# $IgG_3$ as a Biomarker for Distinguishing Recent from Established HIV-1 Infection

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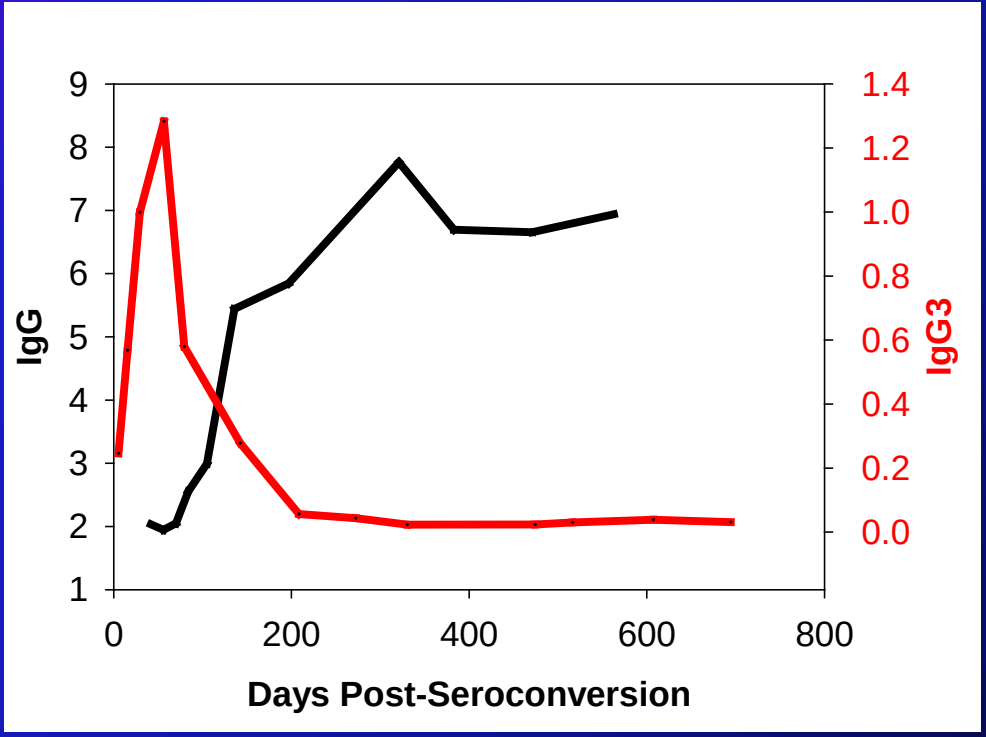
# Serological Assays for Determining Recent HIV Infection

- Advantages:
  - Obvious evolution of antibody response to HIV- antibody quantity and avidity gradually increase as disease progresses.
  - Antibody in plasma samples is relatively stable.
- Challenges:
  - Identify most reliable marker/markers for differentiating early from late infection.
  - Reduce number of false recents due to variations in the antibody response, drug therapy, advanced disease state, etc.
- Approaches:
  - Antibody titer
  - Avidity Index
  - Antibody subclass

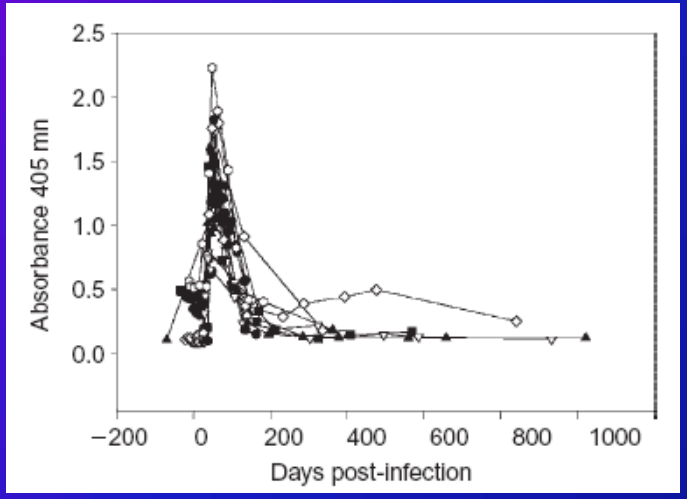
# IgG Subclass

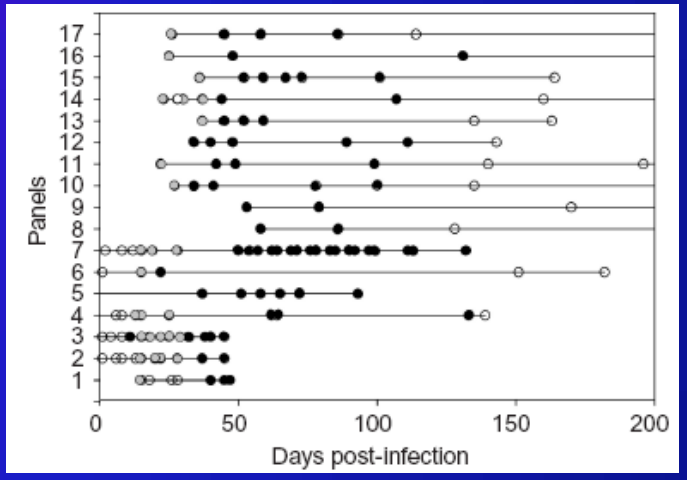
- $IgG_1$:
  - Predominant subclass directed against HIV (major component of total IgG).
  - Increases steadily post-infection.
- $IgG_2$ and $IgG_4$:
  - Not frequently found.
- $IgG_3$:
  - Produced early post-infection.
  - Decreases after initial peak in reactivity.

