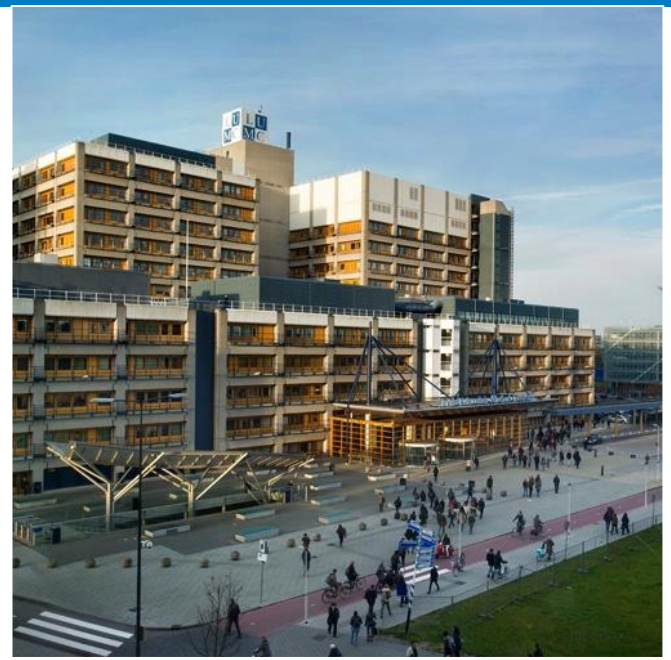
Leiden University
Medical Center

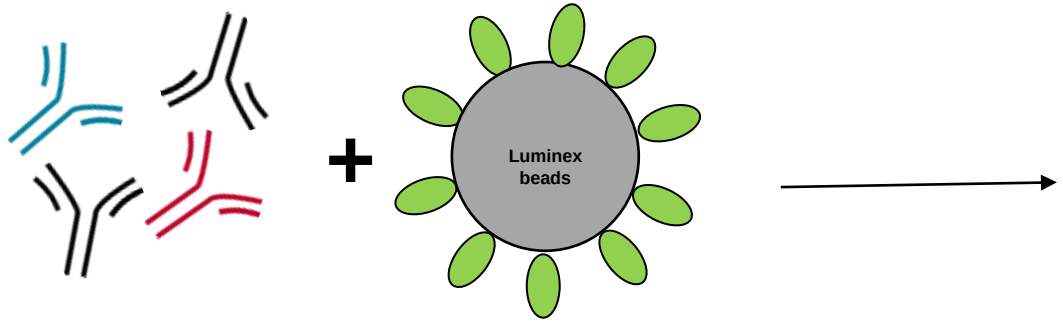
Iki farkli markanin Luminex tek antijen boncuk testlerinin karsilastirilmasi

MFI donusumu icin matematik model



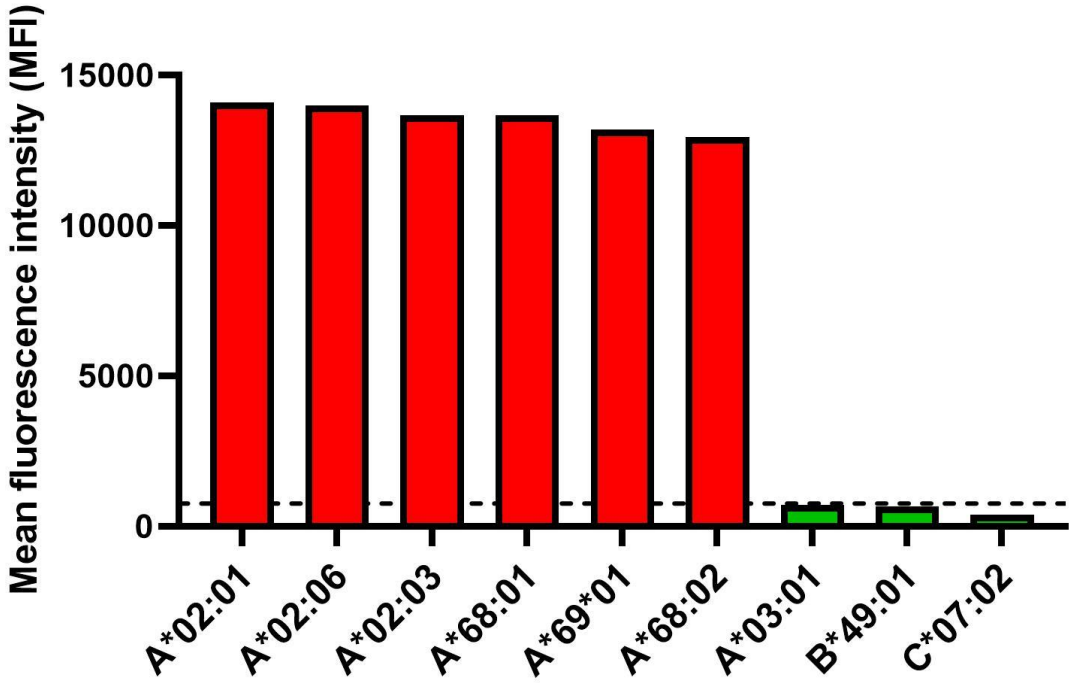
Gonca E. Karahan
TIGED
20-04-2024/ANTALYA

Luminex single antigen bead (SAB) assays are excellent tools to define HLA antibody specificities



Patient serum

Single HLA coated beads



Luminex SAB assays: Discrepancies and challenges

ONE LAMBDA

IMMUCOR

- **Test protocol**

Serum/bead ratio, detection antibody, etc.

- **Analysis software**

Positive/negative antibody assignment criteria

- **Various readout types**

Raw MFI, baseline MFI, background corrected MFI (BCM), MFI/LRA, NBG ratio, AD-BCR …

