Is It Always Necessary for the ARCHITECT 4th-Generation HIV-1/2 Ag/Ab Combo Assay to be Repeatedly Reactive before Moving Forward in the Centers for Disease Control and Prevention (CDC) HIV Screening Algorithm?

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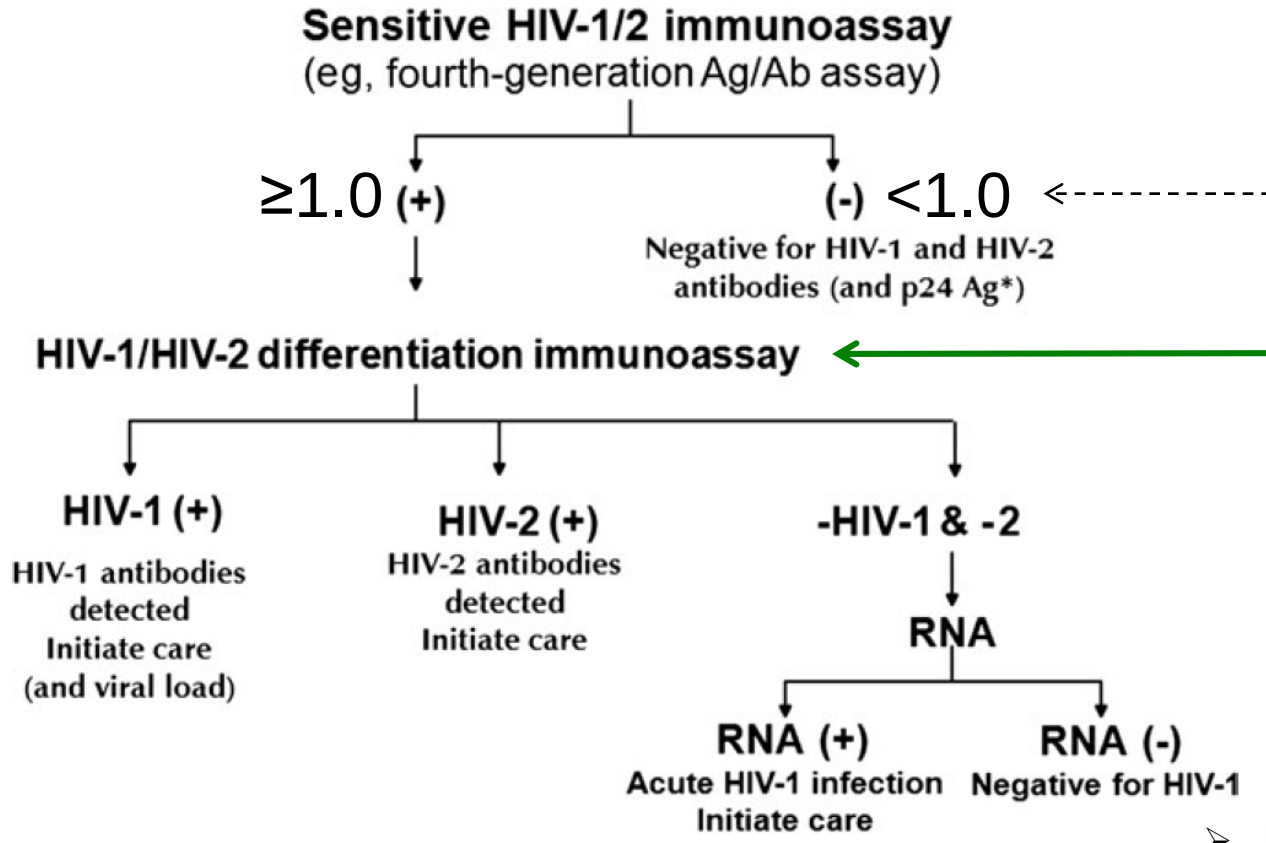
Introduction

Currently, there are three Food and Drug Administration (FDA) approved 4th-generation assays:

- The **Abbott Architect HIV Ag/Ab Combo** assay (June, 2010) Chemiluminescent magnetic microparticle-based immunoassay (CMIA)
- The **Bio-Rad GS HIV Combo Ag/Ab** assay (July, 2011) Enzyme immunoassay (EIA)
- The **ADVIA Centaur HIV Ag/Ab Combo (CHIV)** assay (June, 2015) CMIA

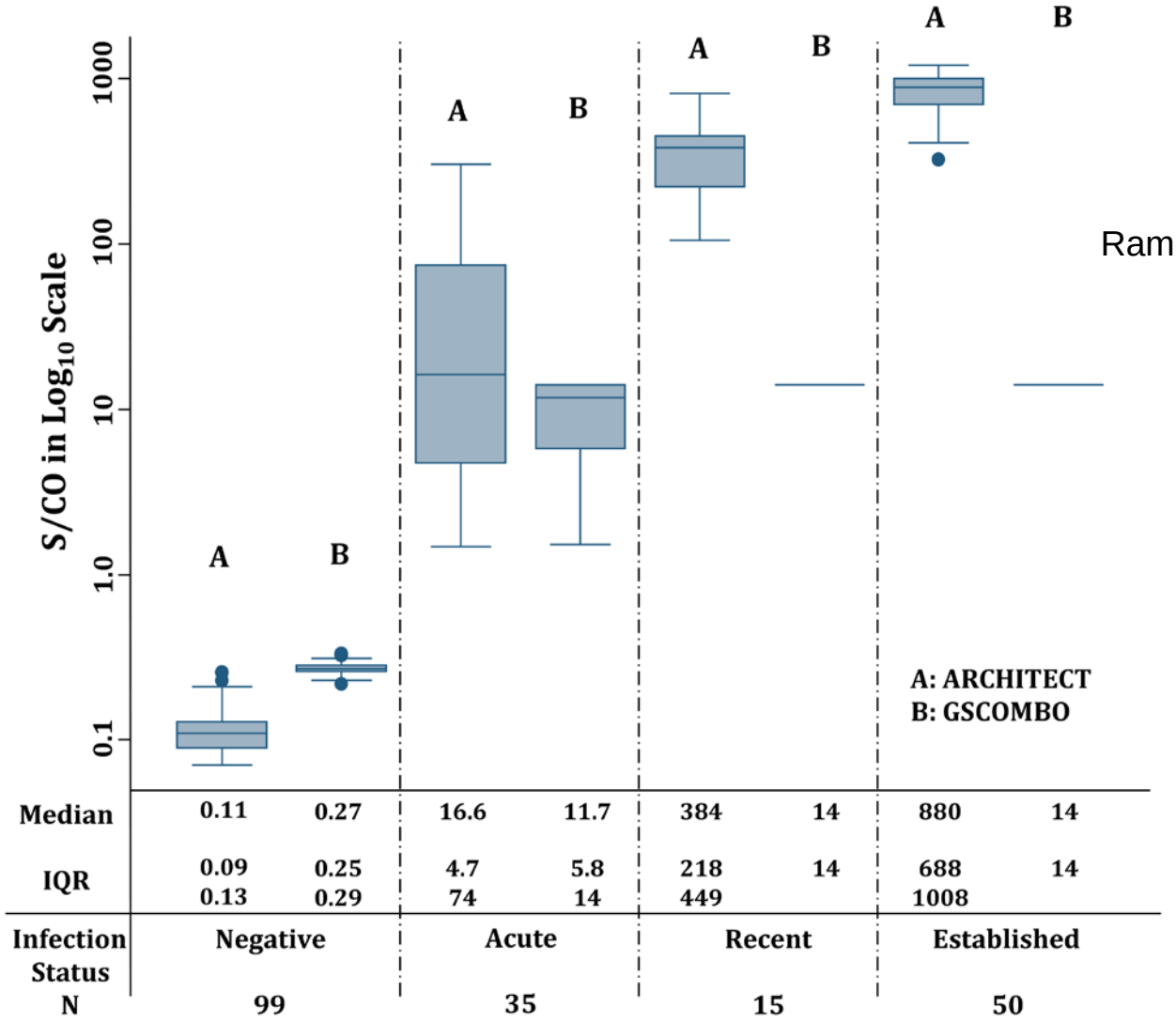
4thGen Testing Algorithm for HIV-1 & HIV-2



