Can Consensus in Single Antigen Testing be Achieved?

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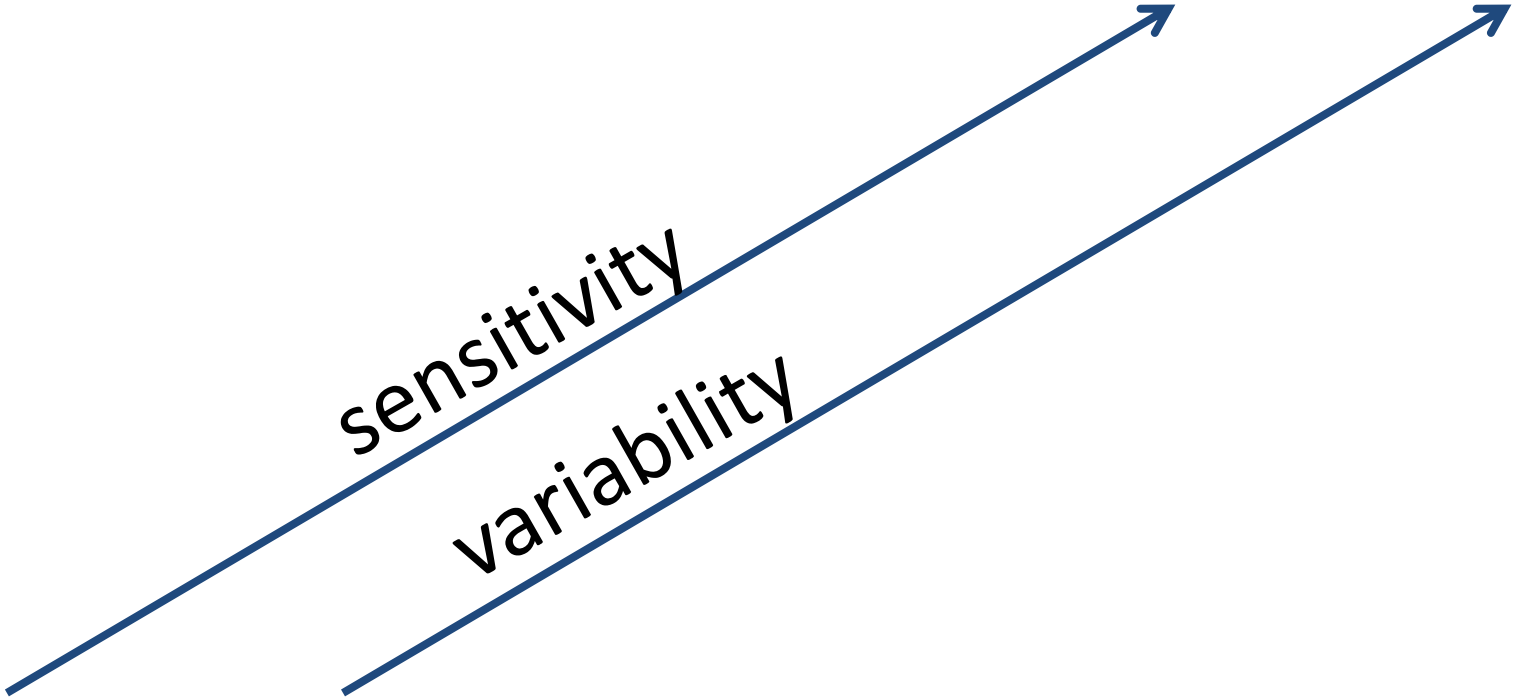
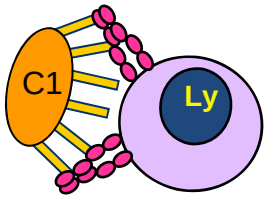
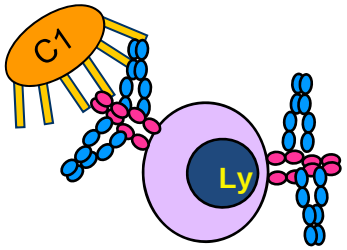
How can you agree that a test result is clinically relevant if you can’t agree on the test result?