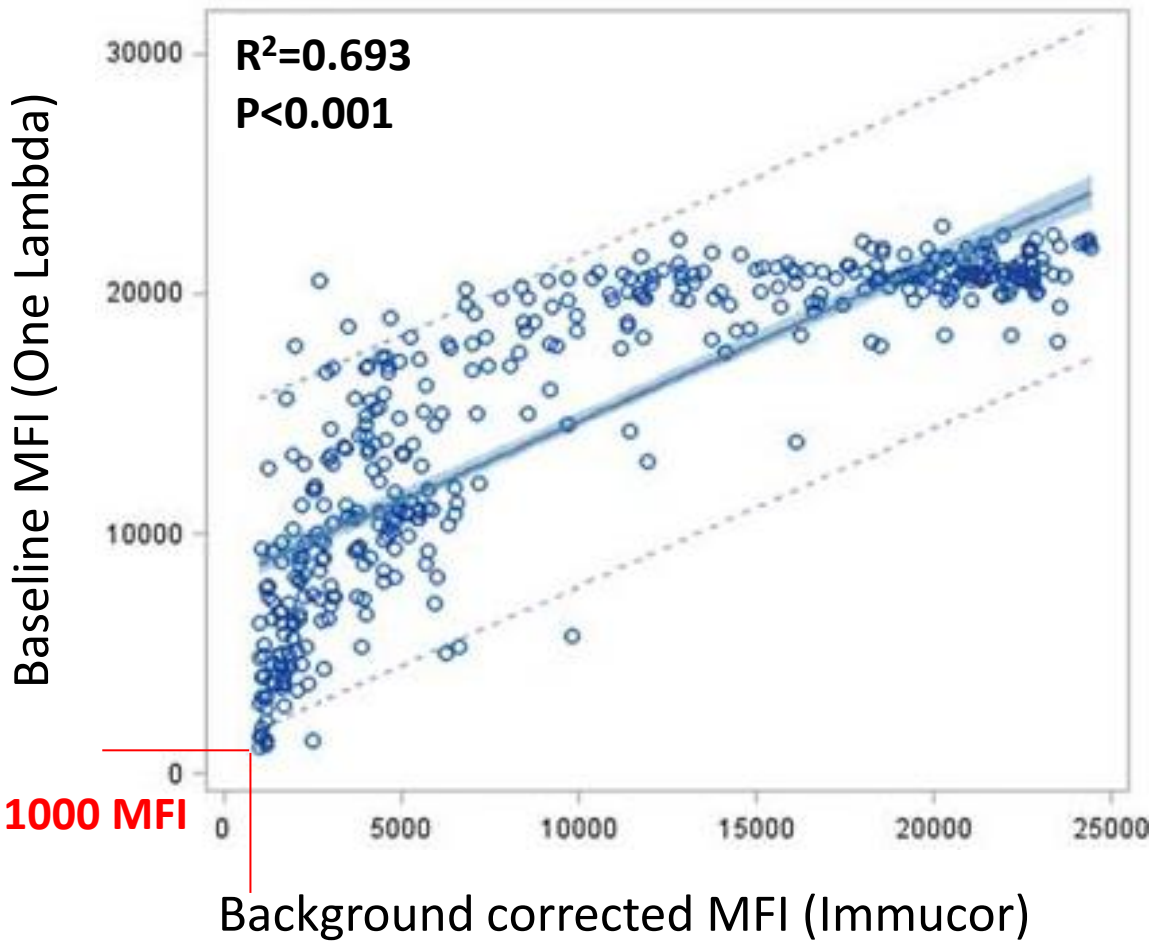
- Antigen panel composition
- Antigen density
- Antigen integrity

- Background noise in sample
- Interference of serum components (and drugs)

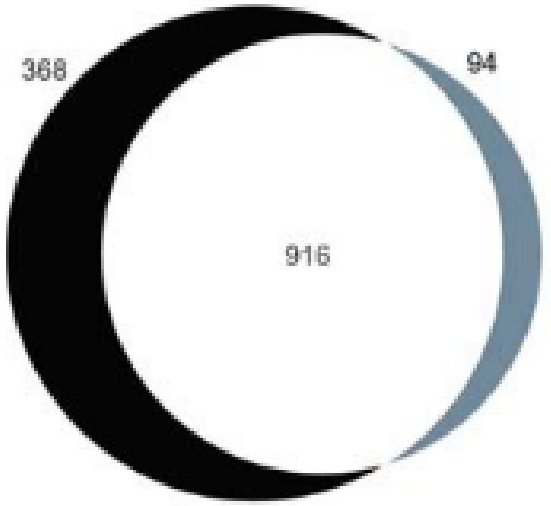
- Lot-to-lot variability
- Day-to-day variability
- Technician variability

Most differences between vendors are observed for low MFI values

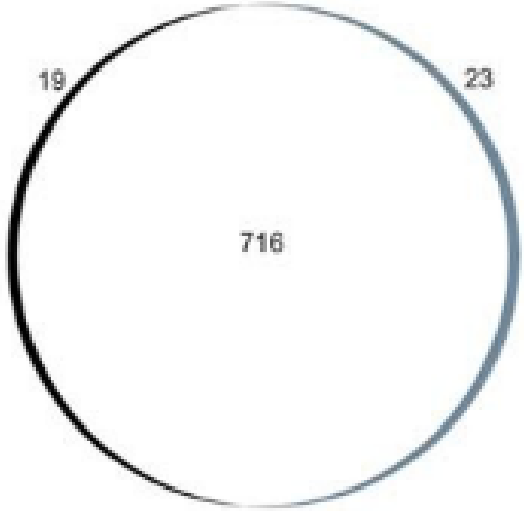
HLA class I



Any MFI



MFI>4000



Black: only One Lambda
Gray: only Lifecodes (Immucor)
White: both

Clerkin et al. Transplant Direct, 2017

Background and aim

- Two vendors (One Lambda=**OL** and Lifecodes/Immucor=**LC**) provide Luminex SAB tests and many centers use both tests simultaneously or switch from one to another
- Due to differences in manufacturing process and design, MFI values between vendors are not comparable
- Cut-offs based on the same (numeric) MFI values cannot be applied for both vendors

AIMS:

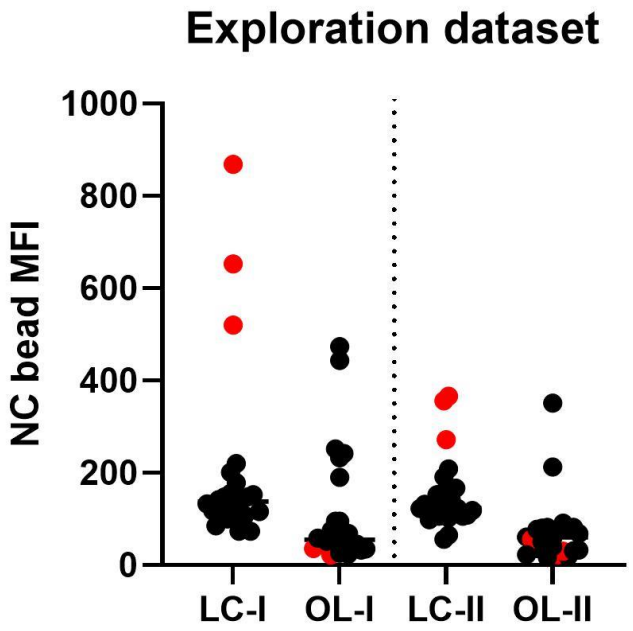
- Generate a model to determine how MFI values from different vendors behave with respect to each other
- Establish data-driven, comparable, vendor-specific cutoffs for analyzing big data sets