"Orthogonal"
e.g., **Multispot test** with consideration for a more rapid turn-around-time; **Geenius** identifies HIV-1 (gp160, gp41, **p31**, p24) and HIV-2 (gp140, gp36)

- Branson, JAIDS 2010;55:S102-5
- *Clinical & Laboratory Standards Institute* 2011, M53-A: Vol.31 No.13
- **MMWR 2013; 62(24): 489-94**

S/CO Distribution by Stage of HIV-1 Infection



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N = 199

Objectives

Since the ARCHITECT S/CO increases initially with viral replication (HIV-1 p24 antigen) and then following the rise in HIV specific antibodies, we sought to:

- Determine the correlation of the ARCHITECT S/CO values between the first, second and third replicates
- Determine whether the ARCHITECT S/CO value could be used to modify this requirement for replicate testing
- Determine a S/CO threshold for a single test without losing ARCHITECT assay sensitivity or specificity, shortening the turn-around time for moving in the HIV screening algorithm.

Methods

- A retrospective analysis was done using ARCHITECT test results obtained from our primary screening HIV diagnostic algorithm between May 2011 to September 2015

- Specimens were obtained from an academic hospital referral laboratory and research HIV-1 vaccine trials

- The testing algorithm interpretation for S/CO:
  - Initially reactive (S/CO ≥1.0) both replicate S/CO <1.0 was considered **non-reactive**;
  - One or both of the replicates ≥1.0 was considered **reactive** and tested with the discriminatory Multispot test, and HIV-1 RNA when indicated.

Results

- From 43,518 specimens tested, 975 were initially S/CO ≥1.0; from these a total of 187 (19.2%) were repeatedly S/CO <1.0 and defined as ARCHITECT non-reactive.

  Median S/CO [interquartile range (IQR); total range] values:

  - Negative results (N=42,543): **0.13** [0.11-0.16 ; 0.07-0.99]
  - First run (N=187): **1.9** [1.4-3.5 ; 1.0-31]
  - Second run (N=187): **0.15** [0.12-0.19 ; 0.07-0.53]
  - Third run (N=187): **0.15** [0.12-0.18 ; 0.07-0.51]

    Bland-Altman analysis (bias±2SD) between first-second: 3.8±8

    Bland-Altman analysis (bias±2SD) between second-third: 0±0.1

    Mann-Whitney-Wilcoxon between second-third, p=0.44

Results

- Of the 788 repeatedly-reactive specimens, 784 specimens were dually reactive and four specimens (0.5%) were discordant among replicates.

  Median S/CO [IQR ; total range] values:

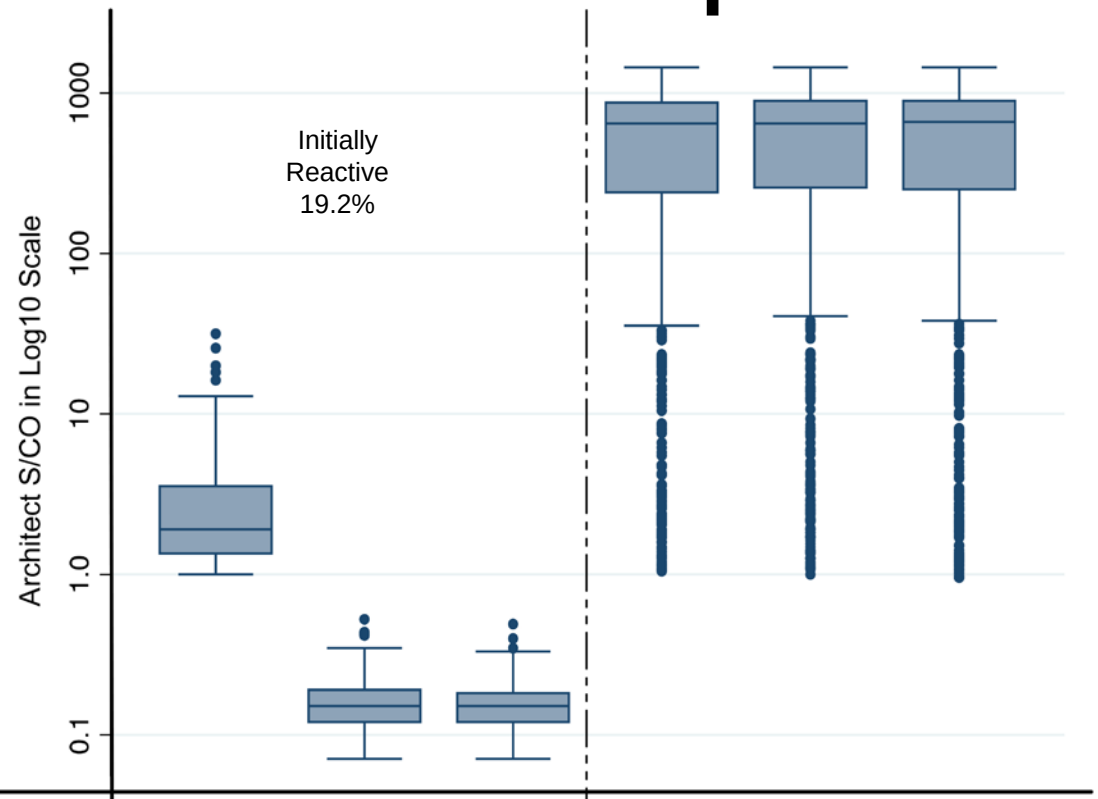
  - First run (N=788): **651** [285-880 ; 1.0-1441]
  - Second run (N=788): **653** [265-889 ; 0.9-1447]
  - Third run (N=788): **656** [264-885 ; 0.9-1454]