# Temporal Relationship Between $IgG_3$ and Total IgG ($IgG_1$)



# $IgG_3$ as a Biomarker for Recent Infection



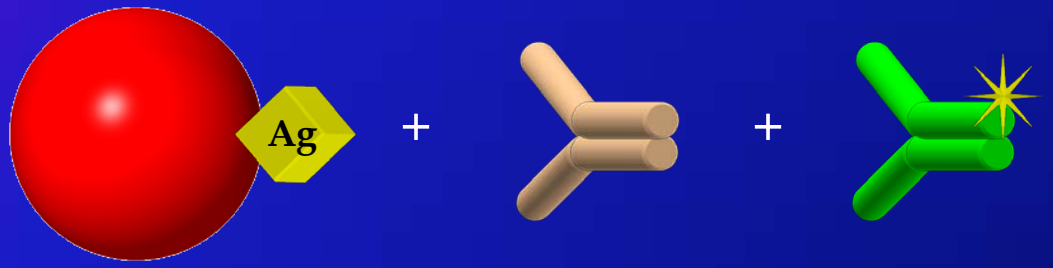

- Developed an $IgG_3$-specific p24 ELISA
- Detectable peak of $IgG_3$ reactivity was observed in 17 seroconversion panels
- Window of detection ranged from 34 to 120 days post-infection

Wilson et al., AIDS, 2004

# Luminex Assay (Bio-Plex System)

- Sensitive, quantitative detection of antibody.
- Detection of multiple analytes in a single well.
- Assay can be customized to detect up to 100 different analytes (100 different microsphere sets).
- Uses extremely small sample volume (1µl/well).

# Assay Procedure



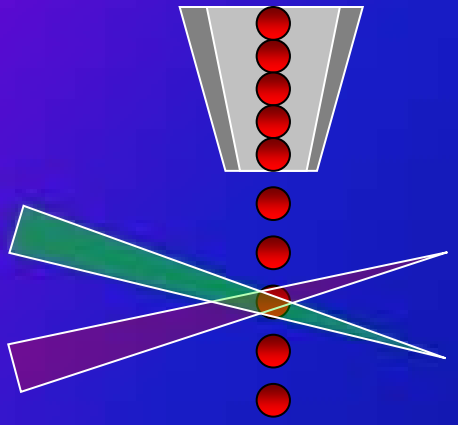

Beads conjugated to specific antigen

**p24**
**p66**
**p31**
**gp120**
**gp160**
**gp41**

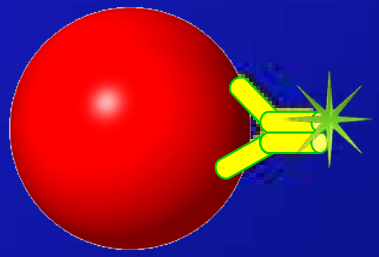
Diluted patient plasma

PE-conjugated anti-human $IgG_3$

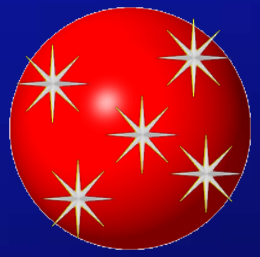
# Bio-Plex Suspension Array System



Microspheres are aligned in single file and passed through 2 lasers



1st laser excites molecular tags- Data output as mean fluorescent intensity (MFI)

