Newborn Screening Quality Assurance Program

2011 ANNUAL SUMMARY REPORT

March 2012

2011 Annual Summary Report for Anti-HIV-1 in Dried-Blood Spots

Introduction

The Newborn Screening Molecular Biology Branch (NSMBB) is within the Centers for Disease Control and Prevention's (CDC) National Center for Environmental Health, Division of Laboratory Sciences. NSMBB's responsibilities include the Newborn Screening Quality Assurance Program (NSQAP), which provides, among other services, quality control (QC) for laboratories and assistance to laboratories that screen newborns for HIV antibodies.

NSQAP operates multi-component, quality assurance programs that assist laboratories in testing dried-blood spots (DBS) for human immunodeficiency virus type 1 (HIV) antibodies. NSQAP's programs help these laboratories achieve high technical proficiency and help them maintain high performance standards. NSQAP also works with those laboratories that need to improve method development and kit validation.

As an integral part of its DBS quality assurance program, each quarter NSQAP distributes a panel of proficiency testing (PT) specimens to participating laboratories. These specimens represent a variety of HIV antibody reactivity. Laboratories then submit their results, which NSQAP evaluates. QC materials are shipped twice a year to all HIV PT-program participating laboratories. The NSQAP is the only source for DBS HIV QC materials—manufacturers do not provide internal QC materials in their kits. NSQAP maintains a consultative service, which is always available to any participant laboratory with quality assurance concerns.

The HIV quality assurance program developed from a 1986 pilot project. The project considered whether residual DBS specimens collected from newborns for metabolic and inherited disease-testing might have other uses. The project found that these DBS specimens could be used to determine the prevalence of HIV infection in childbearing women. CDC then developed and funded the HIV Seroprevalence Survey among Childbearing Women, a national epidemiologic survey that monitored seroprevalence among childbearing women and predicted perinatal transmission rates in new births.[1] Given NSQAP already provided both DBS PT and QC materials to state laboratories for newborn screening metabolic tests, the NSQAP also became creator and distributor of QC and PT materials for use with anti- HIV immunochemical and Western Blot (WB) assays.

Because DBS are an ideal matrix to store and transport whole blood collected from heel sticks or finger sticks,[2] many areas around the world have adapted DBS collection for use in HIV antibody testing. Today, CDC's Quality Assurance Program for Anti-HIV-1 in DBS has 24 participating laboratories.

Methods

NSQAP-prepared DBS materials simulate newborn specimens. Many different assay systems can test them,

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Table 1. Summary of HIV-1 PT Errors by Domestic and Foreign Laboratories in 2011

	Positive Specimens Assayed (N)	False-Negative Errors (%)	Negative Specimens Assayed (N)	False-Positive Errors (%)
Domestic Laboratories	120	0.0%	145	0.0%
Foreign Laboratories	92	0.0%	113	3.5%

and any variance resulting from the manufactured specimens is minimal. An approved national standard governs the collection of DBSs and the handling of the filter paper matrix.[3] Before distribution to participants, DBS QC and PT specimens from various sources are certified for homogeneity, reproducibility, stability, and suitability for performance in enzyme immunoassay (EIA) and WB kits.

Bench-level DBS QC materials are prepared using heat-inactivated, HIV-positive plasma or serum mixed with HIV-negative serum (non-heat inactivated). Preparation next includes the addition of packed red blood cells to achieve a hematocrit of 50% + 1%. HIV-negative, low-positive, and high-positive QC specimens are produced by blending negative serum and HIV- positive serum from a single donor, or by blending the serum of two or more HIV- positive donors to achieve the appropriate EIA target absorbance values (optical density, OD) and banding patterns for WB.[4-6] Each new QC production lot is evaluated relative to the previous two lots to provide linkage across all QC materials. The QC whole blood mixtures are spotted in 110 uL aliquots on Grade 226 filter paper cards (PerkinElmer Health Sciences—formerly Ahlstrom Filtration LLC—Greenville, SC), using a robotics dispensing system (Titertek, Inc., Huntsville, AL). The collection cards are suspended horizontally in specially designed racks. The DBS cards are dried for 24 hours at ambient temperature then placed in low gas-permeable, plastic zip-closure bags containing desiccant packets. The DBS QC materials are stored at -20º C, and the humidity is maintained below 30%. A base pool of whole blood is used to prepare HIV-negative, low-positive, and high-positive QC pools so that all materials are matched to the same homogeneous matrix.