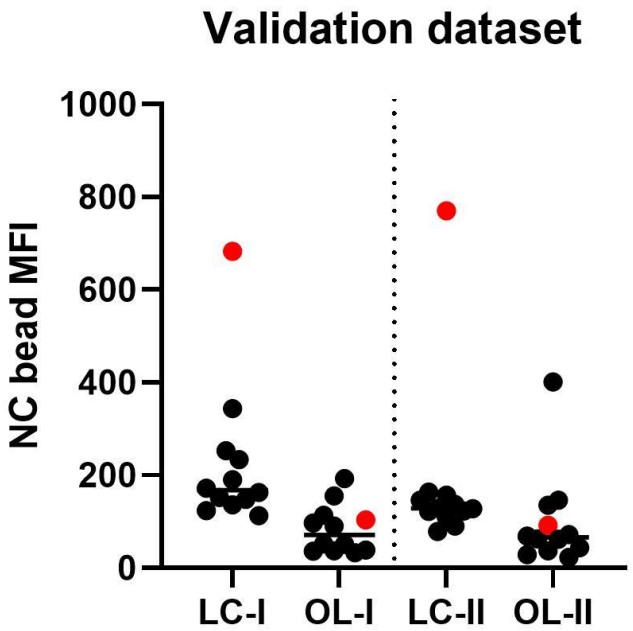
Study cohort – negative control beads

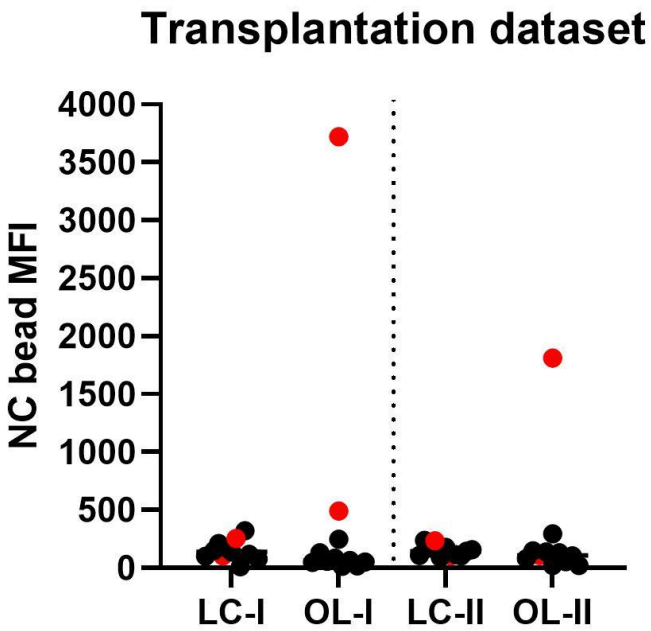
Exploration dataset (n=24)
(Pregnancy-immunized women)

Validation dataset (n=12)
(Pregnancy-immunized women)

Transplantation set (n=11)
(Kidney tx patients, post-tx serum)







Some samples had high negative control bead MFI values but **were not excluded from the study.**

Samples

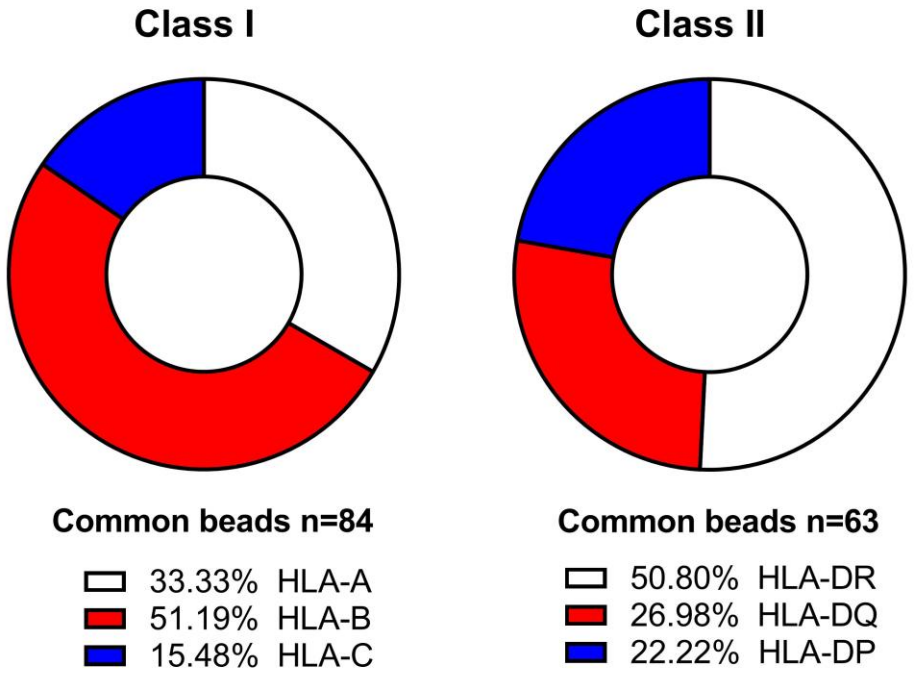
- All serum samples were **EDTA-treated**
- **OL test:** 20 µl serum/4 µl beads
- **LC test:** 7.5 µl serum/30 µl beads[1]

- Luminex SAB tests were performed over a **3-year period** (2019-2021)

- **Various lots** of single antigen beads were used by **different technicians**
- A LabScan 100 device was used for data acquisition

- Only **common beads** were analyzed

- Every common bead had at least **one positive MFI value in the study cohort** (exploration/validation)



[1]*Kamburova et al. HLA, 2016*

Study design-workflow

Step 1: No decision is made

Generation and validation of a model to ‘’convert’’ MFI values of one vendor to other and vice versa

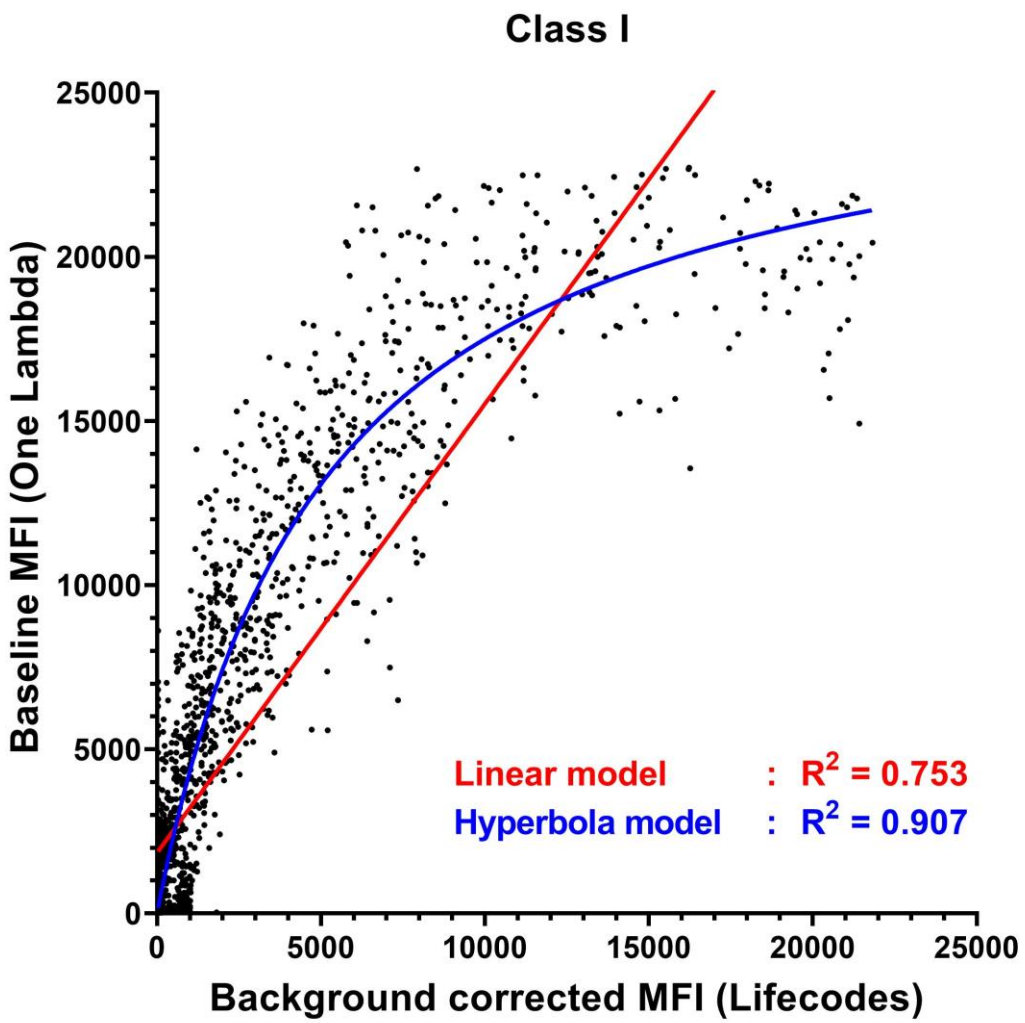
Interpolation of the MFI values when the highest correlation (r^2) is achieved in all cases
Better correlation, better prediction (perfect correlation is impossible!)

