LUMINEX-BASED MULTIPLEX ASSAY FOR HIV INCIDENCE

Principle, Assay Development and Performance

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CENTERS FOR DISEASE CONTROL AND PREVENTION

CDC Developed Incidence Assay

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PLoS one

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

2012

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RESEARCH ARTICLE

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

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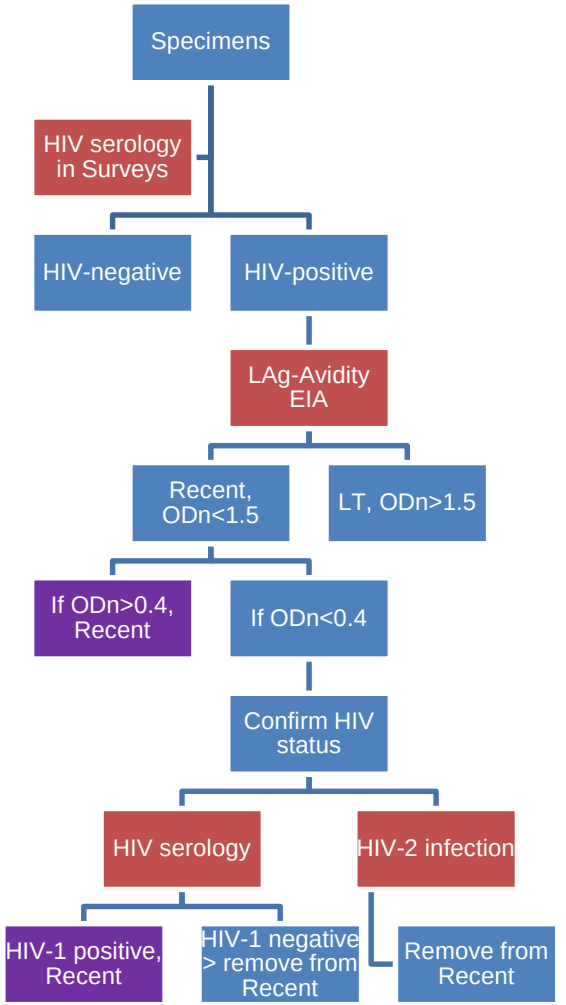
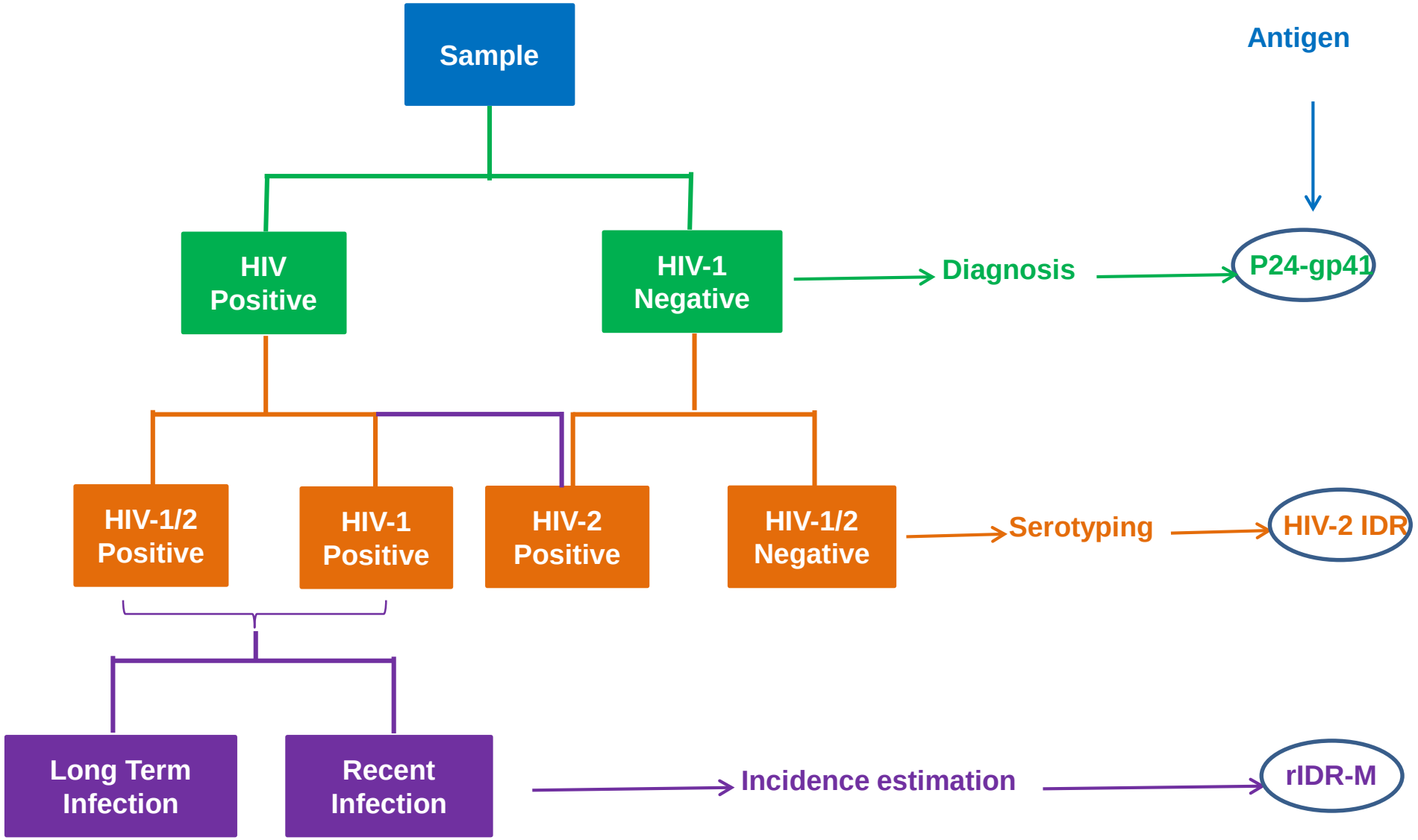
Ab-Based Recent Infection Detection

