Assays to detect and identify HLA antibodies

An overview

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No financial relationships related to this presentation

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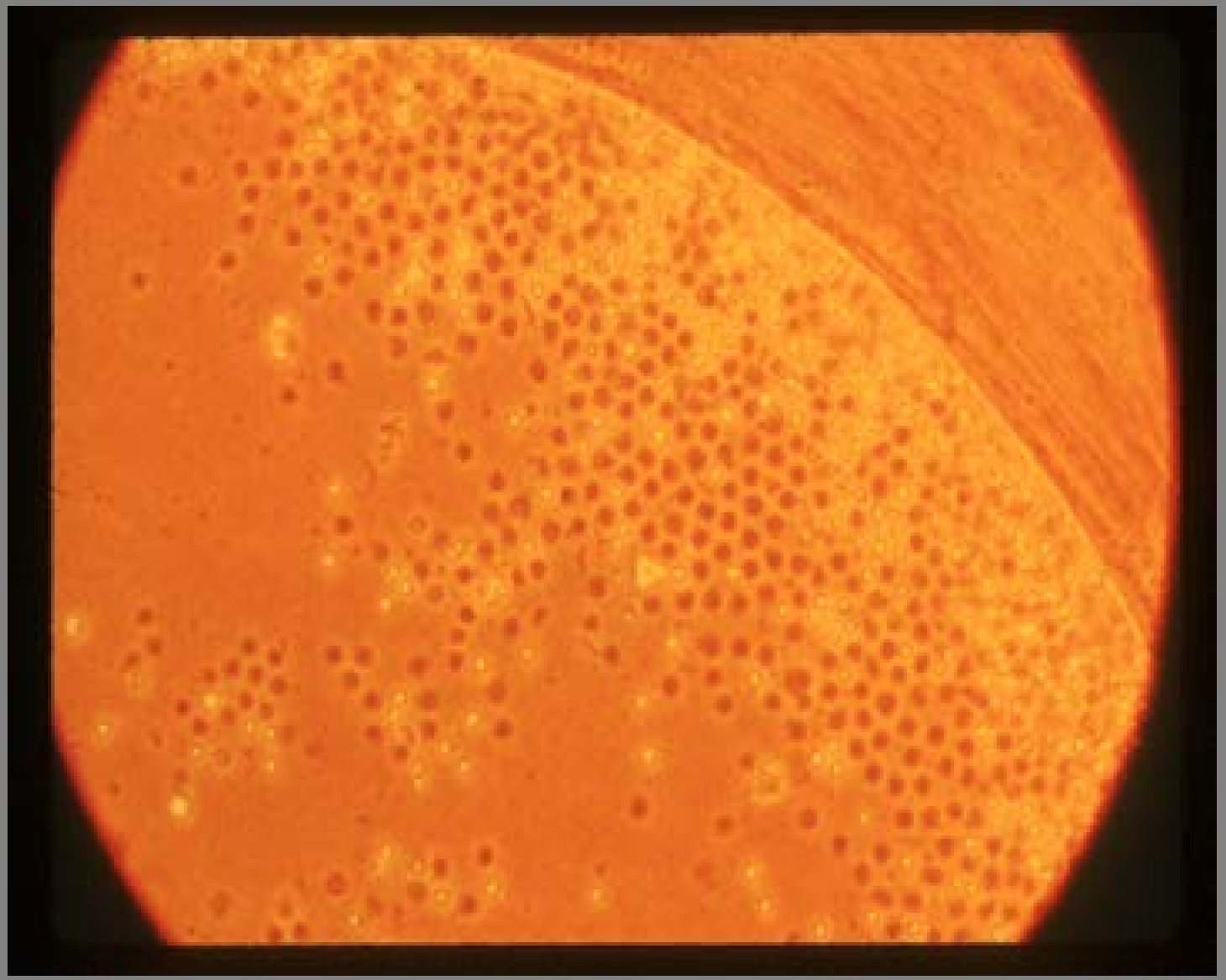
The presentation *does not* include discussion of "off-label" or "investigational" use.



Problems

Sensitivity not optimal (false negatives)

Specificity (false positives)

Panel composition

Viability

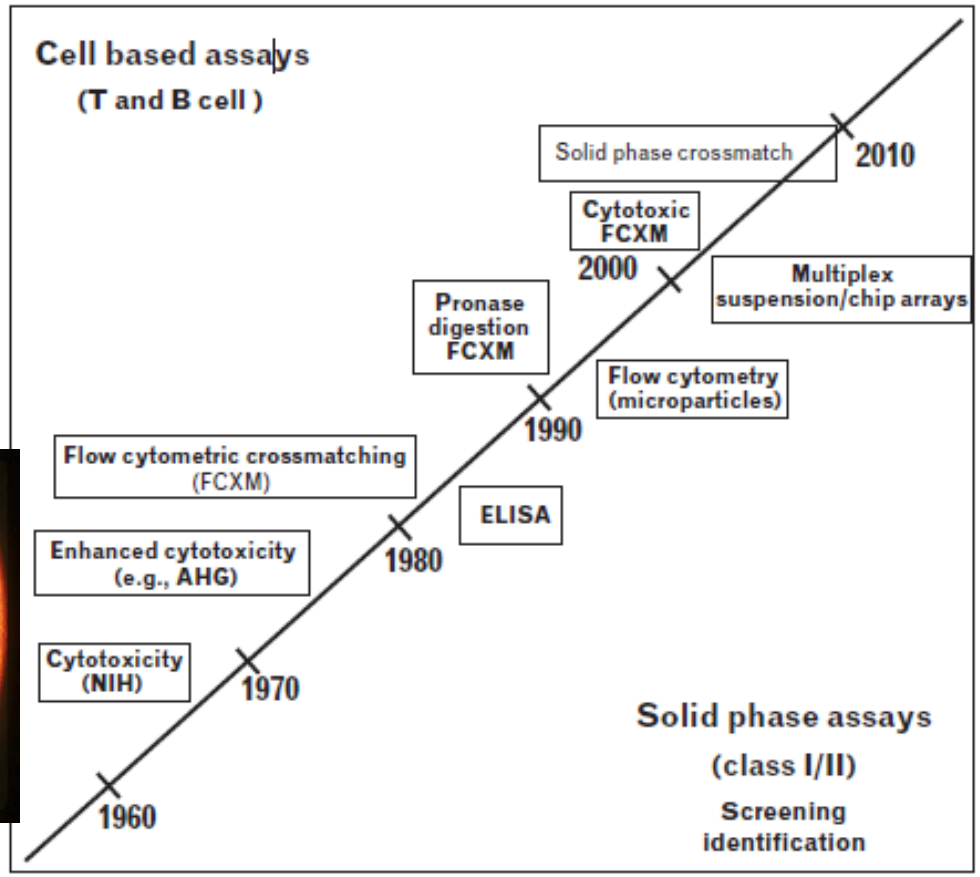
Typically restricted to class I antibodies

The evolution and clinical impact of Human Leukocyte Antigen technology

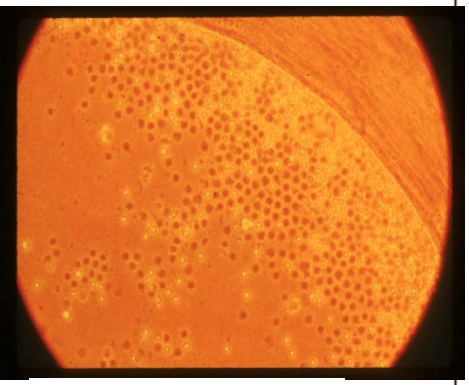
Howard M. Gebel and Robert A. Bray

Current Opinion in Nephrology and Hypertension 2010, 19:598–602

Figure 1 Evolution of human leukocyte antigen antibody testing

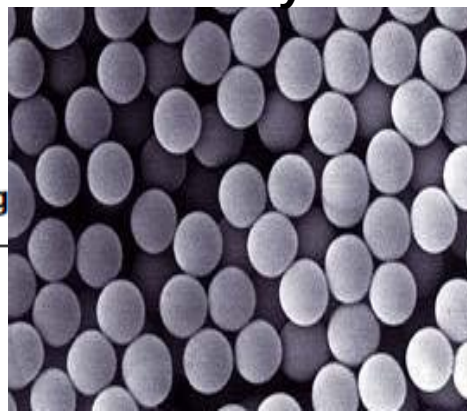


- Cumbersome cell-based assays for XM, anibody ID and HLA typing.