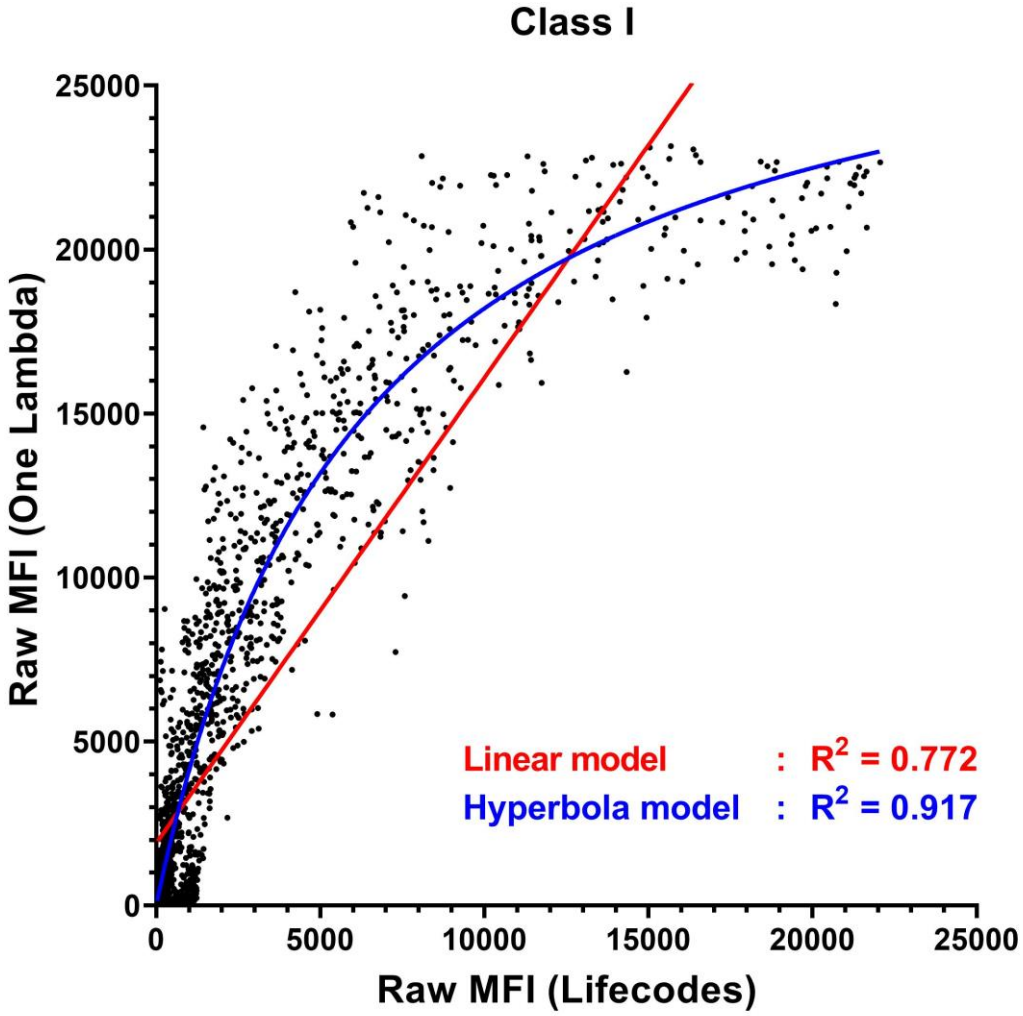
Step 2: Decisions are made

Decision for positive and negative beads with cutoffs based on interpolated values