- 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



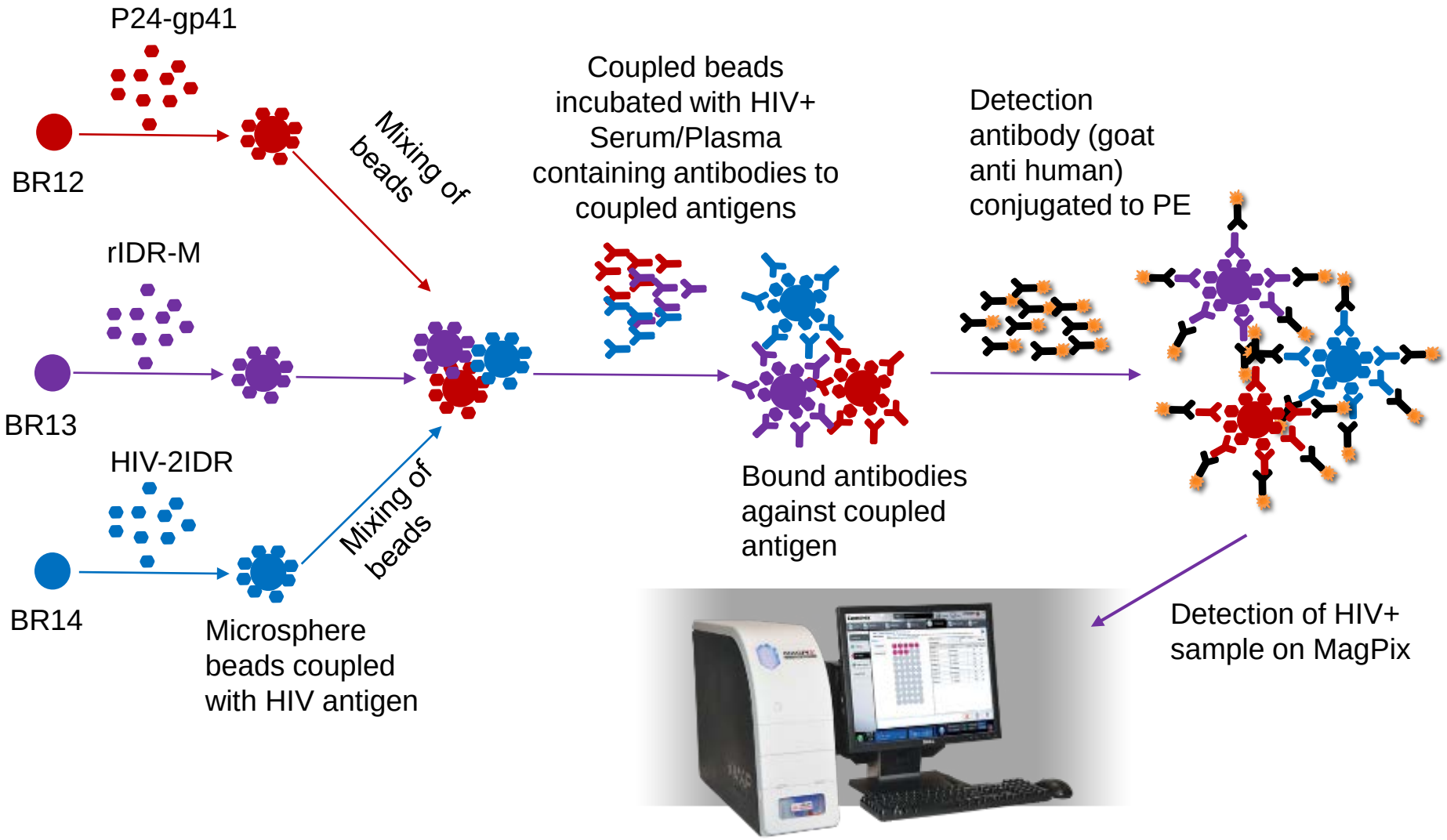
Next Generation Incidence Assay



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

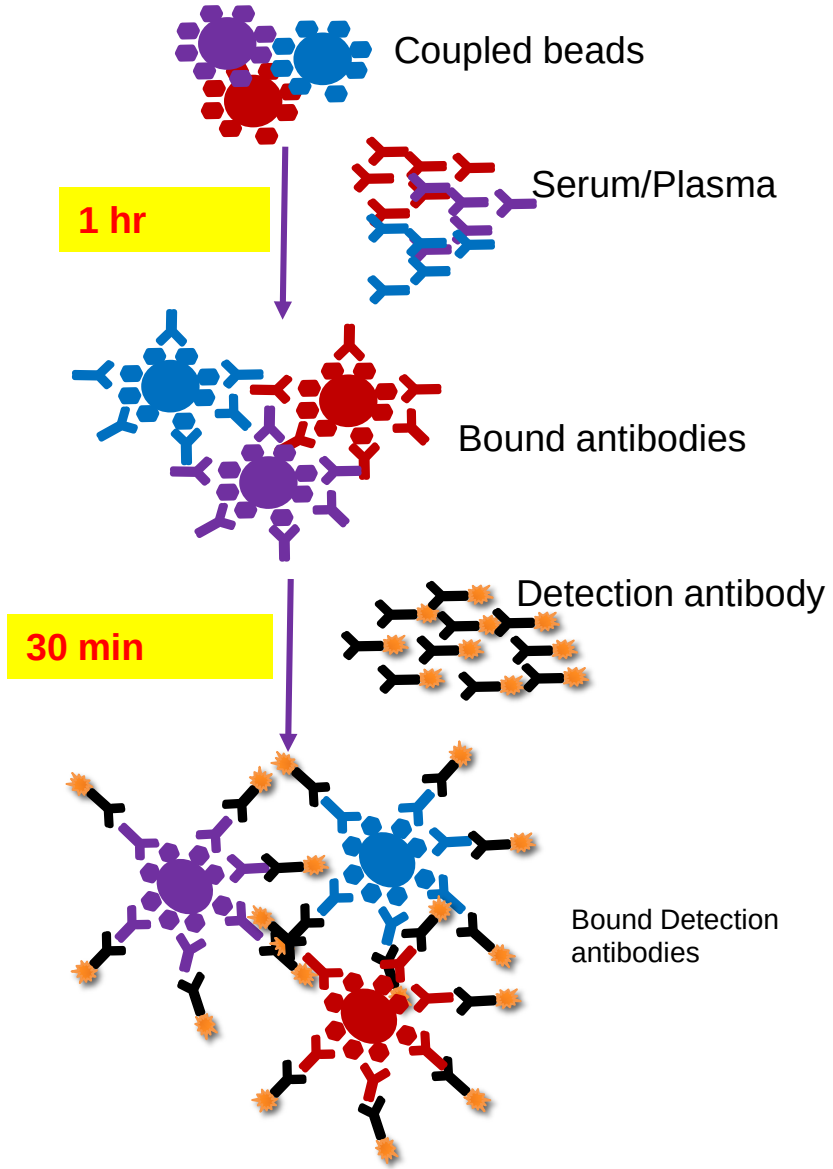
The Art of Multiplexing, Mixing of P24-gp41, rIDR-M and HIV-2 IDR Coupled Beads