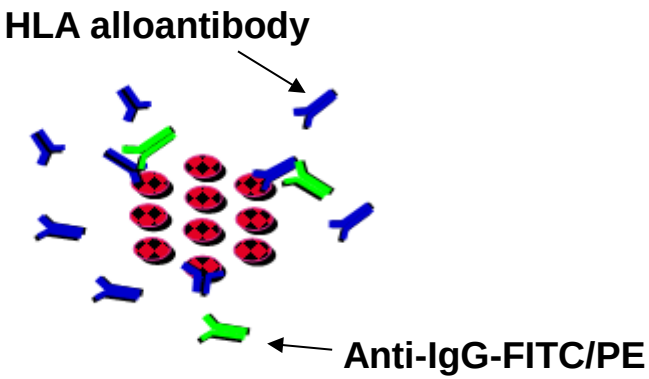
Cytotoxicity

Solid Phase Assays

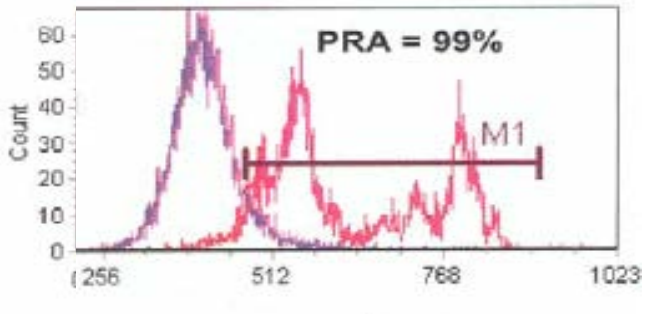


- Highly sensitive and specific bead-based assays for antibody ID and Molecular-based HLA typing.

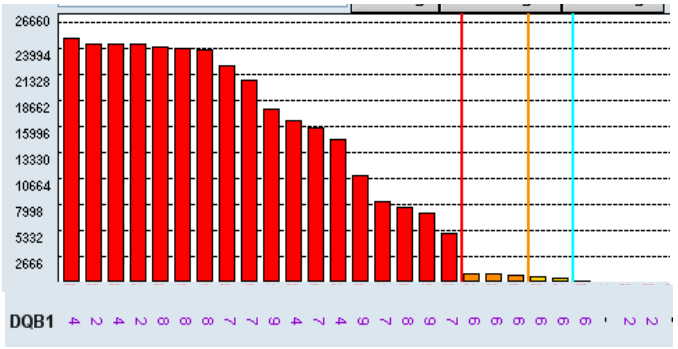
Solid Phase HLA antibody detection



Flow Cytometry



Suspension Arrays



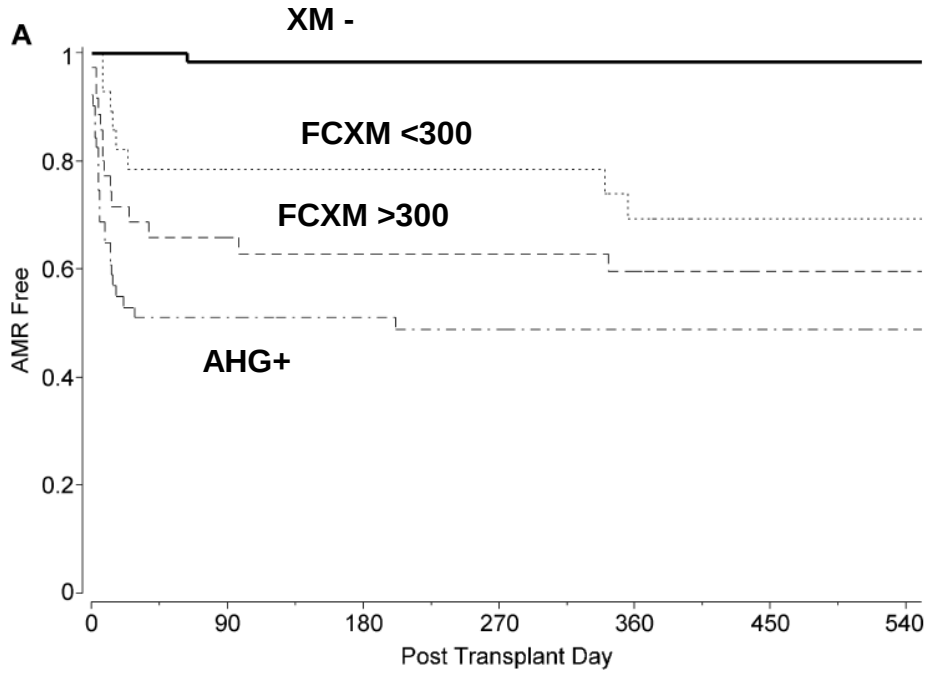
Adapted from Gebel and Bray. Transplantation Reviews 20: 189-194, 2006

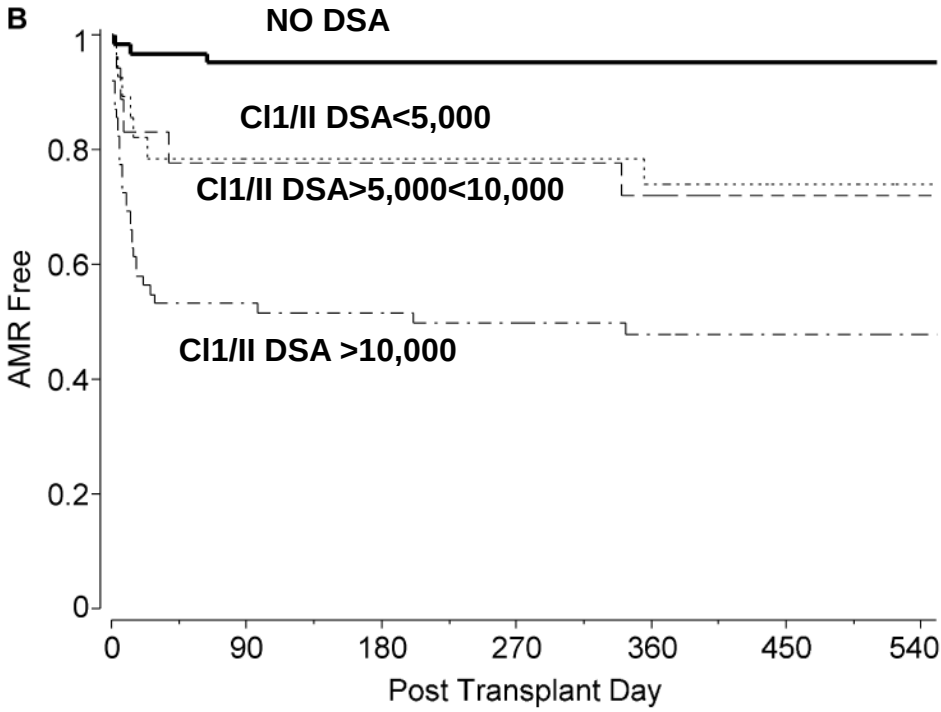
American Journal of Transplantation 2010; 10: 582–589
Wiley Periodicals Inc.

doi: 10.1111/j.1600-6143.2009.02985.x

Baseline Donor-Specific Antibody Levels and Outcomes in Positive Crossmatch Kidney Transplantation

J. M. Gloor[a,*], J. L. Winters[b], L. D. Cornell[b], L. A. Fix[c], S. R. DeGoey[b], R. M. Knauer[b], F. G. Cosio[a], M. J. Gandhi[b], W. Kremers[d] and M. D. Stegall[c]





