LUMINEX-BASED MULTIPLEX ASSAY FOR HIV INCIDENCE

Principle, Assay Development and Performance

Ernest L. Yufenyuy, PhD
ASM/CDC Postdoctoral Research Fellow
International Laboratory Branch, Division of Global HIV/AIDS and TB
CDC Developed Incidence Assay

Detection of Recent HIV-1 Infection Using a New Limiting-Antigen Avidity Assay: Potential for HIV-1 Incidence Estimates and Avidity Maturation Studies

Yen T. Duong, Maofeng Qiu, Anindya K. De, Keisha Jackson, Trudy Dobbs, Andrea A. Kim, John N. Nkengasong, Bharat S. Parekh*

Division of Global HIV/AIDS, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America

Recalibration of the Limiting Antigen Avidity EIA to Determine Mean Duration of Recent Infection in Divergent HIV-1 Subtypes

Yen T. Duong, Reshma Kassanjee, Alex Welte, Meade Morgan, Anindya De, Trudy Dobbs, Erin Rottinghaus, John Nkengasong, Marcel E. Cullen, Chonticha Kittinunvorakoon, Boonyos Raengsakulrach, Michael Martin, Kachit Choopanya, Suphak Vanichseni, Yan Jiang, Maofeng Qiu, Haiying Yu, Yan Hao, Neha Shah, Linh-Vi Le, Andrea A. Kim, Tuan Anh Nguyen, William Ampofo, Bharat S. Parekh*
Ab-Based Recent Infection Detection

- Prior accurate HIV diagnosis needed
- False HIV-1 positives, if present, are classified as recent HIV-1 infections
- HIV-2 infections are classified as recent infections (e.g. BED, LAg-Avidity EIA), critical in some parts of the world
- Contribute to elevated incidence
- Multiple assays required to confirm HIV serology, identify HIV-2 and distinguish recent/LT infections
Next Generation Incidence Assay

Sample

- HIV Positive
  - HIV-1 Positive
  - HIV-1/2 Positive
    - Long Term Infection
    - Recent Infection
  - HIV-2 Positive
  - HIV-1/2 Negative

- HIV-1 Negative
  - Diagnosis
    - P24-gp41
  - Serotyping
    - HIV-2 IDR
  - Incidence estimation
    - rIDR-M
Components of A Next Generation Incidence Assay

• Fulfills most/all the characteristics of LAg
• Has a diagnostic component
  • HIV-1 and HIV-2
  • Negative
• Less time, less complicated, low cost
• Longer Mean duration of recent infection
• High reproducibility and low or no false rates
The Art of Multiplexing, Mixing of P24-gp41, rIDR-M and HIV-2 IDR Coupled Beads

- P24-gp41
- rIDR-M
- HIV-2 IDR

Coupled beads incubated with HIV+ Serum/Plasma containing antibodies to coupled antigens

Bound antibodies against coupled antigen

Detection antibody (goat anti human) conjugated to PE

Detection of HIV+ sample on MagPix
OPTIMIZATION OF ASSAY PARAMETERS
Critical Optimization Parameters

1. Diagnostic antigen=p24-gp41 fusion protein and HIV-2 IDR
2. rIDR-M antigen for separation of recent and long term infection
3. Optimal concentration of Ags on beads
4. Optimal sample dilution
5. Optimal dilution of detection Ab
6. Optimal incubation time
7. MFI signal stability over time and temperature
8. Consistency of coupling method
9. Coupling scale up
10. Wash buffer evaluation (BSA or no BSA)
11. Blocking buffer
12. Stepwise evaluation/performance, n=85 and n=1500
85-Member Panel as Classified by EIA/Western, Multispot and The LAg Assay
1500-Member Panel as Classified by EIA/Western, Multispot and The LAg Assay
Possibility to Extend Window Period
## Data Summary of HIV Diagnosis and Typing

### Reference HIV Status by EIA/Western Blot/Multispot

<table>
<thead>
<tr>
<th>Luminex Multiplex</th>
<th>HIV-1</th>
<th>HIV-2</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1</td>
<td>569</td>
<td>0</td>
<td>3</td>
<td>572</td>
</tr>
<tr>
<td>HIV-2</td>
<td>0</td>
<td>30</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
<td>1</td>
<td>896</td>
<td>898</td>
</tr>
<tr>
<td>Total</td>
<td>570</td>
<td>31</td>
<td>899</td>
<td>1500</td>
</tr>
</tbody>
</table>