Our method for preparation of DBS materials for PT is similar to our method for preparation of the QC

Table 2. HIV Testing Algorithms for DBS in Quarter 4, 2011

Testing Combination	Number of Labs 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

Table 3. Primary and Secondary Screening Methods Used by Participants in 2011

Method Code	Kit Source	# Participants
10	Fujirebio Serodia-HIV 1,2	2
11	In House	1
12	Other	7
15	Genetic Systems rLAV EIA (Bio-Rad) (Discontinued)	5
27	Tecnosuma (Cuba) UMELISA HIV 1+2	3
34	Q-Preven HIV 1+2, DBS, Brazil	1
40	FDA Licensed for DBS-Avioq HIV-1 Microelisa Systems	11
41	Bio-Rad HIV-1/2 plus O EIA	3
	Total	33

Table 4. Confirmatory WB Methods Used By Participants in 2011

Method Code	Kit Source	# Participants
16	***FDA Licensed for DBS - Genetic Systems HIV-1 WB (Bio-Rad)***	12
32	Cambridge Biotech HIV-1 WB Kit (Maxim)	1
35	OraSure HIV-1 WB Kit	1
36	New LAV Blot I (Bio-Rad)	1
37	Genelab diagnostics HIV 2.2 WB	1
42	MP Diagnostics HIV Blot 2.2	1
12	Other	1
	Total	18

materials. We use a single donor's serum without blending or pooling the serum. For 2011 PT materials we used Whatman 903 filter paper cards (GE Healthcare Bio-Sciences Corporation—formerly Whatman Inc.—Westborough, MA). We maintain a library of various individual matrices for PT testing.

Results

Each year we produce and certify approximately 30,000 DBS for HIV QC and PT. We distribute the DBS to participating laboratories worldwide. We assess the analytical performance of immunochemical, EIA, and WB methods and monitor any noticeable problems or trends. Some laboratories might report analytical or specimen classification errors. If so, those laboratories receive feedback to help them identify how the error occurred and how to avoid similar errors in future.

Table 1 summarizes false-positive and false-negative rates for domestic and foreign laboratories in 2011. Over the year, foreign laboratories reported four false-positive results. During 2011, domestic laboratories reported no errors.

Proficiency Testing

Laboratories participating in PT are located throughout the world and use a variety of Anti-HIV-1 testing algorithms. Table 2 lists the combinations of HIV testing algorithms that laboratories reported for DBS PT specimens. The 2011 quarter 4 data were used to create this table because that quarter had the largest number of laboratories reporting results.

PT Material and Laboratory Performance

Tables 3 and 4 list the names of the screening and confirmatory methods used by participants in 2011 for the PT quarterly events. Since Bio-Rad discontinued the Genetics Systems rLAV EIA early this year, the only kit available with Food and Drug Administration (FDA) approval for a DBS protocol is the Avioq HIV-1 Microelisa System (Avioq Inc., Research Triangle