American Journal of Transplantation 2013; 13: 1859–1870
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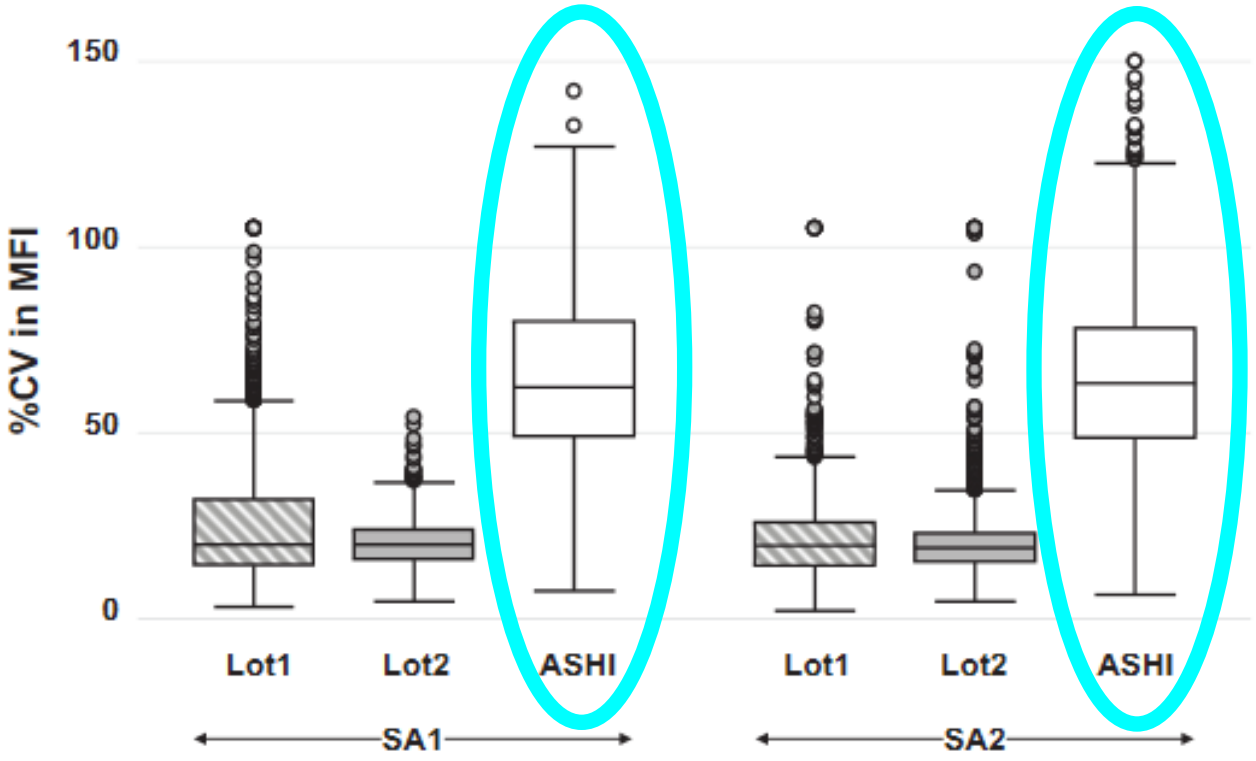
Comprehensive Assessment and Standardization of Solid Phase Multiplex-Bead Arrays for the Detection of Antibodies to HLA

E. F. Reed[1,*], P. Rao[1], Z. Zhang[1], H. Gebel[2], R. A. Bray[2], I. Guleria[3], J. Lunz[4], T. Mohanakumar[5], P. Nickerson[6], A. R. Tambur[7], A. Zeevi[4], P. S. Heeger[8] and D. Gjertson[1]



Why laboratories don't get identical results when testing the same sample

1) Vendors
2) Antigen source/type
    a) Native
    b) Recombinant
3) Antigen expression
    a) Conformationally correct
    b) Amount
4) Interfering factors
5) Reagents
6) Tech-tech variation
7) Protocols
8) Assay conditions

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

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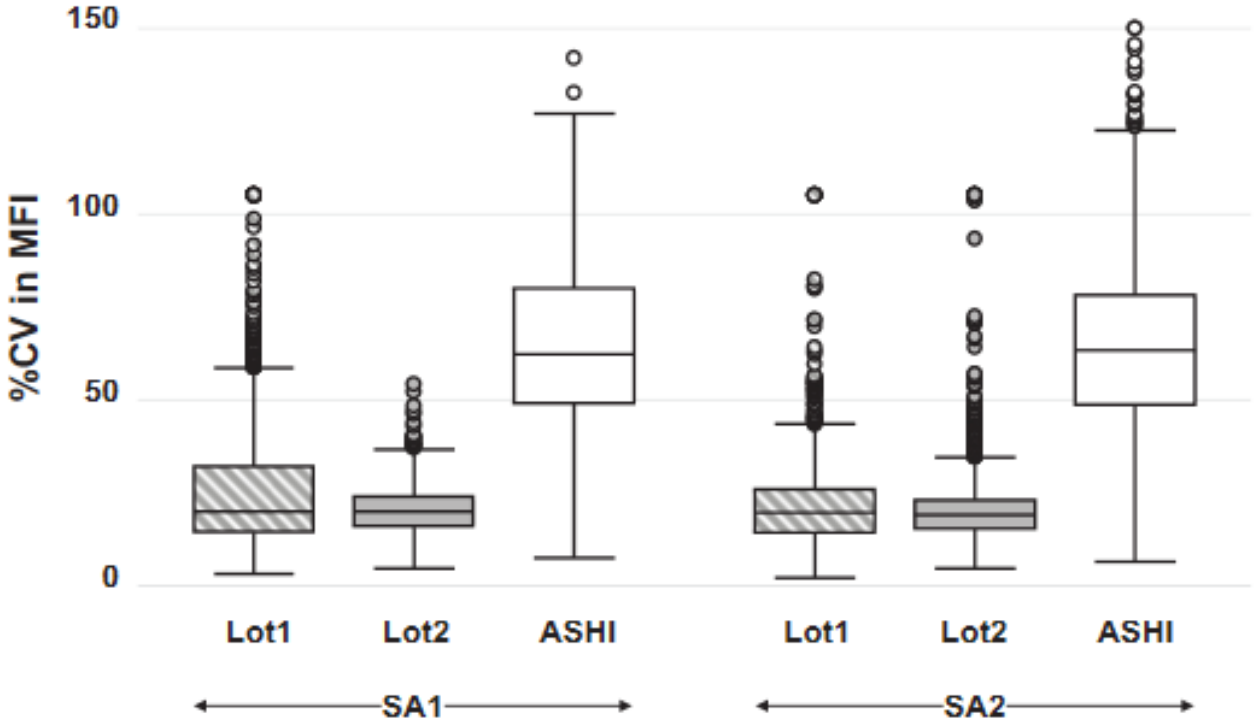


Interfering Factors

Detection of Immunoglobulin G Human Leukocyte Antigen-Specific Alloantibodies in Renal Transplant Patients Using Single-Antigen-Beads is Compromised by the Presence of Immunoglobulin M Human Leukocyte Antigen-Specific Alloantibodies

Vasilis Kosmoliaptsis,[1,2] J. Andrew Bradley,[2] Sarah Peacock,[1] Afzal N. Chaudhry,[3] and Craig J. Taylor[1,4]

Transplantation 87:813-820; 2009.

Naturally occurring interference in Luminex® assays for HLA–specific antibodies: Characteristics and resolution

Andrea A. Zachary [a,*], Donna P. Lucas [a], Barbara Detrick [b], Mary S. Leffell [a]

[a] *Departments of Medicine, The Johns Hopkins University School of Medicine, Baltimore, Maryland, USA*
[b] *Department of and Pathology, The Johns Hopkins University School of Medicine, Baltimore, Maryland, USA*

Hu. Immunol 70:496-501, 2009.

INTERNATIONAL JOURNAL OF
IMMUNOGENETICS

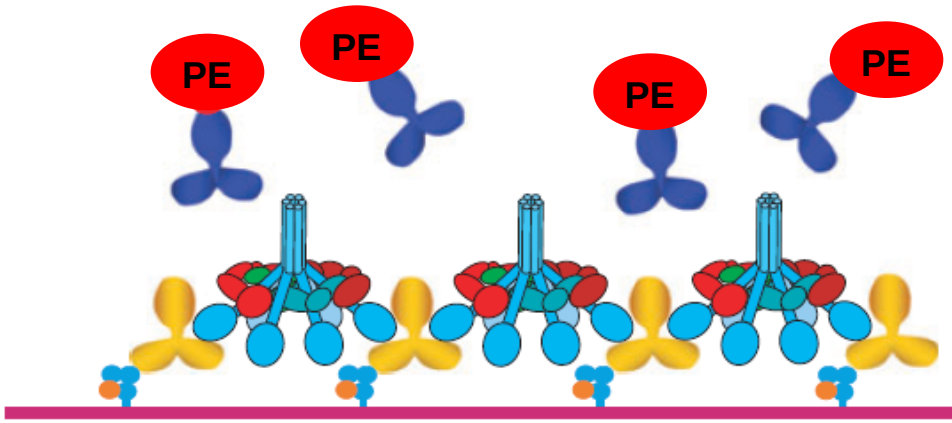
Review

doi: 10.1111/j.1744-313X.2012.01147.x

The complement-mediated prozone effect in the Luminex single-antigen bead assay and its impact on HLA antibody determination in patient sera

C. Weinstock* & M. Schnaidt†

International Journal of Immunogenetics, 2013, **40**, 171–177



PE ~250,000 d

Figure 2. The complement-mediated prozone effect: C1 is thought to bind between the HLA antibodies and to interfere with binding of the anti-IgG detection antibodies.