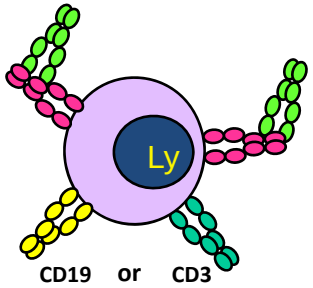

sensitivity
variability

Sources of Variability in HLA Antibody Detection - Cellular

Cytotoxicity (CDC) → **AHG Enhanced Cytotoxicity** → **Flow Cytometry**





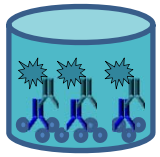


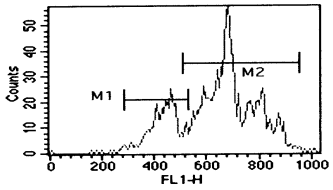
Cytotoxicity (CDC)	AHG Enhanced Cytotoxicity	Flow Cytometry
cell panel composition linkage disequilibrium number of washes autoantibodies condition of cells effects of drugs*	same as for CDC but also: source and lot of AHG can't use AHG for B cells	Fc receptors pronase – HLA expression serum:cell ratio condition of cells effects of drugs* variability in cytometers, fluorochromes, reagents

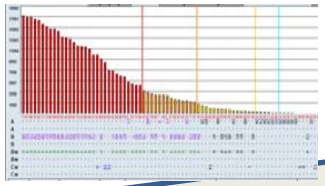
*steroids, rituximab, IVIg, ATG, etc.

