Assuring the Quality of Dried-Blood Spot Assays for Anti-HIV-1 Antibodies; Laboratory and Method Performance

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Objective

To track the long-term laboratory and method performance of Anti-HIV-1 dried-blood spot (DBS) serological assays.

Methods

- The Newborn Screening Quality Assurance Program (NSQAP) provides quality control (QC) and proficiency testing (PT) DBS materials for HIV serology
- DBS materials were made by
  - Blending HIV-positive donor serum with HIV-negative serum and washed red blood cells to create whole blood matrices of varying HIV reactivities
  - Blood pools were spotted onto FDA-approved filter paper, dried overnight, and stored at -20 ºC with desiccant until shipment.
  - QC materials were sent laboratories 2 times per year
  - PT materials were sent 4 times per year.
  - False-positive and false-negative results were tracked over time

Results: Participant and Method Demographics

- PT participation averaged 58 laboratories over an 8-year period from 2005-2012 and ranged from 28 (2012 enrollment) to a high of 83 (2009 enrollment) laboratories (Figure 1)
  - Domestic laboratories ranged from 13 to 18 laboratories; 15 laboratories currently participate
  - Foreign laboratory participation reached a high of 70 laboratories in 2009, but due to a lack of funding, laboratories supported by the Center for Global Health were dropped
    - Quality assurance for these labs is now provided the Center for Global Health
- The false-positive rate for domestic laboratories for the years 2009-2011 was 0.0% and the false-negative rate ranged from 0.0% to 2.1% (Figure 2)
- The false-positive rate for foreign laboratories for the same years ranged from 1.4% to 3.5% and the false-negative rate ranged from 0.0% to 5.8% (Figure 3)
- The greatest number of errors came from foreign laboratories (Figures 1 - 3)
- Tables 1 and 2 list the HIV enzyme immunoassay (EIA) and western blot (WB) methods reported to NSQAP in 2011
  - Only one domestic EIA method is FDA-approved for DBS
  - Only one WB is FDA-approved for DBS
- Table 3 lists the HIV algorithms used by laboratories testing DBS

Figure 1. Participation in HIV Antibody Quality Assurance in Dried Blood Spots

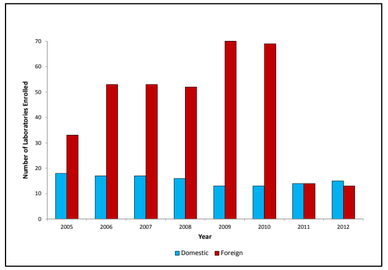

Figure 2. Anti-HIV-1 in Dried Blood Spots Proficiency Testing Domestic Errors (%) from 2005-2011

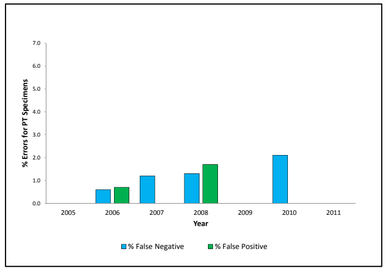

Figure 3. Anti-HIV-1 in Dried Blood Spots Proficiency Testing Foreign Errors (%) from 2005-2011

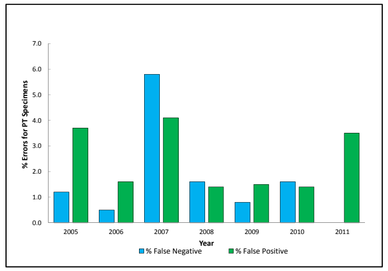

Figure 4. Reproducibility of HIV DBS QC Lots 111-114

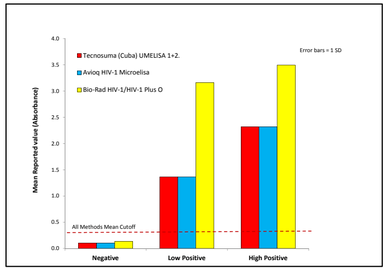

Figure 5. Reproducibility of HIV-Reactive PT Specimen Lot 1004 Over 3 Quarters in 2011