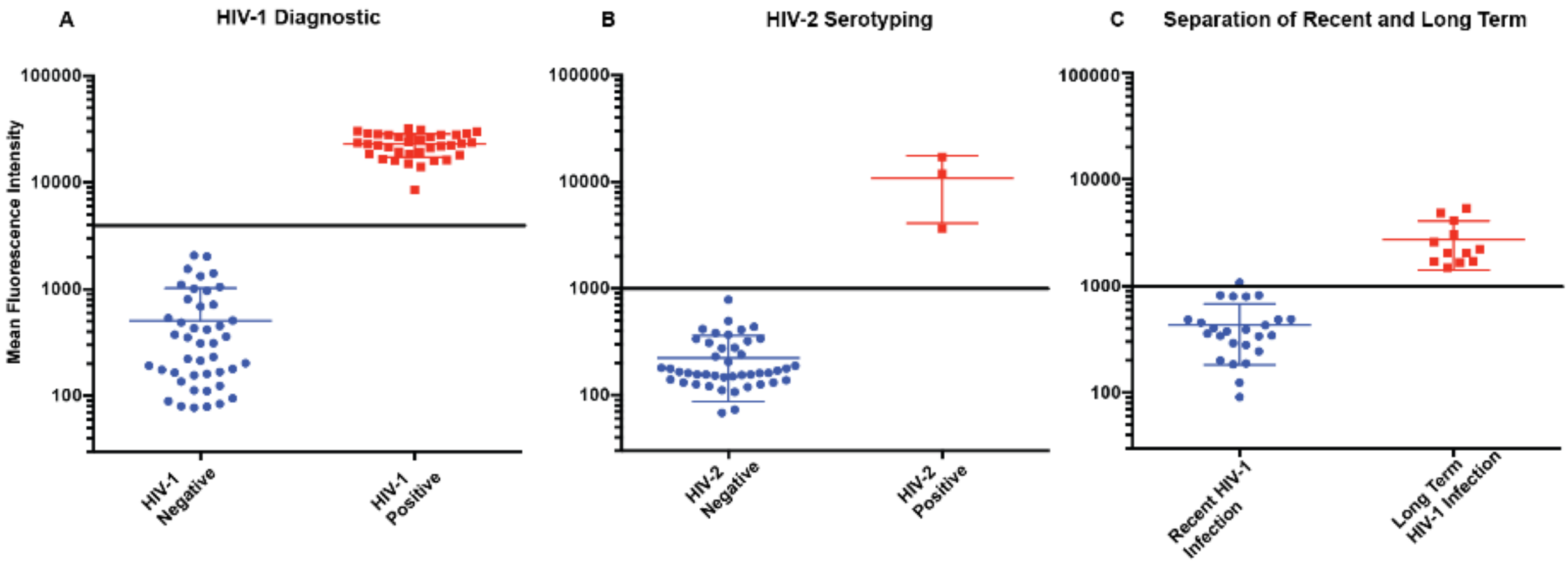
OPTIMIZATION OF ASSAY PARAMETERS

Critical Optimization Parameters

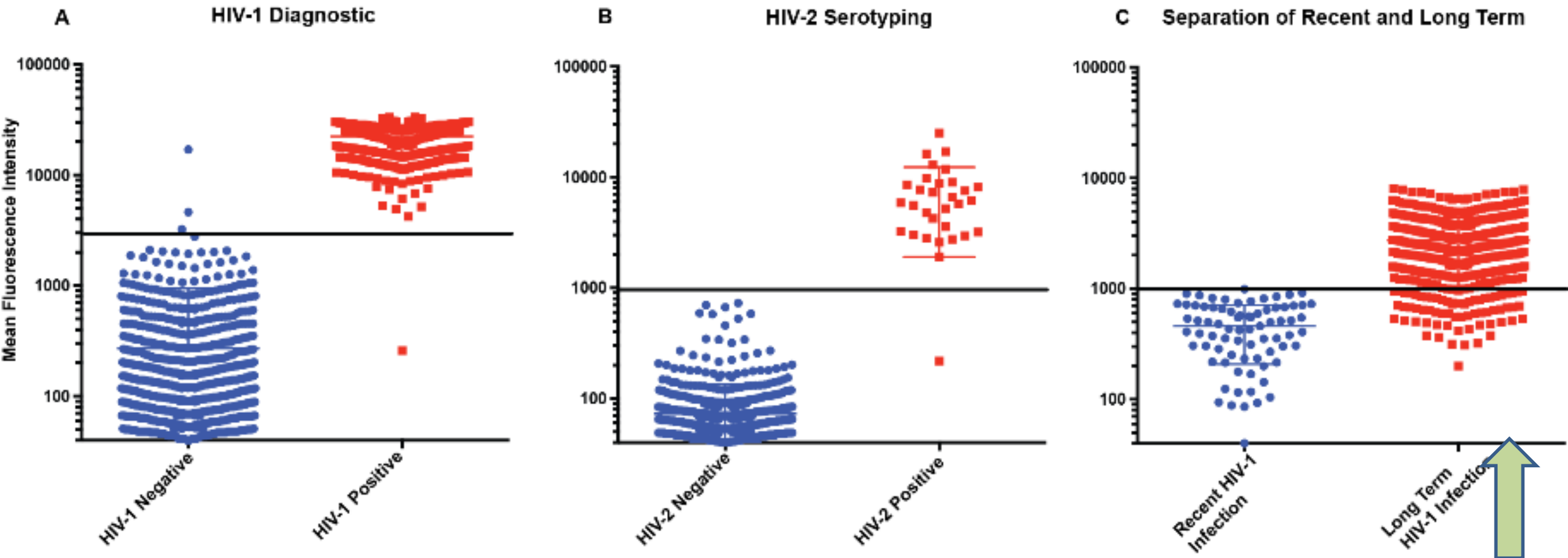
1. Diagnostic antigen=p24-gp41 fusion protein and HIV-2 IDR
2. rIDR-M antigen for separation of recent and long term infection
3. Optimal concentration of Ags on beads
4. Optimal sample dilution
5. Optimal dilution of detection Ab
6. Optimal incubation time
7. MFI signal stability over time and temperature
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