Sources of Variability in HLA Antibody Detection – Solid Phase

ELISA → **Flow Bead Array** → **Luminex Bead Array**



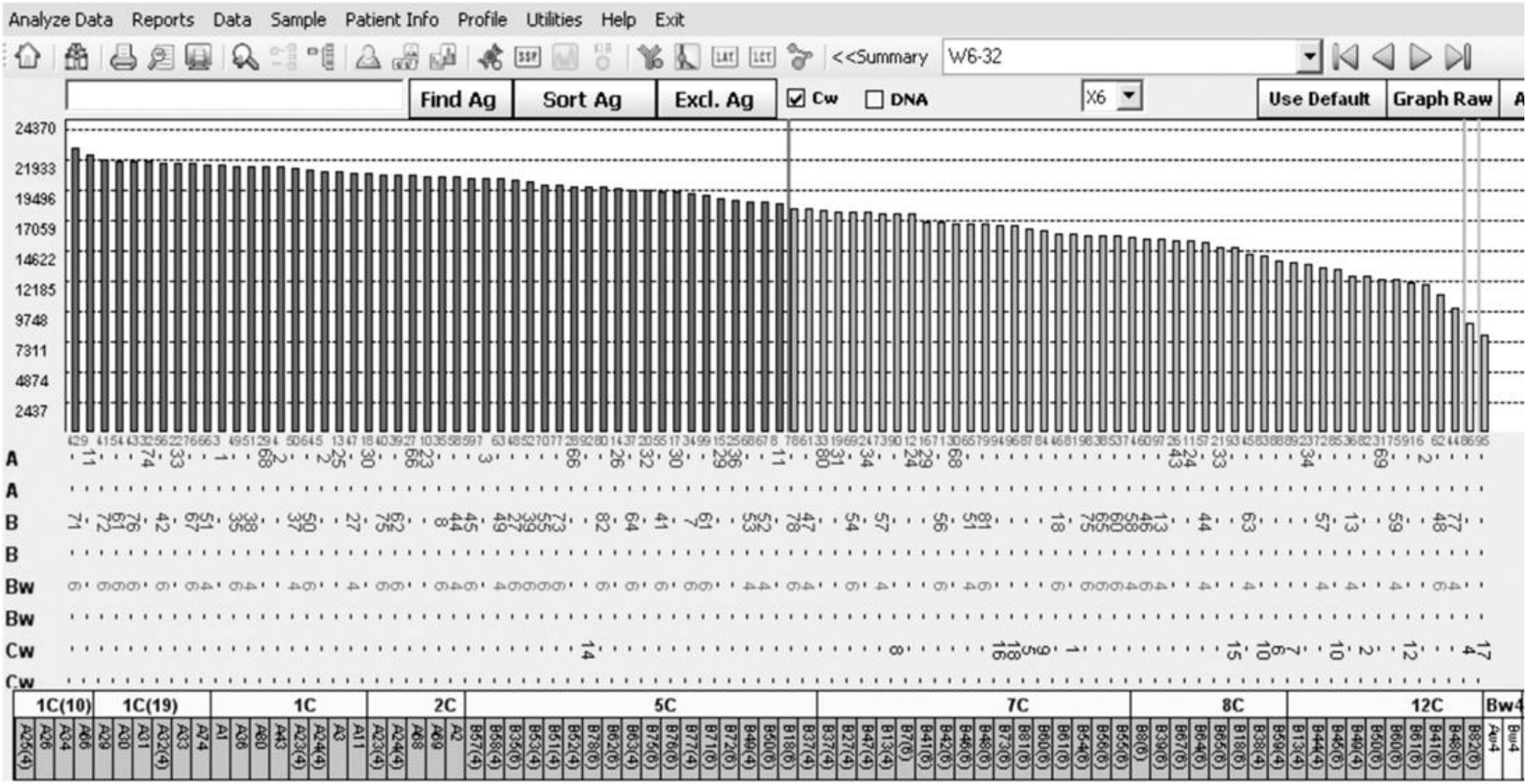




ELISA	Flow Bead Array	Luminex Bead Array
antigen source	epitopes spread across a few beads	epitopes spread across many beads
antigen density	antigen source	denatured antigen
Ig isotype and subclass	Ig isotype and subclass	interfering factors*
interfering factors*	interfering factors*	latex antibodies
no single antigen for class II – linkage disequilibrium	manual vs automation	antigen source
	number of washes	Ig isotype and subclass
	serum:bead ratio	manual vs automation
	variable antigen density	number of washes
		serum:bead ratio
		variable antigen density on bead
		exaggerated antigen density
		DQA and DPA chain effects

*IgM, complement C1, immune complexes, IVIg

variable antigen density on beads (staining with anti-class I mAb W6/32)

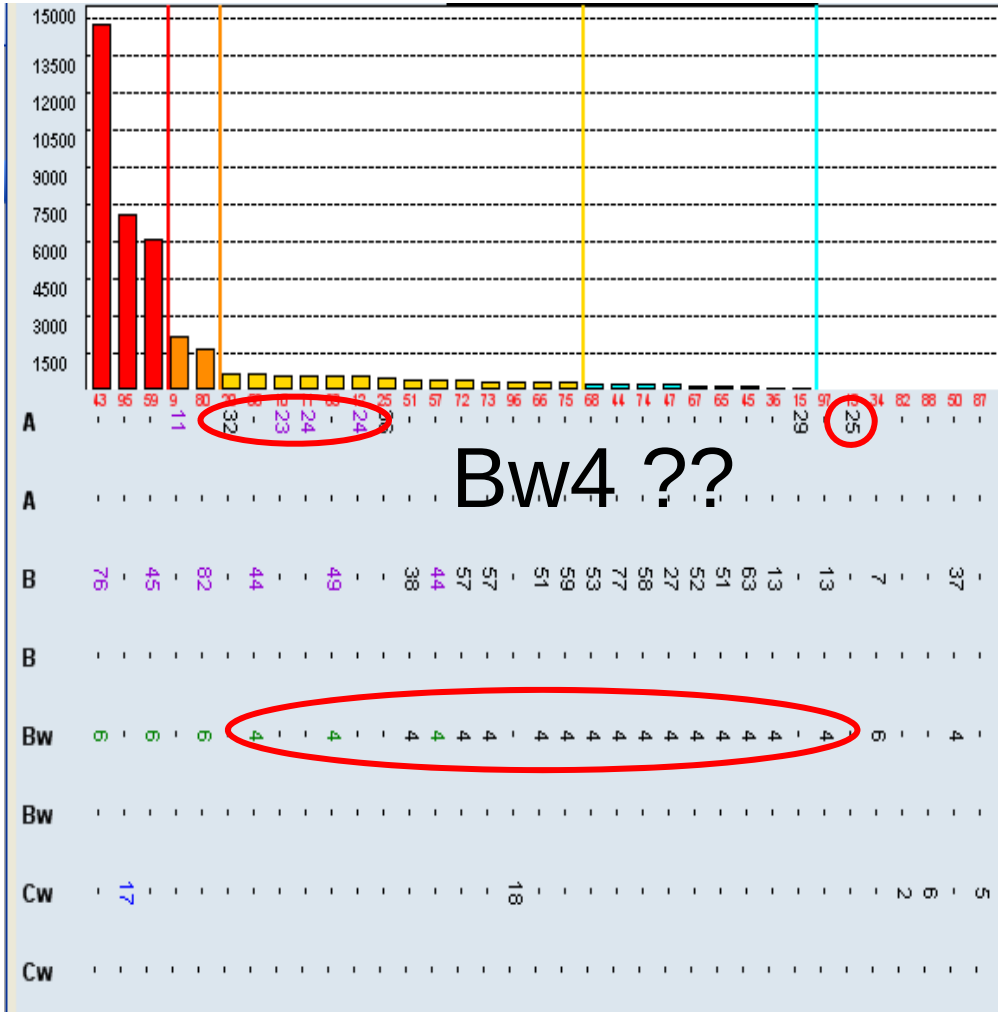


Note the range in fluorescence intensities. Y-axis indicates the MFI values.

