Evaluation of the LAg-Avidity Assay for Estimating HIV Incidence

Oliver Laeyendecker, MS/MBA/PhD
Senior Research Assistant, LIR/NIAID/NIH
Instructor of Medicine, ID/SOM/JHU
Director, HPTN Laboratory Center Incidence Core

Special thanks to Susan Eshleman, Director of the HPTN LC
What is Cross-Sectional HIV Incidence Testing?

Laboratory method that can reliably discriminate between recent and non-recent infection
How Do You Measure HIV Incidence in a Cross-Sectional Cohort?

- **HIV Uninfected**
- **Assay/MAA positive**
- **Assay/MAA negative**

### Incidence Estimate

1. Determine mean window period using numeric integration.
2. Reversion

\[
\text{Incidence estimate} = \frac{\# \text{ Assay/MAA Positive}}{\# \text{ HIV Uninfected} \times \text{Mean window period}}
\]

Brookmeyer and Quinn, 1995. AJE. 141:166
Theoretical Framework for Cross-Sectional Incidence Testing

**Individual Time Varying**
- AIDS (Hayashida ARHR 2008, Longosz AIDS 2014)
- ART (Marinda JAIDS 2010, Longosz AIDS 2014)
- Viral breakthrough (Wendel PLoS One 2013)

**Population**
- Duration of epidemic (Hallett PLoS One 2009)
- Access to ART
- Current state of epidemic (Laeyendecker PLoS One 2013)

**Individual Fixed**
- Race (Laeyendecker ARHR 2012)
- Gender (Mullis ARHR 2013)
- Geography (Laeyendecker ARHR 2012)
- Infecting subtype (Parekh ARHR 2011, Longosz JAIDS 2014)
- Viral load set-point (Laeyendecker JAIDS 2008)

**Graph Details**
- Probability vs. Duration of Infection
- The graph shows the probability of recent vs. recent infections over time.

**Graph Notes**
- Different lines represent varying levels of recent infections.
Rationale for Evaluating the LAg-Avidity Assay

• Developed by the CDC
• Commercially-available
• Easy to use
• Promoted for use
  – Wall Street Journal
  – CROI 2012
  – IAS 2012
• BED-CEIA being phased out
Factors Associated with “False-Recent” Misclassification using LAg

- Samples were obtained from US cohorts MACS & ALIVE
  - 1089 samples from 667 individuals; 595 samples 2-4 years + 494 samples 4-8 years post-SC

### Percent Misclassified

Factors associated with misclassification (adjusted odds)
- Viral load < 400 c/mL
  - 3.7 (1.6-8.6)
- CD4 < 50 cells/µL
  - 5.4 (1.9-15.7)
- Misclassified at earlier time point
  - 5.6 (1.6-20.3)
- Not misclassified at earlier time point
  - 0.3 (0.1-0.6)

---

Longosz et al., 2014. AIDS. Feb 6.
Affect of Viral Suppression and Breakthrough on LAg-Avidity Results

![Graph showing the affect of viral suppression and breakthrough on LAg-Avidity Results. The graph plots normalized optical density on the y-axis against years of follow-up on the x-axis. Three lines are shown: BED, LAg, and Viral Load. The BED line shows an initial rise, followed by a decline, while the LAg and Viral Load lines show a more gradual decrease.]
Changes in assay values at paired time points based on VL status

- 20 subjects (179 samples) from JHU Moore Clinic
- Evidence of viral breakthrough followed by viral suppression

![Graph showing changes in viral load over time points](image-url)
Change in LAg-Avidity Values in Paired Time-points by Viral Load Status

<table>
<thead>
<tr>
<th>Viral load status</th>
<th>Time point 1</th>
<th>Time point 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>suppressed</td>
<td>Suppressed</td>
<td>Suppressed</td>
</tr>
<tr>
<td>breakthrough</td>
<td>Breakthrough</td>
<td>Suppressed</td>
</tr>
</tbody>
</table>
Evaluation of LAg-Avidity in the US (Clade B)

Evaluate assay performance
Evaluate MAAs that include the LAg-Avidity assay
  • 1,782 samples from individuals with known duration of infection 0.1-8+ years (HIVNET 001, ALIVE, MACS)
  • 500 additional samples from individuals infected 8+ years

Evaluate accuracy of incidence estimates obtained using the optimized MAAs in three longitudinal cohorts with observed incidence
  – HPTN 064 (low)
  – HIVNET 001 (medium)
  – HPTN 061 (high)

Why use Multi-Assay Algorithms (MAAs)

- MAAs provide more accurate discrimination between recent and non-recent infection

- We use higher cutoffs for serologic assays to increase identification of new infections

- We use two serologic assays in combination, along with non-serologic biomarkers, to drive the false-recent rate to zero and provide accurate cross-sectional HIV incidence estimates

Methods

Evaluated > 500,000 testing algorithms
  – LAg-Avidity assay alone or with 1-4 other biomarkers
    (BED-CEIA, BioRad-Avidity, CD4 count, viral load)
  – Modeled the probability of being assay/MAA positive as a function of duration of infection

Calculated the mean window period as the area under the probability curve

The following criteria were used to select optimized testing algorithms:
  – Probability curve converges to zero
  – Longest mean window period
  – Shadow < 1 year