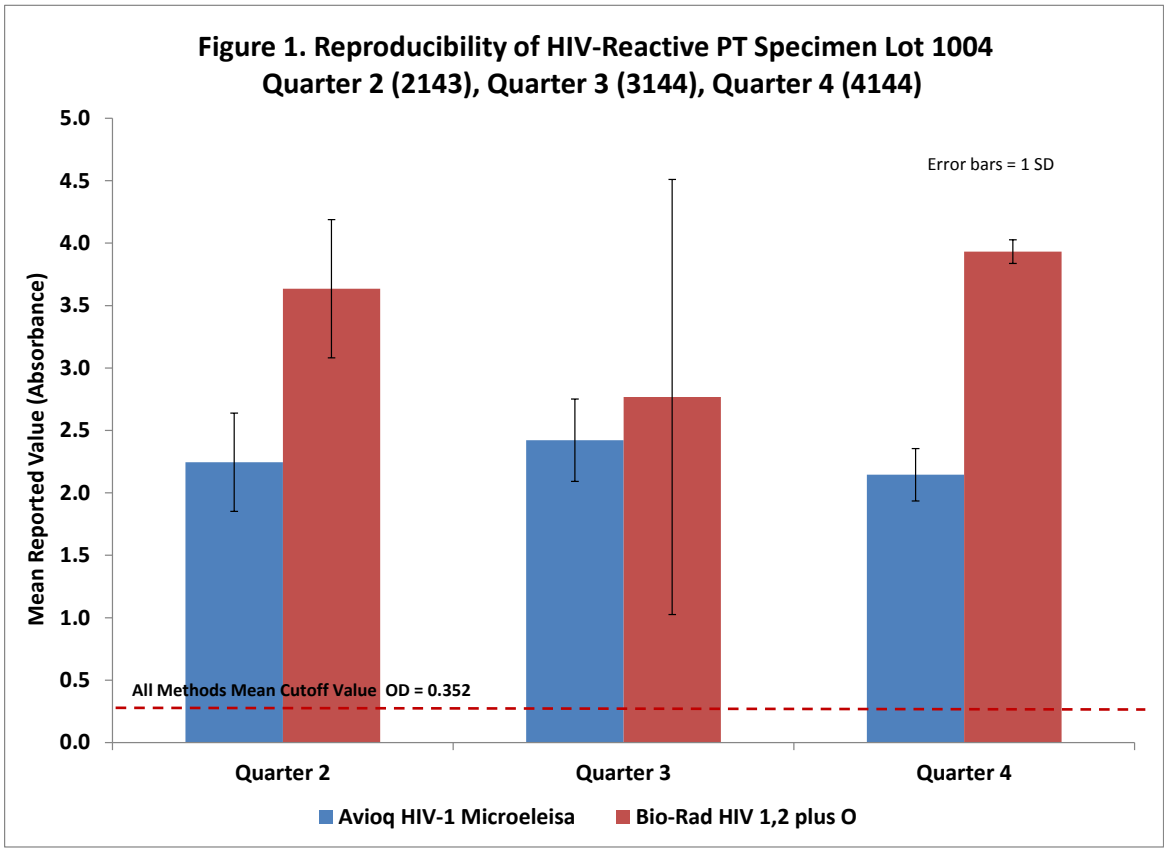
Figure 1. Reproducibility of HIV-Reactive PT Specimen Lot 1004
Quarter 2 (2143), Quarter 3 (3144), Quarter 4 (4144)
Mean Reported Value (Absorbance)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Error bars = 1 SD
All Methods Mean Cutoff Value OD = 0.352
Quarter 2
Quarter 3
Quarter 4
Avioq HIV-1 Microeleisa
Bio-Rad HIV 1,2 plus O