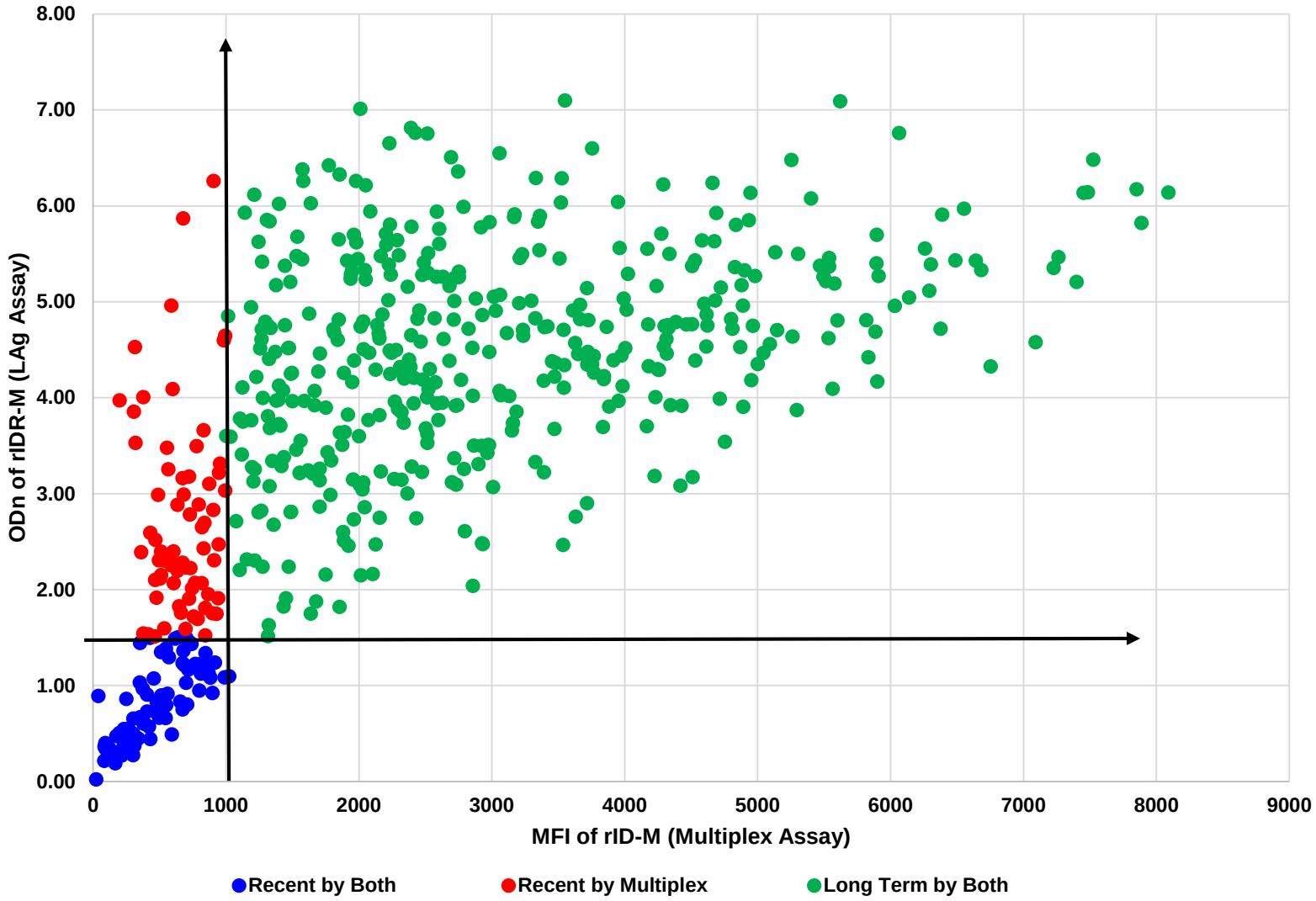
85-Member Panel as Classified by EIA/Western, Multispot and The LAg Assay