LAg-Avidity assay alone

Probability Curves

Manufacturer's Protocol:
LAg-Avidity assay cutoff of < 1.5 with VL > 1000 and CD4 > 200

Results:
- The curve converges to zero
- Window period: 85 days (not 130)
- Shadow: 158 days

LAG-Avidity based Multi-Assay Algorithms

MAA #1

BioRad-Avidity

≥40 → MAA Negative

<40

LAG-Avidity

≥2.8 → MAA Negative

<2.8

MAA Positive

Mean window period: 119 days (94, 144)  
Shadow: 247 days (160, 339)
Probability Curves

LAG-Avidity assay alone

Comparison of Cross-Sectional Incidence Testing to Observed Incidence: Model

Longitudinal cohort

Enrollment 6 months 12 months

HIV-  HIV+

Observed HIV incidence between survey rounds (HIV seroconversion)

Perform cross-sectional incidence testing at end of study

Compare the cross-sectional incidence estimate to incidence observed in the longitudinal study (based on HIV seroconversion)
## Performance Comparisons

MAA performance assessed in three clinical cohorts

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th># enrolled</th>
<th>Person-Yrs follow-up</th>
<th># SC</th>
<th>Observed HIV incidence (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPTN 064</td>
<td>Women at risk for HIV acquisition</td>
<td>HIV pos: 33 HIV neg: 1947</td>
<td>1639</td>
<td>4</td>
<td>0.24% (0.07, 0.62)</td>
</tr>
<tr>
<td>HIVNET 001</td>
<td>Men and women with varied risk factors for HIV acquisition</td>
<td>HIV pos: 90 HIV neg: 4175</td>
<td>2304</td>
<td>24</td>
<td>1.04% (0.70, 1.55)</td>
</tr>
<tr>
<td>HPTN 061</td>
<td>African American MSM</td>
<td>HIV pos: 246 HIV neg: 872</td>
<td>926</td>
<td>28</td>
<td>3.02% (2.01, 4.37)</td>
</tr>
</tbody>
</table>

SC = Seroconversion

Koblin, 2013. PLoS One. 8:e70413
## Incidence Estimation in Three Clinical Cohorts

<table>
<thead>
<tr>
<th></th>
<th>Longitudinal cohort</th>
<th>2-assay MAA</th>
<th>4-assay MAA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean window period</strong></td>
<td>--</td>
<td>119 (94, 144)</td>
<td>146 (122, 170)</td>
</tr>
<tr>
<td><strong>Shadow</strong></td>
<td>--</td>
<td>247 (160, 339)</td>
<td>180 (144, 235)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Incidence estimate</strong></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HPTN 064</strong></td>
<td>0.24% (0.07, 0.62)</td>
<td>0.32% (0.04, 1.17)</td>
<td>0.26% (0.03, 0.95)</td>
</tr>
<tr>
<td><strong>HIVNET 001</strong></td>
<td>1.04% (0.70, 1.55)</td>
<td>0.92% (0.45, 1.73)</td>
<td>1.09% (0.60, 1.84)</td>
</tr>
<tr>
<td><strong>HPTN 061</strong></td>
<td>3.02% (2.01, 4.37)</td>
<td>4.57% (2.37, 8.24)</td>
<td>3.44% (1.75, 6.20)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Percent difference</strong></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HPTN 064</strong></td>
<td>--</td>
<td>33.3%</td>
<td>8%</td>
</tr>
<tr>
<td><strong>HIVNET 001</strong></td>
<td>--</td>
<td>-12%</td>
<td>5%</td>
</tr>
<tr>
<td><strong>HPTN 061</strong></td>
<td>--</td>
<td>51.3%</td>
<td>14%</td>
</tr>
</tbody>
</table>

Summary

• The LAg-Avidity assay alone does not provide accurate incidence estimates
• Addition of VL and CD4 at appropriate cutoffs significantly reduces false-recent misclassification
• Optimized MAAs that include the LAg-Avidity assay accurately estimate HIV incidence in clade B settings
• An optimized 2-assay MAA is simpler and has lower testing costs, but has a shorter mean window period and requires larger surveys to obtain the same precision as the 4-assay MAA
Acknowledgements

LAB OF IMMUNOREGULATION
THOMAS QUINN
ANDY LONGOSZ
SARAH WENDEL
JORDYN GAMIEL
AMY OLIVER
CAROLINE MULLIS

UCLA
RON BROOKMEYER
JACOB KONIKOFF

JOHNS HOPKINS UNIVERSITY
MACS, ALIVE, MOORE CLINIC
COHORTS
LISA JACOBSON
JOSEPH MARGOLICK
GREG KIRK
SHRUTI MEHTA
JACQUIE ASTEMBORSKI
RICHARD MOORE

HPTN LABORATORY CENTER AT JOHNS HOPKINS UNIV.
SUSAN ESHLEMAN
MATT COUSINS
ESTELLE PIWOWAR-MANNING

HIVNET 001/1.1
CONNIE CELUM
SUSAN BUCHBINDER
GEORGE SEAGE
HAYNES SHEPPARD

HPTN 064
SALLY HODDER
JESSICA JUSTMAN

Sponsored by NIAID, NIDA, and NIMH: UM1-AI068613, 1R01-AI095068 and NIH Intramural