# Performance Evaluation of HIV Supplemental Assays

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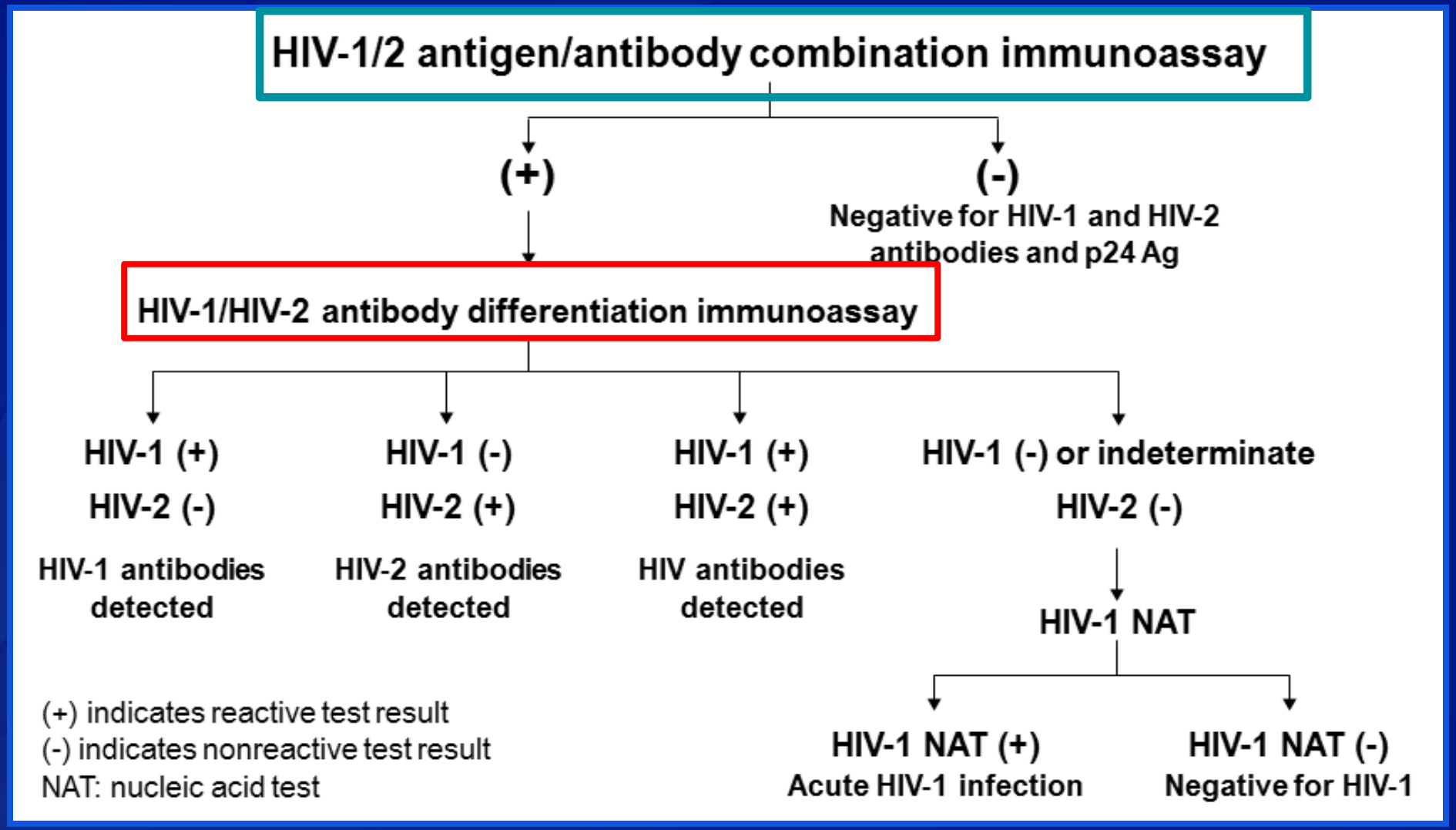
2016 HIV Diagnostic Conference

