Optimization and Calibration of Less Sensitive and Avidity Modified Protocols for the Vitros Immunodiagnostic Products Anti-HIV-1+2 Assay for Detection of Early HIV Infections

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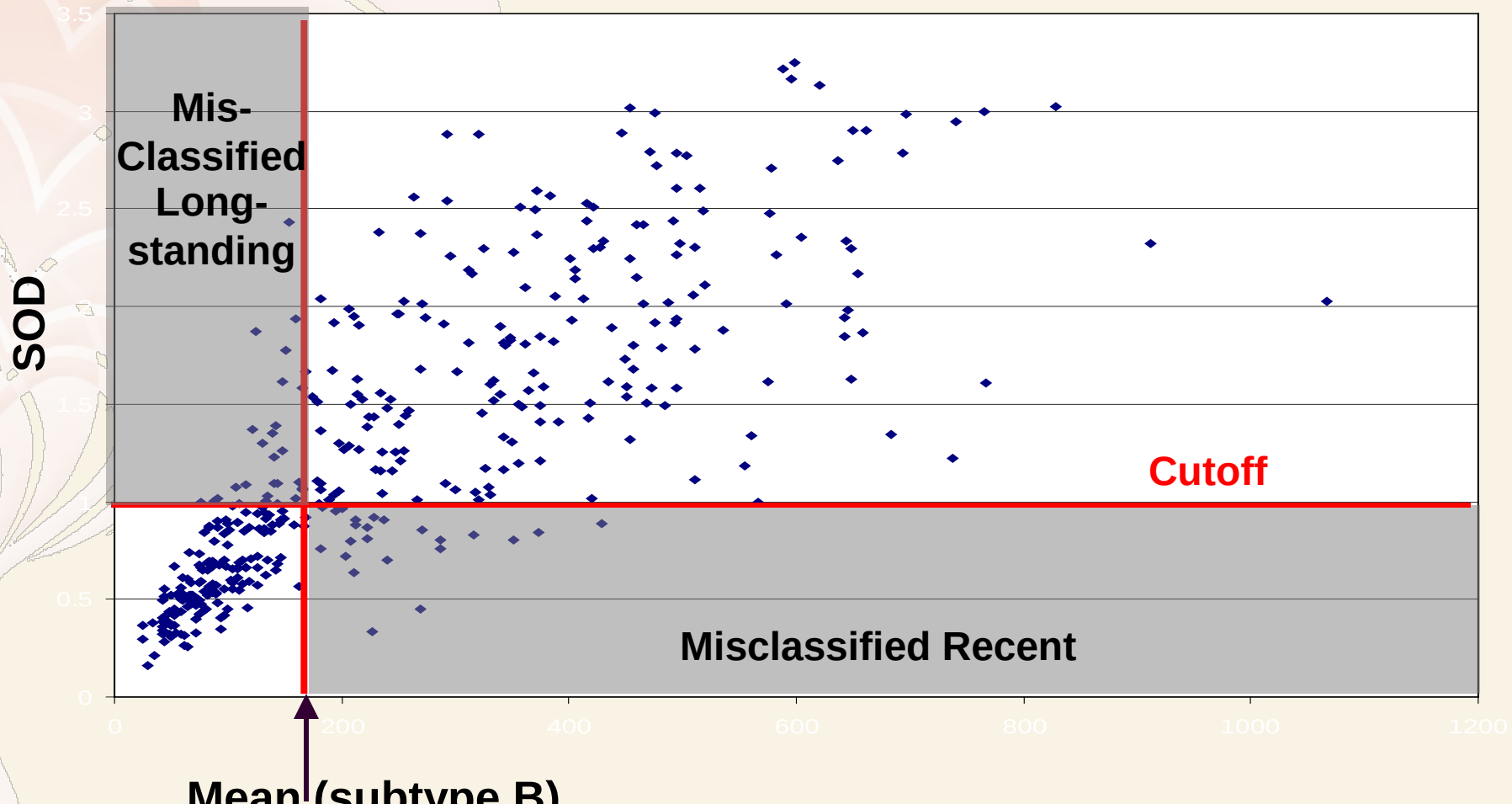
Blood Systems

Background

- Serologic Testing Algorithms for Recent HIV Seroconversion (STARHS) assays, also termed Recent Infection Testing Algorithms (RITAs), are used to distinguish recent from long-standing HIV infections.
  - Perform a sensitive HIV Ab detection assay to screen population
  - Test confirmed HIV Ab+ samples by a less-sensitive (detuned) avidity modified HIV Ab assay
  - If negative on the less/sensitive/avidity assay, the individual is within a recent infection “window period”.
- High titer and avidity HIV-1 IgG immune responses to the STAHRS/RITA assays are usually complete by one year following HIV infection; dynamic period is from 2-11 months following infection
- New STARHS/RITA assays (less-sensitive/avidity/other) requires well characterized specimens collected in the dynamic period following HIV infection, as well as samples from infected subjects with long-standing infections including categories prone to “false recent” results (AIDS; HAART, EC).

STARHS/RITA and Misclassification



Courtesy B. Branson, CDC

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Vironostika HIV-1 EIA System

- Uses a viral lysate as the capture antigen.
- Low sensitive version uses a difficult, 2-step 1:20,000 dilution.
- Inter assay calibration done by using a calibrator standard supplied by the CDC.
  - Screening:
    - (Sample OD-Negative OD)/(CDC calibrator OD-Negative OD)
  - Confirmatory for samples under a result of 2.0
    - (Median sample OD-median negative OD)/(CDC calibrator OD-Negative OD)
- Window period at 1.0 SOD is 170 days (95% CI:163-183 days)
- BioMerieux sold its license to manufacture this kit to Avioq which recently received FDA approval.