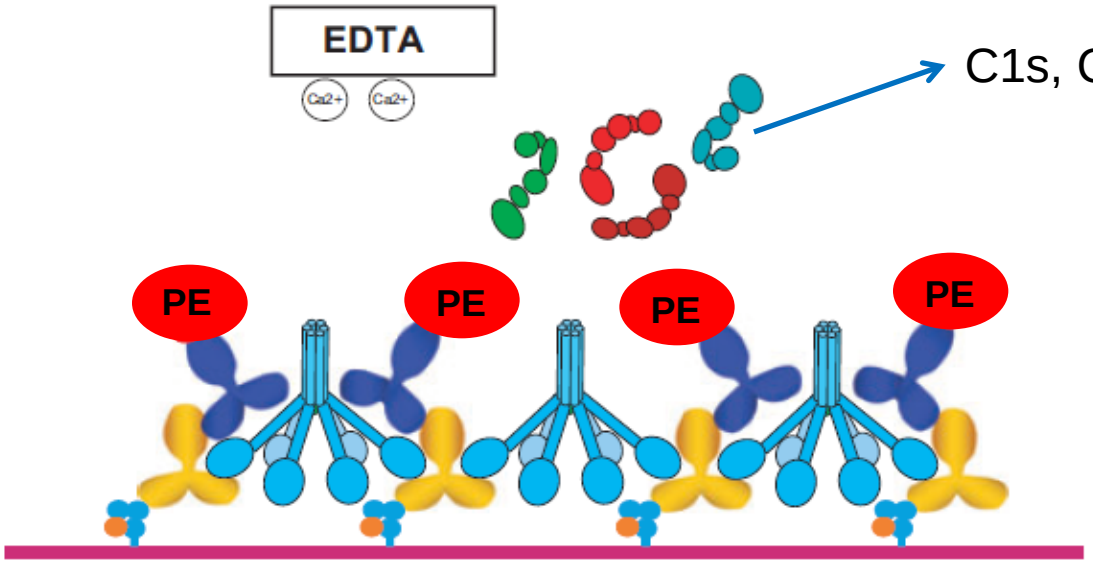


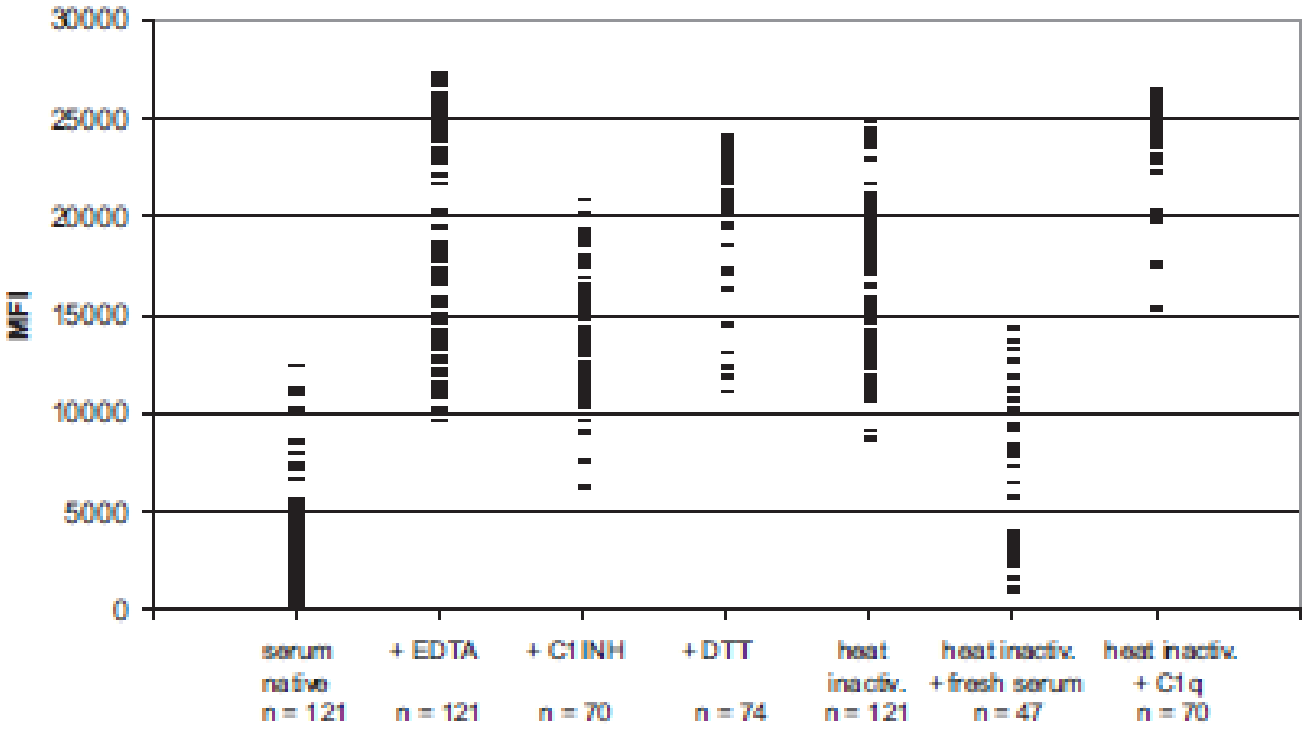
Figure 3. The complement-mediated prozone effect: EDTA binds Ca^{2+} ions, resulting in the dissociation and cleavage of the C1s-C1r-C1r-C1s tetramer. PE-conjugated anti-IgG antibodies can now bind to the HLA antibodies.

International Journal of Immunogenetics, 2013, **40**, 171–177

HLA Antibody Specification Using Single-Antigen Beads—A Technical Solution for the Prozone Effect

Martina Schnaidt,[1,4] Christof Weinstock,[2] Marija Jurisic,[1] Barbara Schmid-Horch,[1] Andrea Ender,[3] and Dorothee Wernet[1]

Transplantation 2011;92: 510–515)



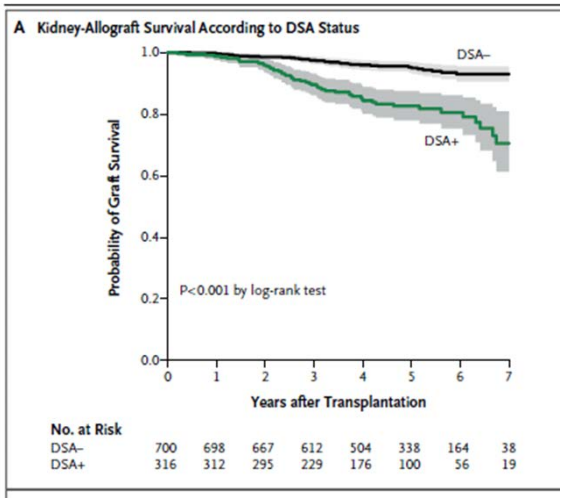
serum from one patient containing antibodies against HLA class II (B) were tested after 1:10 dilution, confirming the presence of a prozone effect for some specificities. In parallel, EDTA plasma from the same venipuncture was tested. MFI, mean fluorescence intensity; EDTA, ethylenediaminetetraacetic acid.

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Complement-Binding Anti-HLA Antibodies and Kidney-Allograft Survival

Alexandre Loupy, M.D., Ph.D., Carmen Lefaucheur, M.D., Ph.D., Dewi Vernerey, M.P.H., Christof Prugger, M.D., Jean-Paul Duong van Huyen, M.D., Ph.D., Nuala Mooney, Ph.D., Caroline Suberbielle, M.D., Ph.D., Véronique Frémeaux-Bacchi, M.D., Ph.D., Arnaud Méjean, M.D., François Desgrandchamps, M.D., Dany Anglicheau, M.D., Ph.D., Dominique Nochy, M.D., Dominique Charron, M.D., Ph.D., Jean-Philippe Empana, M.D., Ph.D., Michel Delahousse, M.D., Christophe Legendre, M.D., Denis Glotz, M.D., Ph.D. Gary S. Hill, M.D.,* Adriana Zeevi, Ph.D., and Xavier Jouven, M.D., Ph.D.