03/22/2016

# CDC HIV guidelines for HIV diagnosis in laboratory settings

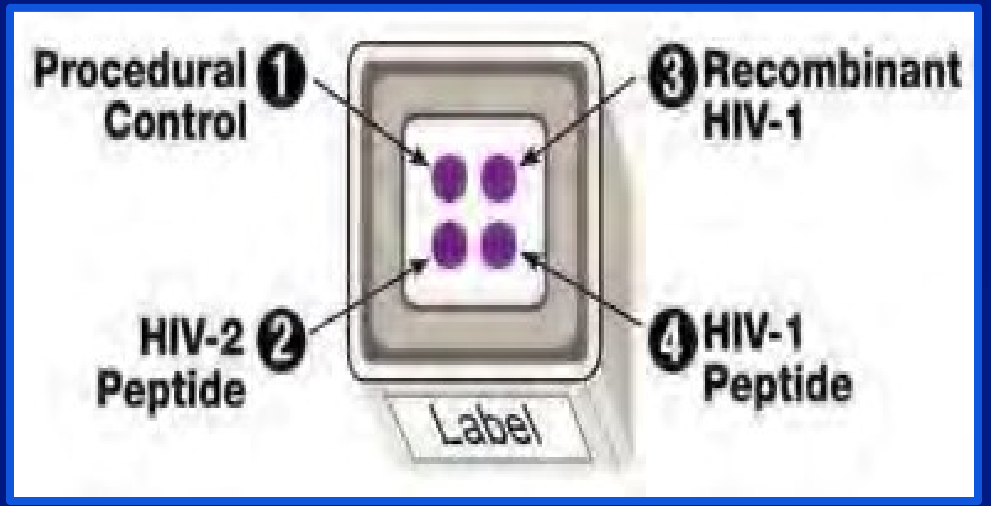


# Objective

- Compare the performance of two FDA-approved HIV1/2 differentiation assays within the algorithm
  - Bio-Rad Multispot HIV1/ HIV2 Rapid Test
  - Bio-Rad Geenius HIV1/ HIV2 Supplemental Assay

# Bio-Rad Multispot HIV1/HIV2 Rapid Test

- 30 ul of plasma/serum
- Reactivity to:
  - gp41 HIV-1 envelope recombinant protein
  - gp41 HIV-1 envelope peptide (IDR)
  - gp36 HIV-2 envelope peptide
- Operator interpretation
- Screening or supplemental
- Interpretations as supplemental test:
  - HIV-1 or HIV-2 positive
  - HIVundifferentiated
  - HIV-1 indeterminate
  - Negative




- Dilution protocol
  - 1:10 or 1:100 dilution for undifferentiated
- Discontinued in 2016