- variability bead-to-bead, lot-to-lot
- low expression loci (Cw, DQ, DP) at high density on SAB
- saturation of beads with high titer serum

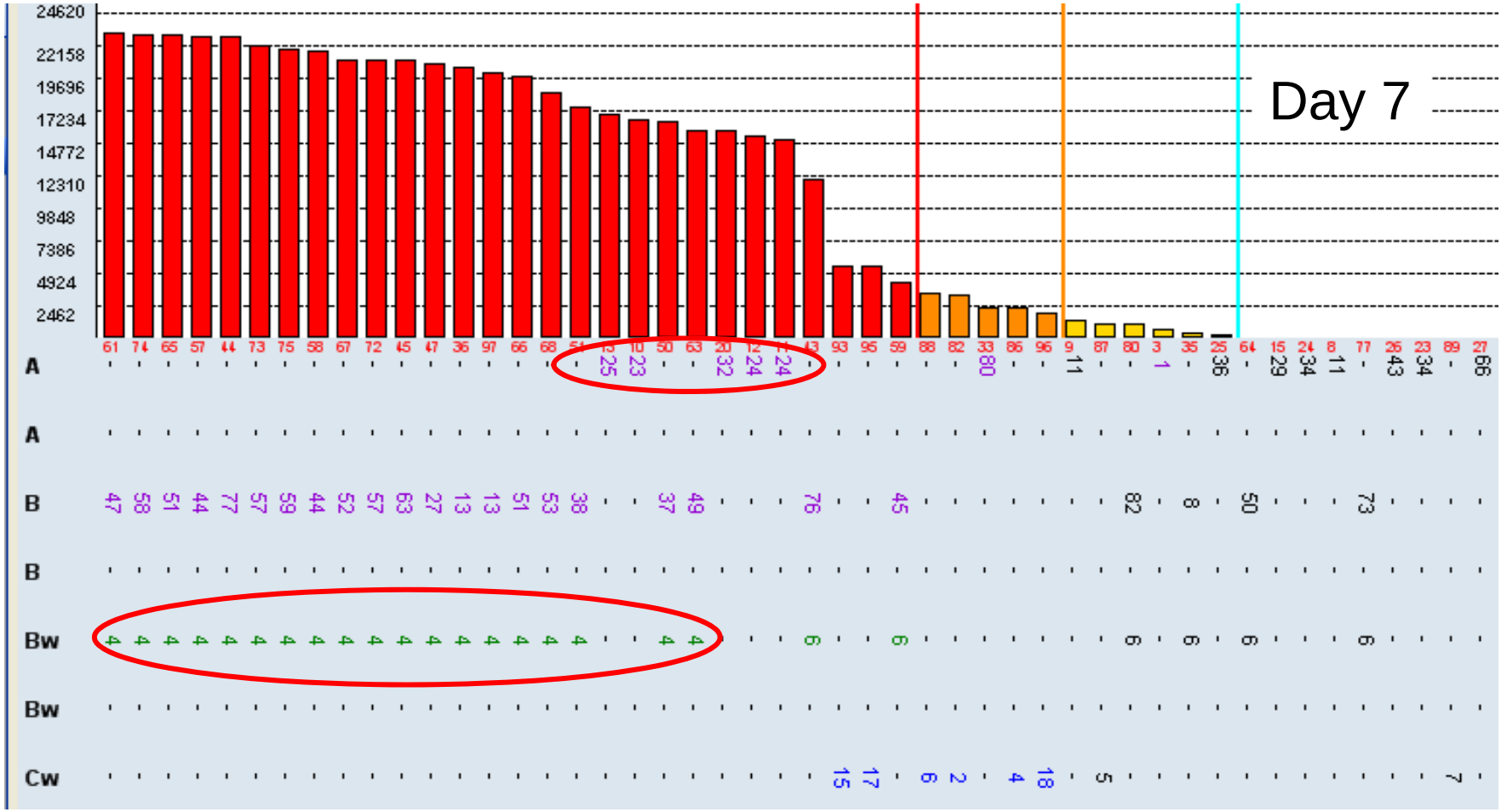
Bray, Gebel. Curr Opin Organ Transp. 2009;14:392.

epitope spread across many beads



	dMCF(T/B)	dMESF(T/B)
Pre-transplant	10 / 20	537 / 548

Bob Bray, Emory



Serum date	dMCF(T/B)	dMESF(T/B)
pre-txp	10 / 20	537 / 548
day 7 post-txp	281 / 294	32,389 / 66,439