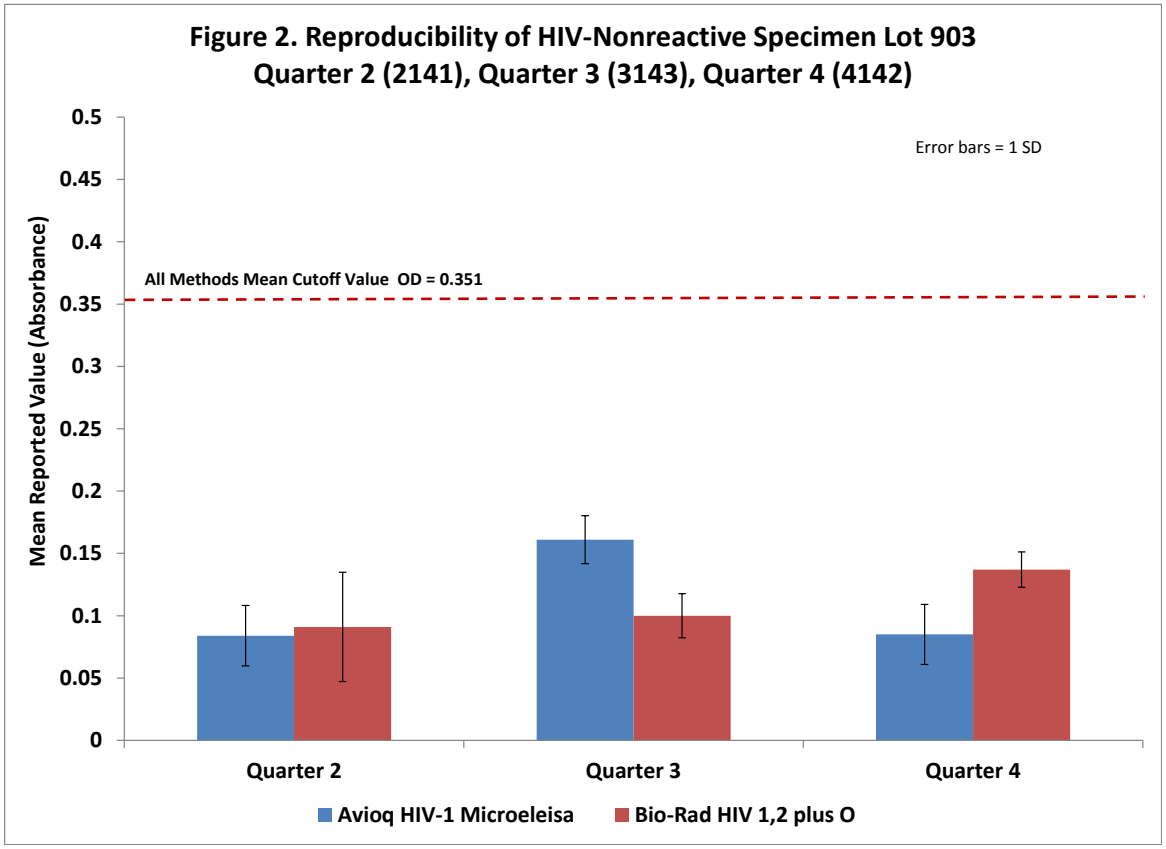
Figure 2. Reproducibility of HIV-Nonreactive Specimen Lot 903
Quarter 2 (2141), Quarter 3 (3143), Quarter 4 (4142)
Mean Reported Value (Absorbance)
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Error bars = 1 SD
All Methods Mean Cutoff Value OD = 0.351
Quarter 2
Quarter 3
Quarter 4
Avioq HIV-1 Microeleisa
Bio-Rad HIV 1,2 plus O

Park, NC). Table 3 shows the number of participants and the methods used over the course of the year. Several participants used EIA screening both as the primary method and confirmatory method. Two laboratories used WB only as their primary method for testing the PT materials. These laboratories were grouped with other methods.

Reproducibility of PT materials is often tested throughout the year when choosing the specimens that make up each quarter's PT panel. Past data have shown that reproducibility was good between methods when comparing overall mean values for the same specimen tested several times throughout the year. But within methods, a large amount of absorbance variability often appears. Figure 1 illustrates reproducibility of PT Specimen Lot 1004 (reactive) EIA methods between quarters. Figure 2 shown the reproducibility of PT Specimen Lot 903 (non-reactive) EIA methods.

WB Method Performance with PT Materials

In 2011, PT Specimen lot 1004 (reactive) was distributed over three quarters. Figure 3 shows the reproducibility of Western Blot protein molecular-weight bands reported for one method. Very little difference appeared between quarters. Those differences that did appear can be attributed to kit-lot differences and to the subjective nature of WB pattern interpretation. The Genetic System's HIV-1 Western Blot (Bio-Rad) is the only method that carries an FDA approved DBS testing protocol.