Non-linear hyperbola model fits the data better than the linear model

Exploration set n=24



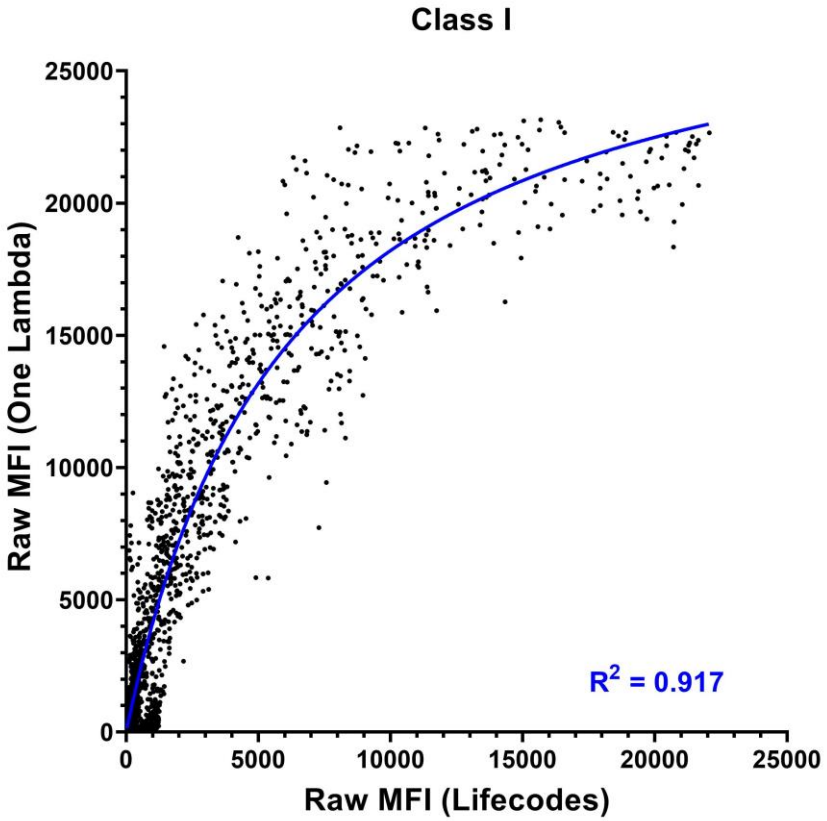


Karahan et al. HLA, 2023

Correction for self HLA improves the goodness of the fit of the model

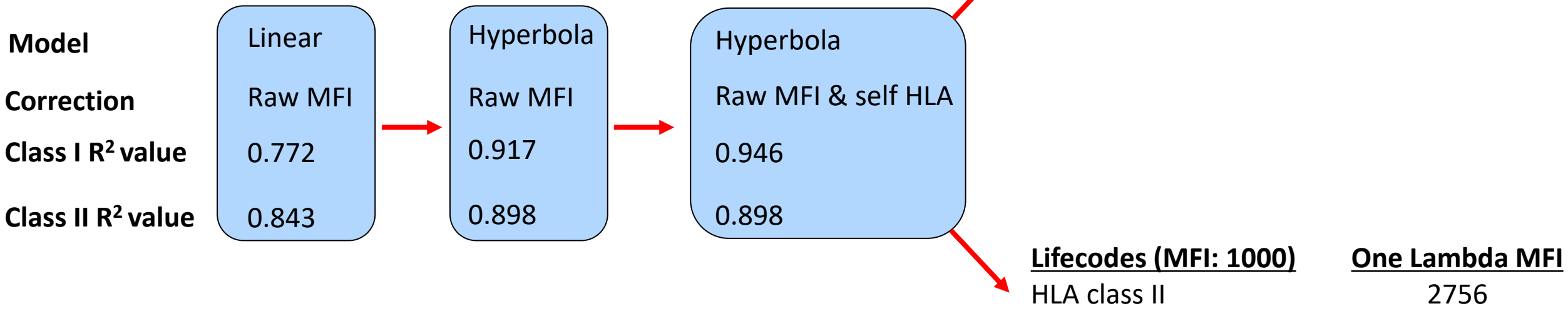
Exploration set n=24

Correction self-HLA: Locus-specific highest self HLA MFI was subtracted from raw MFI of specific bead



Exclude samples with background : R^2 =0.943

Interpolations from one vendor to the other



Locus-specific interpolations

HLA locus	Correction	Model	Exploration Cohort			
					Interpolated MFI OL	
			N	R^2	LC=1000	LC=3000
HLA Class I	Raw MFI & Self-HLA	Hyperbola	2016	0,946	**5044**	**10830**
HLA-A	Raw MFI & Self-HLA	Hyperbola	672	0,933	4942	10768
HLA-B	Raw MFI & Self-HLA	Hyperbola	1032	0,953	5010	10759
HLA-C	Raw MFI & Self-HLA	Hyperbola	312	0,889	**6647**	**12505**

✓ **Similar trends** were observed for interpolations **in both exploration and validation** datasets

Workflow-Step 2

- What happens if we use these interpolated values as cutoff values to assign a bead positive/negative?

  - How good is the quality of these assignments? (**statistically**)
  - Are there any (interesting) outliers?

Model: Hyperbola, self-HLA corrected raw MFI, locus-specific

Quality of assignments: Lifecodes → One Lambda

Exploration Cohort											
MFI LC	**MFI OL**	**locus**	**LC/OL neg/neg**	**LC/OL pos/neg**	**LC/OL neg/pos**	**LC/OL pos/pos**	**specificity (%)**	**sensitivity (%)**	**accuracy (%)**	**r_φ**	**N**
1000	4942	HLA-A	408	16	13	235	96.2	94.8	95.7	0.908	672

Specificity: True negatives

Sensitivity: True positives

Accuracy: Proportion of true results

Quality of assignments: One Lambda → Lifecodes

	Exploration Cohort										
MFI OL	MFI LC	locus	LC/OL neg/neg	LC/OL pos/neg	LC/OL neg/pos	LC/OL pos/pos	specificity	sensitivity	accuracy	r_{φ}	N
3000	556	HLA-A	384	6	8	274	98,5%	97,2%	97,9%	0.957	672
8000	1890	HLA-A	465	26	26	155	94,7%	85,6%	92,3%	0.803	672
3000	545	HLA-B	539	12	20	461	97,8%	95,8%	96,9%	0.937	1032
8000	1874	HLA-B	680	19	19	314	97,3%	94,3%	96,3%	0.915	1032
3000	336	HLA-C	269	3	11	29	98,9%	72,5%	95,5%	0.786	312
8000	1317	HLA-C	295	3	2	12	99,0%	85,7%	98,4%	0.819	312
3000	1435	HLA-DR	560	13	19	176	97,7%	90,3%	95,8%	0.889	768
8000	4447	HLA-DR	637	14	5	112	97,8%	95,7%	97,5%	0.908	768
3000	531	HLA-DQ	301	11	8	88	96,5%	91,7%	95,3%	0.872	408
8000	1869	HLA-DQ	331	10	5	62	97,1%	92,5%	96,3%	0.870	408
3000	1517	HLA-DP	286	1	0	49	99,7%	100,0%	99,7%	0.988	336
8000	4346	HLA-DP	317	3	0	16	99,1%	100,0%	99,1%	0.913	336

Outliers: Beads outside 95% prediction intervals

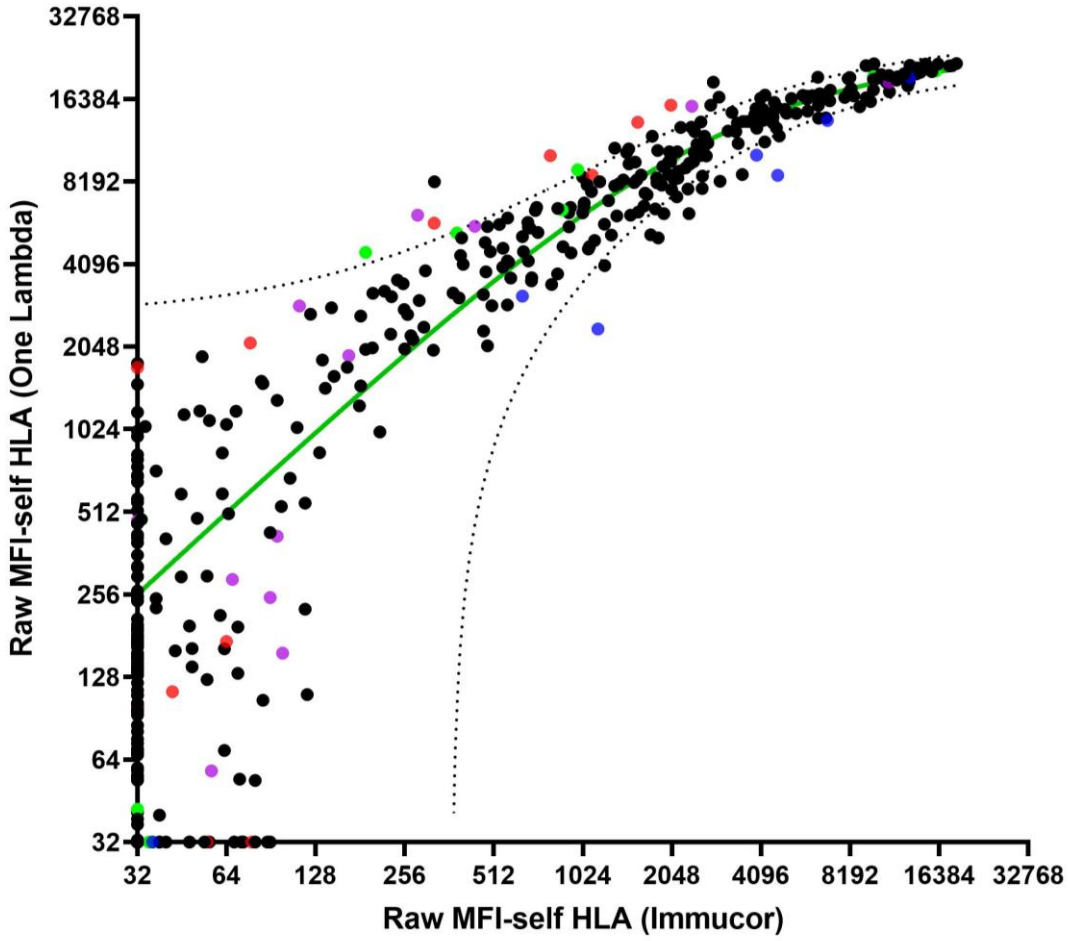
Approach: For a given MFI value in one vendor, we compared the observed MFI with the expected (predicted) MFI for the other vendor.