# Bio-Rad Geenius HIV1/HIV-2 Supplemental Assay

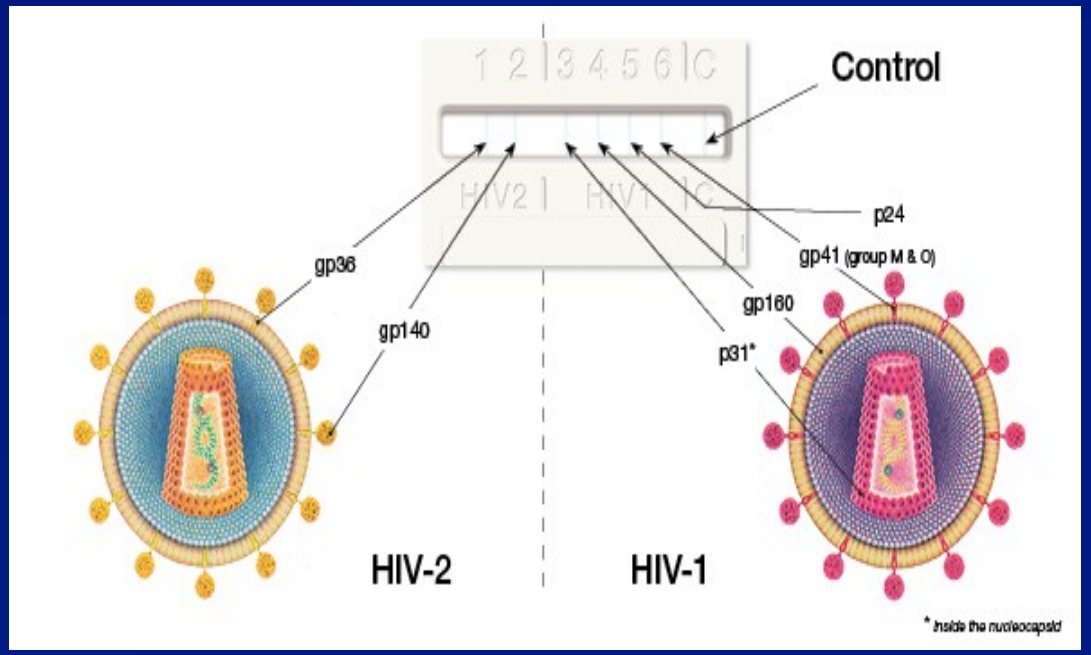
Approval Date: October 24, 2014

Indications:

- Single-use immunochromatographic assay for the confirmation and differentiation of individual antibodies to Human Immunodeficiency Virus Types 1 and 2 (HIV-1 and HIV-2) in fingerstick whole blood, venous whole blood, serum, or plasma samples (EDTA, heparin, and sodium citrate).
- Intended for use as an additional, more specific test to confirm the presence of antibodies to HIV-1 and HIV-2 for specimens found to be repeatedly reactive by diagnostic screening procedures. The assay may also be used to confirm the presence of antibodies to HIV-1 and/or HIV-2 in pediatric subjects (i.e., children as young as 2 years of age).

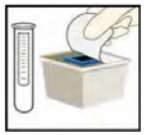
# Bio-Rad Geenius HIV1/HIV-2 Supplemental Assay

- 5ul of plasma/serum, 15ul whole blood
- In the USA, interpretation by reader
- Assay interpretation by the Geenius software :
  - ❖HIV Negative
  - ❖HIV-1 Indeterminate
  - ❖HIV-2 Indeterminate
  - ❖HIV Indeterminate
  - ❖HIV-1 Positive
  - ❖HIV-2 Positive
  - ❖HIV-2 Positive with HIV-1 cross-reactivity
  - ❖HIV Positive Untypeable