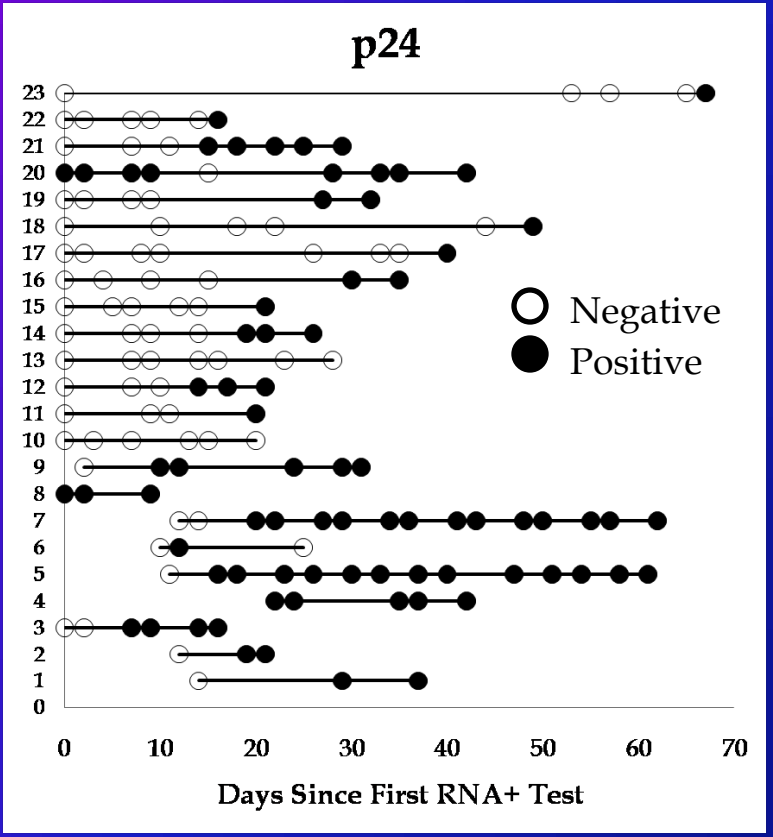


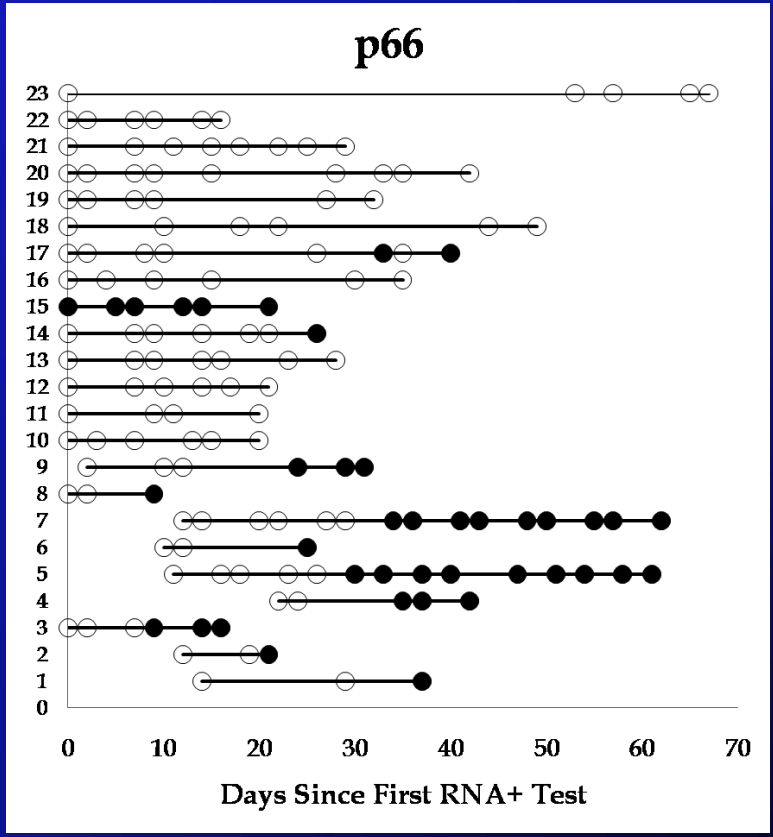
2nd laser excites microsphere and identifies dye signature

# Samples Tested

- Seroconversion Panels/Longitudinal Specimens:
  - Total- 1377 specimens/ 382 subjects
    - Includes subtypes- B, B′, E, A, and G
    - Timing of $IgG_3$ detection- 23 panels (148 specimens)
    - Peak $IgG_3$ detection- 18 panels (177 specimens)
    - Longitudinal detection- 1200 specimens/ 332 subjects
- ART:
  - Total- 557 specimens/ 104 subjects
- AIDS and LT Non-Progressors:
  - Total- 350 specimens/ 138 subjects