Bob Bray, Emory

Influence of Test Technique on Sensitization Status of Patients on the Kidney Transplant Waiting List.

tested pretransplant sera of 534 patients using CDC, ELISA and SAB (1λ)

Table 1: Detection of HLA antibodies using different test techniques in patients on the kidney transplant waiting list

	Positive patients							
		ELISA				SAB		
	CDC	Class I	Class II	Class I or II	ELISA or CDC	Class I	Class II	Class I or II
All patients[1] (n = 534)	28 (5%)	48 (9%)	54 (10%)	73 (14%)	78 (15%)	392 (73%)	246 (46%)	435 (81%)
Without history of immunization (n = 133)	2 (2%)	0 (0%)	1 (1%)	1 (1%)	3 (2%)	93 (70%)	45 (34%)	102 (77%)
With history of immunization (n = 286)	22 (8%)	39 (14%)	47 (16%)	61 (21%)	63 (22%)	221 (77%)	150 (52%)	240 (84%)

[1]In 115 patients, the information on immunization history was not available.
Luminex cutoff MFI ≥1000.

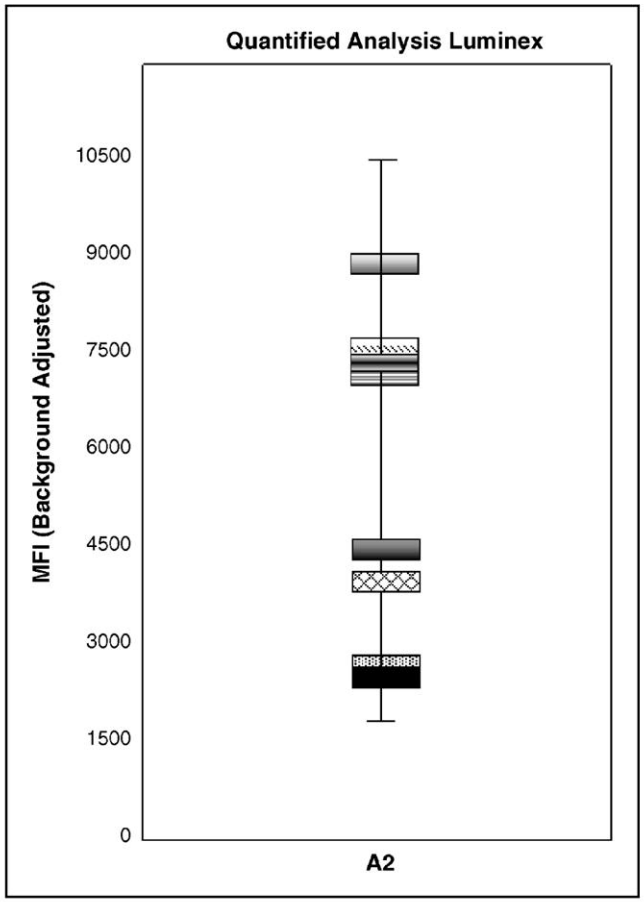
antibodies in non-immunized patients:

- 25% >5,000 MFI (up to 14,400)
- some against common antigens (A*2402, B*0801, etc.)
- most not present in SAB assay by other vendor (Lifecodes)
- most not present on PRA bead assay (natural antigens – not recombinant)

Gombos, et al. Am J Transp. 2013; 13:2075

Strategies for human leukocyte antigen antibody detection.

8 labs tested one serum by same method, looked at one bead (A*02:01)