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VITROS Anti-HIV 1+2 Assay

Product Overview

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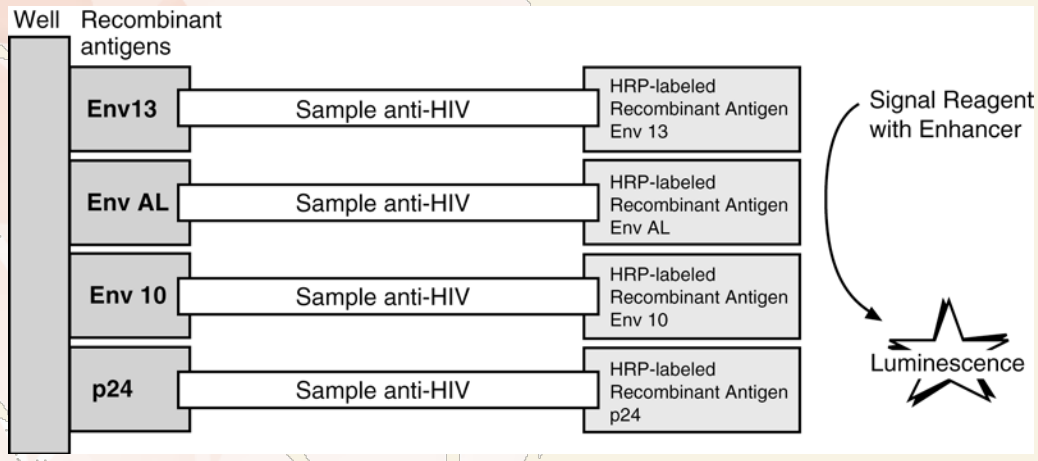
VITROS® HIV 1+2

Principle of the Procedure



4 Antigens Coat Well

4 Ag-conjugated to HRP

- 3 Ag for HIV-1

  (Env 13, Env 10, p24)

- 1 Ag for HIV-2

  (Env AL)

Principles of Procedure: Two stage reaction

1) HIV antibody present in the sample binds with HIV recombinant antigen coated on the wells. Unbound sample is removed by washing.

2) horseradish peroxidase (HRP)-labeled recombinant HIV antigens are added in the conjugate reagent. The conjugate binds specifically to any human anti-HIV-1 or anti-HIV-2 (IgG and IgM) captured on the well in the first stage. Unbound conjugate is removed by washing.

The bound HRP conjugate is measured by a luminescent reaction.

The amount of HRP conjugate bound is indicative of the level of anti-HIV-1 and anti-HIV-2 present.

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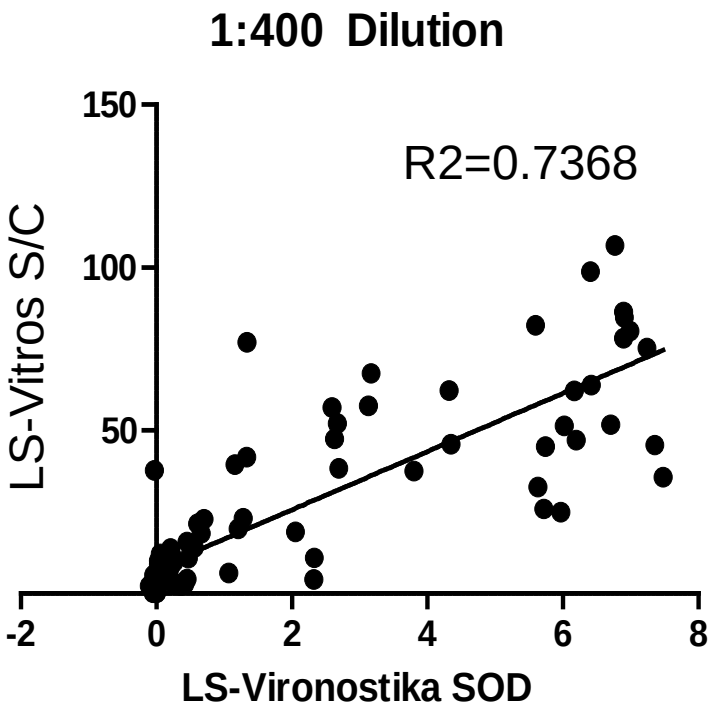
Ortho Vitros Assay