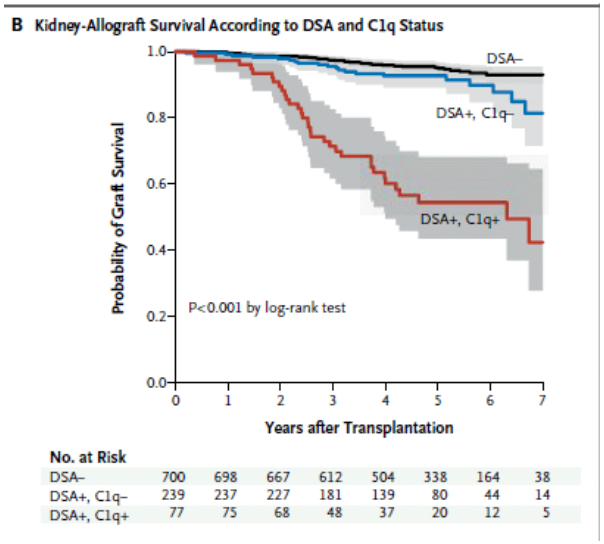
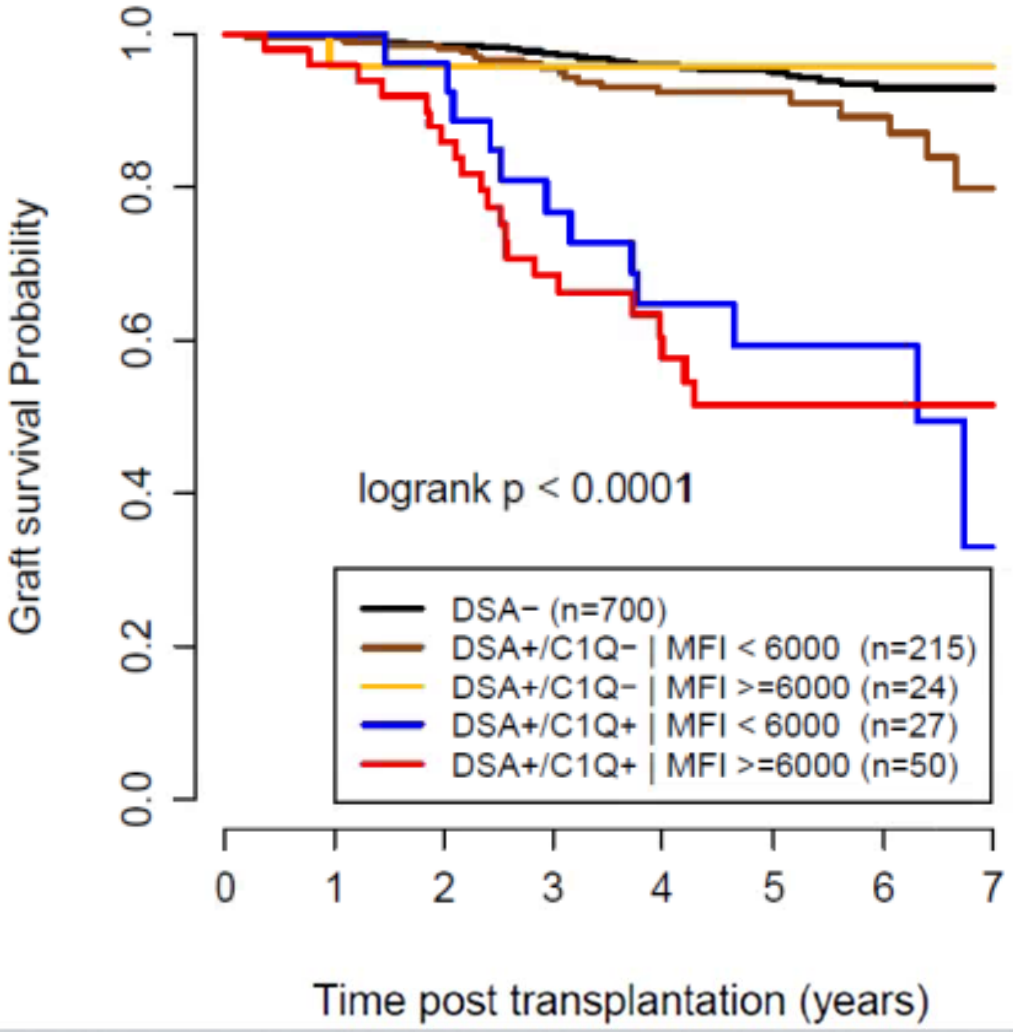


Figure S2: Kaplan Meier Analysis of graft outcome according to post-transplar DSA-MFI and complement-binding status



Supplement to: Loupy A, Lefaucheur C, Vernerey D, et al. Complement-binding anti-HLA antibodies and kidney-allograft survival. N Engl J Med 2013;369:1215-26. DOI: 10.1056/NEJMoa1302506

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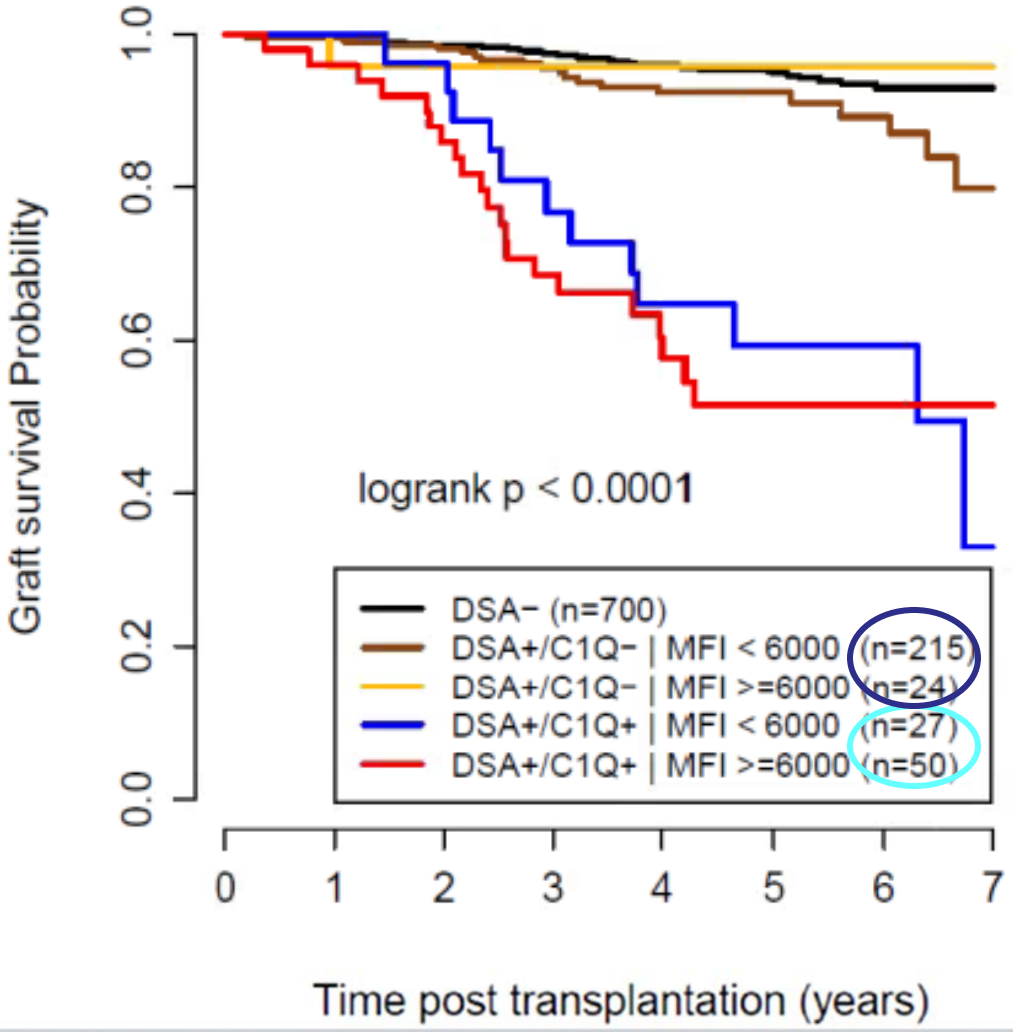

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C1q Binding Activity of De Novo Donor-specific HLA Antibodies in Renal Transplant Recipients With and Without Antibody-mediated Rejection

Maggie Yell, MD,[1] Brenda L. Muth, RN, MS,[2] Dixon B. Kaufman, MD, PhD,[3] Arjang Djamali, MD,[2] and Thomas M. Ellis, PhD[1]

TABLE 5.

Effects of normalization of C1q + DSA MFI values to levels comparable C1q – on Luminex-C1q activity

	N	MFI	Luminex-C1q
C1q + DSA	12	18,233 + 4268	+
C1q + DSA-diluted	12	6784 + 3386	–
C1q – DSA	22	5864 + 2686	–

TABLE 6.