### Test Sensitivity and Specificity

<table>
<thead>
<tr>
<th>Test</th>
<th>HIV-1</th>
<th>HIV-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>99.8</td>
<td>96.7</td>
</tr>
<tr>
<td>Specificity</td>
<td>99.7</td>
<td>100</td>
</tr>
</tbody>
</table>

### Notes
- The table above summarizes the data of HIV diagnosis and typing using Luminex Multiplex, EIA, and Western Blot/Multispot methods.
- The sensitivity and specificity of the test are calculated based on the reference status from the EIA/Western Blot/Multispot method.
- The values indicate the accuracy of the Luminex Multiplex test compared to the reference method.
Multiplex Assay Algorithm

Sample

- MFI > 3000
  - HIV Positive
    - MFI > 1000
      - HIV-1/2 Positive
      - Long Term Infection
    - MFI < 1000
      - HIV-1 Positive
      - Recent Infection
  - MFI < 1000
    - HIV-2 Positive
    - Incidence estimation

- MFI < 3000
  - HIV-1 Negative
    - Diagnosis
    - P24-gp41
    - Serotyping
      - HIV-2 IDR
    - rIDR-M
ASSAY REPRODUCIBILITY PARAMETERS
<table>
<thead>
<tr>
<th>Specimen No</th>
<th>HIV Status</th>
<th>D1</th>
<th>D2</th>
<th>D3</th>
<th>Mean</th>
<th>STD</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>VP1</td>
<td>Neg</td>
<td>62.0</td>
<td>62.0</td>
<td>57.0</td>
<td>60.3</td>
<td>2.9</td>
<td>4.8</td>
</tr>
<tr>
<td>VP2</td>
<td>HIV-1</td>
<td>25139.0</td>
<td>25075.0</td>
<td>23445.0</td>
<td>24553.0</td>
<td>960.1</td>
<td>3.9</td>
</tr>
<tr>
<td>VP3</td>
<td>HIV-1</td>
<td>31215.5</td>
<td>30878.0</td>
<td>29840.5</td>
<td>30644.7</td>
<td>716.6</td>
<td>2.3</td>
</tr>
<tr>
<td>VP4</td>
<td>Neg</td>
<td>61.0</td>
<td>65.0</td>
<td>62.0</td>
<td>62.7</td>
<td>2.1</td>
<td>3.3</td>
</tr>
<tr>
<td>VP5</td>
<td>Neg</td>
<td>47.0</td>
<td>50.0</td>
<td>51.0</td>
<td>49.3</td>
<td>2.1</td>
<td>4.2</td>
</tr>
<tr>
<td>VP6</td>
<td>HIV-1</td>
<td>23469.0</td>
<td>22880.5</td>
<td>21994.0</td>
<td>22781.2</td>
<td>742.5</td>
<td>3.3</td>
</tr>
<tr>
<td>VP7</td>
<td>Neg</td>
<td>53.0</td>
<td>55.0</td>
<td>61.0</td>
<td>56.3</td>
<td>4.2</td>
<td>7.4</td>
</tr>
<tr>
<td>VP8</td>
<td>HIV-1</td>
<td>28656.5</td>
<td>28951.0</td>
<td>28541.0</td>
<td>28716.2</td>
<td>211.4</td>
<td>0.7</td>
</tr>
<tr>
<td>VP9</td>
<td>HIV-1</td>
<td>27917.0</td>
<td>27130.0</td>
<td>26731.0</td>
<td>27259.3</td>
<td>603.5</td>
<td>2.2</td>
</tr>
<tr>
<td>VP10</td>
<td>Neg</td>
<td>92.0</td>
<td>92.0</td>
<td>86.0</td>
<td>90.0</td>
<td>3.5</td>
<td>3.8</td>
</tr>
</tbody>
</table>
Assay Reproducibility Parameters

Intra-Assay Reproducibility (p24-gp41 and HIV-2 IDR)

- Linear regression equation: $y = 0.9849x + 0.6465$
- $R^2 = 0.995$
- Linear regression equation: $y = 1.0081x - 8.5353$
- $R^2 = 0.997$

Intra-Assay reproducibility (rIDRM)