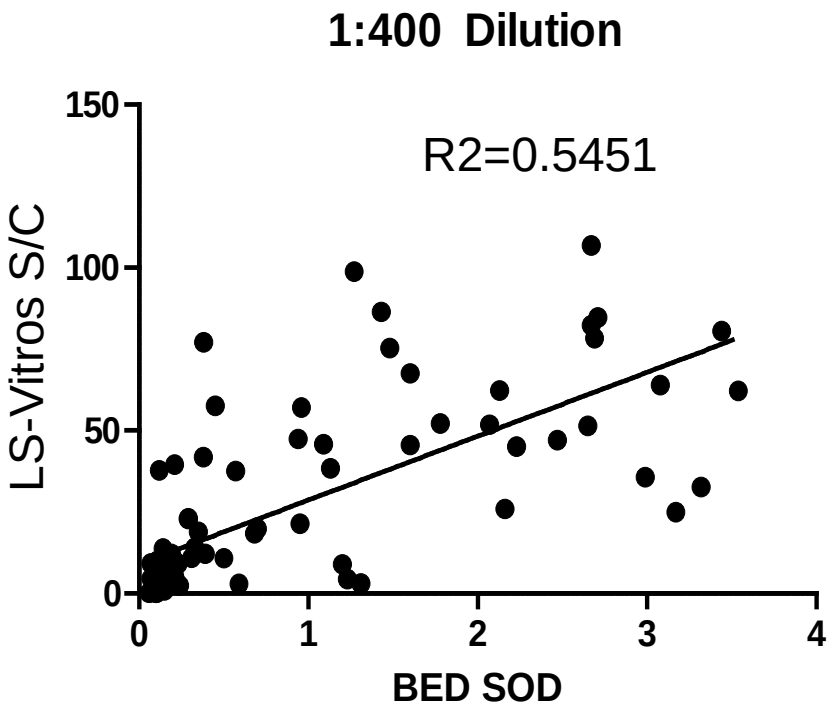
- Currently there are 1450 VITROS ECi customers in the US, 350 using their HIV detection system.
- This can be used in a low sensitive (diluted in plasma) version or an avidity (chaotropic agent incubation step).
- There is potential for doing the dilution step on board the machine. For now, the dilution is being done off board.

LS-VITROS and Avidity Development

- Optimize dilution factor for LS assays by calibration to Vironostika LS-EIA.
- Test samples in avidity assay (Chawla et al.) of the assay.
- Investigate proficiency and precision of assay using CDC proficiency panels.
- Calculate window periods at different cut-offs.

1:400 dilution





y=8.337x+11.643
y=8.337(1)+11.643
LS-Ortho (y)=20

y=8.7572x+14.133
y=8.7572(0.8)+14.133
LS-Ortho (y)=21

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Vitros Avidity Studies

Previous studies have been done using the Axsym platform to look at HIV-1 avidity.
(Chawla et al. J Clin Micro Feb 2007)

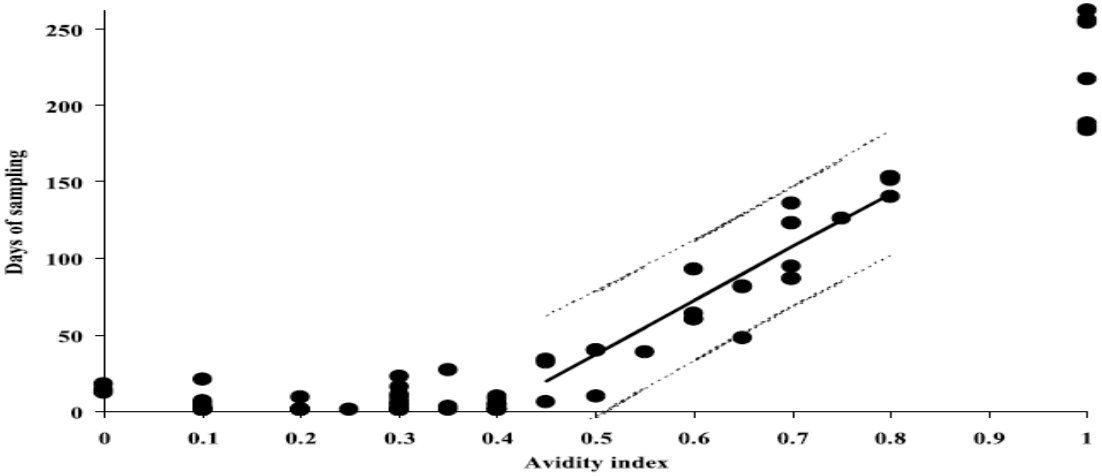

FIG. 2. Avidity index values observed with 72 serum samples collected from 23 HIV-1-infected persons at multiple points between day 0 and day 262 after HIV antibody seroconversion and tested by a guanidine-based HIV antibody avidity assay. The data points spanning the range of day 32 to day 153 (17 points) were analyzed by linear regression. The regression line (solid line) and 95% confidence prediction intervals (dotted lines) are shown.

$$\frac{\text{1:10 plasma dilution with 1M Guanidine}}{\text{1:10 plasma dilution with PBS}} = \text{Avidity Index}$$