    Bland-Altman analysis (bias±2SD) between first-second: -4.0±31

    Bland-Altman analysis (bias±2SD) between second-third: 0.5±30

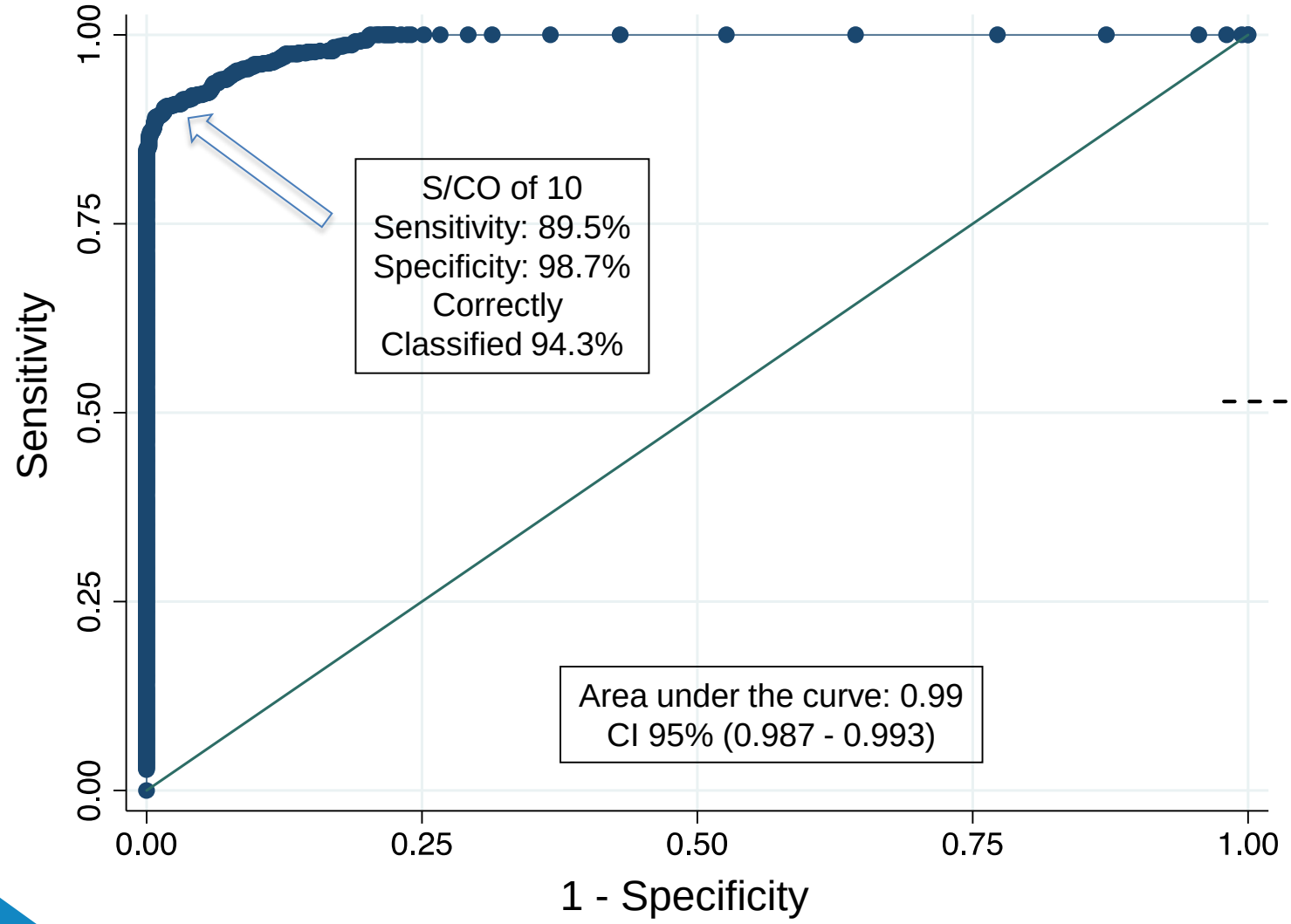
    Kruskal Wallis, p=0.97

ARCHITECT S/CO Distribution Between Replicates



Median	1.9	0.15	0.15	651	653	656