Effects of serum concentration on C1q-binding activity of C1q – DSA

	Neat		Concentrated	
Sample	MFI	Luminex-C1q	MFI	Luminex-C1q
1	5489	Neg	12,243	Pos
2	4924	Neg	10,125	Pos
3	6985	Neg	13,112	Pos
4	5573	Neg	11,832	Pos
5	6323	Neg	7125	Neg
6	3794	Neg	5793	Neg

Transplantation 2015; 99:1151-1155

IgG Donor-Specific Anti-Human HLA Antibody Subclasses and Kidney Allograft Antibody-Mediated Injury

Carmen Lefaucheur,*† Denis Viglietti,*† Carol Bentlejewski,‡ Jean-Paul Duong van Huyen,†§ Dewi Vernerey,‖ Olivier Aubert,† Jérôme Verine,¶ Xavier Jouven,† Christophe Legendre,** Denis Glotz,* Alexandre Loupy,†** and Adriana Zeevi‡

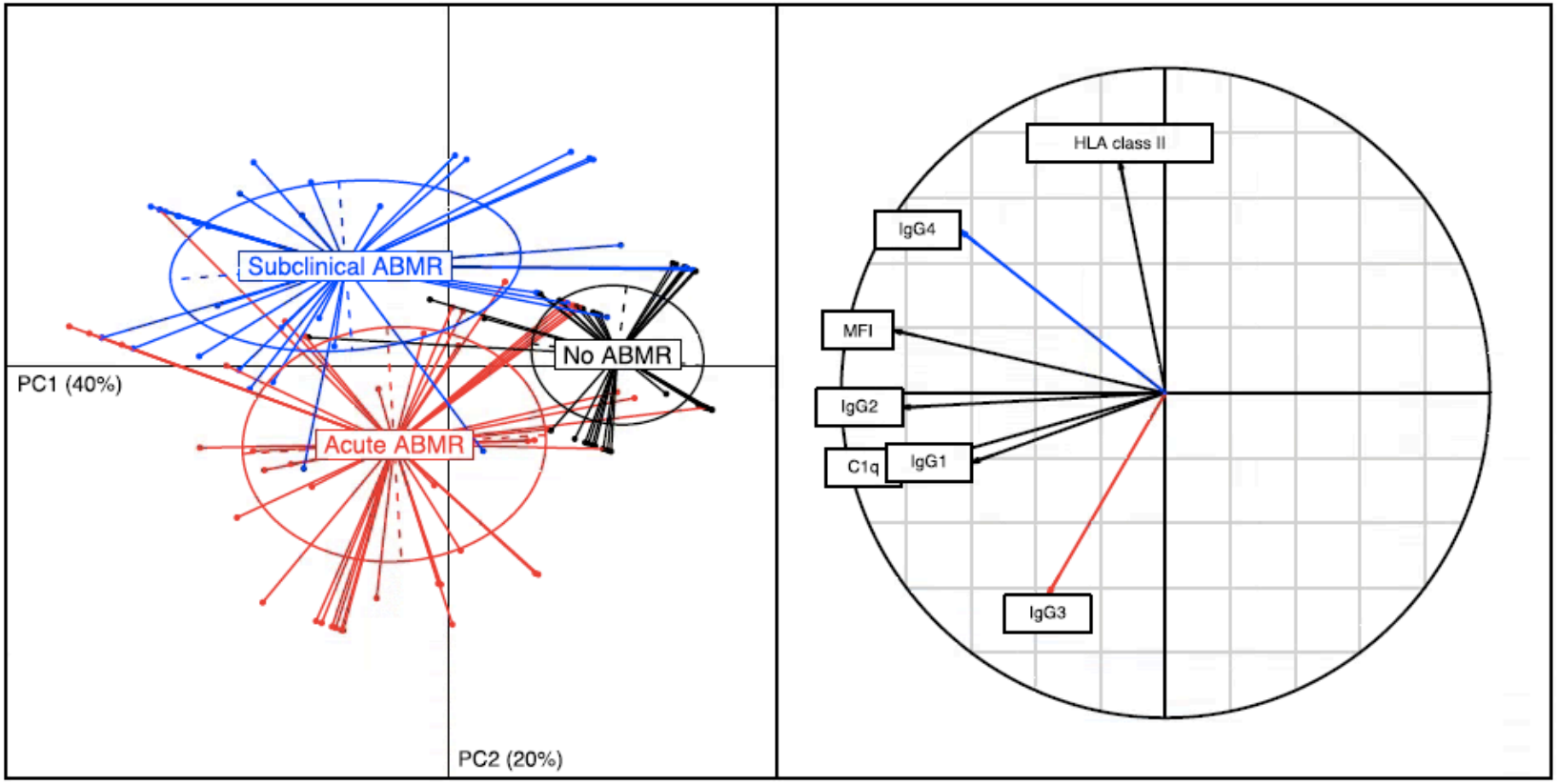

Figure 2. Identification of the three distinct rejection phenotypes according to the characteristics of the dominant donor-specific anti HLA antibody (MFI, HLA class specificity, C1q-binding capacity, and IgG1–4).

IgG Donor-Specific Anti-Human HLA Antibody Subclasses and Kidney Allograft Antibody-Mediated Injury

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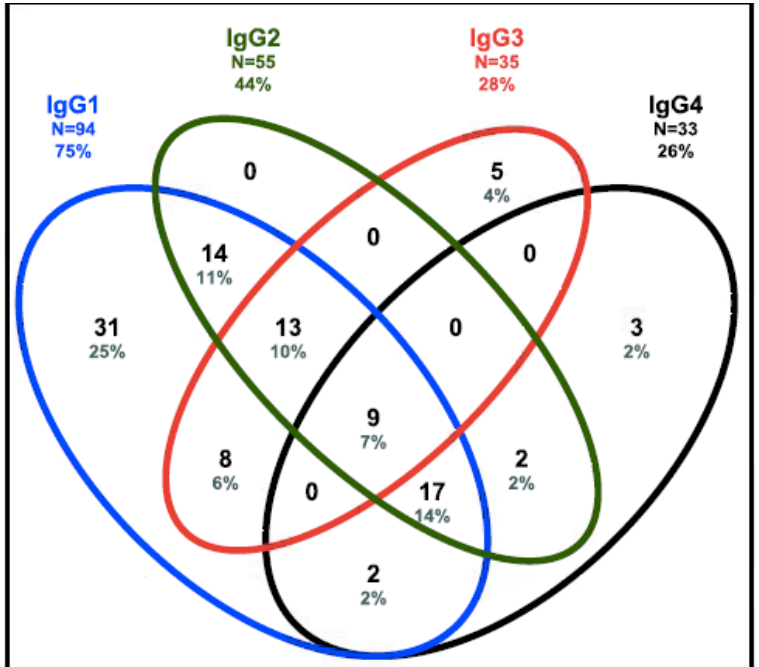

IgG1: N=31 (25%)	IgG1+3: N=8 (6%)
IgG1+2: N=14 (11%)	IgG1+4: N=2 (2%)
IgG1+2+3: N=13 (10%)	IgG2+4: N=2 (2%)
IgG1+2+3+4: N=9 (7%)	IgG3: N=5 (4%)
IgG1+2+4: N=17 (14%)	IgG4: N=3 (2%)

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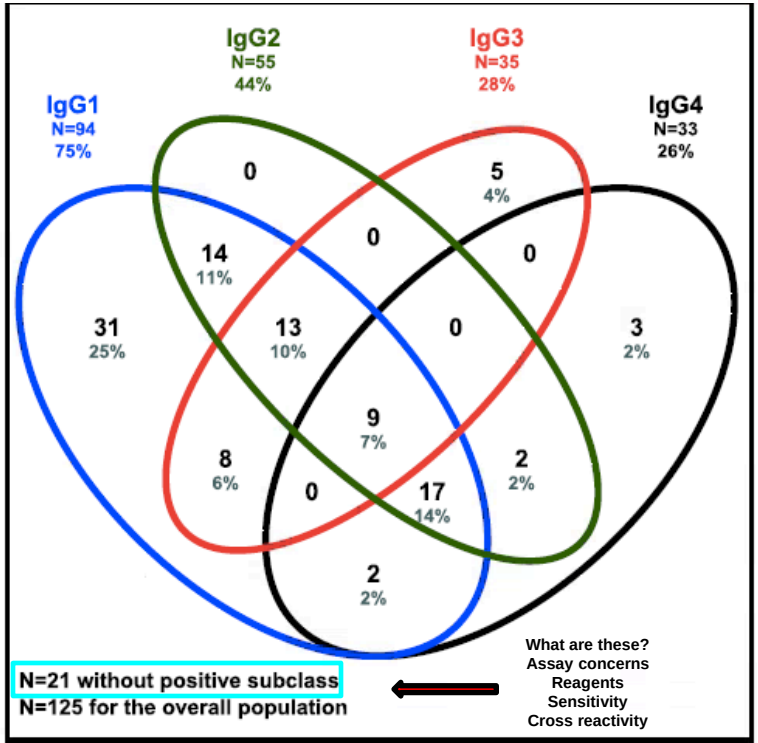