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Blood Donors

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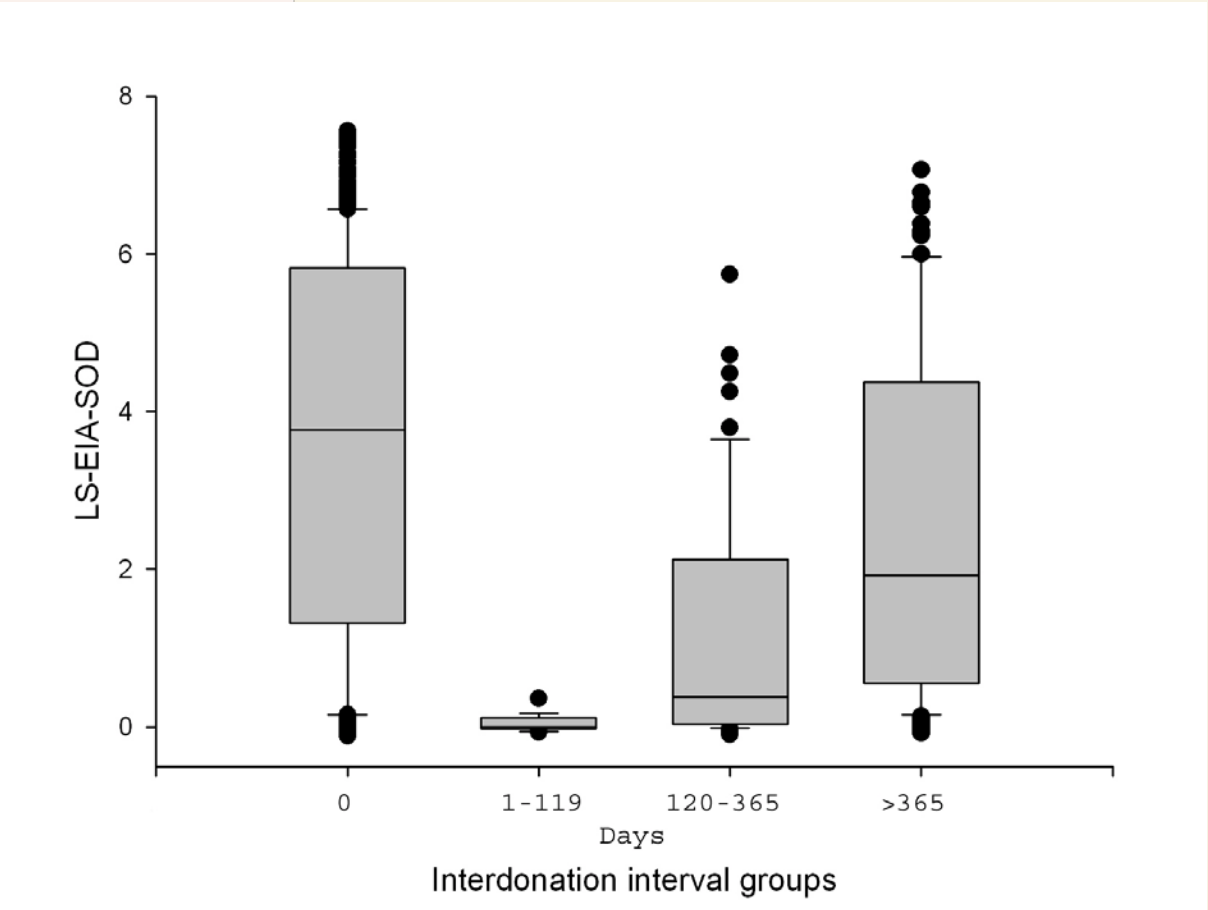
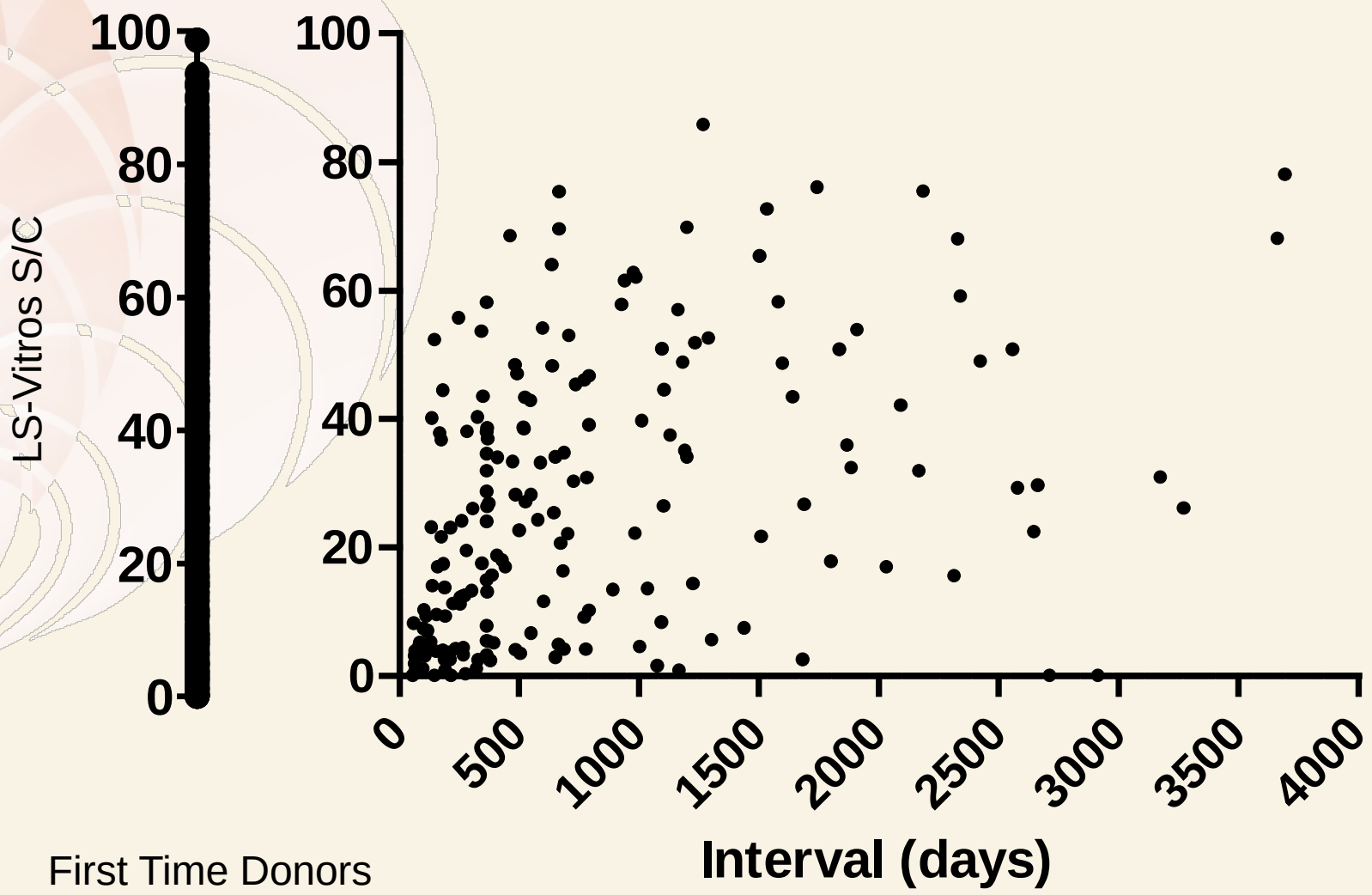
2007 ARC Interdonation Intervals