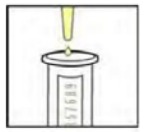
# Multispot vs. Geenius

- Protocols take about the same time, but Multispot has many more steps

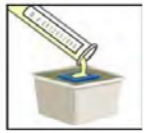


1. Remove foil; press prefilter down. Label cartridge and specimen or control test tubes.

2. Add 2 full droppers of Specimen Diluent to each test tube.



3. Add one drop of each sample or control to each labeled tube using a transfer pipette. Mix well.



4. Pour each sample into the prefilter of the labeled cartridge. Wait 2 minutes.

5. Remove and discard prefilter.

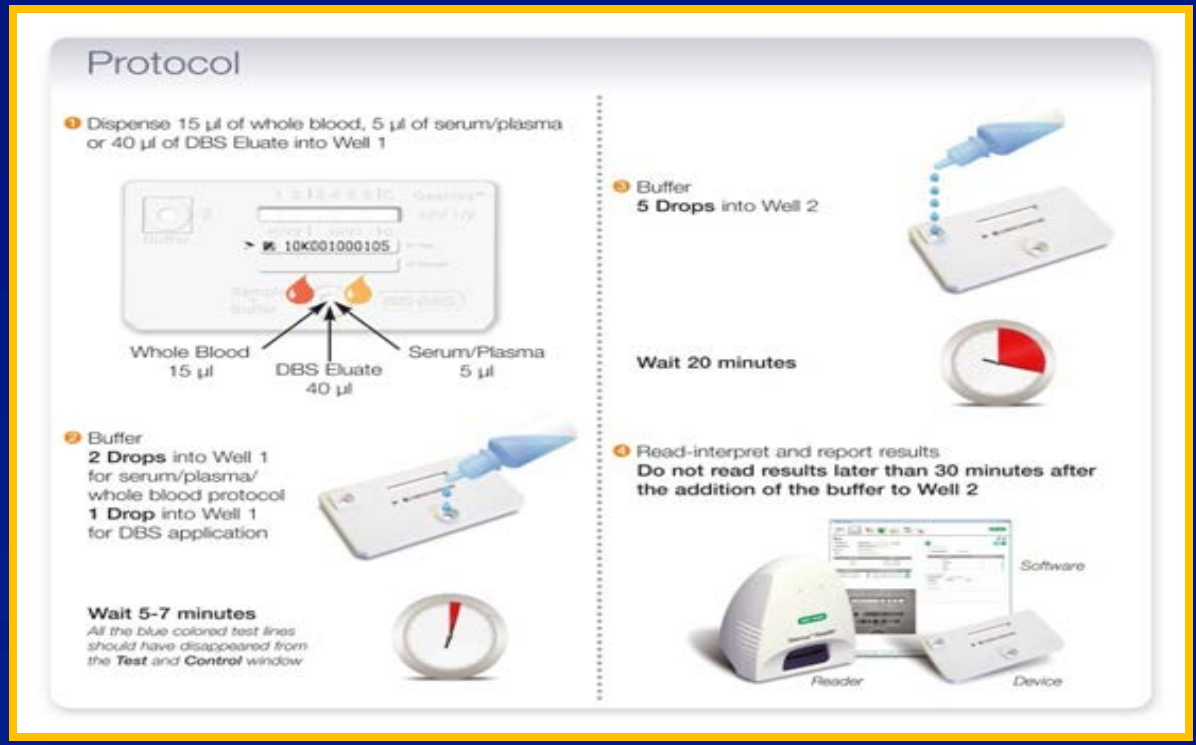
6. Fill the central well of each cartridge with Wash Solution.