- Linear regression equation: $y = 0.9494x + 16.69$
- $R^2 = 0.9953$

Inter-Assay Reproducibility (p24-gp41 and HIV-2 IDR)

- Linear regression equation: $y = 0.9898x - 32.243$
- $R^2 = 0.997$
- Linear regression equation: $y = 0.8325x + 7.6466$
- $R^2 = 0.9607$

Inter-Assay reproducibility (rIDRM)

- Linear regression equation: $y = 0.9557x + 26.611$
- $R^2 = 0.9727$
Assay Reproducibility Parameters

**Inter-Coupling Consistency**

\[ y = 1.0784x + 78.843 \]

\[ R^2 = 0.993 \]

**Multiplex Vs Monoplex**

\[ y = 0.9554x - 66.385 \]

\[ R^2 = 0.9967 \]
Signal Stability at 4°C and at RT

Stability of Signal Intensity over time at 4 deg (p24-gp41 and HIV-2 IDR)

\[ y = 0.9552x - 40.349 \]
\[ R^2 = 0.9996 \]

Stability of Signal Intensity over time at 4 deg (rIDR-M)

\[ y = 0.9075x - 147.68 \]
\[ R^2 = 0.999 \]

Mean Fluorescence Intensity at 72 hr

Mean Fluorescence Intensity at 0 hr

Stability of Signal Intensity over time at RT (p24-gp41 and HIV-2 IDR)

\[ y = 0.9654x - 21.923 \]
\[ R^2 = 0.999 \]

Stability of Signal Intensity over time at RT (rIDR-M)

\[ y = 0.8118x - 22.834 \]
\[ R^2 = 0.9955 \]
Summary of Assay Parameters

- Precision
  - Intra-Assay CV <10%
  - Inter-Assay CV <10%

- Signal Stability up to 8 hrs at RT in the dark

- Sample Working dilution 1:50 for a net dilution of 1:100

- Detection antibody titration: 1:250 dilution which gives a concentration of 2 μg/ml

- For diagnostic antigen, optimal coupling at 1 μg of antigen/1.5 millions beads, 0.04 μg for incidence and 10 μg for serotyping
The Next Steps

• Develop QC standards and evaluate on an ongoing basis
• Develop a data management file
• Evaluation with longitudinal specimens of known recency to establish MDRI
• Evaluation with specimens from long-term infections to determine FRR
• Evaluate the influence of ART on recency classification
• Evaluation with other collaborators to compare and refine MDRI and FRR
• Field validation using cross sectional survey specimens to calculate incidence estimates and conduct risk-factor analysis
## Multiplex Assay Summary

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Multiplex Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomarker Concept</td>
<td>Antibody Titer and Avidity</td>
</tr>
<tr>
<td>Development Stage</td>
<td>Development/Performance Evaluation</td>
</tr>
<tr>
<td>Intended Use</td>
<td>For Estimation of Incidence</td>
</tr>
<tr>
<td>Specimen Types</td>
<td>Plasma, Serum</td>
</tr>
<tr>
<td>Sample Requirement</td>
<td>1 µL</td>
</tr>
<tr>
<td>Stability of Coupled Beads</td>
<td>1 year</td>
</tr>
<tr>
<td>Equipments Needed</td>
<td>MagPix, Magnetic Plate Washer</td>
</tr>
<tr>
<td>Applicability</td>
<td>HIV-1 and HIV-2, All Subtypes</td>
</tr>
<tr>
<td>MDRI</td>
<td>To be Determined</td>
</tr>
<tr>
<td>FRR</td>
<td>To be Determined</td>
</tr>
<tr>
<td>Influence of ART</td>
<td>To be Determined</td>
</tr>
<tr>
<td>Cost</td>
<td>To be Determined</td>
</tr>
</tbody>
</table>
Acknowledgements

Incidence/Serology Team
Bharat Parekh
Yen Duong
Trudy Dobbs
Mervi Detorio
Mireille Kalou
Nnaemeka Iriemenam*
Hetal Patel
Keisha Jackson
Ebenezer David
Vedapuri Shanmugam
Audrey White
Eucaris Torres
Erin Rottinghaus*

ASM Office
Irene Hulede
Tiffani Fonseca
Ruth Pruitt
Angela Slaughter

Funding
ASM/CDC Postdoctoral Fellowship