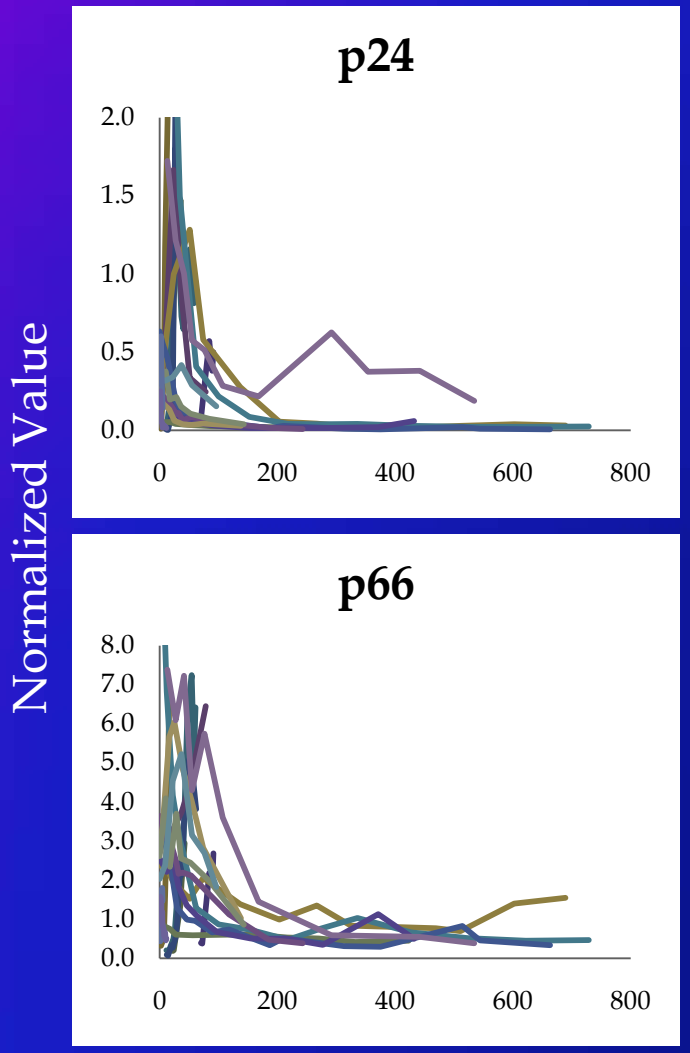
# $IgG_3$ Detection




Median time to detection=
19 days for p24 and 26 days for p66

# Seroconversion Panels

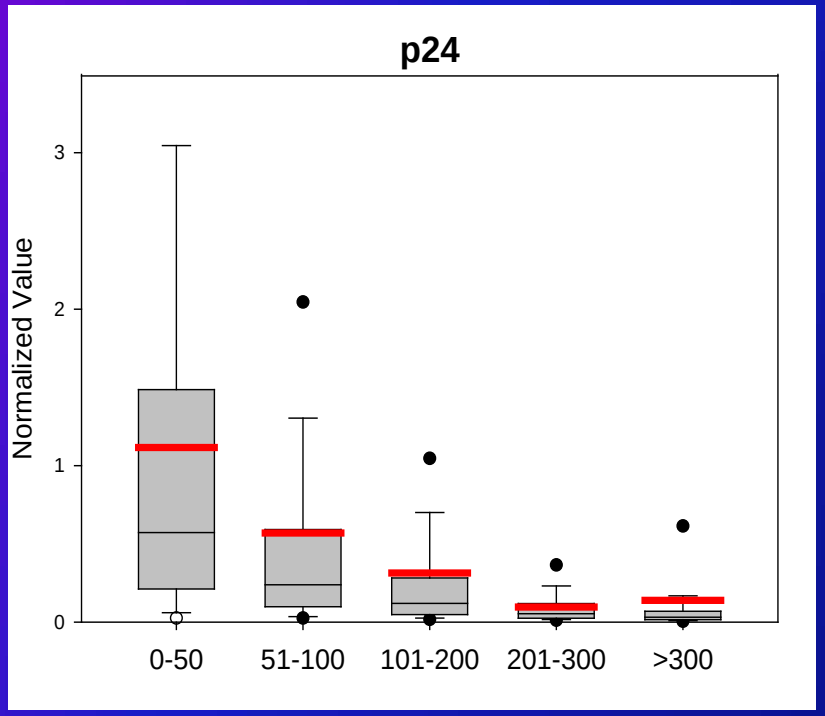


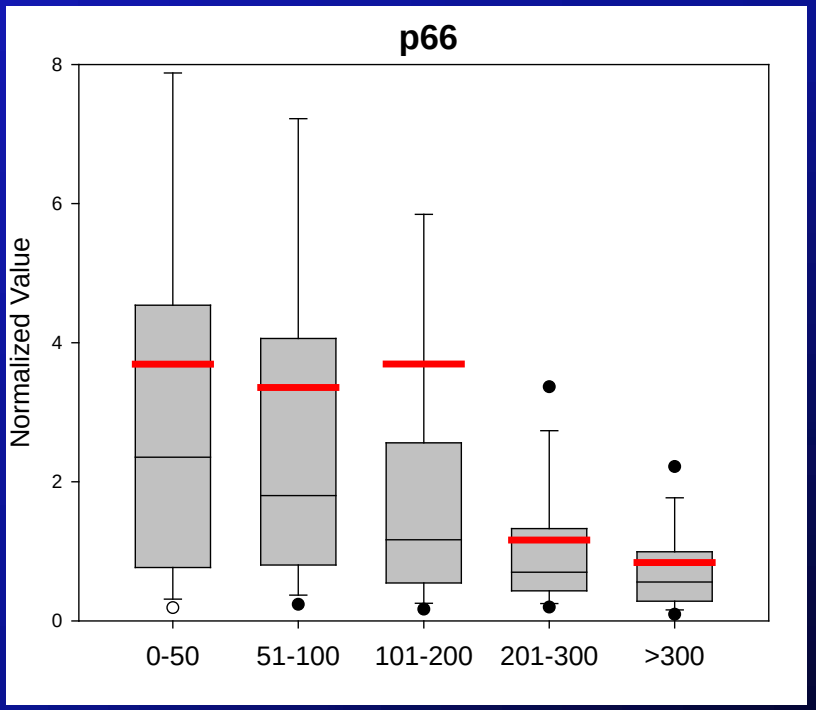

- Evaluated 18 seroconversion panels
- Peak of $IgG_3$ reactivity was observed shortly after initial detection
  - Median= 26 days (p24)
  - Median= 37 days (p66)