QC Material Performance–EIA

Table 5 illustrates the overall performance of CDC QC materials used with each EIA method. This illustration does not show the methods listed as in-house or other methods. The wide variability of measured absorbance units among kits for the CDC negative, low positive, and high positive control materials makes difficult any certification of an overall QC range to fit all methods. Each laboratory must establish its own mean and acceptable range limits based on its method of choice. Figure 4 shows the mean of the CDC QC Lots 91–93 materials for each of the most commonly reported EIA methods during quarters 1 and 2 of 2011. Figure 5 shows CDC QC Lots 111–114 for quarters 3 and 4 of 2011. The mean cutoff of all methods, as represented by the

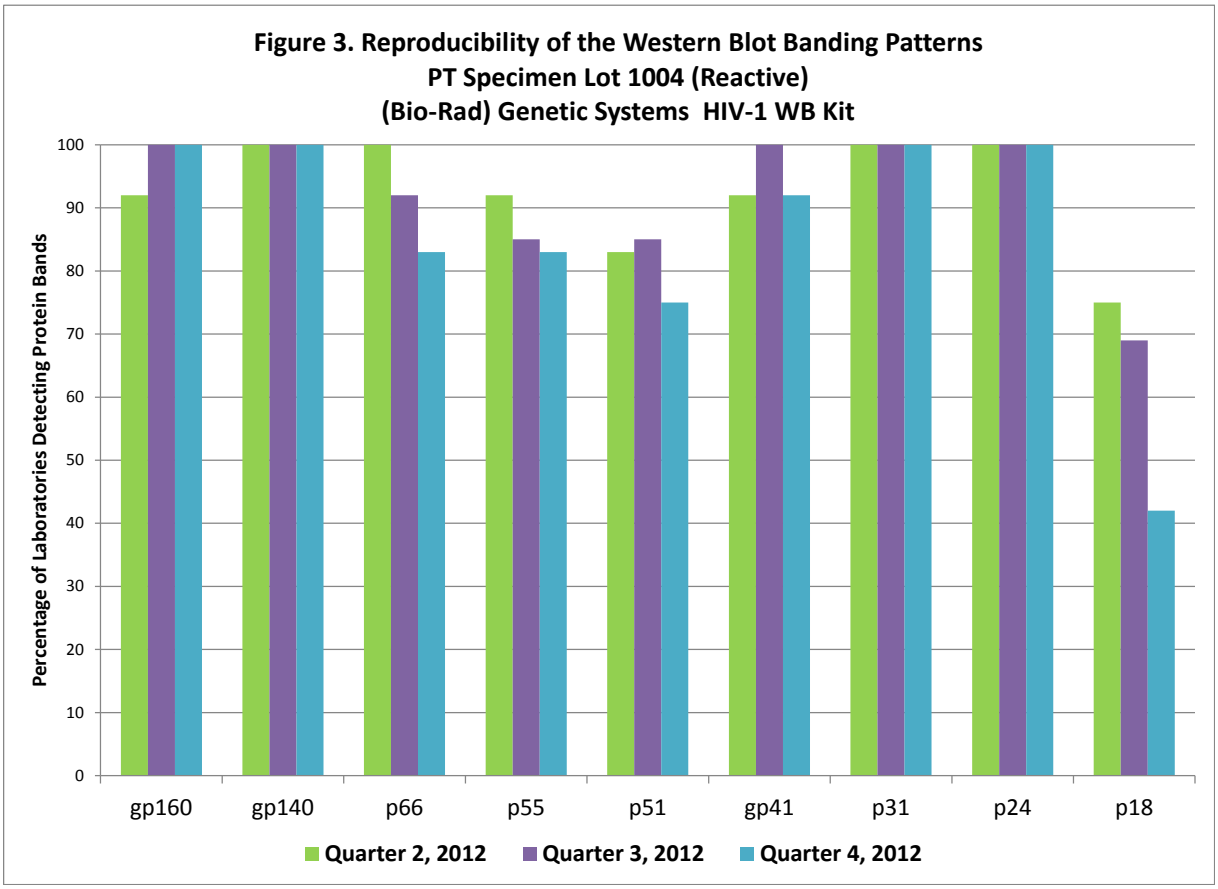

Table 5. 2011 Anti-HIV-1 Quality Control Lots 91-93

	Genetics System rLav			
	CDC QC Lot 91	**CDC QC Lot 92**	**CDC QC Lot 93**	**Cutoff**
MEAN	0.118	0.559	1.216	0.272
Min	0.087	0.373	0.968	0.262
Max	0.175	0.929	2.099	0.280
	Tecnosuma UMELISA 1,2			
	CDC QC Lot 91	**CDC QC Lot 92**	**CDC QC Lot 93**	**Cutoff**
MEAN	0.169	0.732	1.141	0.382
Min	0.108	0.503	0.522	0.280
Max	0.224	1.260	1.980	0.646
	Q-Preven HIV 1+2			
	CDC QC Lot 91	**CDC QC Lot 92**	**CDC QC Lot 93**	**Cutoff**
MEAN	0.147	0.574	1.578	0.308
Min	0.110	0.061	1.013	0.293
Max	0.163	0.650	2.170	0.323
	Avioq HIV-1			
	CDC QC Lot 91	**CDC QC Lot 92**	**CDC QC Lot 93**	**Cutoff**
MEAN	0.094	1.733	2.486	0.370
Min	0.061	0.972	1.763	0.339
Max	0.152	3.000	3.010	0.415
	BioRad HIV-1,2 Plus O EIA			
	CDC QC Lot 91	**CDC QC Lot 92**	**CDC QC Lot 93**	**Cutoff**
MEAN	0.076	2.981	3.474	0.284
Min	0.013	1.019	2.179	0.265
Max	0.141	4.000	4.000	0.305

2011 Anti-HIV-1 Quality Control Lots 111-114

	Genetics System rLav			
	METHOD DISCONTINUED			
	Tecnosuma UMELISA 1,2			
