BLINDED EVALUATION OF ULTRASENSITIVE ASSAYS OF HIV VIRAL LOAD IN PLASMA

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INTRODUCTION

- Investigations into new therapies for reducing or eradicating the HIV reservoir are underway. Although there is sustained progress in developing approaches for quantitation of replication competent provirus and expressed RNA and proteins in cells in peripheral blood and tissues, it is also important to measure residual viral load (VL) in plasma by ultrasensitive assays to determine post-treatment changes in systemic virus production.
- A major barrier in these studies is the lack of validated assays that reliably quantify plasma HIV below the limit of detection of current clinical assays. We sought to objectively assess the performance characteristics of previously established and newer, more sensitive assays to quantify plasma VL.

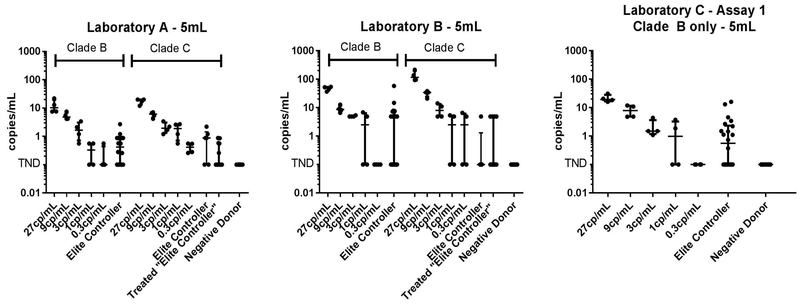
METHODS

- The HIV Reservoir Assay Validation and Evaluation Network (RAVEN) project was created to assess the sensitivity, specificity, limits of detection and quantitative ranges of blood-based assays of HIV persistence.
- HIV Clade B and C plasma qualification panels were constructed that contained 50 coded duplicate samples including:
  - Virus-spiked analytic standards: 2 subtype-specific 5-step, 3-fold dilution series with highest concentrations ranging from 45 to 6 copies/mL to lowest concentrations ranging from 0.33 to 0.07 copies/mL
  - Clinical samples with expected low-level viremia and
  - Negative controls
- Original stocks quantified by Roche and Hologic Panther VL assays.
- Panels were distributed to 8 laboratories for HIV quantification using 9 assays, including:
  - Four labs that used ultra-centrifugation to concentrate virus prior to HIV RNA nucleic acid extraction and PCR amplification
  - Two labs used replicate testing using diagnostic HIV RNA VL assay platforms
  - Two assays used ultrasensitive detection of p24 Ag without virus enrichment
- Volumes provided for testing are reported for each assay with the RNA assays using 5-10mL. The volume used for the p24 assays was substantially less.
- Results were analyzed for sensitivity, specificity, reproducibility, and ability to accurately quantify HIV VL in analytical standards

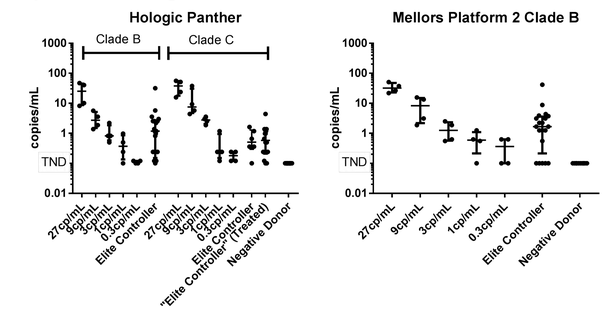
RESULTS- I

- Data from six laboratories using 7 assays were included in this analysis.
- All labs received Clade B and C panels except for one (Laboratory C only tested the Clade B panel)
- Four of five RNA-amplification based assays detected virus in the standards down to ~1 copy/mL in at least 1 of the 2 replicates.
- Negative controls were all negative.
- Ultrasensitive p24 Ag assays were not able to quantitatively measure HIV in the diluted standards and one p24 Ag assay had a specificity of 90%.

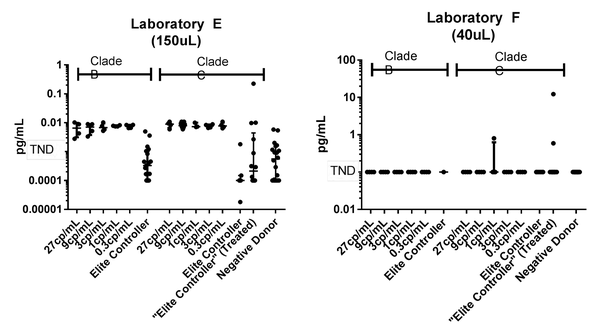
Ultracentrifugation



Replicate Testing

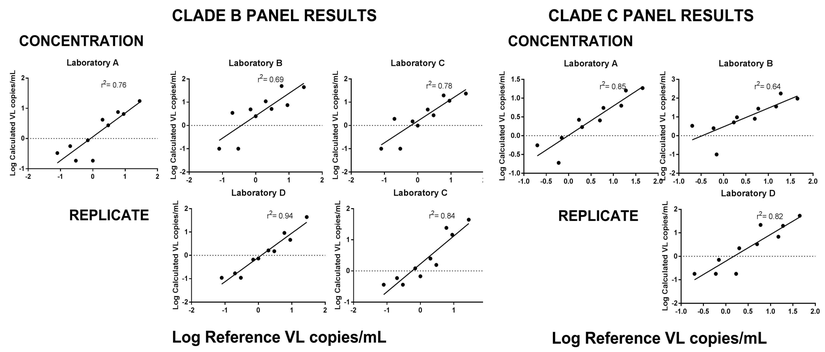


Ultrasensitive P24 Assays



RESULTS-II

- All RNA-amplification-based assays had strong correlations between replicates across the standards ($p<0.05$, rho>0.8).
- For Clade B r^2 results were higher for replicate testing platforms
  - Replicate Testing: Lab D (0.94)>Lab C (0.84)
  - Ultracentrifugation: Lab C (0.78)> Lab A (0.76)> Lab B (0.69)
- For Clade C r^2 results were: Lab A (0.85)> Lab D (0.82) > Lab B (0.64)
- Four assays quantified standards with little bias (mean recovery 69-218% of nominal HIV [RNA]) whereas one assay overestimated copies/mL by >300%.



CONCLUSIONS

- Ultrasensitive plasma VL measurements can be accurately measured on large volumes of plasma by concentrating virus using ultracentrifugation or by replicate testing on diagnostic VL platforms.
- Ultracentifugation- or replicate RNA-amplification assays can quantitatively measure HIV RNA down to ~1 copy/mL, which is necessary to assess the impact of experimental curative interventions on residual viremia.
- One p24 assay could detect virus in seronegative plasma without virus enrichment, but dynamic sensitivity was lacking at <45 copies/mL.

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