Inter-donation interval	Number of subjects	Mean EIA (Standard deviation)
0	535	3.59 (2.36)
<120	18	0.04 (0.10)
120-365	54	1.11 (1.50)
>365	109	2.49 (2.16)

OTRHO VITROS LS-Eci



First Time Donors

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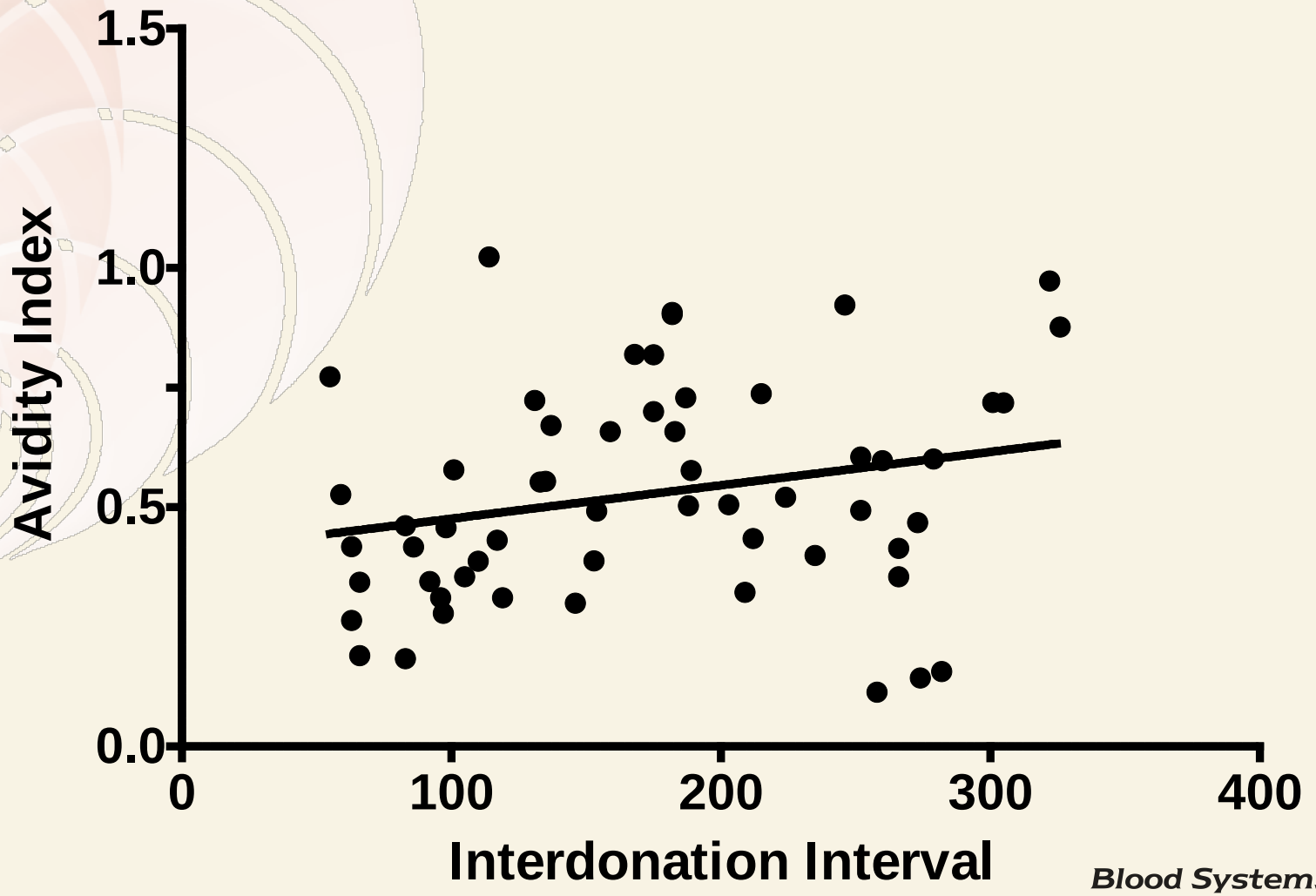
Vitros Avidity