	CDC QC Lot 111	**CDC QC Lot 112**	**CDC QC Lot 113**	**Cutoff**
MEAN	0.115	0.691	1.304	0.329
Min	0.084	0.581	1.006	0.300
Max	0.120	0.929	1.618	0.357
	Q-Preven HIV 1+2			
MEAN	NO DATA WERE REPORTED			
Min				
Max				
	Avioq HIV-1			
	CDC QC Lot 111	**CDC QC Lot 114**	**CDC QC Lot 113**	**Cutoff**
MEAN	0.103	1.365	2.332	0.365
Min	0.061	0.645	1.773	0.148
Max	0.181	3.000	3.000	0.432
	BioRad HIV-1,2 Plus O EIA			
	CDC QC Lot 111	**CDC QC Lot 114**	**CDC QC Lot 113**	**Cutoff**
MEAN	0.135	3.163	3.732	0.282
Min	0.095	1.595	3.058	0.267
Max	0.190	4.000	4.000	0.304

dotted line, clearly shows that our QC materials perform as expected with all methods reported by participants.

Discussion

NSQAP supplies unique materials for those laboratories that test DBS for HIV antibodies. The materials provide a level of confidence for those testing methods that do not provide DBS QC materials as part of their kits. Three levels of QC materials are distributed to participants to cover a range of HIV reactivity. By tracking the performance of the CDC QC materials, laboratories can monitor their methods and identify shifts in values over time. The materials are also available for method evaluation and validation.

Although laboratories might use common methods, results can vary widely. Still, despite such within-method variability, the majority of laboratories correctly identified PT specimens as nonreactive or HIV-reactive (Table 1). Testing algorithms for DBS also varied widely, with most labs using a combination of EIA and WB testing for their algorithm (Table 2). There remains only one EIA method and one WB method that have protocols approved specifically for DBS. These products are primarily available in North America. Nevertheless, laboratories outside North America have adapted serum-based methods to the filter paper DBS matrix (Tables 3 and 4) with good results.