    (7 and 11 days post initial detection)

# Longitudinal Specimens

1200 samples from 332 subjects



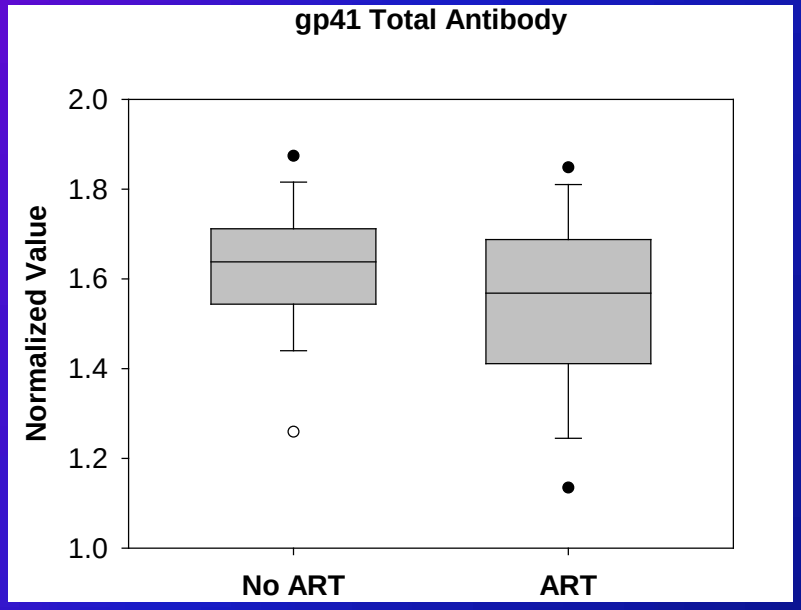



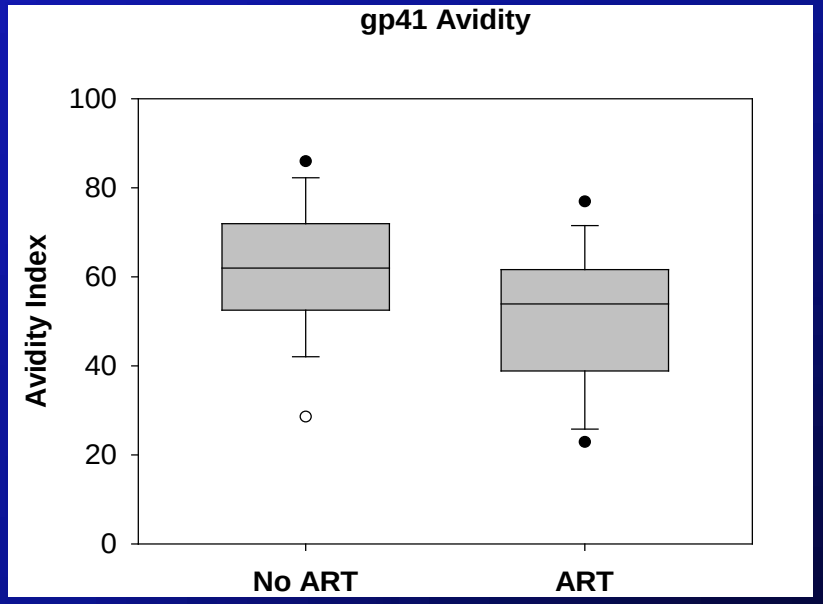

Days Since Seroconversion

- p24- Reactivity drops drastically after 50 days post-seroconversion.
- p66- Reactivity begins to decline after 100 days.

# Effects of ART on Serological Assays

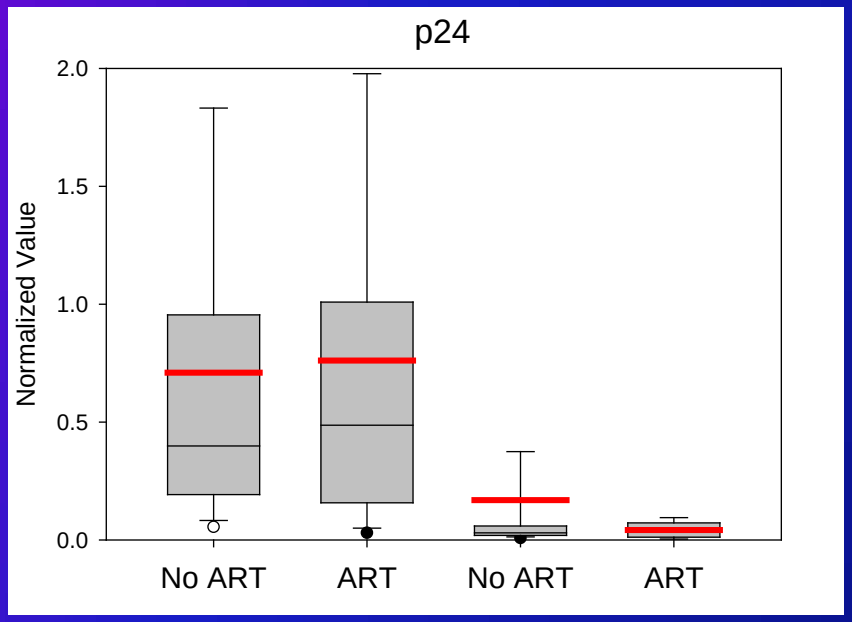
Total IgG response to gp41 in individuals infected >365 days