Early view

American Journal of Transplantation 2017; XX: 1–7
Wiley Periodicals Inc.

doi: 10.1111/ajt.14229

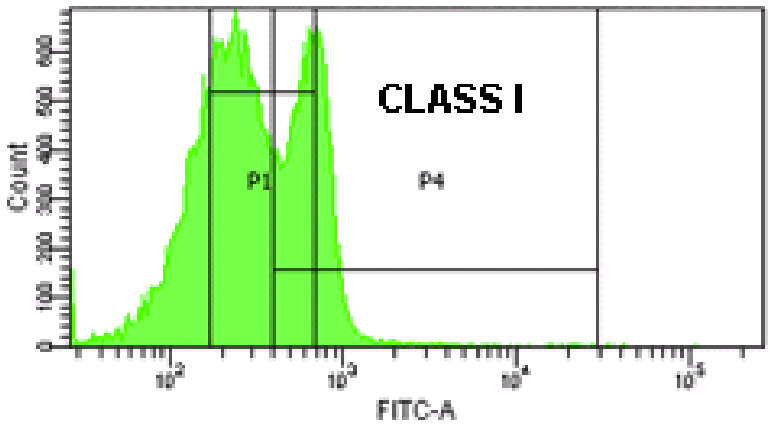
Personal Viewpoint

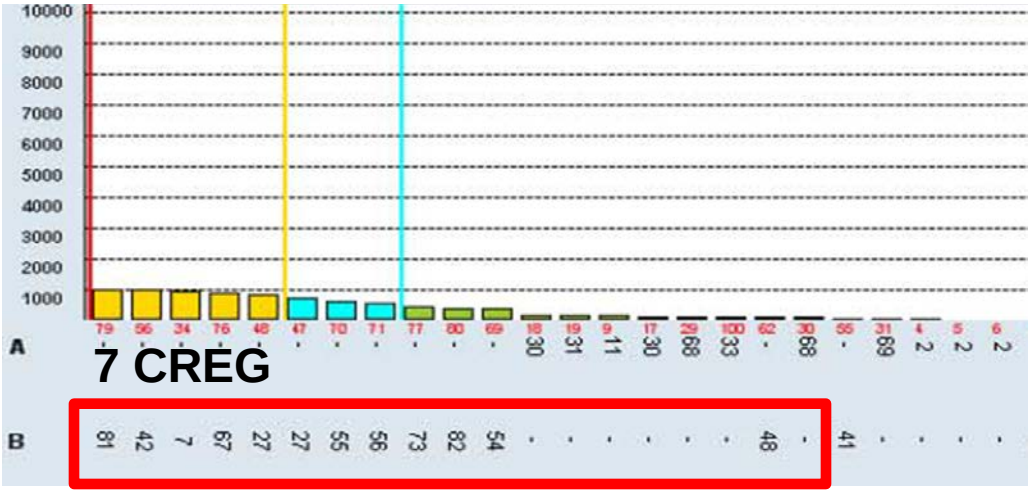
The Road to HLA Antibody Evaluation: Do Not Rely on MFI

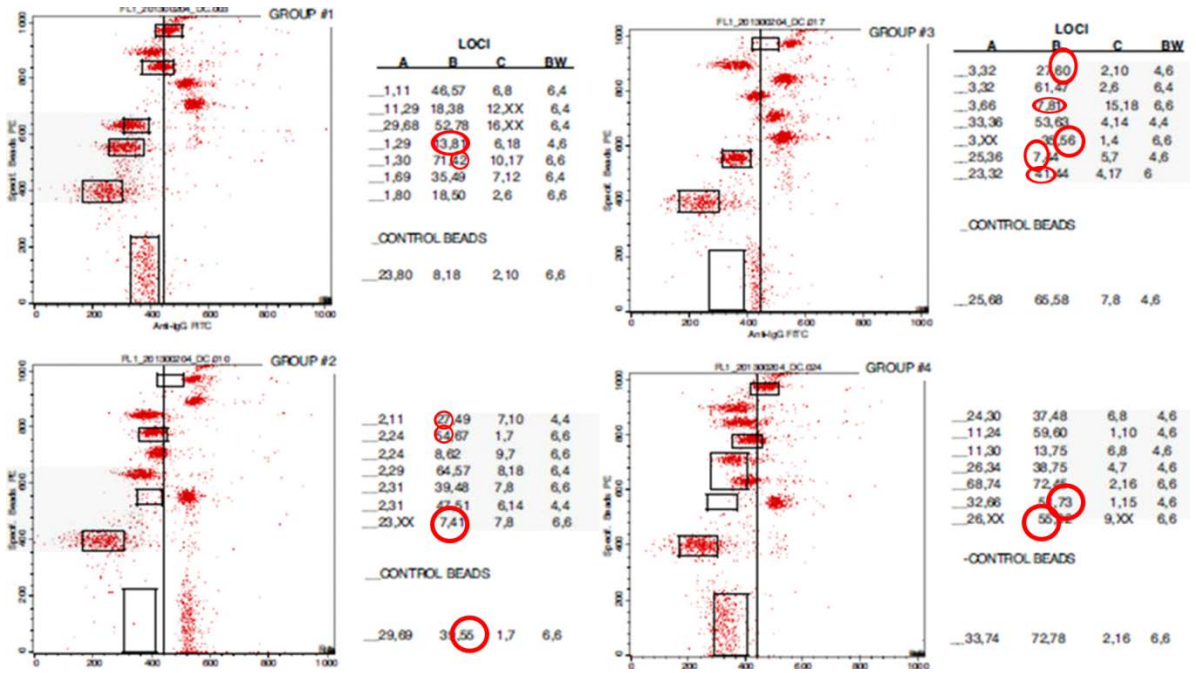
H. C. Sullivan[1], R. S. Liwski[2], R. A. Bray[1] and H. M. Gebel[1,*]

