HLA antibody screening and identification
Should we use kits from both vendors?
Why consider to test kits from another provider?

- Financial incentive
- The benefit of to approaches
Questionnaire

• Eight labs use OneLambda products for HLA antibody screening and identification

• Uppsala, Oslo and (Aarhus) has validated the Immucor product for routine usage

• Uppsala uses Immucor for routine analyses?
<table>
<thead>
<tr>
<th></th>
<th>Oslo</th>
<th>Helsinki</th>
<th>Uppsala</th>
<th>Aarhus</th>
<th>Stockholm</th>
<th>Skåne</th>
<th>Reykjavik</th>
<th>Tartu</th>
<th>Copenhagen</th>
<th>Gothenburg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lifecodes screen and identification</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
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<tr>
<td>Lifecodes LSA Single Antigen</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
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<tr>
<td>Lifecodes C3d Detection</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Lifecodes Donor specific antibodies</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Have you validated the Immucor products for routine usage?</td>
<td>Yes</td>
<td>No</td>
<td>Yes, but will re-evaluate with the by H Otten in Transplantation proposed cut off</td>
<td>Yes, not comprehensively</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Have you run EPT samples on the Immucor products?</td>
<td>No</td>
<td>No</td>
<td>Yes, but not submitted</td>
<td>Yes, but not submitted</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>
• Lifecode screen and identification

• Lifecode LSA single antigen
Aarhus experience

• Technically:
  – easy to use
  – Visually difficult to pipette
  – Different conjugate for mixed and single
  – Cuts of 1 hour of the hands-on time
  – Lesser variation in the washing procedure

• Software:
  – Cut-off 1,000 MFI
  – Overview could be better
  – The graphical overview is inferior
  – Not possible to sort donor-specific antibodies

• Comparison of EPT samples between Immucor and OneLambda
EPT samples

- Last round of EPT samples from ETRL were analysed with both Immucor and OneLambda

- The results from the OneLambda was acceptable results, no false negative and no false positives in regards to consensus results
EPT samples

• Serum A:
  – One lambda
    • 44 HLA cl I antibody specificities
    • HLA cl II negative
  – Immucor
    • 40 HLA cl I antibody specificities (2 neg, 2 no bead B75,B71)
    • HLA cl II negative
• Serum B:
  – One lambda
    • 16 HLA cl I antibody specificities
    • 13 HLA cl II antibody specificities
  – Immucor
    • 9 HLA cl I antibody specificities
    • 13 HLA cl II antibody specificities
Serum D:
- One lambda
  - 39 HLA cl I antibody specificities
  - 1 HLA cl II antibody specificity
- Immucor
  - 15 HLA cl I antibody specificities
  - negative
Leiden experience

- Uses both vendors alternating for routine screening of waiting list patients
- Lower background with Immucor kit
- EPT samples:
  - samples with moderate to high MFI values the two kits are rather identical, with
  - more variation in samples with antibodies at low MFI levels
  - in the most recent EPT rounds samples were tested with Immucor not missing any specificity
Leiden experience

• AM program:
  – Now changing to use Immucor
  – For all AM patients, the reference lab does check the unacceptable by Labscreen
Comparative Assessment of Anti-HLA Antibodies Using Two Commercially Available Luminex-Based Assays

Kevin J. Clerkin, MD, MSc; Sarah B. See, PhD; Maryane A. Farr, MD, MSc; Susan W. Restano, MD; Gee Sertcan, PhD; Farhana Latif, MD; Lingzhi Li; Paolo C. Colombo, MD; George Vlad, PhD; Bryan Ray, PhD; Elena R. Vasilescu, MD; and Emmanuel Zion, PhD.

Background. Alloreactive anti-HLA antibodies (Abs) are associated with rejection of solid organ grafts. The 2 manu.

Results. Most HLA class I (64.2%) and class II (90.6%) Abs detected with moderate- to high MI values were detected by both assays. A moderate correlation was observed between MI values obtained from the 2 assays for both class I (r = 0.8) and class II Abs (r = 0.6, P = 0.01). Both assays detected anti-class I and II Abs that the other did not. However, no specific HLA allele was detected preferentially by either of the 2 assays. For a limited number of coreactant serum, a distinction of more immune-reactive profiles between the 2 platforms. Conclusion. Immunor and One Lambda/ThermoFisher assays have a similar, albeit non-normally, ability to detect anti-HLA Ab. Although the correlation between the assays was present, significant variations exist, some of which can be explained by a dilution-sensitive "prezone" effect.

(Transplantation Direct 2017;3:e216; doi:10.1097/TP.0000000000000734. Published online 2 October 2017.)


Dominique Bertrand1*, Fabienne Farce2, Charlotte. Laurent1, Frédérique Hamelin7, Arnaud François5, Dominique Guevron1, Isabelle Etienne1, Françoise Hau2

Toward a sensible single antigen bead cut-off based on kidney graft survival


• 125 serum samples from heart (120) and lung (5) transplant recipients

• Sera drawn
  – pre-transplant (N=17)
  – post-transplant (N=108)
• Prozone effect accounts for some antibodies identified by Immucor and not One Lambda (Immucor protocol has a 1:5 dilution of serum)
FIGURE 3. Correlation for all class II Ab.

FIGURE 2. Correlation for (A) class I Ab.
• We do not know if all the "weak" antibody specificities found by the OL kit are relevant

• Need to look into sensitivity and specificity

Dominique Bertrand1*, Fabienne Farco2, Charlotte Laurent1, Frédérique Hamelin2, Arnaud François3, Dominique Guerrot1, Isabelle Ettienne1, Françoise Hau2

Post transplant monitoring in 100 kidney-transplanted patients

- Group 1 (N=50): biopsy highly suspected for rejection
- Group 2 (N=50): biopsy with no rejection
• OneLambda:
  – Cut-off 500 MFI

• Immucor:
  – Cut-off
    • BCM >1,500 (background corrected MFI)
      – (background MFI – raw MFI)
    • BCR >4.0 (BCM/MFI for CalcCon for each locus)
    • AD-BCR >5.0 (normalization of BCR to the amount of antigen on each bead- from the Lot-specific recording sheet)
  – 2 out of 3 criteria fulfilled -> positive result
Correlation and agreement between MFI and BCM

HLA cl I

$\text{r} = 0.63 \ (0.53 - 0.70)$
$p < 0.0001$

$\text{ICC} = 0.61 \ (0.51 - 0.83)$

HLA cl II

$\text{r} = 0.80 \ (0.75 - 0.84)$
$p < 0.0001$

$\text{ICC} = 0.79 \ (0.73 - 0.83)$
Sensitivity and specificity for ABMR diagnosis

<table>
<thead>
<tr>
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<th>OneLambda</th>
<th>Immucor</th>
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<tbody>
<tr>
<td>Sensitivity</td>
<td>82%</td>
<td>78%</td>
</tr>
<tr>
<td>Specificity</td>
<td>82%</td>
<td>86%</td>
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</tbody>
</table>

Table 1: Sensitivity and specificity to confirm ABMR with One Lambda provider according different cut off of MFI used.

<table>
<thead>
<tr>
<th>Cut off value (MFI)</th>
<th>Sensitivity</th>
<th>Specificity</th>
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<tbody>
<tr>
<td>500</td>
<td>88%</td>
<td>68%</td>
</tr>
<tr>
<td>1000</td>
<td>82%</td>
<td>82%</td>
</tr>
<tr>
<td>1500</td>
<td>78%</td>
<td>90%</td>
</tr>
<tr>
<td>2000</td>
<td>78%</td>
<td>90%</td>
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Experience from Uppsala and Oslo