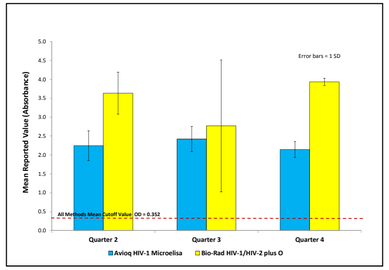

Figure 6. Reproducibility of HIV-Nonreactive PT Specimen Lot 903 Over 3 Quarters in 2011

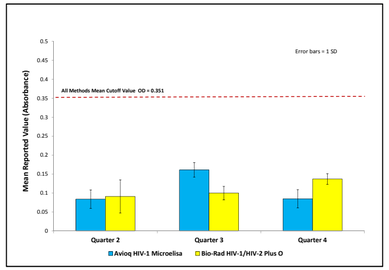

Figure 7. Reproducibility of the Western Blot Banding Patterns PT Specimen Lot 1004 (Reactive) (Bio-Rad) Genetic Systems HIV-1 WB Kit

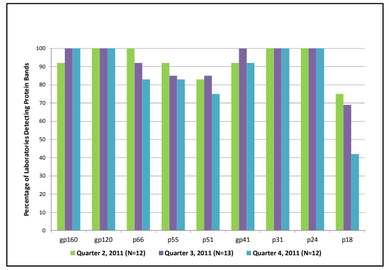

Table 1. HIV EIA Methods Used to Test Dried Blood Spot Quality Assurance Materials in 2011

Kit Source	Number of Participants
FDA Licensed for DBS-Avioq HIV-1 Microelisa Systems	11
Genetic Systems rLAV EIA (Bio-Rad) (Discontinued)	5
Bio-Rad HIV-1/HIV-2 plus O EIA	3
Fujirebio Serodia-HIV 1,2	2
Tecnosuma (Cuba) UMELISA HIV 1+2	3
Q-Preven HIV 1+2, DBS, Brazil	1
In House	1
Other	7
Total	33

Table 2. WB Methods Used to Test Dried Blood Spot Quality Assurance Materials in 2011

Kit Source	Number of Participants
FDA Licensed for DBS - Genetic Systems HIV-1 WB (Bio-Rad)	12
Cambridge Biotech HIV-1 WB Kit (Maxim)	1
OraSure HIV-1 WB Kit	1
New LAV Blot I (Bio-Rad)	1
Genelab diagnostics HIV 2.2 WB	1
MP Diagnostics HIV Blot 2.2	1
Other	1
Total	18

Table 3. HIV Algorithms Used by Laboratories Testing DBS, Quarter 4, 2011

Testing Combination	Number of Laboratories Reporting
Agglutination/WB	1
Agglutination Only	1
EIA/Agglutination/WB	1
EIA/WB	13
EIA Only	5
EIA/EIA	2
EIA//EIA/WB	1
WB Only	2
Luminex Multiplex	2

Results: Method Performance

- Method reproducibility for QC and PT materials varied within and between methods, and between laboratories using the same method (Figures 4-6)
  - Bio-Rad (Genetic Systems) HIV-1/HIV-2 Plus O method had the greatest variability for QC materials (Figure 4)
    - Low-Positive and High-Positive Bio-Rad QC values were close, indicating a lack of optimization for use with DBS
  - Reproducibility for HIV-Reactive PT specimens also showed greater variability for the Bio-Rad method (Figure 5)
  - Absorbance values for HIV-Nonreactive PT specimens showed more uniformity between methods (Figure 6)
- All western blot (WB) methods for HIV-reactive DBS detected gp160, p24 over the period from 2009-2011(data not shown)
  - Bio-Rad (Genetic Systems) HIV-1 WB method varied in its ability to detect gp120, p66, p51, p55, gp41, p31, and p18 as shown by the reproducibility of the same PT specimen in 2011 (Figure 7)
    - Twelve laboratories reported using this method in Quarters 2 and 4
    - Thirteen laboratories reported using this method in Quarter 3

Conclusion

- NSQAP provides evaluations of laboratory PT performance and tracks yearly error rates
- Domestic laboratories reported no false-positive errors for 2009-2011 and no false-negative errors in 2009 and 2011
- Using both DBS PT and QC materials, differences in EIA and WB method performance was observed
- PT and QC are important parts of a quality management system
- PT provides a snap shot of laboratory performance at one point in time while QC illustrates method performance over time
- Continued independent assessments of laboratory performance are needed for laboratory certification and for continuous quality improvement

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