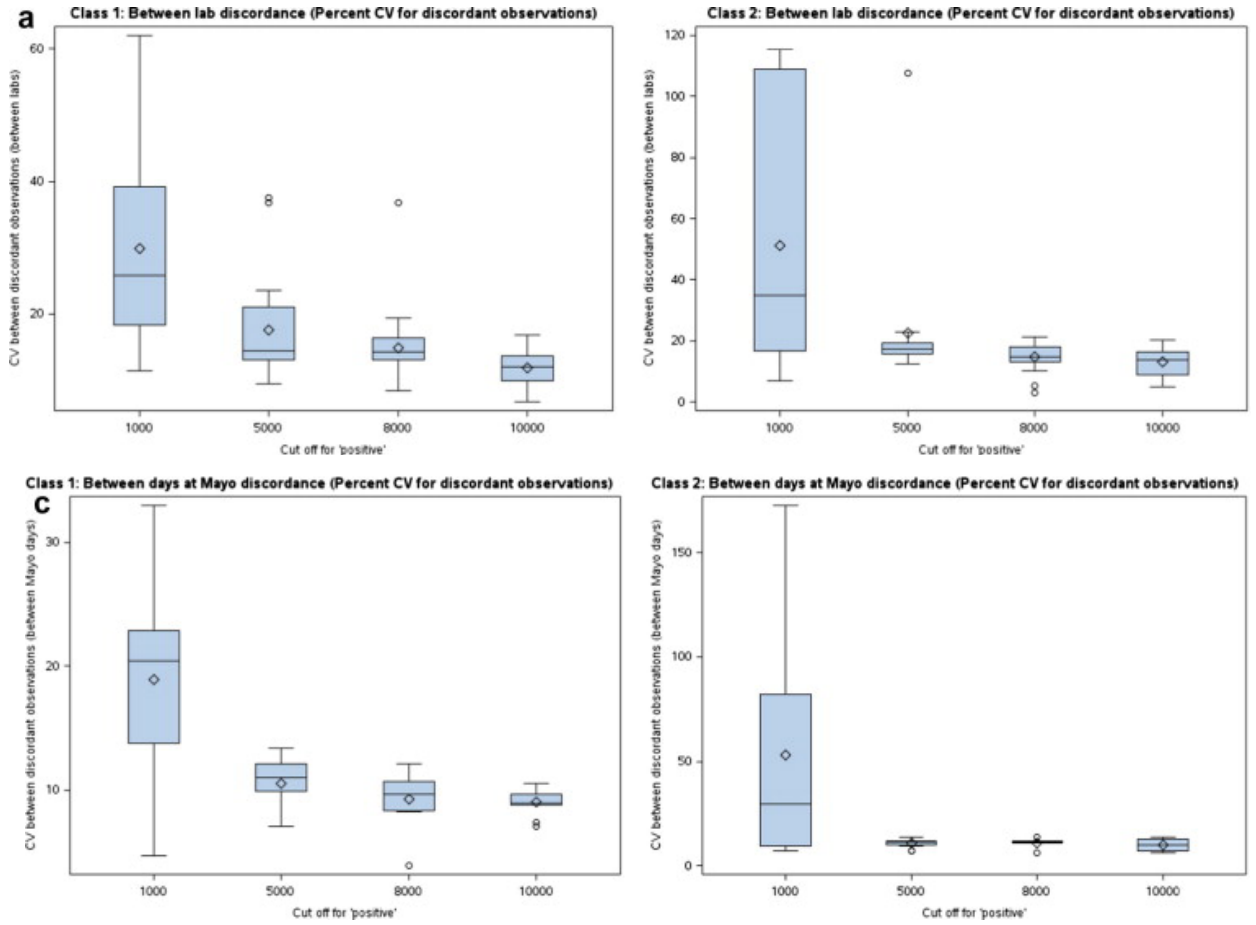


Bars indicate the MFI (median fluorescence intensity) reported by each of the eight participating laboratories for the single-antigen bead containing A∗0201. Note: although all laboratories agreed on the antigen assignment (HLA- A∗02), there was a wide range of values reported.

Bray, Gebel. Curr Opin Organ Transp. 2009;14:392.

Inter and intra laboratory concordance of HLA antibody results obtained by single antigen bead based assay.