1500-Member Panel as Classified by EIA/Western, Multispot and The LAg Assay



Possibility to Extend Window Period

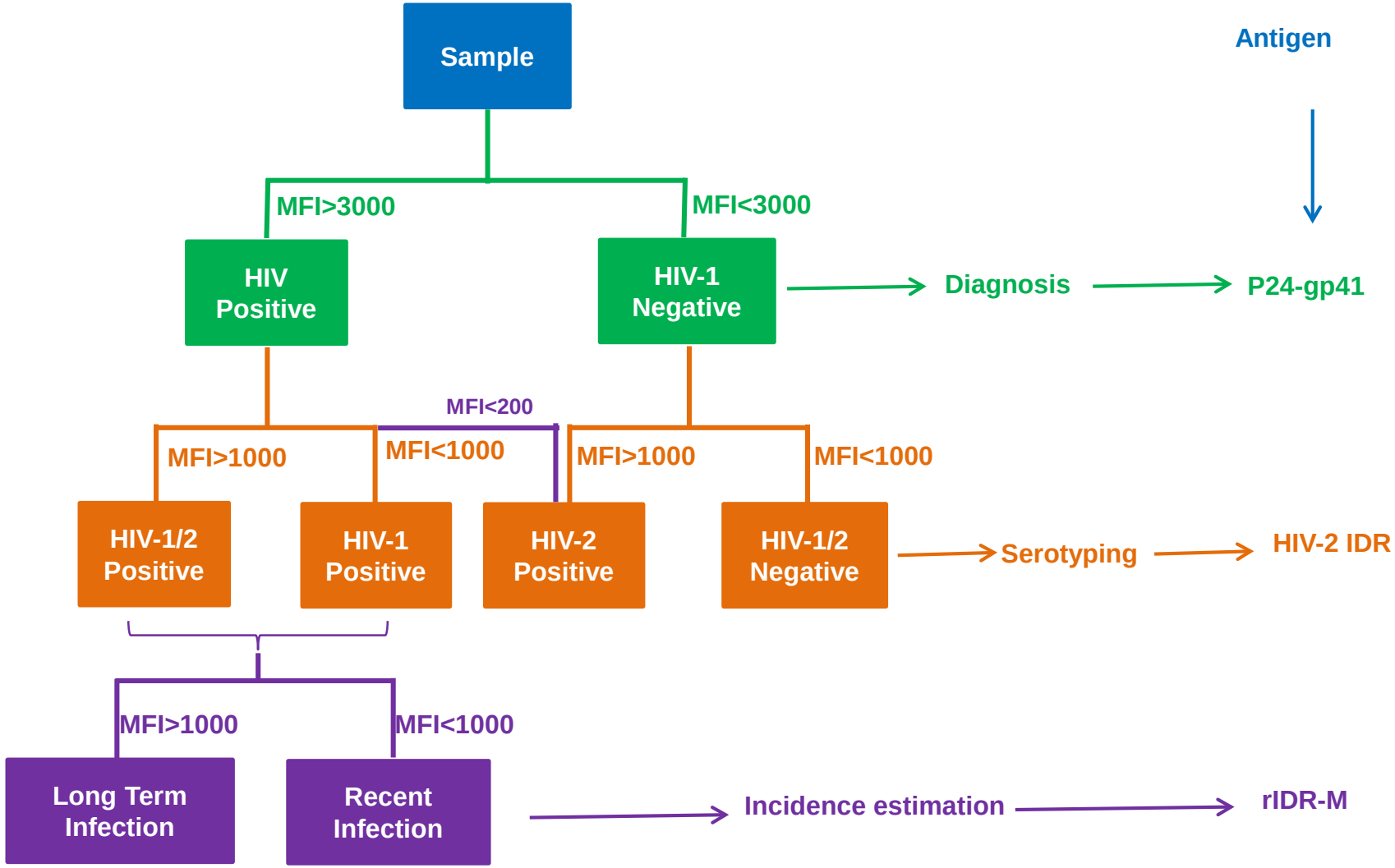


Data Summary of HIV Diagnosis and Typing

	Reference HIV Status by EIA/Western Blot/Multispot			
Luminex Multiplex	HIV-1	HIV-2	Negative	Total
HIV-1	569	0	3	572
HIV-2	0	30	0	30
Negative	1	1	896	898
Total	570	31	899	1500

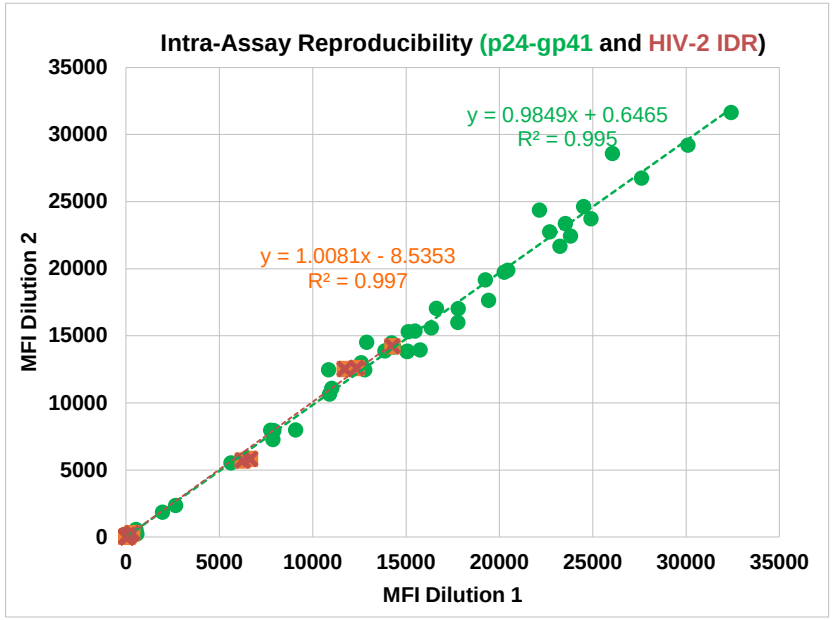
n=1500	Test	HIV-1	HIV-2
	Sensitivity	99.8	96.7
	Specificity	99.7	100