IQR	1.4 3.5	0.12 0.19	0.12 0.18	285 880	265 889	264 885
Sample Repetition	1st	2nd	3rd	1st	2nd	3rd
Architect Status	Non-Reactive			Reactive		
N	187			788		

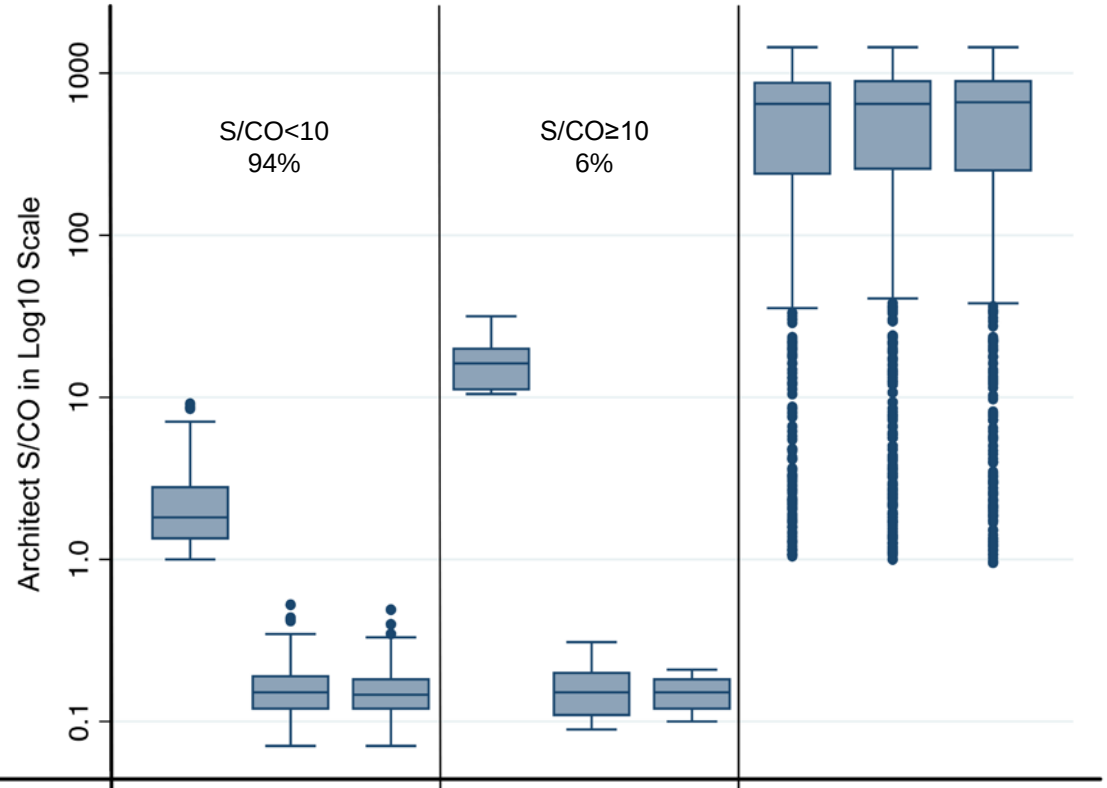
Receiver Operator Curve for ARCHITECT S/CO Initially Reactive Specimens