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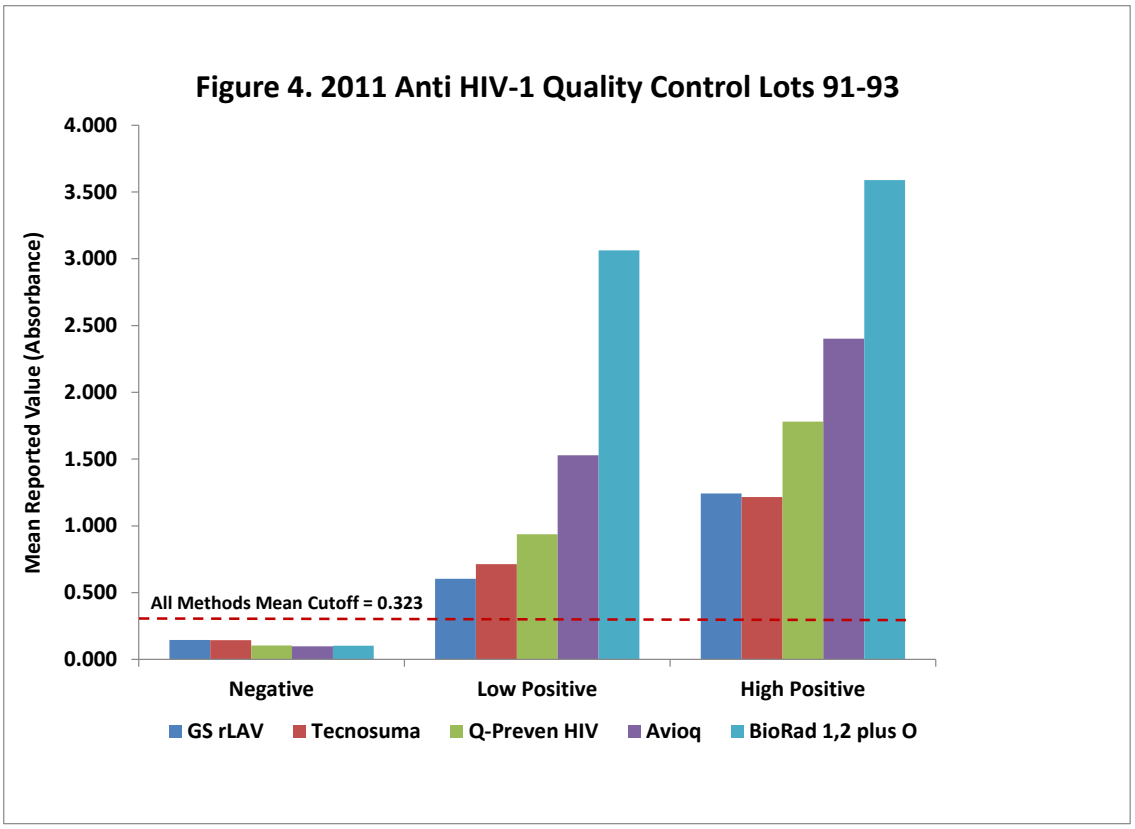
Figure 4. 2011 Anti HIV-1 Quality Control Lots 91-93
Mean Reported Value (Absorbance)
4.000
3.500
3.000
2.500
2.000
1.500
1.000
0.500
0.000
All Methods Mean Cutoff = 0.323
Negative
Low Positive
High Positive
GS rLAV
Tecnosuma
Q-Preven HIV
Avioq
BioRad 1,2 plus O

