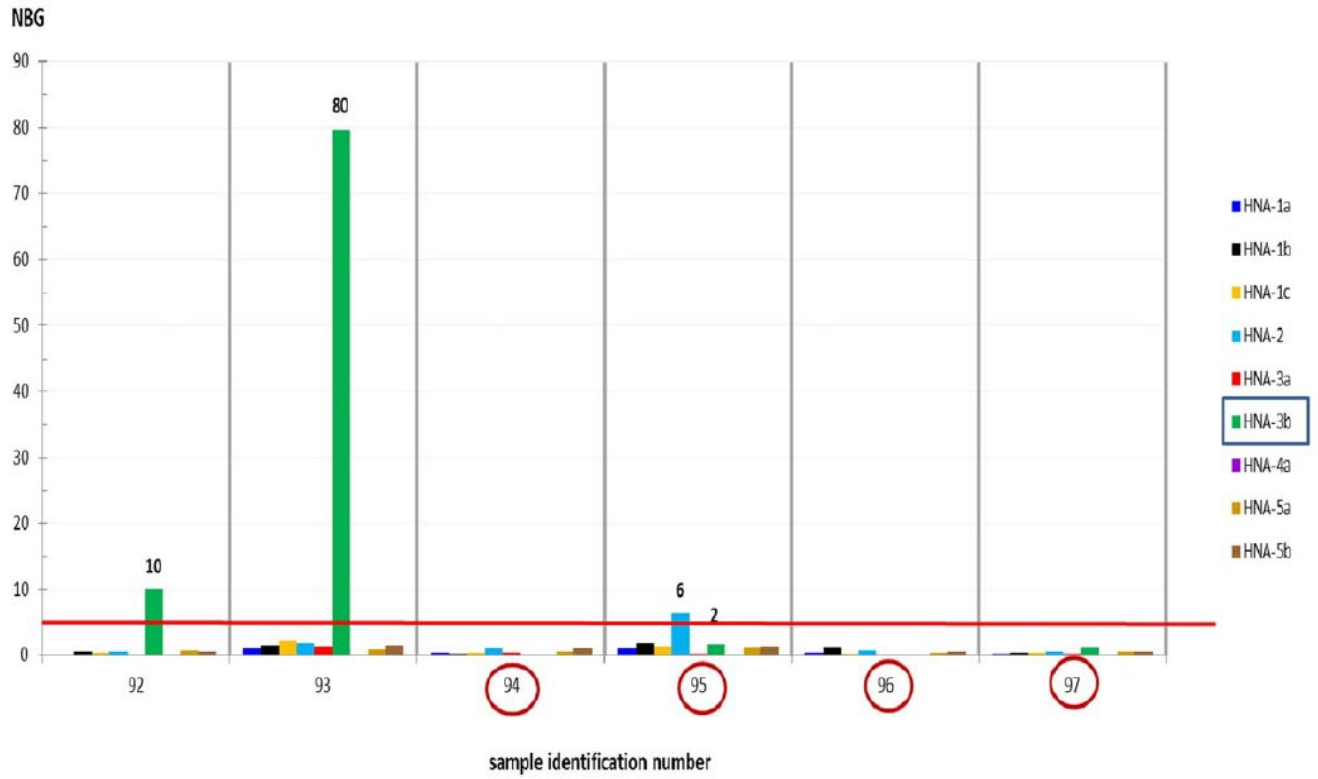


Courtesy of Kaspar René Nielsen, Aalborg University, Denmark

Performance of positive controls (alloantibodies) in LSM in our laboratory

Spec	Bead#	NIBSC HNA-1a	HNA-1b	HNA-2	HNA-3a NIBSC	HNA-3b	HNA-4a
NC	1	58	79	45	9	19	157
PC	2	6002	2226	5320	6313	4081	6145
HNA-1a	35	5928	139	140	98	57	163
HNA-1b	39	121	6665	128	71	80	168
HNA-1c	40	545	4938	124	66	49	149
HNA-2	41	117	157	23632	61	48	255
HNA-3a	42	151	177	129	11034	37	150
HNA-3b	45	176	208	158	2926	274	183
HNA-4a	49	145	66	41	17	151	9936
HNA-5a	50	127	228	143	45	131	213
HNA-5b	53	124	127	125	70	64	153

Many false negatives for HNA-3b also in published material



Schultz et al., Transfusion, 57, 70, 2017

LSM for alloantibody detection

- Detects almost all known HNA-1, HNA-2 and HNA-4 alloantibodies identified using other methods.
- 10% of known HNA-3a and 67% of known HNA-3b antibodies are missed.
- Crossreactivity is often seen between HNA-3a and HNA-3b
- GAT is still be needed as a complement for TRALI investigations.

LSM in patients with neutropenia: preliminary conclusions from our laboratory

- Individual cut-offs must be determined in a large number of healthy blood donors
- LSM has a rather good correspondens with the MAIGA test for HNA-1 in a large cohort of previously analysed AIN patients.
- Correspondence for HNA-2, HNA-4 and HNA-5 is much poorer and LSM does not detect these weaker antibodies.

LSM – summary

- LSM is a fast, easy-to-use, high-throughput kit.
- It tests for HLA class I, class II and HNA antibodies at the same time and uses only a very small sample volume (20 μl).
- Cut-offs based on samples from non-immunized male donors and (if possible) healthy children should be individualized for each lab.
- At the Karolinska, we have performed LSM for all neutropenia investigations since February 2020. Follow-up is in progress, including stratification for adult and pediatric samples and correlation to other tests.
- LSM has been tested also for screening assay blood donors. 2,3 % were positive for HNA antibodies and many more for HLA antibodies (close to 20%). NBG ratios were between 8 and 22. Follow-up is ongoing with repeated testing.

Reflections on the diagnostics of autoantibodies in suspected AIN

- The presence of autoantibodies supports a diagnosis of AIN but their absence does not exclude it.
- It is important not to mislead clinicians (and possibly delayíng further investigations) by answering non-specific reaction as positive. There is no need to strive for maximum sensitivity at the cost of specificity. Better to identify too few than too many antibody-positive samples!
- There is no ”answer section” you can look up in the back of the book to find out if a specific sample contains real anti-neutrophil antibodies or not. Thus, we cannot à priori know which method most adequately reflect the clinical entity. Clinical studies are required!
- My opinion: The best method for autoantibody detection is the one that reproducibly identifies positive results in a fraction of samples from patients with a clinical history of suspected acquired neutropenia. At the same time, the method should be cost-efficient, off the shelf, easy-to-perform and (preferrably) independent from blood donors.
- LSM is an interesting new method, with the drawback of lower sensitivity compared to classical methods. Comparative studies are needed, including clinical follow-ups.

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