cohort	bead	observed LC	observed OL	expected OL
exploration	A*80:01 17V	8260	21671	17236
validation	A*80:01 19A	958	12334	5796
validation	A*80:01 17C	913	11606	5580

Criteria:

-Always in one direction in both cohorts

-Occurring ≥ 2 times in both cohorts



Agreement of two vendors for outliers

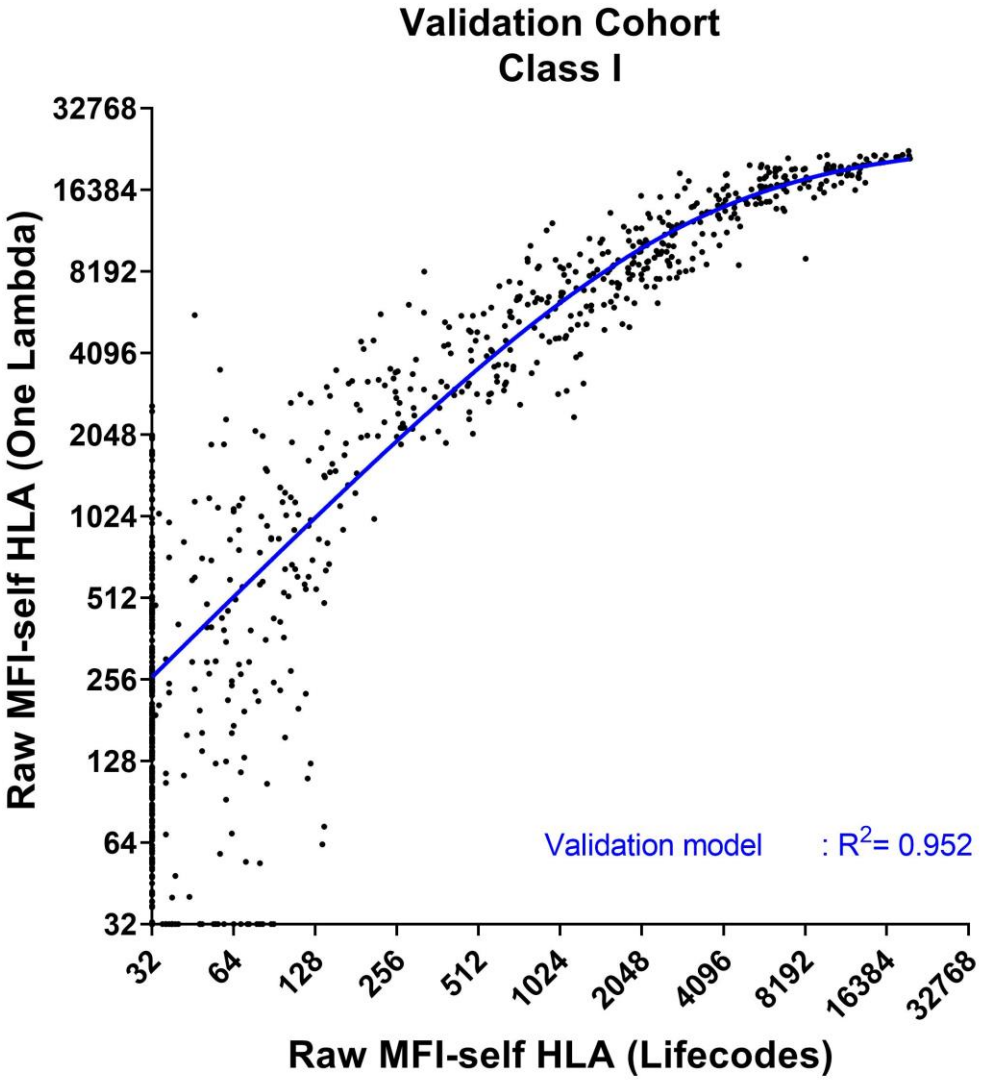
Bead	Values outside prediction intervals			
	exploration set		validation set	
	lower	higher	lower	higher
B*47:01	0	8	0	4
B*48:01	7	0	4	0
B*42:01	3	0	2	0
B*46:01	2	0	2	0
C*05:01	3	0	3	0
DQB1*05:01-DQA1*01:01	5	0	2	0
DQB1*06:01-DQA1*01:03	4	0	2	0
DRB1*01:01	2	0	2	0

	Cut-off MFI LifeCodes = 1000, OneLambda MFI = interpolated			
	exploration set		validation set	
	LC/OL neg/pos	LC/OL pos/neg	LC/OL neg/pos	LC/OL pos/neg
	0	2	0	1
	2	0	1	0
	1	0	1	0
	0	0	1	0
	0	0	0	0
	1	0	1	0
	0	0	1	0
	0	0	0	0

	Cut-off OneLambda = 3000, MFI Lifecodes = interpolated			
	exploration set		validation set	
	LC/OL neg/pos	LC/OL pos/neg	LC/OL neg/pos	LC/OL pos/neg
	0	1	0	1
	0	0	1	0
	1	0	1	0
	1	0	1	0
	2	0	2	0
	1	0	0	0
	0	0	1	0
	0	0	0	0

Not all outliers resulted in disagreement!