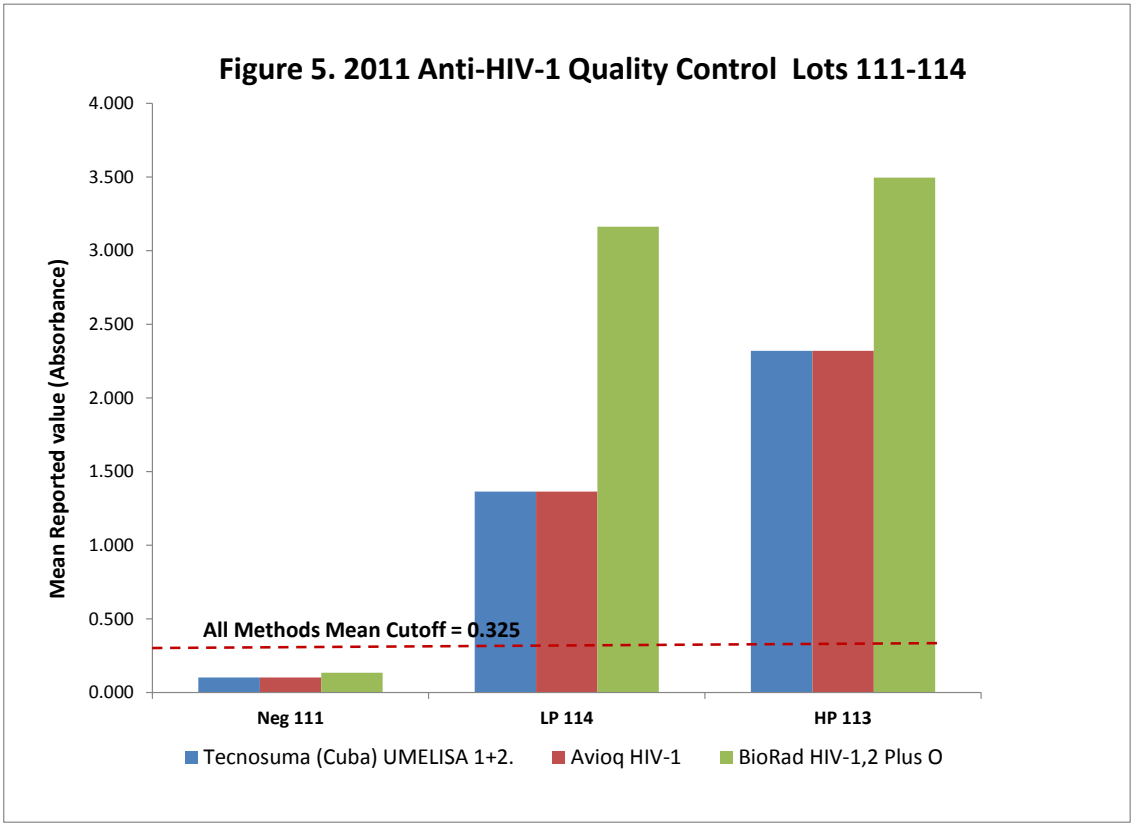
Figure 5. 2011 Anti-HIV-1 Quality Control Lots 111-114
Mean Reported value (Absorbance)
4.000
3.500
3.000
2.500
2.000
1.500
1.000
0.500
0.000
All Methods Mean Cutoff = 0.325
Neg 111
LP 114
HP 113
Tecnosuma (Cuba) UMELISA 1+2.
Avioq HIV-1
BioRad HIV-1,2 Plus O

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CENTERS FOR DISEASE CONTROL AND PREVENTION (CDC)
ATLANTA, GA 30341

Director
Thomas R. Frieden, M.D., M.P.H.
Director
National Center for Environmental Health
Christopher J. Portier, Ph.D.
Director
Division of Laboratory Sciences
James L. Pirkle, M.D., Ph.D.
Chief
Newborn Screening and Molecular Biology Branch
Carla Cuthbert, Ph.D.

Contributors:
Barbara W. Adam
Carol Bell
Dana Chafin
Paul Dantonio
Victor R. De Jesus, Ph.D.
Marie C. Earley, Ph.D.
Sharon Flores
Tamara Ganka
Elizabeth M. Hall
Christopher Haynes, Ph.D.
Kevin Lanza
Francis Lee, Ph.D.
Lixia Li, Ph.D.
Timothy Lim, Ph.D.
Joanne Mei, Ph.D.
Nancy Meredith
Tracey Myers
Jennifer Taylor
Robert Vogt, Ph.D.
Golriz Yazdanpanah
Hui Zhou, Ph.D.
Sherri Zobel

Production:
Sarah Brown
Felicia Manning
Teresa Moore
Connie Singleton

ASSOCIATION OF PUBLIC HEALTH LABORATORIES
SILVER SPRING, MD 20910

President
Victor Waddell, Ph.D.
Chairman, Newborn Screening and Genetics in Public Health Committee
Susan M. Tanksley, Ph.D.
Chairman, Newborn Screening Quality Assurance Quality Control Subcommittee
Patrick Hopkin, B.S.

INQUIRIES TO:

Nancy Meredith, Editor • Centers for Disease Control and Prevention (CDC)
Newborn Screening Quality Assurance Program • Mailstop F-43
4770 Buford Highway, N.E. • Atlanta, GA 30341-3724
Phone (770) 488-4582 • FAX (770) 488-4255 • E-mail: NMeredith@cdc.gov