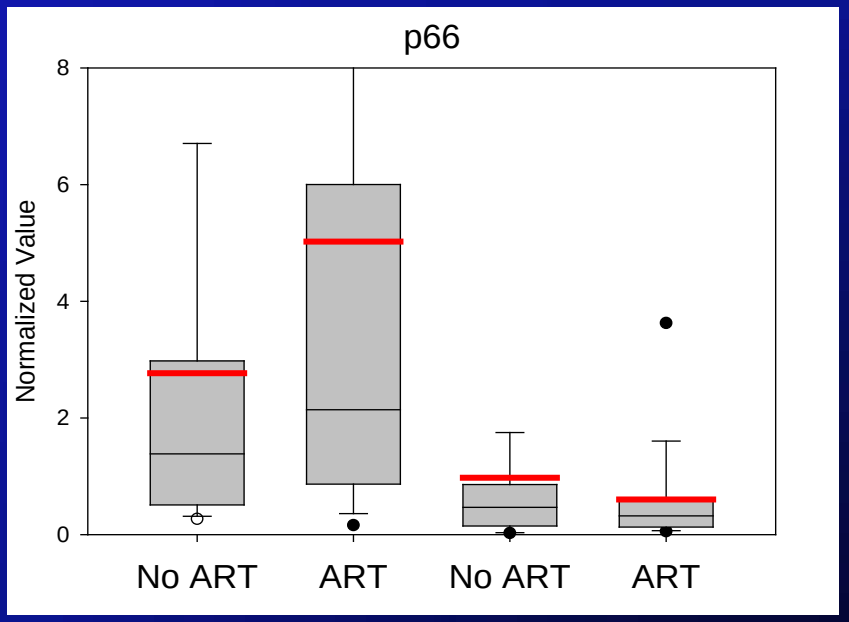



*Reduction in antibody levels and avidity, especially if ART is initiated early post infection