Consistency of the model was verified in an independent validation dataset



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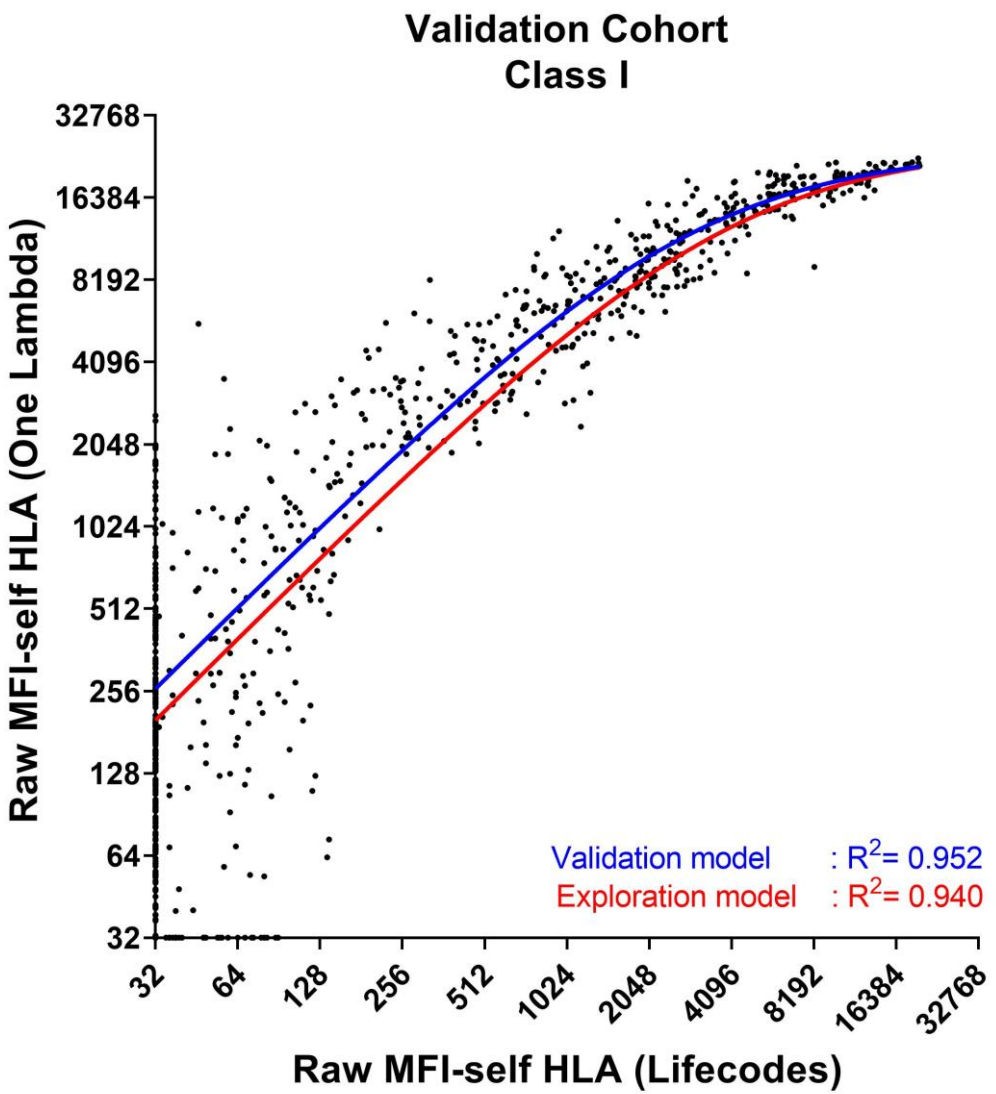
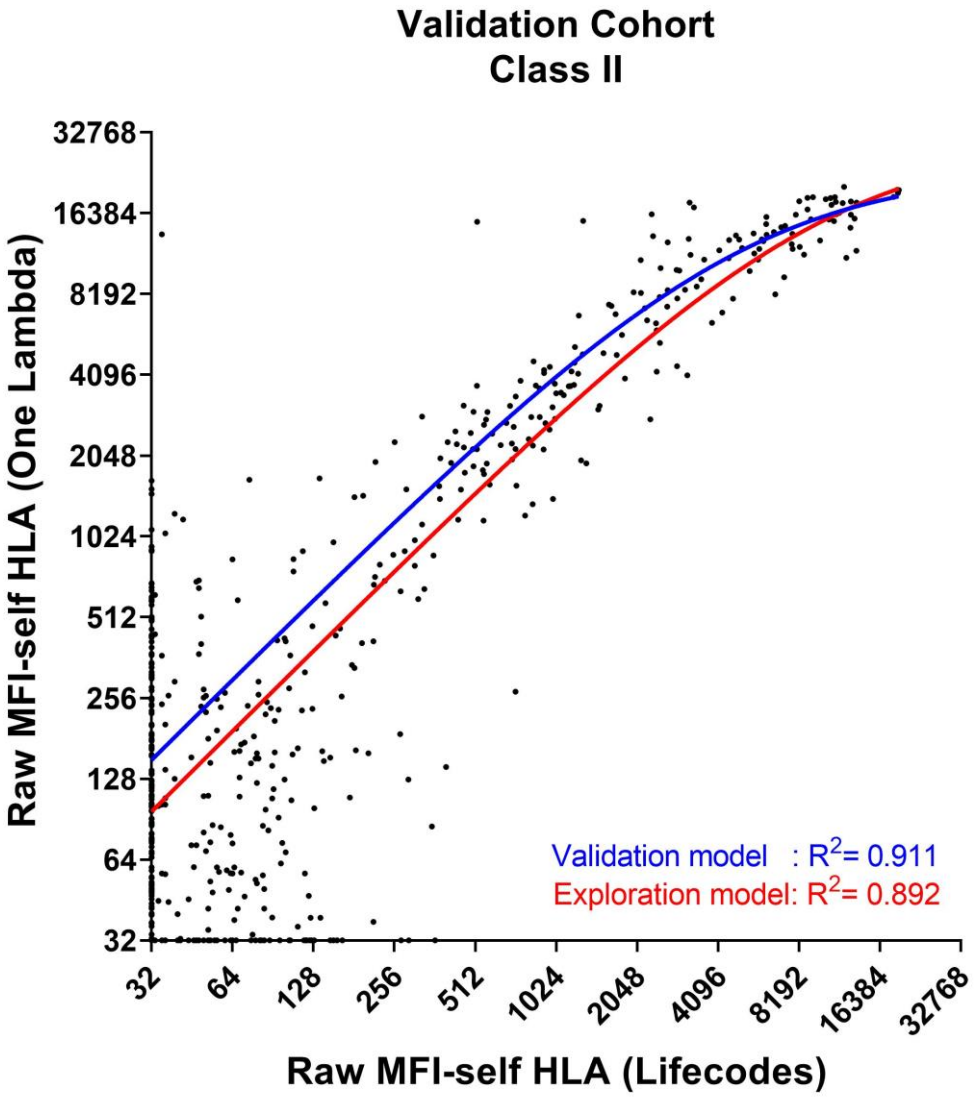
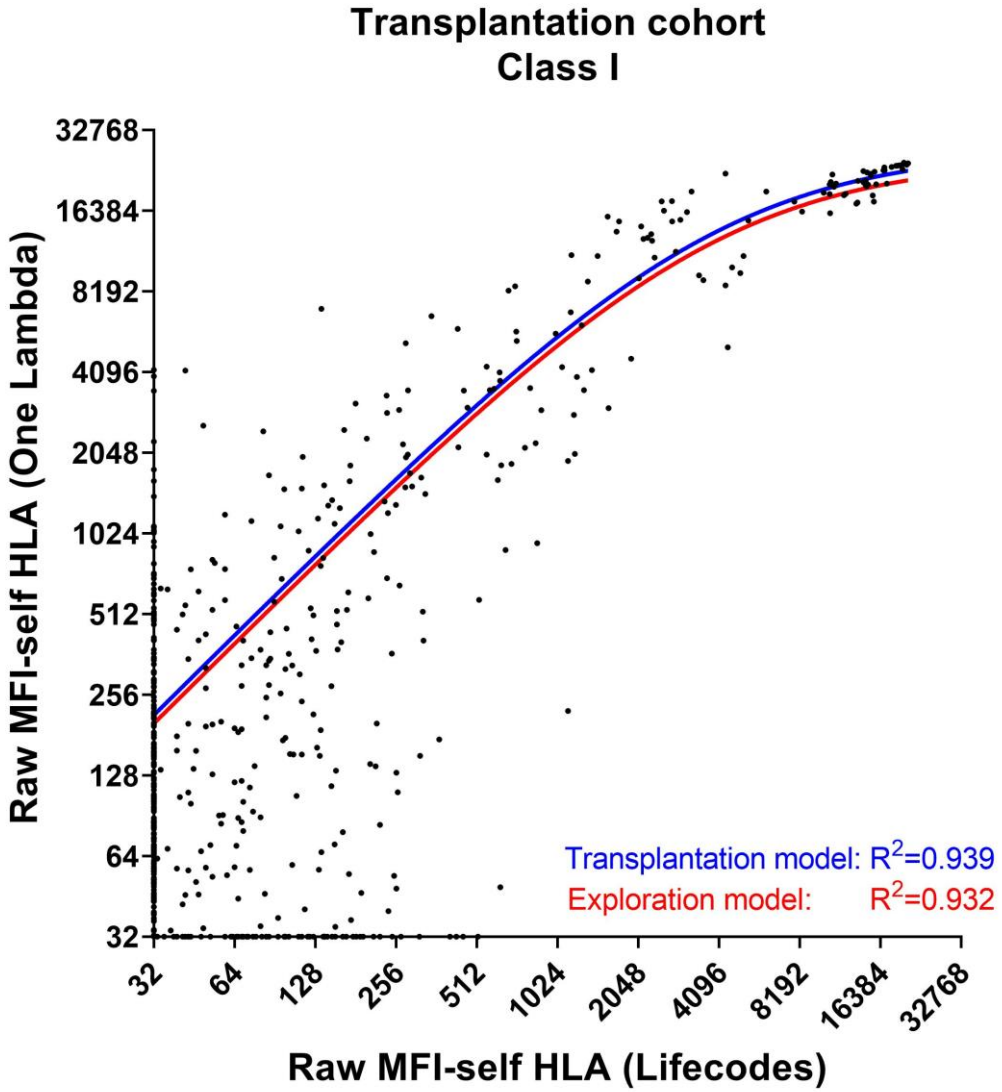
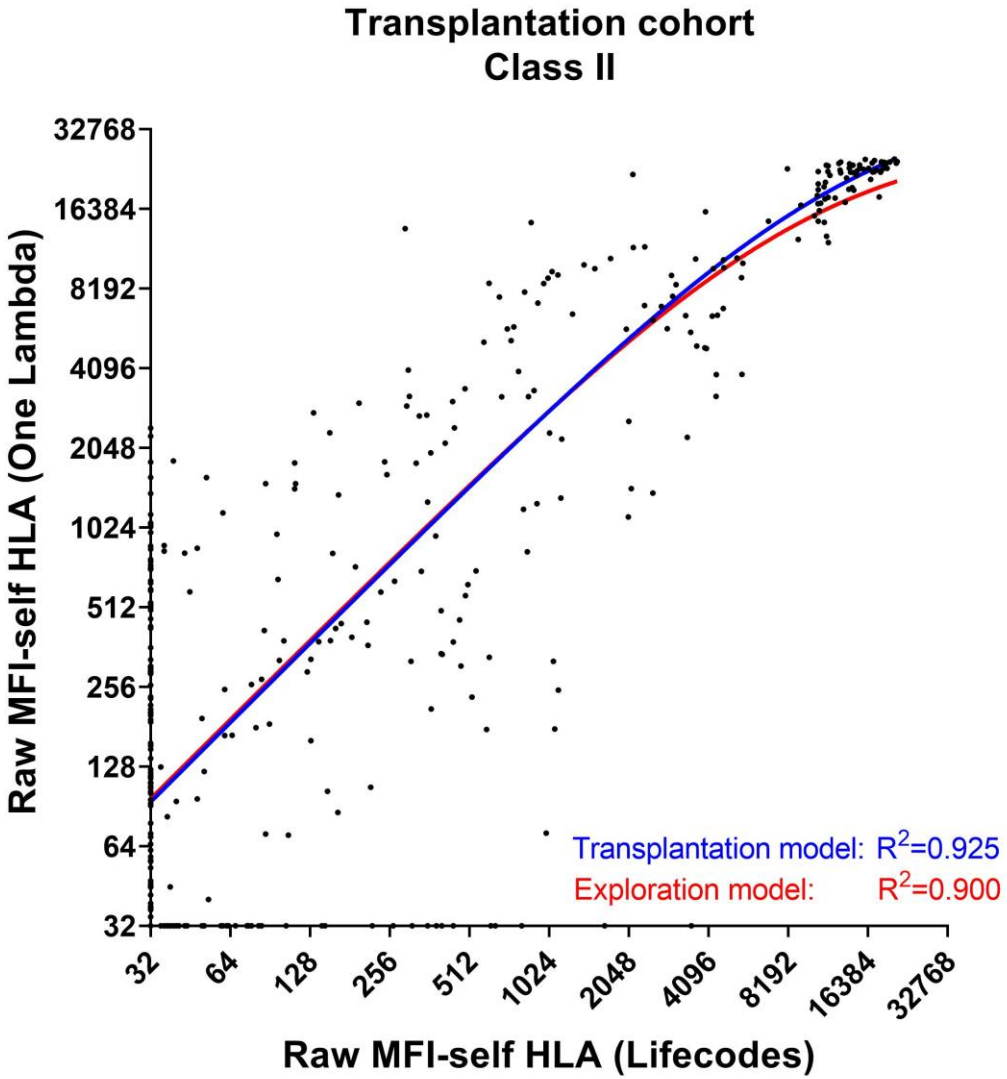