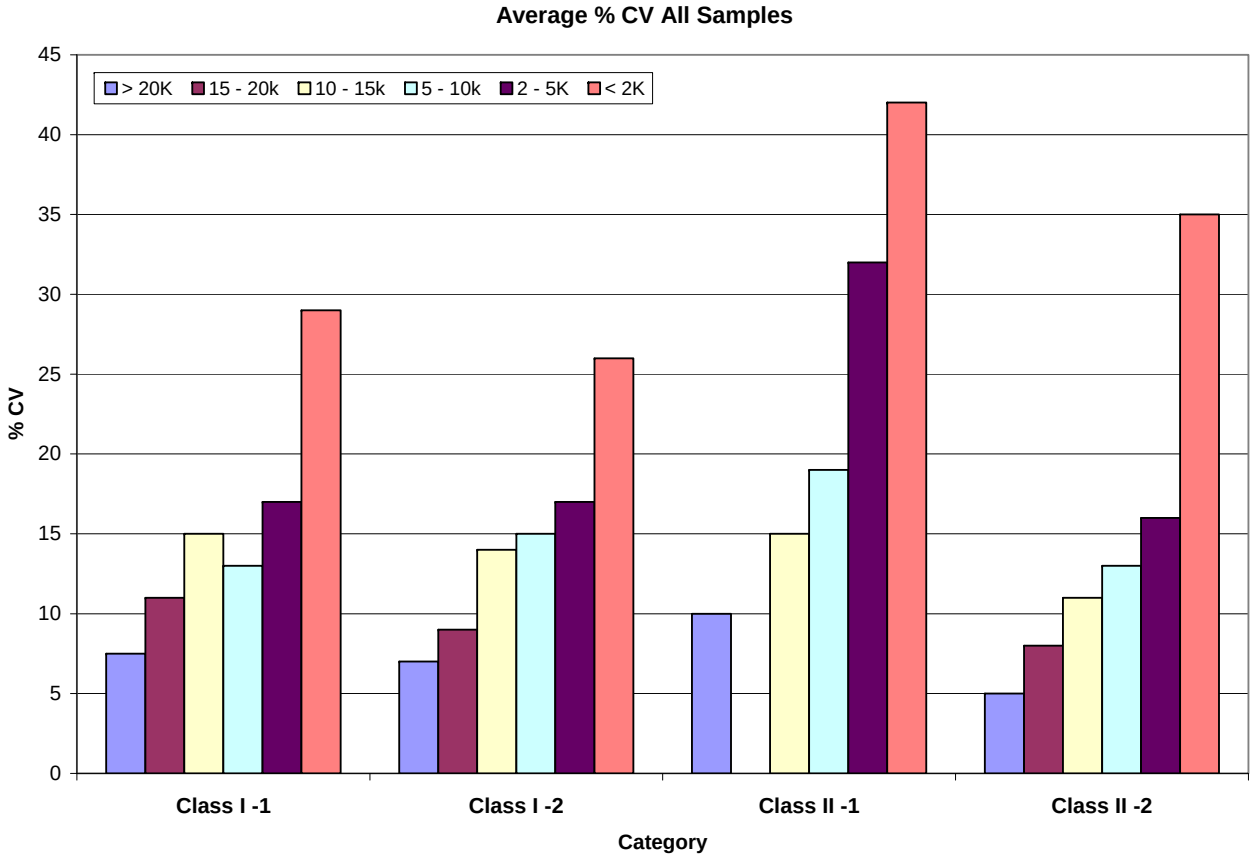
4 labs used class I and II SAB from a single manufacturer to test 10 patient sera
single lot, same SOP, looked at 4 different MFI cutoffs
originating lab also tested same samples on four consecutive days



Gandhi et al. Hum Immunol. 2013;74:310

Standardization of Microparticle-Based, Solid Phase, HLA Antibody Identification Assays

SENDOUT ONE
- 21 Laboratories enrolled (UK, US, France, Germany, Italy) (data from 19 using one vendor)
- Testing performed on 5 well characterized sera using laboratory's standard methods
- First round of data analysis to identify parameters leading to non-consensus
- SENDOUT TWO
- fewer labs (N=8) using a standardized protocol and same lot of reagents to test 4 sera

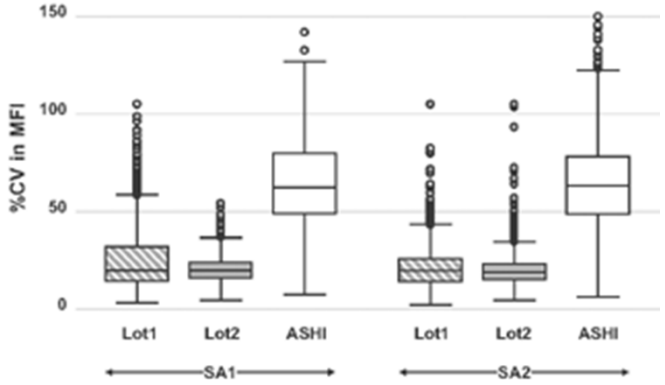


- %CV decreases with standard protocol
- Factors NOT correlating with results:
  - Filter vs flick/spin
  - Washes – 3 to 5; Avg: 3.5
  - serum-to-cell ratio
- still differences between labs
- converting to SFI?
- lower MFI – higher CV