# ART and the $IgG_3$ Response

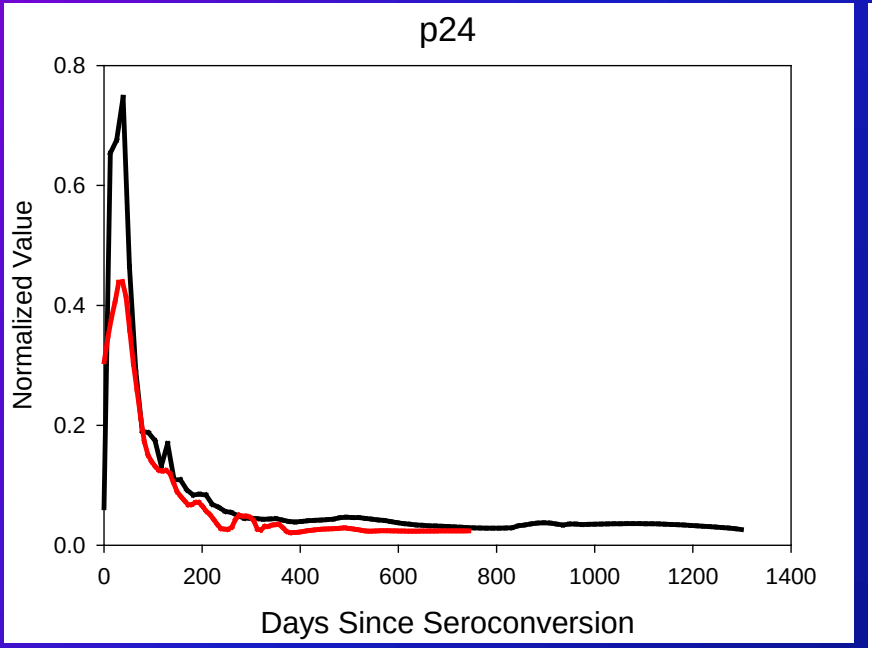


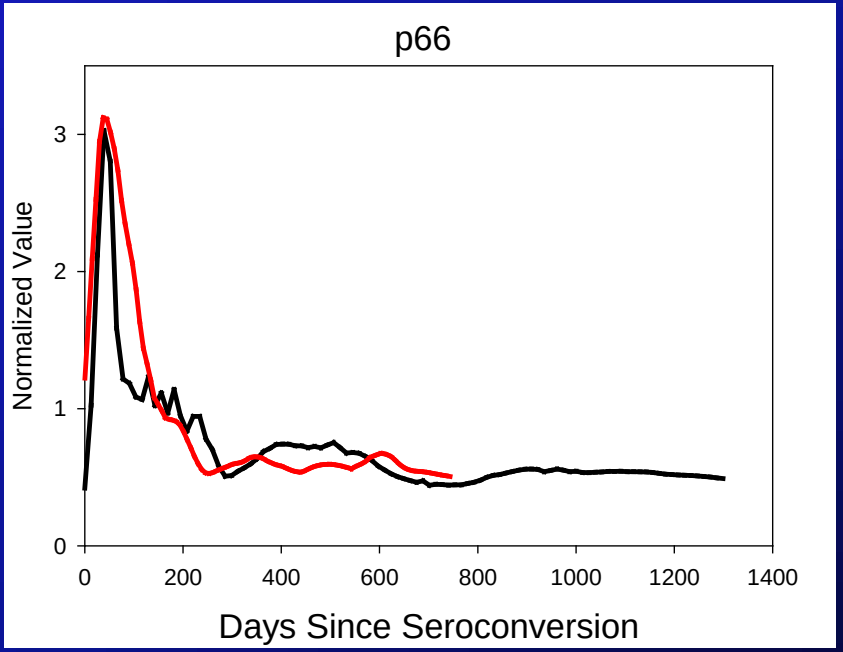




**0-100 days** **>300 days** **0-100 days** **>300 days**

*Lower likelihood of false recents caused by ART

# HIV-1 Subtype

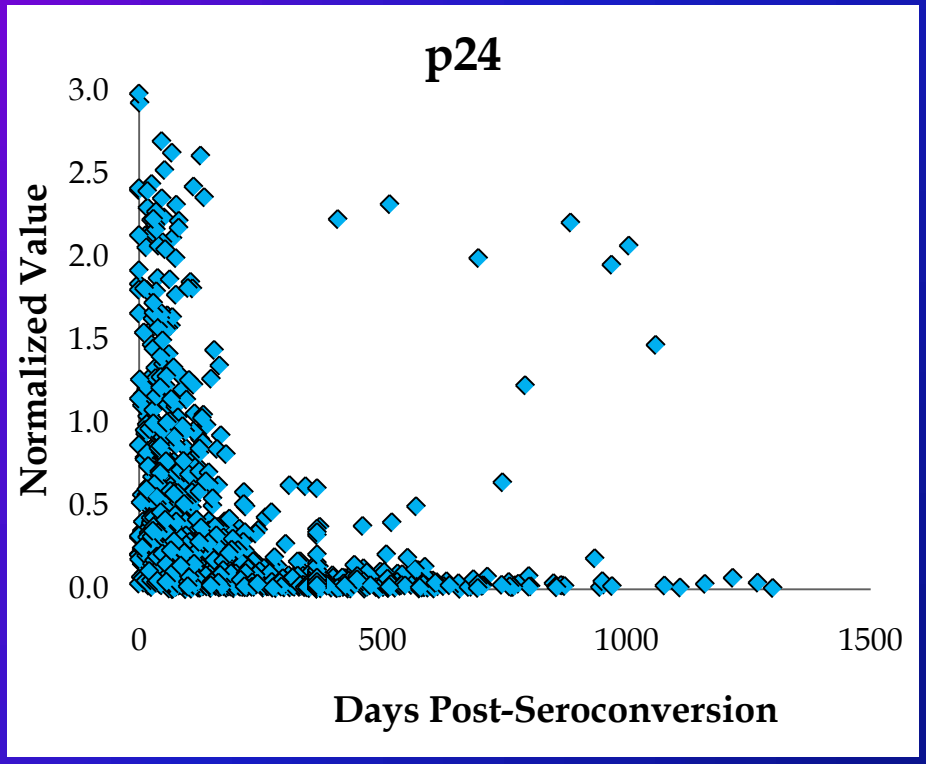



Subtype B (645 specimens/143 subjects)
Subtype A/G (131 specimens/13 subjects)