7. Once absorbed, add 3 drops of Conjugate. Wait 2 minutes.

8. Fill well with Wash Solution and let absorb. Repeat.

9. Add 3 drops of Development Reagent. Wait 5 minutes.

10. Fill well with Stop Solution. Allow to absorb and read results.



# Sample sets and analysis I

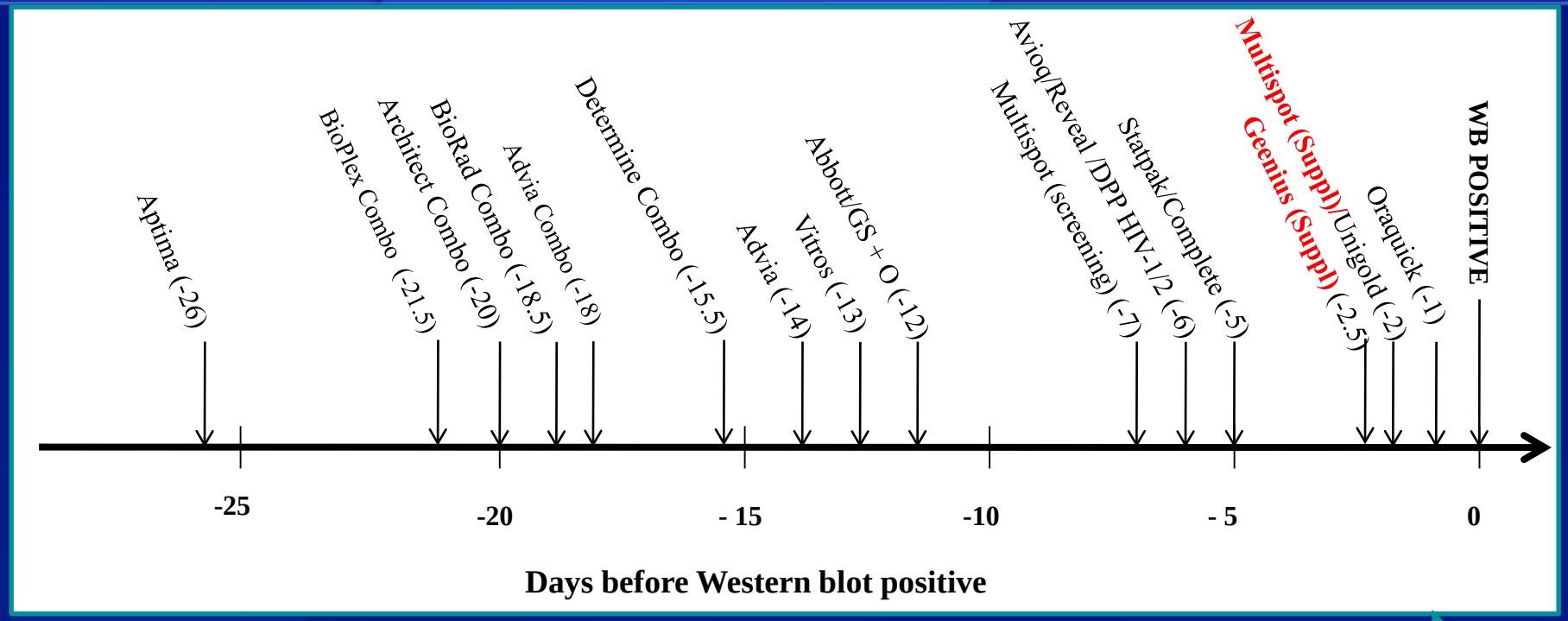
- **Assay performance in early HIV-1 infections**
  - Relative sensitivity of assay's performance with 50% cumulative frequency analysis
    - 17 commercial seroconverters with HIV-1 WB positive

- **Plasma specimens from STOP study**
  - Screening Targeted Populations to Interrupt On-going Chains of HIV transmission with Enhanced Partner Notification (STOP) study
  - Subset of 158 tested with Abbott Architect, Bio-Rad Multispot, and Abbott m2000 viral load (San Francisco) were tested with Geenius
  - Architect-false reactive (32 SF, 37 NY, 23 NC)
    - 87 were tested with GS HIV Ag/Ab Combo and Geenius at CDC

# Sample sets and analysis II

- **Analysis was performed considering previous testing**
  - 85 Early HIV-1 infection: Ag/Ab Combo-pos/MS-neg or –ind/NAT-pos
  - 41 Established HIV-1 infection: Ag/Ab Combo-pos/MS-pos or -undiff
  - 92 False reactive: Architect-pos/NAT-neg
- **HIV-2 established infections characterized at CDC**
  - 16 plasma specimens from USA
  - 25 plasma specimens from Ivory Coast

# Results of the Performance Evaluation of HIV Supplemental Assays




IgG immunoassays

IgG and/or IgM rapid tests

IgG/IgM immunoassays

Antigen/Antibody Combo assays/rapid test

Nuclei Acid test

# HIV-1 Infections

| HIV-1 infections | Total | Geenius results: HIV negative | HIV-1 indeter | HIV-1 positive | HIV-2 indeter |
|---|---|---|---|---|---|
| ARC-positive/MS-negative | 74 | 65 | 4 | 2 | 3 |
| ARC-positive/MS-indeterminate | 11 | 5 | 3 | 3 | |
| ARC-positive/MS-HIV-1 positive | 40 | 3 | 5 | 32* | |
| ARC-positive/MS-undifferentiate | 1 | | | 1 | |

HIV infections were confirmed with nucleic acid testing ; indeter: indeterminate
* eight required 1:10 diluation with MS