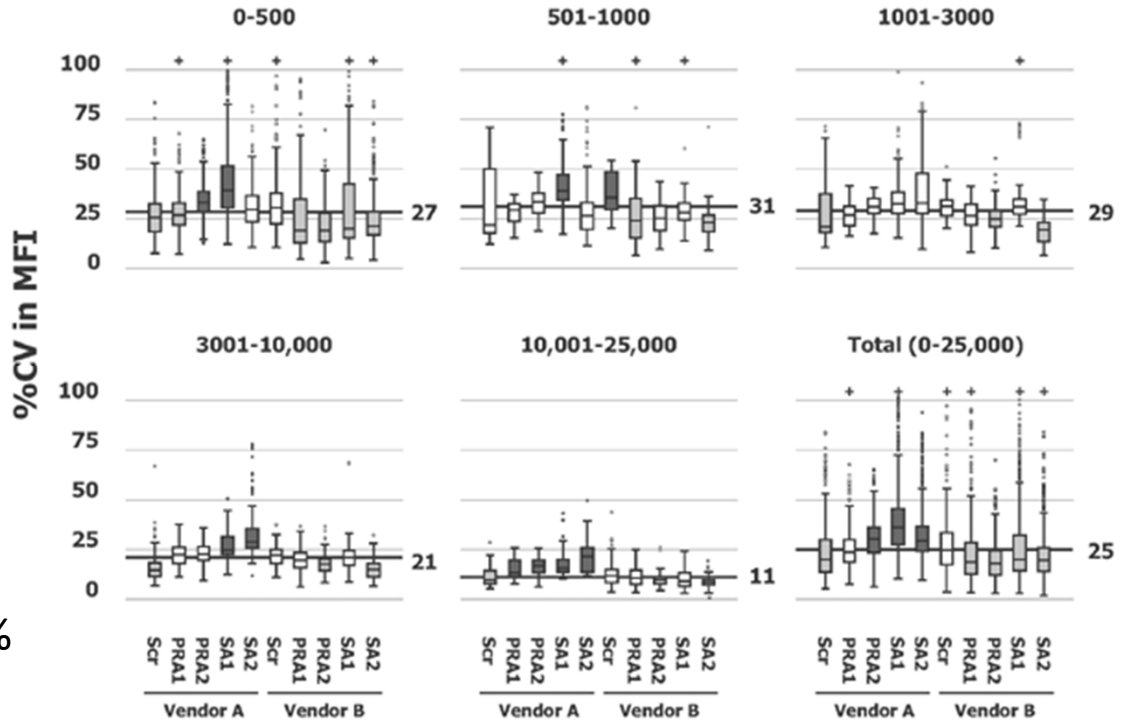
16th IHWS Project – Bray and Gebel

Comprehensive Assessment and Standardization of Solid Phase Multiplex-Bead Arrays for the Detection of Antibodies to HLA.

CTOT: Clinical Trials in Organ Transplantation Antibody Core Laboratories
7 labs used 10 kits from 2 manufacturers to test 20 reference sera



SOP vs iHOP



- single SOP reduces variation
- still have manufacturer variation
- most variation at low MFI
- MFI cutoff of 1000-1500 had >90% agreement in antigen assignment
- overall CV=25% {so 50% change in MFI (2xCV) means real change in Ab level}

Reed, et al. Am J Transp. 2013; 13:1859

Antibody Consensus Conference – May 2012 recommendations toward standardization

- standardize critical components
  - HLA source and preparation method
  - panel composition
    - appropriate allele coverage, including DQA, DPA, DPB
  - antigen density on bead
  - antigen integrity
    - native vs denatured
  - anti-human Ig detection reagents

Tait, et al. Transplantation 2013;95:19

Antibody Consensus Conference – May 2012 recommendations toward standardization

- standardize operating procedure
  - type of plastic trays used
    - V-bottom vs. U-bottom
  - serum volume to bead ratio
  - washing methods
    - spin/flick vs. filter tray
  - vortexing methods
  - unified approach to sample preparation to minimize interference
    - EDTA, DTT, hypotonic dialysis, spin column
  - automated processing equipment

Tait, et al. Transplantation 2013;95:19

Antibody Consensus Conference – May 2012 recommendations toward standardization

- standardize interpretation and reporting
  - calibration of fluoroanalyzers and flow cytometers using control particles
  - use of standard fluorescent intensity (SFI) and MESF
  - defined reporting algorithms
  - background normalization
  - reports should include assay type, criteria for positive/negative results, Ig isotype, serum modification, factors that affect test values and interpretation

Tait, et al. Transplantation 2013;95:19

Antibody Consensus Conference – May 2012 recommendations toward standardization

- standardized reference reagents should be developed
  - repository of well-characterized HLA polyclonal and monoclonal reference reagents
    - to all HLA class I and II antigens
    - different titers and isotypes
    - validated in national and international exchange
    - validated on cell panels and solid phase immunoassays
    - validated for all available techniques
  - ongoing technique and reagent validation
  - monitoring interlaboratory variability
  - reproducible quantification of fluorescence values

Tait, et al. Transplantation 2013;95:19


thank you
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