*Evaluation of multiple subtypes is needed

# Summary



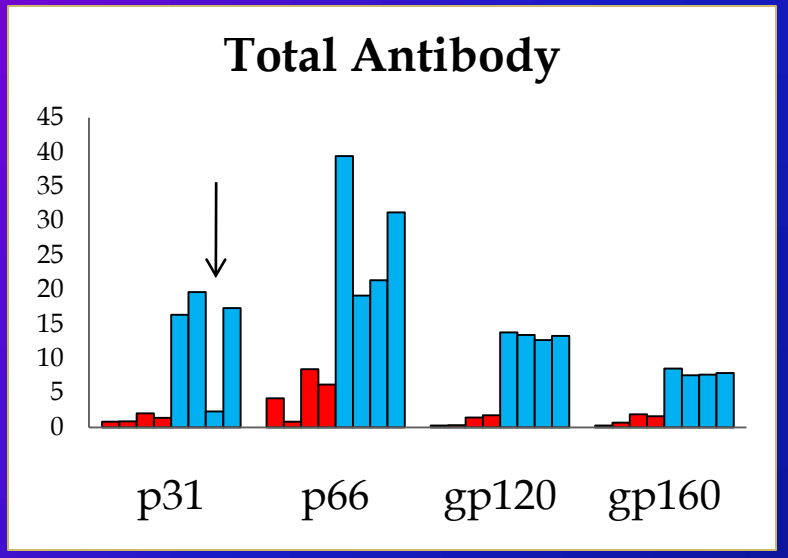

1200 samples from 332 subjects

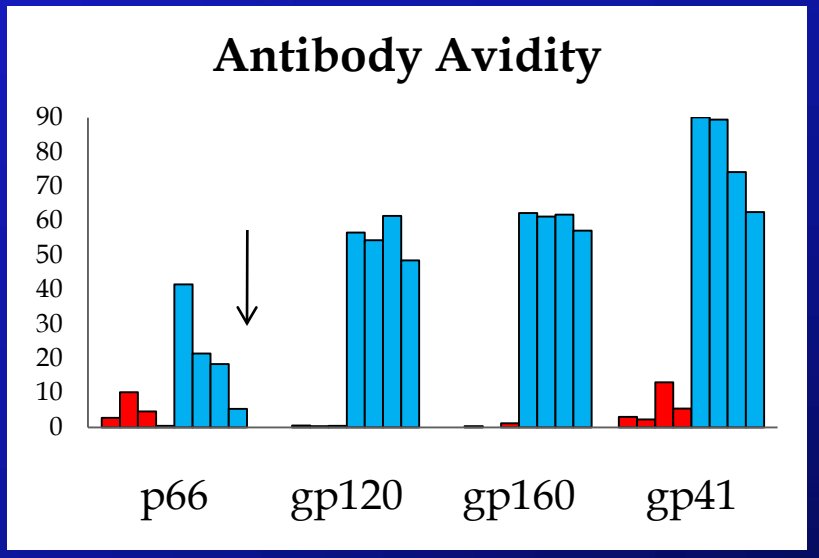
- $IgG_3$ directed against p24 and p66 appears to be a good indicator for early infection.
- As a single determinant of recent infection, there will still be some misclassification due to individuals that do not follow the rule. (*Similar outliers were observed with AIDS/LT samples)

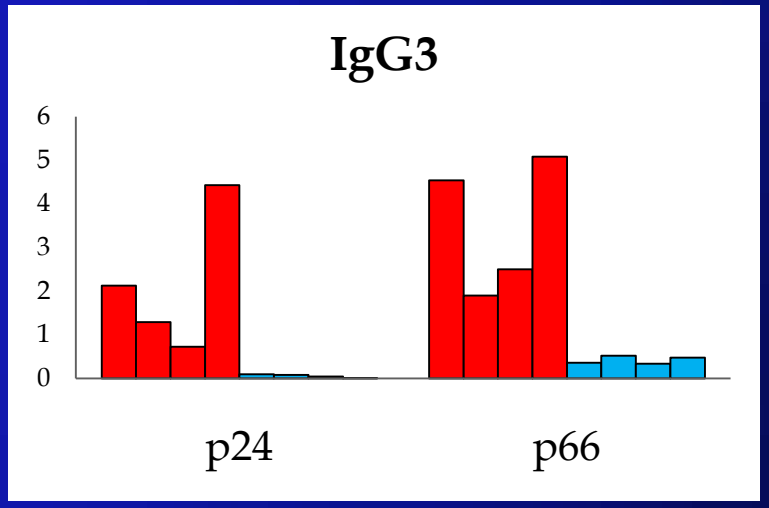
# Algorithms for Determining Recent HIV Infection

- Increasing number of studies evaluating algorithms of different tests for estimating HIV incidence.
- Bio-Plex assay incorporates multiple analyses in 1 test.
  - Currently developing an assay that determines recent infection based on 8 different antibody measures (poster)
    - Antibody quantity and avidity for total IgG
  - Evaluating feasibility of incorporating $IgG_3$ subclass into multiplexed assay
  - Determining recent infection based on an antibody profile as opposed to a single measure

# Identification of Recent Infection Based on Antibody Profile




Known recent

Known LT

*Each bar represents 1 individual

# Next Steps

- Statistics, statistics, statistics.
  - Results from ~2500 specimens.
  - Assay cutoffs.
  - Window period of detection.
  - Assay validation with additional samples- LT samples are underrepresented.
- Feasibility of multiplexing IgG3 with total IgG measures.
- Feasibility of $IgG_3$ as a stand-alone assay- ratio of $IgG_3$ to total IgG
- Evaluation of additional analytes.

# Acknowledgements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  - Lab Branch
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