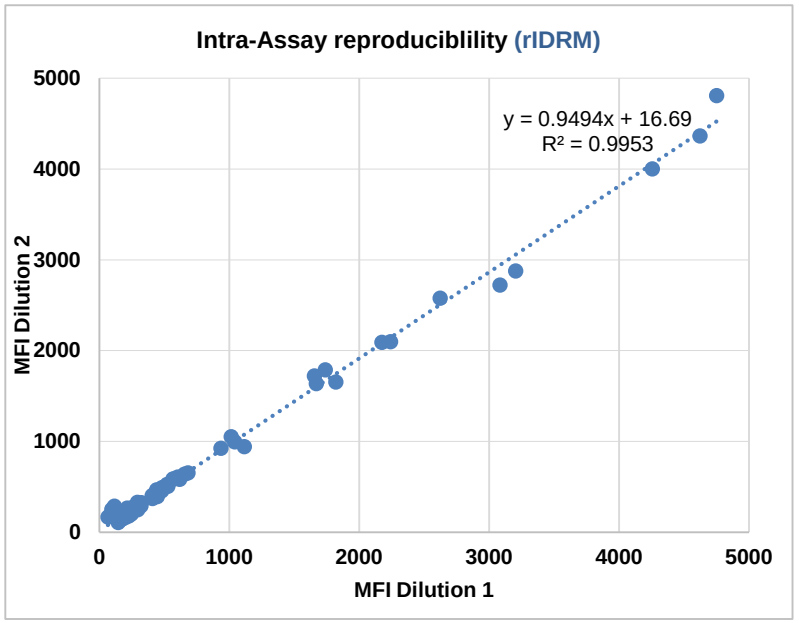
Multiplex Assay Algorithm



ASSAY REPRODUCIBILITY PARAMETERS

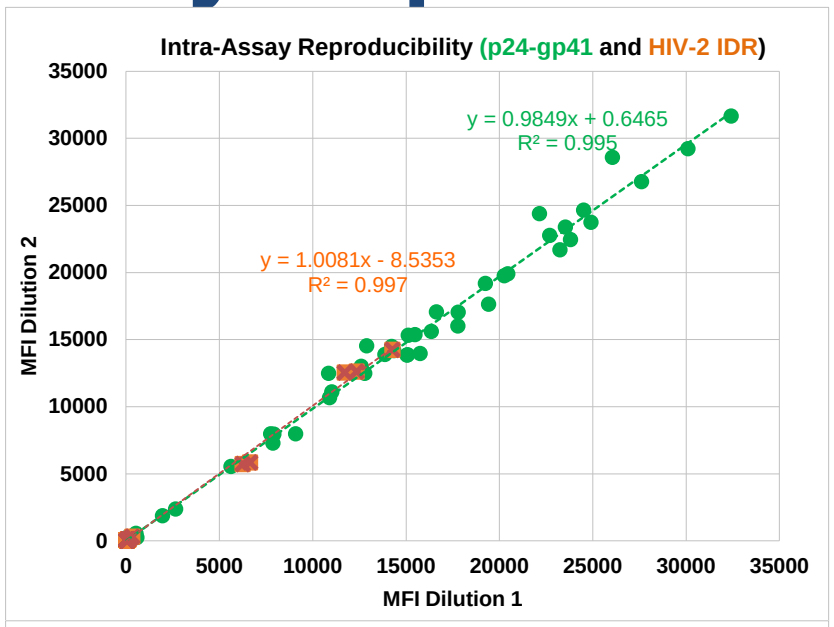
Assay Reproducibility Parameters

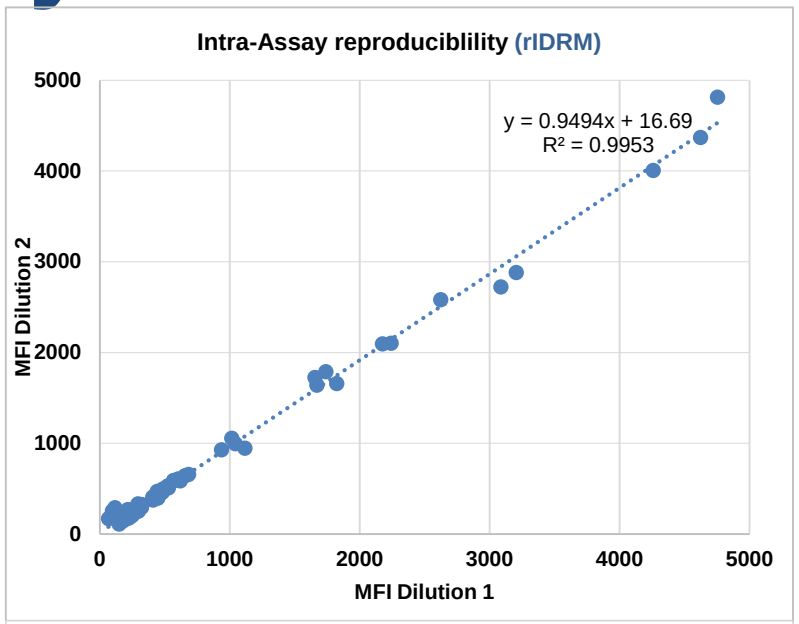


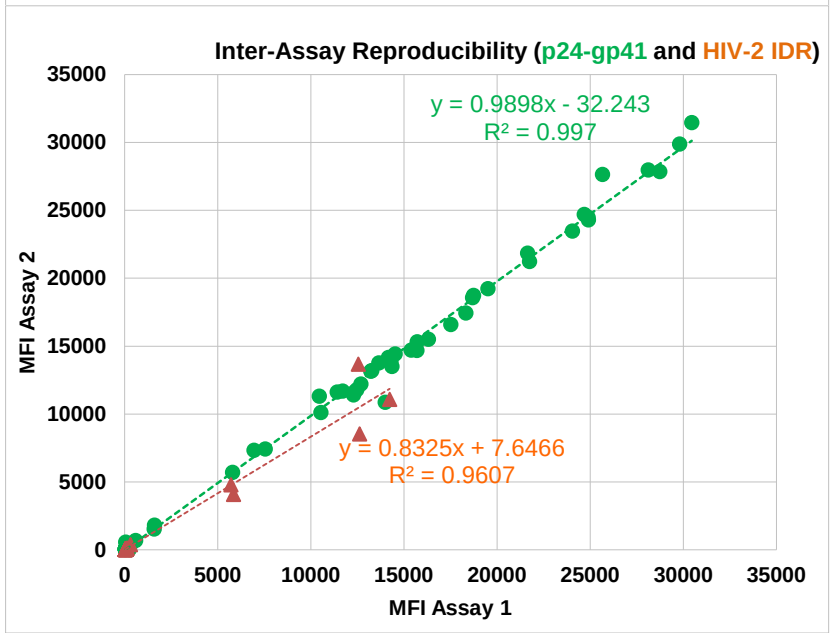


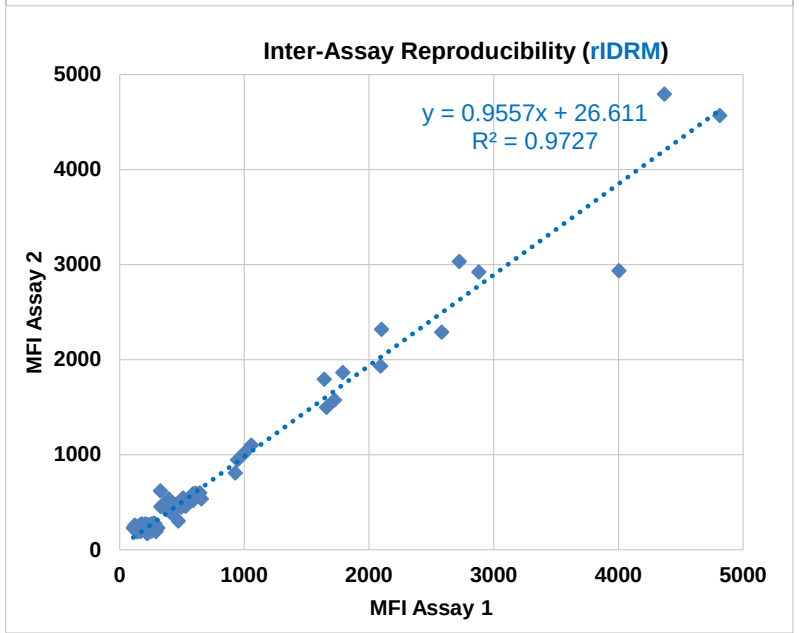
Specimen No	HIV Status	D1	D2	D3	Mean	STD	%CV
VP1	Neg	62.0	62.0	57.0	60.3	2.9	4.8
VP2	HIV-1	25139.0	25075.0	23445.0	24553.0	960.1	3.9
VP3	HIV-1	31215.5	30878.0	29840.5	30644.7	716.6	2.3
VP4	Neg	61.0	65.0	62.0	62.7	2.1	3.3
VP5	Neg	47.0	50.0	51.0	49.3	2.1	4.2
VP6	HIV-1	23469.0	22880.5	21994.0	22781.2	742.5	3.3
VP7	Neg	53.0	55.0	61.0	56.3	4.2	7.4
VP8	HIV-1	28656.5	28951.0	28541.0	28716.2	211.4	0.7
VP9	HIV-1	27917.0	27130.0	26731.0	27259.3	603.5	2.2
VP10	Neg	92.0	92.0	86.0	90.0	3.5	3.8