Consistency of the model was verified in a transplantation dataset





Disagreements in bead-specific reactivity assignments in transplantation dataset

- Post-tx serum from kidney transplant recipients (n=11)
- Bead-specific reactivity for a total of 87 HLA mismatches (HLA-A, -B, -C, -DR, -DQ, -DP) was assessed
- Overall agreement: 94 %

Cutoff: Lifecodes (1000 MFI) and interpolated OL MFI		MFI observed	
Patient #	Bead	LC	OL
7	A*01:01	1121	223
4	C*01:02	1260	6122
3	DRB1*07:01	1028	2328
9	DRB3*01:01	3529	32
7	DQB1*02:01-DQA1*05:01	293	13775

Cut-off	
LC	int OL (tx)
1000	3479
1000	6403
1000	3073
1000	3073
1000	4083

Bead-specific reactivity	
LC	OL (tx)
pos	neg
pos	neg
pos	neg
pos	neg
neg	pos

Cutoff: One Lambda (3000 MFI) and interpolated LC MFI		MFI observed	
Patient #	Bead	OL	LC
7	A*01:01	223	1121
11	C*03:04	2952	892
3	DRB1*07:01	2328	1028
9	DRB3*01:01	32	3529
7	DQB1*02:01-DQA1*05:01	13775	293
7	DQB1*05:02-DQA1*01:02	32	807

Cut-off	
OL	int LC (tx)
3000	621
3000	331
3000	738
3000	738
3000	662
3000	662

Bead-specific reactivity	
OL	LC (tx)
neg	pos
neg	pos
neg	pos
neg	pos
pos	neg
neg	pos

Karahan et al. HLA, 2023

Concluding remarks

- ✓ The current model (non-linear hyperbola with self HLA correction) will help to establish comparable MFI cutoffs for the two different kits, **at least on the population level** (**big data analyses)**

- ✓ For **individual patients**, variation in the two assays per specificity **precludes MFI conversion** from one vendor to the other

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