S/CO of 1.0
Sensitivity: 100%
Specificity: 78.5%
Correctly
Classified 88.8%

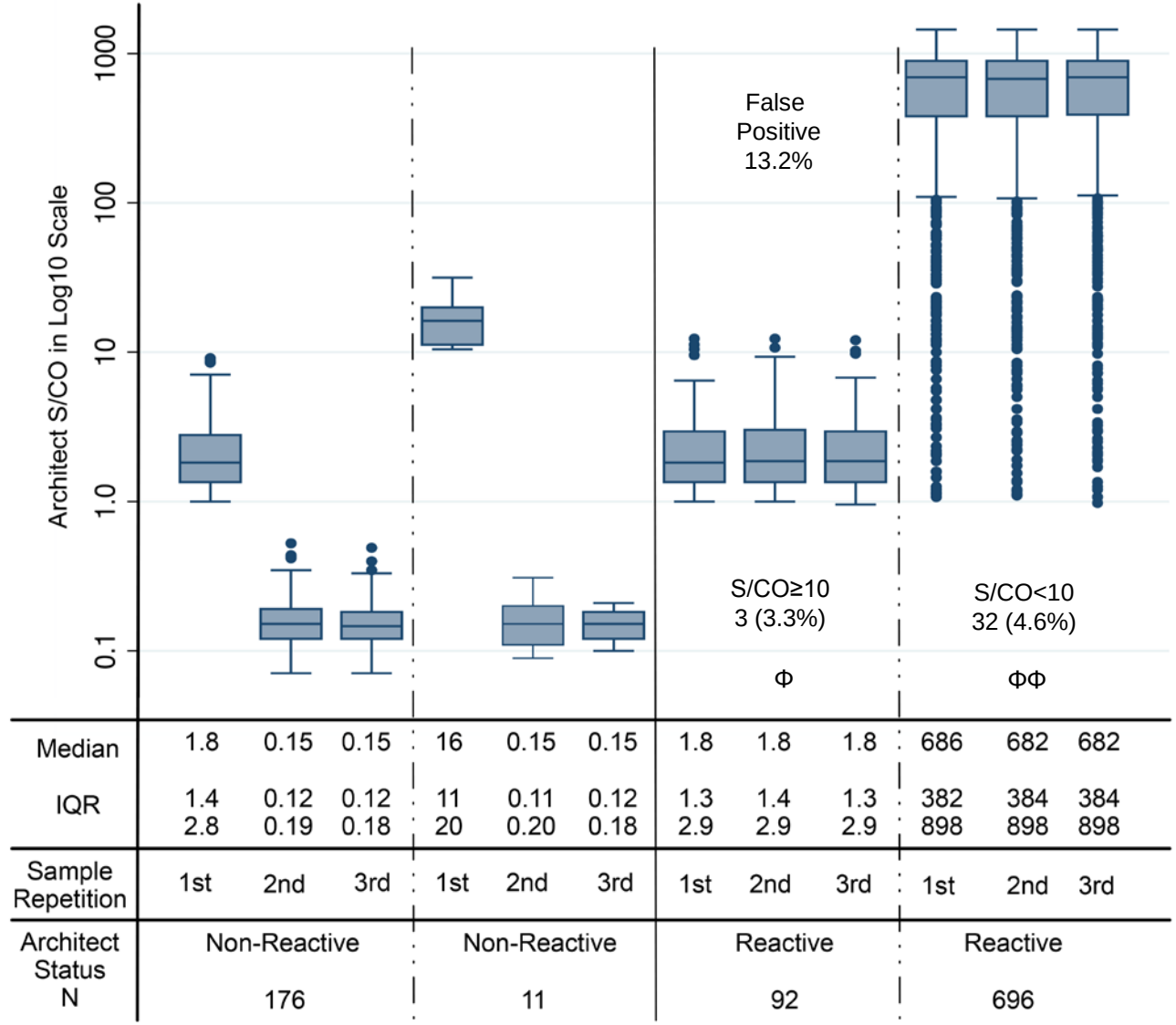
S/CO of 32
Sensitivity: 84.8%
Specificity: 100%
Correctly
Classified 92.7%

