Assays to detect and identify HLA antibodies
An overview

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Emory University Hospital
Atlanta, GA
No financial relationships related to this presentation

AND

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

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

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

Figure 1: Evolution of human leukocyte antigen antibody testing.
Solid Phase HLA antibody detection

Flow Cytometry

Suspension Arrays

Adapted from Gebel and Bray. Transplantation Reviews 20: 189-194, 2006
Baseline Donor-Specific Antibody Levels and Outcomes in Positive Crossmatch Kidney Transplantation

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

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

Why laboratories don’t get identical results when testing the same sample
Comprehensive Assessment and Standardization of Solid Phase Multiplex-Bead Arrays for the Detection of Antibodies to HLA

E. F. Reed¹,², P. Rao¹, Z. Zhang¹, H. Gebel²,
R. A. Bray², I. Guleria³, J. Lunz⁴,
T. Mohanakumar⁵, P. Nickerson⁶,
A. R. Tambur⁷, A. Zeevi⁴, P. S. Heeger⁸
and D. Gjertson¹
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. Taylor1,4

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 Pathology, The Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

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†

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

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

Alexandre Loupy, M.D., Ph.D., Carmen Lefaurheur, 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 Frémaux-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.

A  Kidney-Allograft Survival According to DSA Status

B  Kidney-Allograft Survival According to DSA and C1q Status

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N ENGL J MED 369;13   NEJM.ORG   SEPTEMBER 26, 2013
Figure S2: Kaplan Meier Analysis of graft outcome according to post-transplant DSA-MFI and complement-binding status

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

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, PhD¹

TABLE 5.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>MFI</th>
<th>Luminex-C1q</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1q + DSA</td>
<td>12</td>
<td>18,233 + 4268</td>
<td>+</td>
</tr>
<tr>
<td>C1q + DSA-diluted</td>
<td>12</td>
<td>6784 + 3386</td>
<td>-</td>
</tr>
<tr>
<td>C1q – DSA</td>
<td>22</td>
<td>5864 + 2666</td>
<td>-</td>
</tr>
</tbody>
</table>

TABLE 6.

Table of effects of serum concentration on C1q-binding activity of C1q – DSA

<table>
<thead>
<tr>
<th>Sample</th>
<th>Neat MFI</th>
<th>Neat Luminex-C1q</th>
<th>Concentrated MFI</th>
<th>Concentrated Luminex-C1q</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5489</td>
<td>Neg</td>
<td>12,243</td>
<td>Pos</td>
</tr>
<tr>
<td>2</td>
<td>4924</td>
<td>Neg</td>
<td>10,125</td>
<td>Pos</td>
</tr>
<tr>
<td>3</td>
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<td>Neg</td>
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<tr>
<td>4</td>
<td>5573</td>
<td>Neg</td>
<td>11,832</td>
<td>Pos</td>
</tr>
<tr>
<td>5</td>
<td>6323</td>
<td>Neg</td>
<td>7125</td>
<td>Neg</td>
</tr>
<tr>
<td>6</td>
<td>3794</td>
<td>Neg</td>
<td>5793</td>
<td>Neg</td>
</tr>
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</table>
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 Loubry.†*** 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

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

<table>
<thead>
<tr>
<th>IgG1: N=31 (25%)</th>
<th>IgG1+3: N=8 (6%)</th>
</tr>
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<tr>
<td>IgG1+2: N=14 (11%)</td>
<td>IgG1+4: N=2 (2%)</td>
</tr>
<tr>
<td>IgG1+2+3: N=13 (10%)</td>
<td>IgG2+4: N=2 (2%)</td>
</tr>
<tr>
<td>IgG1+2+3+4: N=9 (7%)</td>
<td>IgG3: N=5 (4%)</td>
</tr>
<tr>
<td>IgG1+2+4: N=17 (14%)</td>
<td>IgG4: N=3 (2%)</td>
</tr>
</tbody>
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N=21 without positive subclass
N=125 for the overall population
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‡

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<td>IgG1+4: N=2 (2%)</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>IgG2</th>
<th>N=55</th>
<th>44%</th>
<th>IgG3</th>
<th>N=35</th>
<th>26%</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG4</td>
<td>N=33</td>
<td>26%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

What are these?
Assay concerns
Reagents
Sensitivity
Cross reactivity
The Road to HLA Antibody Evaluation: Do Not Rely on MFI

H. C. Sullivan¹, R. S. Liwski², R. A. Bray¹ and H. M. Gebel¹,*

**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
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.
A TALE OF TWO CITIES
OF CHARLES DICKENS

LONDON: CHAPMAN AND HALL, 193, PICCADILLY;
AND "ALL THE YEAR ROUND" OFFICE, 12, FINSBURN STREET, BLACKFRIARS, E.

O'E The Author reserves the right of Translation.
"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