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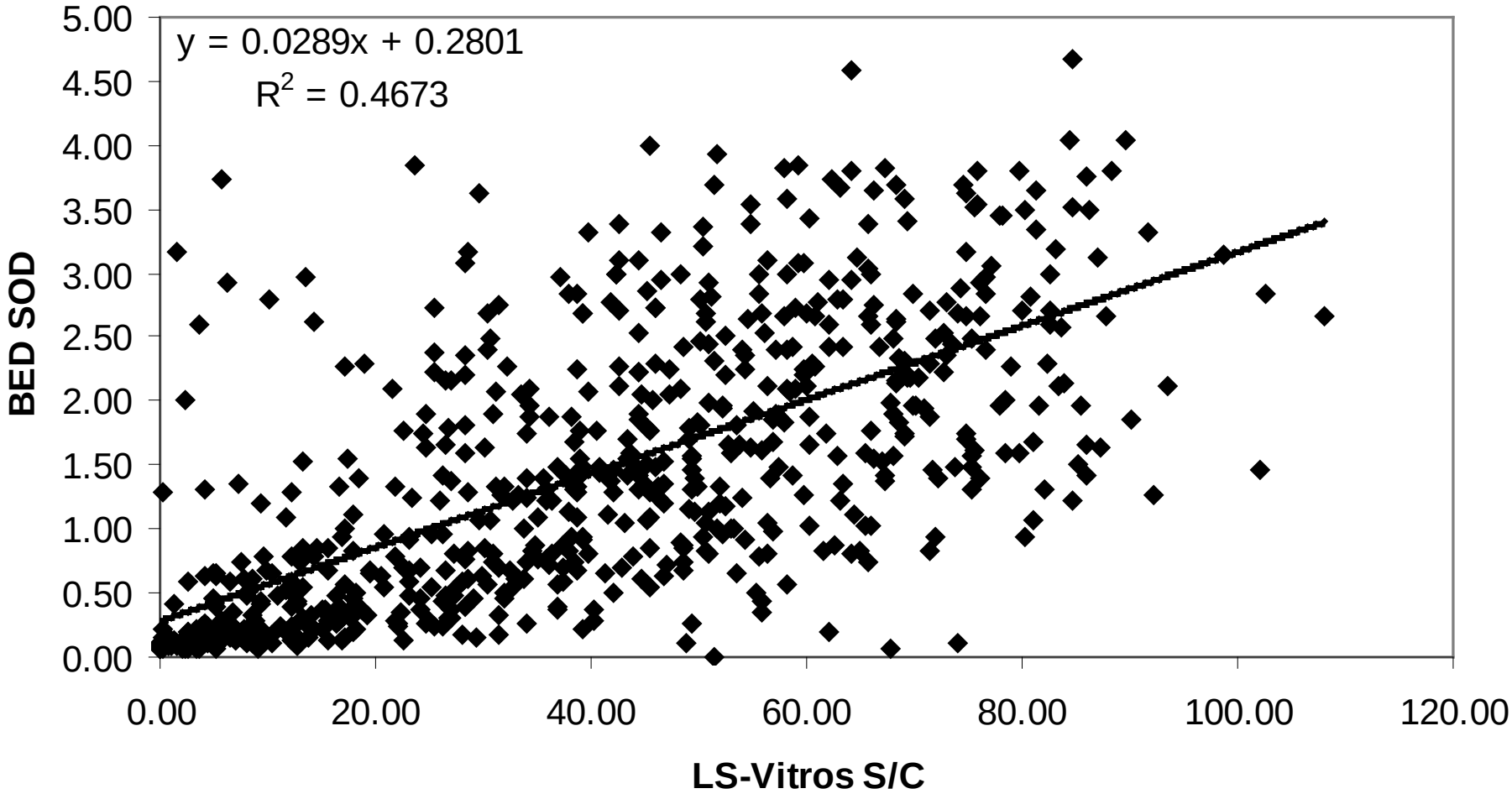
Vitros vs BED