Assay Reproducibility Parameters

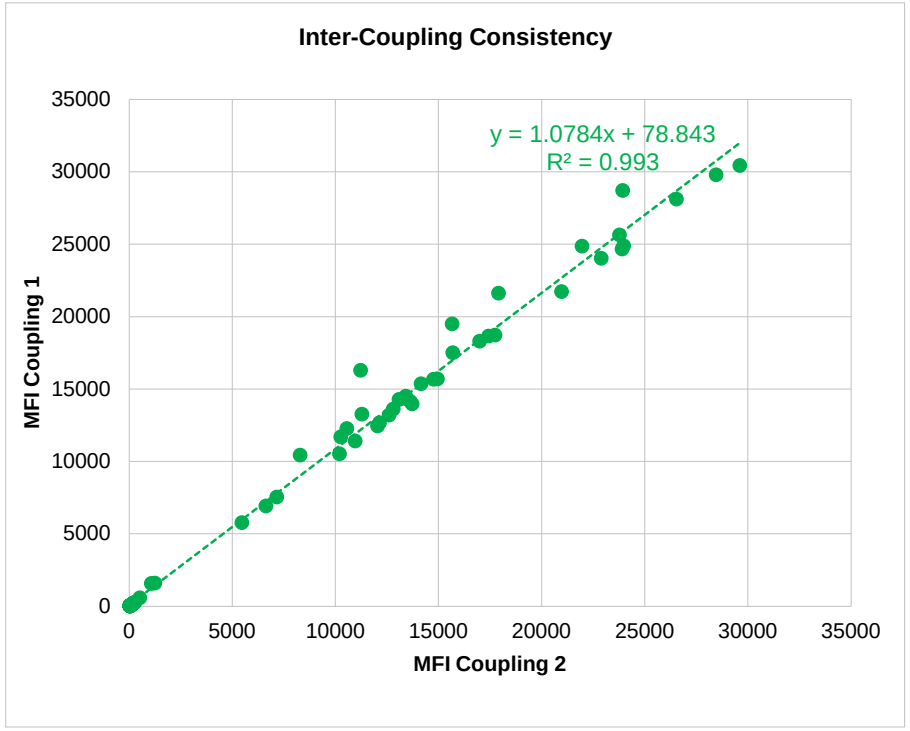


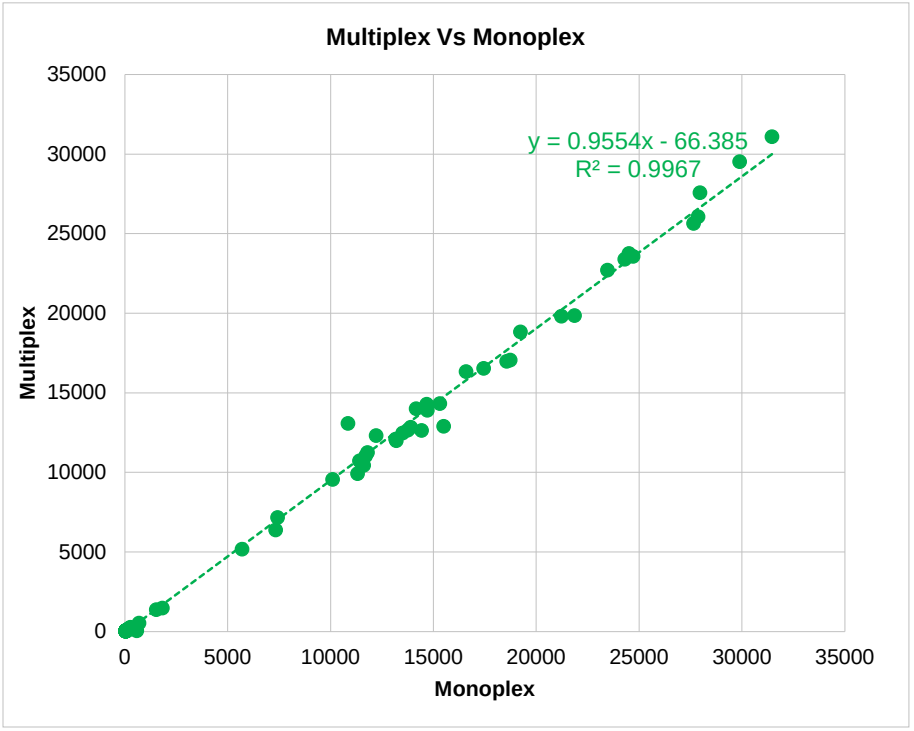




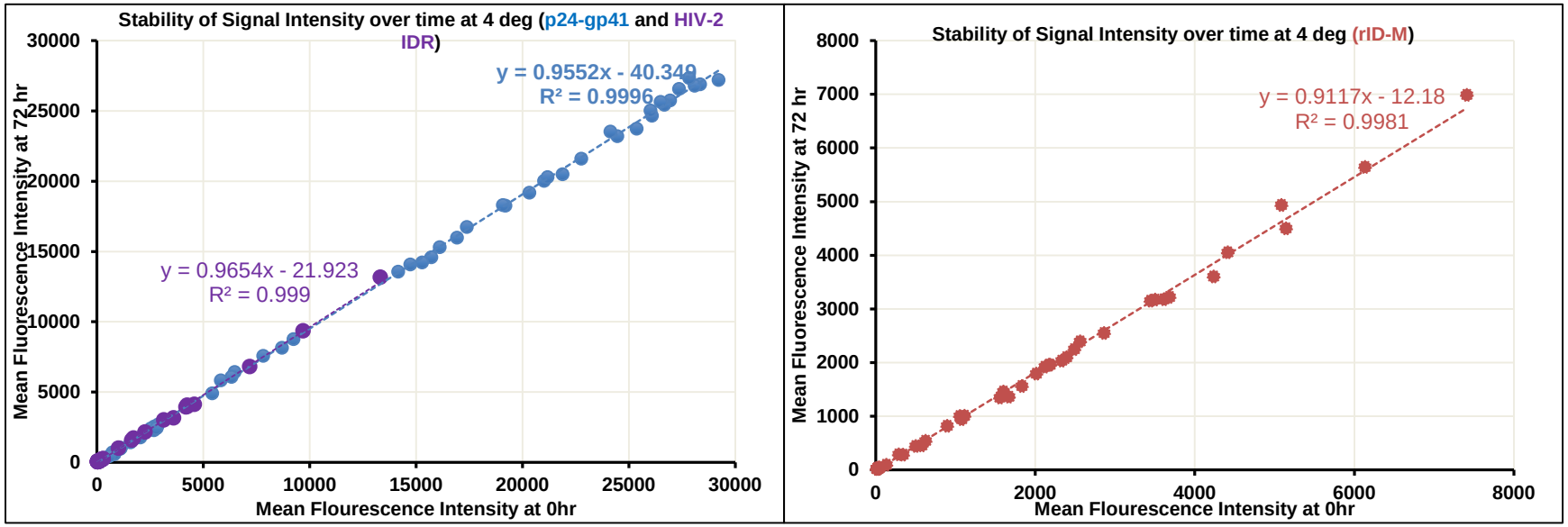


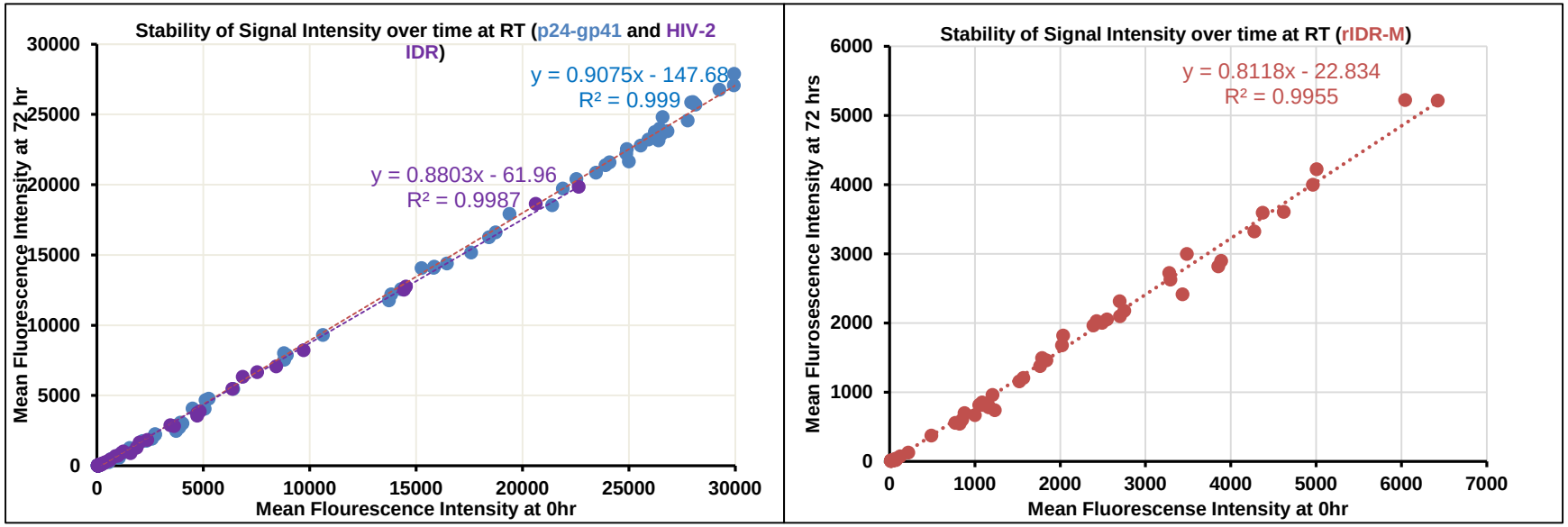
Assay Reproducibility Parameters





Signal Stability at 4ºC and at RT





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

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

Acknowledgements

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