Hard Hats Required: Controversies in HLA Antibody Assessment

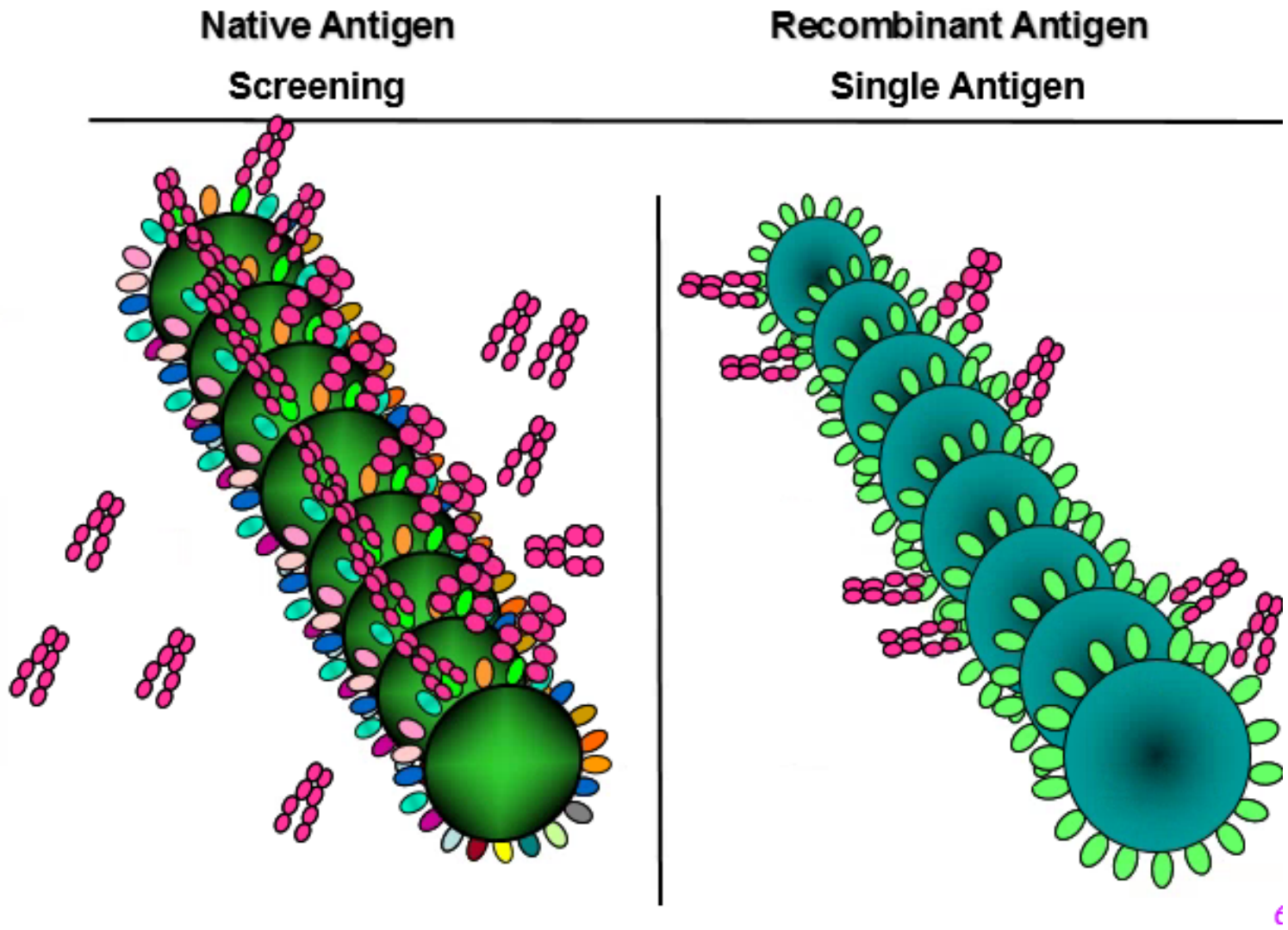
While multiplex Luminex technology (Luminex Corporation, Austin, TX) has provided a specific and sensitive platform to identify HLA antibodies, it is not flawless. A major point of contention revolves around results from SAB testing being reported as a numerical value referred to as mean fluorescence intensity (MFI). It is natural to think of a number as a quantitative assessment, but MFI values were never intended to quantify antibodies, nor was the Luminex-based test approved as a quantitative assay by the US Food and Drug Administration (2). Instead, MFI values reflect a given bead's relative fluorescence without reference to a standard. It is important to recognize that relative fluorescence can be affected by many variables. Nevertheless, MFI values have consistently been used as a quasiquantitative assessment of antibody strength by both laboratorians and clinicians. The tendency is to correlate MFI values with clinical outcomes and to serially monitor their fluctuations as a measure of clinical status. Decreas-







Gebel et al., Current Opin Organ Transplant 18:455-462, 2013



Adapted from Gebel and Bray, Am J Transplant 14:1964-1975, 2014

Transplantation Reviews 27 (2013) 108–111

Interpretation of HLA single antigen bead assays

Thomas M. Ellis *

Department of Pathology and Laboratory Medicine, School of Medicine and Public Health, University of Wisconsin-Madison, Madison, WI 53792-0428

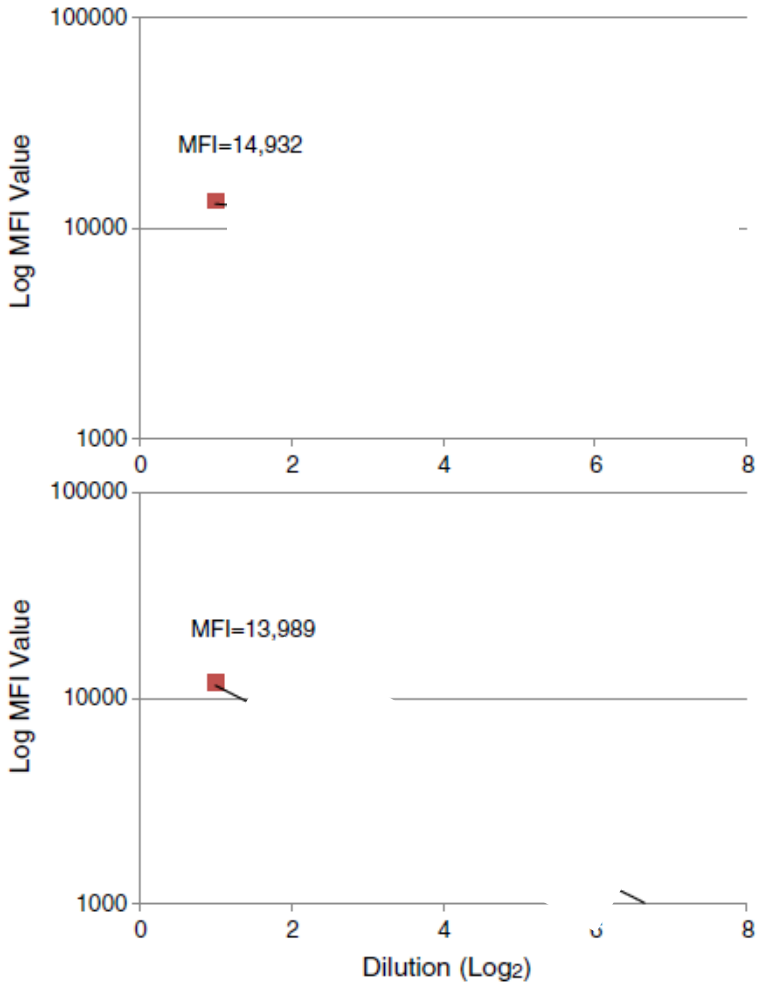


Fig. 2. Titration of 2 anti-HLA-A2 alloantisera with comparable MFI values when run undiluted in the standard SAB assay.

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A TALE OF TWO CITIES

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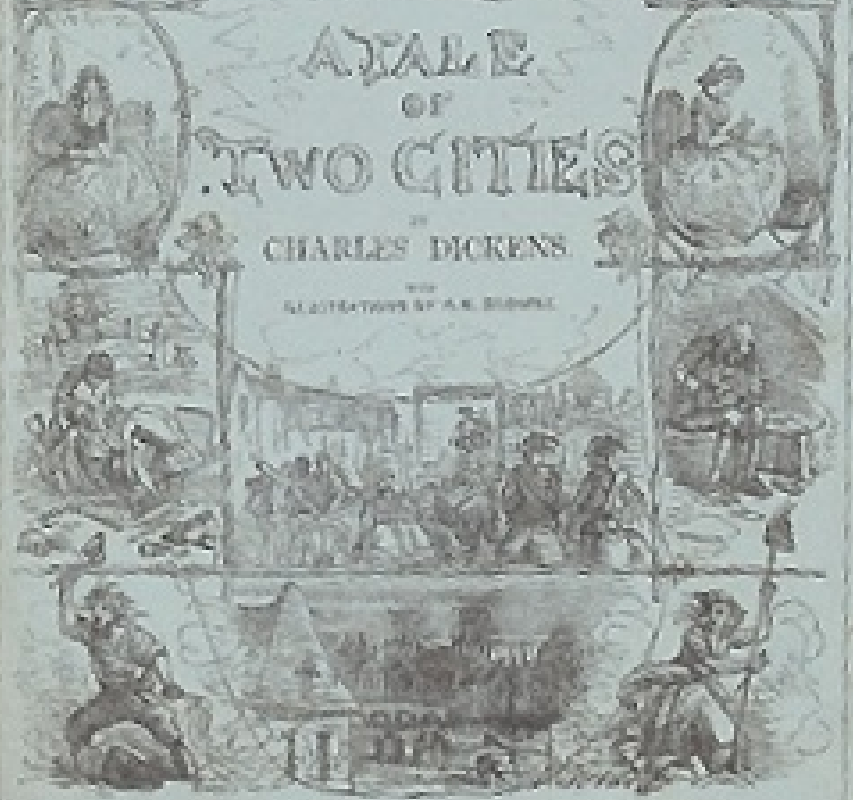

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AND "ALL THE YEAR ROUND" OFFICE, 11, WELLINGTON STREET NORTH, STRAND.

"It was the best of times, it was the worst of times; it was the age of wisdom, it was the age of foolishness; it was the epoch of belief, it was the epoch of incredulity; it was the season of Light, it was the season of Darkness; it was the spring of hope, it was the winter of despair; we had everything before us, we had nothing before us; we were all going directly to Heaven, we were all going the other way."

-- Charles Dickens

"It was the best of tests, it was the worst of tests; it was the test of wisdom, it was the test of foolishness; it was the test of belief, it was the test of incredulity; it was the test of Light, it was the test of Darkness; it was the test of hope, it was the test of despair; we had everything before us, we had nothing before us; we were all going directly to Heaven, we were all going the other way."

-- with apologies to Charles Dickens