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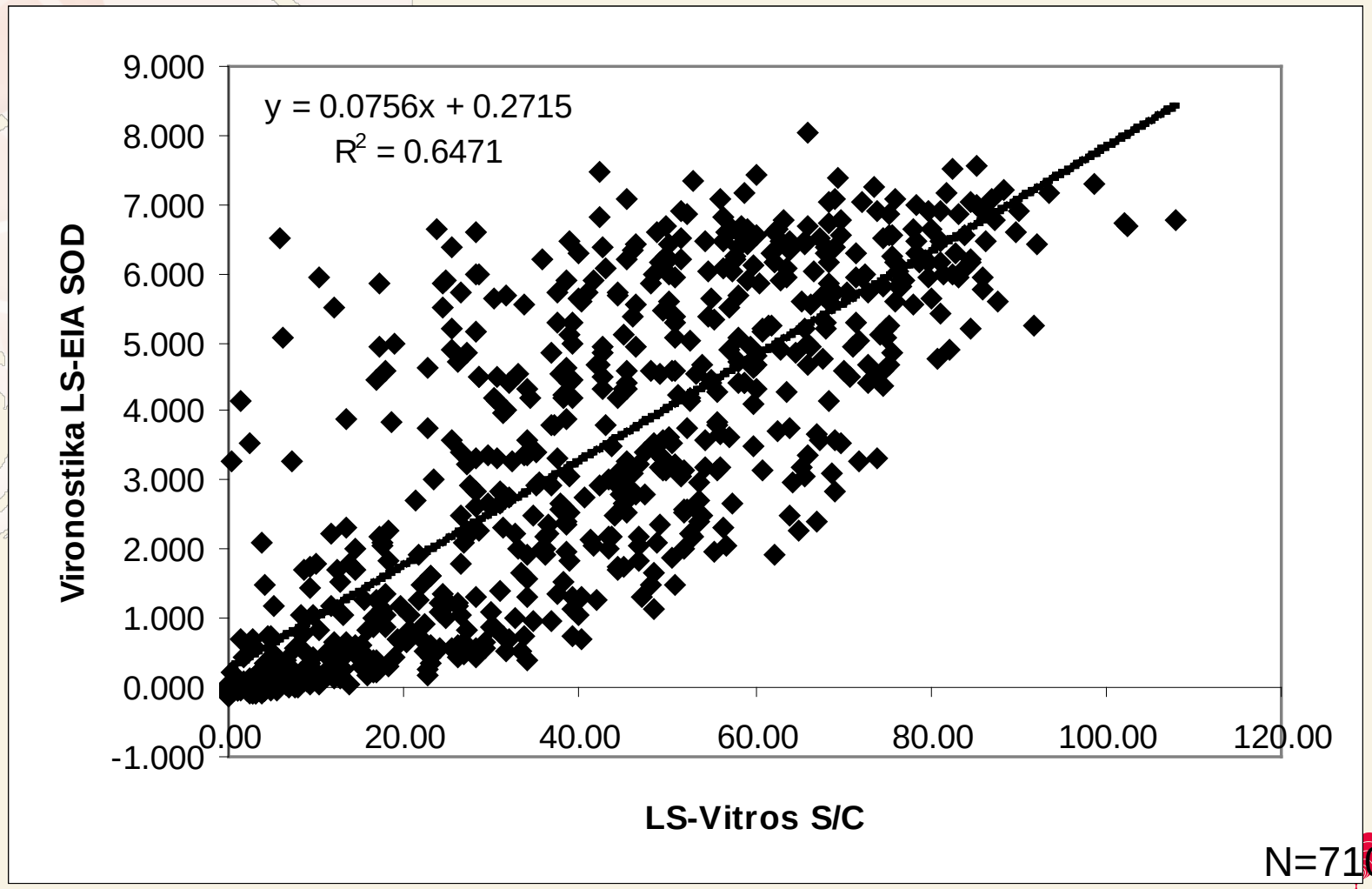
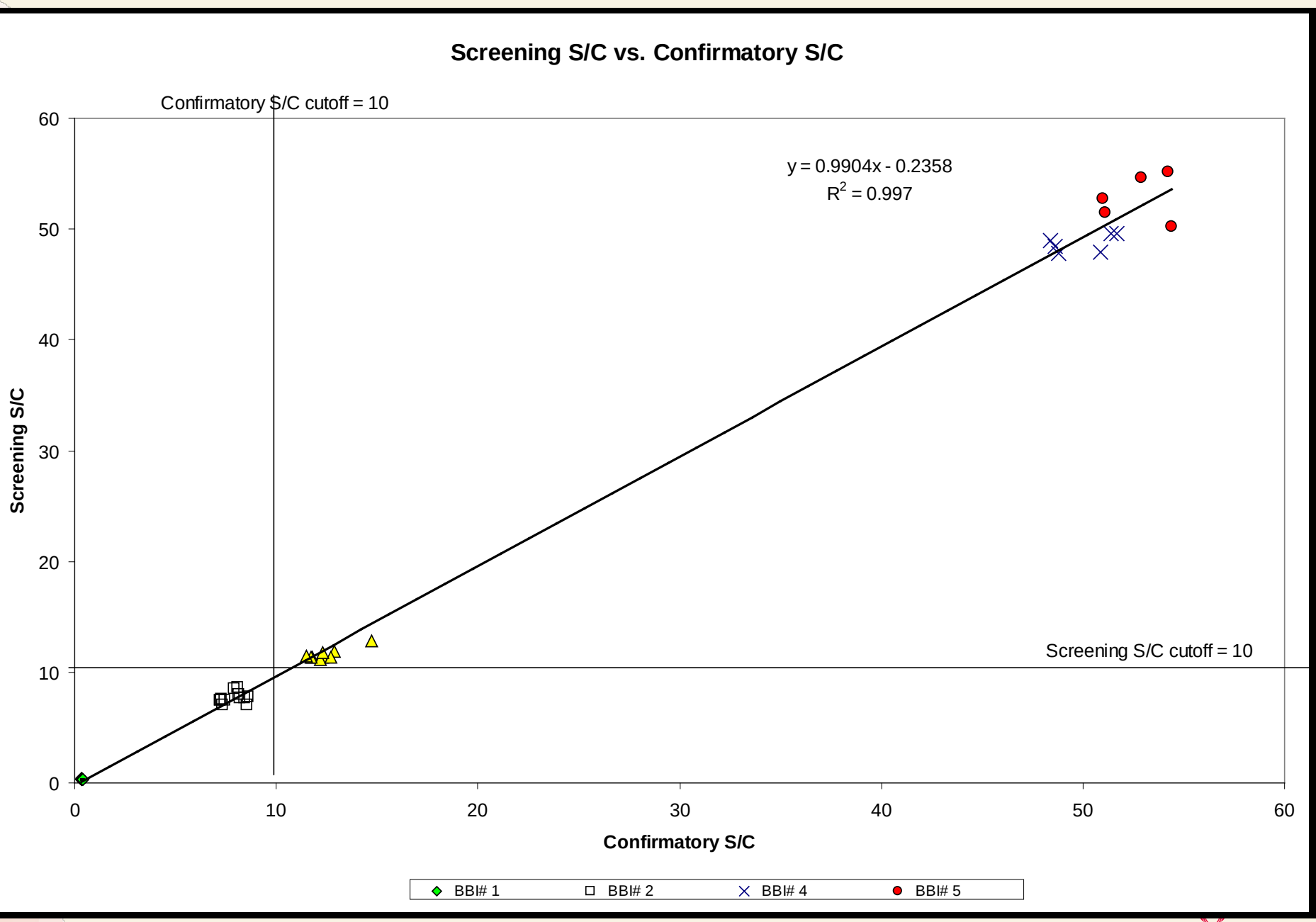
N=710