ARCHITECT S/CO Initially Reactive at S/CO ≥10



Median	1.8	0.15	0.15	16	0.15	0.15	651	653	656
IQR	1.4 2.8	0.12 0.19	0.12 0.18	11 20	0.11 0.20	0.12 0.18	285 880	265 889	264 885
Sample Repetition	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
Architect Status	Non-Reactive			Non-Reactive			Reactive		
N	176			11			788		

ARCHITECT S/CO Replicates Reactive with Negative Viral Load (False Positive)

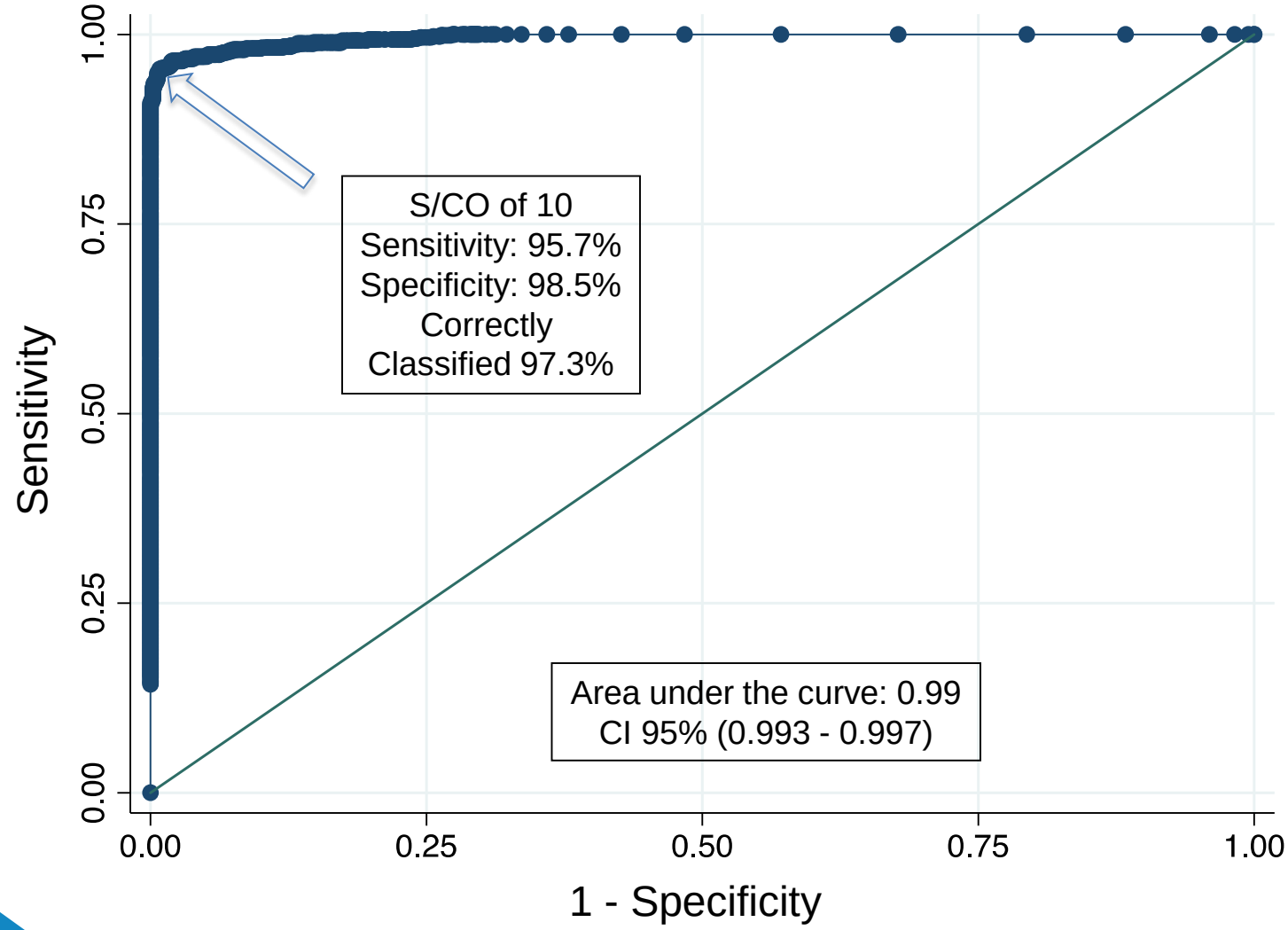


Median	1.8	0.15	0.15	16	0.15	0.15	1.8	1.8	1.8	686	682	682
IQR	1.4 2.8	0.12 0.19	0.12 0.18	11 20	0.11 0.20	0.12 0.18	1.3 2.9	1.4 2.9	1.3 2.9	382 898	384 898	384 898
Sample Repetition	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
Architect Status	Non-Reactive			Non-Reactive			Reactive			Reactive		
N	176			11			92			696		

Φ: Negative Viral load

ΦΦ: Positive Viral load or Reactive Multispot

Receiver Operator Curve for ARCHITECT S/CO Replicates Reactive and HIV Confirmed



S/CO of 1.0
Sensitivity: 100%
Specificity: 71.1%
Correctly
Classified 83.7%

S/CO of 32
Sensitivity: 91%
Specificity: 100%
Correctly
Classified 96.1%

Results

- Turnaround time of the ARCHITECT from 942 repeatedly-reactive specimens was:

  Median time [IQR ; total range] values:

  - First/Second run (N=942): **74 min** [57-98 ; 34-198]
  - Second/Third run (N=942): **1 min** [1-1 ; 1-33]

Conclusions

- For samples with an initial ARCHITECT S/CO ≥10, the false-reactive duplicate rate was 1.3% with a sensitivity of 85.5% while the false HIV infection rate was 1.5% with a sensitivity of 95.7%.
- Most of the samples initially ARCHITECT reactive as well as ARCHITECT false positive (negative viral load) were S/CO <10.
- To decrease turnaround time and total screening costs for research testing, all initially reactive research specimens with a S/CO ≥10 could reflex directly to Multispot discriminatory testing and HIV-1 RNA as indicated, while initially reactive specimens with a S/CO between 1 and 10 could be rerun only in singleton after centrifugation.

Acknowledgements

- Robert Coombs, MD, PHD, FRCPC
- Joan Dragavon MLS, Socorro Harb & the UW Retrovirology laboratory staff
- Paul Swenson PhD (DHSKC) and Joanne Stekler MD, MPH from Seattle King County Department of Public Health
- Funding support:
  - ACTG Virology Specialty Laboratory (UM1-AI-068636; UM1-AI-106701)
  - HVTN HIV Diagnostic Laboratory (UM-AI-068618)
  - UW CFAR Clinical Research and Retrovirology Core (P30-AI-027757)