- Among early infections, Geenius confirmed five more infections than MS, but eight established HIV-1 infections required NAT with Geenius
- Geenius confirmed eight infections that required 1:10 dilution and one undifferentiated with MS
- Three specimens with Geenius HIV-2 indeterminate results would have needed HIV-2 NAT
- McNemar's analysis showed no significant difference *(p=0.7893)* between MS and Geenius (NAT vs. no NAT required or positive result)

# HIV-2 infections

| | | Geenius results | | | | |
|---|---|---|---|---|---|---|
| | Total | HIV negative | HIV-2 indeter | HIV-2 positive | HIV-2 pos (HIV-1 XR) | HIV untyp |
| **HIV-2 infections** | | | | | | |
| BRC-positive/MS-HIV-2 positive | 40 | 1 | 2** | 15 | 15 | 7*** |
| BRC-positive/MS-undifferentiate | 1 | | | | 1 | |

indeter: indeterminate; HIV-1 XR: cross-reactivity with HIV-1; untyp: untypable

HIV infections were confirmed with nucleic acid testing

** one required 1:10 dilution with MS

*** four required 1:10 dilution and two 1:100 diluation with MS

- Ten HIV-2 infections required NAT for confirmation with Geenius while only one with MS
- Seven of those ten required the dilution protocol with MS
- McNemar's analysis showed significant differences between MS and Geenius (*p=0.0159*)

# False reactive specimens

| | | Geenius results | | | |
|---|---|---|---|---|---|
| | Total | HIV negative | HIV-1 indeter | HIV-1 positive | HIV-2 indeter |
| **HIV-negative (false reactive)** | | | | | |
| ARC-positive/MS-negative | 88 | 84 | 1 | | 3 |
| ARC-positive/MS-indeterminate | 2 | | 1 | 1 | |
| ARC-positive/MS-HIV-2 positive | 2 | 2 | | | |

HIV infections were confirmed with nucleic acid testing; indeter: indeterminate

- Of ARC-false reactive specimens (HIV NAT negative)
  - 95.6% were MS-negative
  - 93.5% were Geenius-negative
- HIV-2 reactivity contributed to decrease specificity
- Of 87 available ARC-false reactive
  - 6.9% (6) were Bio-Rad Combo-repeatedly reactive
    - 1 MS-indeterminate/Geenius HIV-1 indeterminate
    - 1 MS-indeterminate/Geenius HIV-1 positive

# Geenius HIV-2 indeterminate results

- Among HIV-1 and ARC-false reactive samples, gp140 only reactivity was observed

- HIV-2 indeterminate result showed different reactivity among HIV-2 infections

| Boca Biolistics Data | | | MP diagnostics |
|---|---|---|---|
| Multispot HIV-2 | Geenius conclusion | Detected Bands Geenius | HIV-2 WB - 125, 80, 68. 56. 53. 36. 26 |
| POSITIVE | HIV-2 INDETERMINATE | gp36 CTRL | 80,68,56trace,53,36,27,26 |
| POSITIVE | HIV-2 INDETERMINATE | gp36 CTRL | 80,68,56,53,36 |

# Summary

- Overall, MS confirmed 40 HIV-1 and 40 HIV-2 infections, while Geenius confirmed 38 HIV-1 and 31 HIV-2 infections
- Due to the higher HIV-2 indeterminates, more NAT testing is required with Geenius compared to MS
- High number of HIV-2 indeterminate results with Geenius among HIV-1 and HIV-false reactive specimens (gp140 reactivity); while true HIV-2 positive specimens were due to gp36
- MS showed slightly better specificity than Geenius

# Conclusions

- MS and Geenius had comparable performance in identifying HIV-1 infections using the recommended laboratory diagnostic algorithm

- Increased the number of HIV-2 indeterminate results with Geenius may require more nucleic acid testing

# Acknowledgements

HIV Diagnostics and Incidence Team

- Krystin A. Price
- William Fowler
- Vickie Sullivan
- Sarah Adams
- Michele Owen
- Silvina Masciotra

STOP Team

- Cindy Gay
- Emily Westheimer
- Stephanie Cohen
- Lisa Hightow-Weidman
- Philip Peters