Vironostika vs Vitros




Screening S/C vs. Confirmatory S/C
Confirmatory S/C cutoff = 10
y = 0.9904x - 0.2358
R2 = 0.997
Screening S/C cutoff = 10
Screening S/C
Confirmatory S/C
0
10
20
30
40
50
60
BBI# 1
BBI# 2
BBI# 4
BBI# 5

Vitros Detuned and Avidity Assays
Window period calculations from Seroconversion panels

- 357 (LS-Vitros) and 350 (Vitros Avidity) longitudinal observations from 70 subjects were used in the window period estimation.
- Days since seroconversion was assumed to be the midpoint between last negative and first positive test dates when the interval was ≤120 days.
- Slope & intercept from a random effects regression, and LS S/C or avidity index were the explanatory variables in the multiple imputation regression model.
- The date that the subject reaches cut-off value was linearly interpolated from the last known date below the first known date above the avidity index cut-off value. If the subject does not reach the cut-off value, the observation is censored at the last date above the cut-off.

Statistical Analysis done by Debra Hanson.

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LS-Vitros

Mean Window Period Estimates

Assay, Cutoff Value	Mean Window Period	Standard Deviation	95% Confidence Limits
S/C Ratio, 5.0	61.0	8.7	43.9, 78.1
S/C Ratio, 10.0	115.0	18.1	79.6, 150.4
S/C Ratio, 15.0	178.2	29.1	121.1, 235.4
S/C Ratio, 20.0	238.8	38.6	163.1, 314.5
S/C Ratio, 30.0	387.5	65.2	259.8, 515.2

Seroconversion panels 357 samples from 70 subjects

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Ortho Vitros

Mean Window Period Estimates

Assay, Cutoff Value	Mean Window Period	Standard Deviation	95% Confidence Limits
Avidity, 0.50	145.3	25.0	96.4, 194.3
Avidity, 0.60	179.8	26.8	127.3, 232.3
Avidity, 0.70	243.0	29.7	184.8, 301.2
Avidity, 0.80	368.2	42.9	284.2, 452.2

Seroconversion panels 350 samples from 70 subjects

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Caveat: False Incident Cases

- False-positive EIAs not confirmed with an HIV-1 Western blot or IFA (i.e., diagnostic algorithms with poor specificity)
- Poor specimen handling during processing or shipping
- Chronic infection, inflammation (too much antibody)
- HIV subtype heterogeneity
- Persons with advanced HIV disease (AIDS)
- Persons who have taken antiretroviral (ARV) agents 6 months before test

Validation Panels

- Sensitivity and window period calculations.
  - Clade B Seroconversion panels from HIVNet (collaboration with the CDC)
  - Clade C Seroconversion panels from Caprisa Studies in South Africa (collaboration with Salim Kalim).
  - Multi-clade Seroconversion panels from Nigerian cohort (collaboration with Kevin Delaney at CDC)
- Specificity
  - Long term non progressors/Elite controllers
  - JHU Specificity Panels
    - JHU ER study: predominantly chronically infected individuals
    - AIDS patients with low CD4 counts
    - HAART treated patients with low viral load

Validation Panels

Cutoff and false Incidence comparison of specificity panels (% Incident):

False Recency Rates	Vitros LS-Eci S/C				Vitros Avidity Index		BED SOD			Vironostika LS-EIA SOD			BioRad Avidity Index	
Cut-Off:	**13**	**16**	**18**	**20**	**60**	**35**	**0.60**	**0.80**	**1.00**	**0.50**	**0.75**	**1.00**	**75**	**35**
JHU ER Study (n=297; Vitros avidity n=247)	12%	15%	16%	18%	21%	5%	14%	19%	23%	9%	12%	13%	2%	0%
CD4<50 (n=140)	6%	7%	11%	14%	6%	1%	22%	26%	31%				1%	1%
HAART CD4>400 VL<50 (n=134)	17%	23%	25%	29%	42%	4%	12%	21%	31%				1%	0%

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Conclusions

- Calibrated the LS-Vitros assay to the Vironostika.
- Calculated the window period for LS- and avidity Vitros.
- Investigated false recency rates of challenge panels.

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Work to do

- Window periods for combined assays.
- Investigate other chaotropic agents for avidity assay.
- Test with other clades to look at clade variation.

Thank you!

BSRI

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