Newborn Screening Quality Assurance Program

2009 ANNUAL SUMMARY REPORT

June 2010

2009 Annual Summary Report for Anti-HIV-1 in Dried-Blood Spots

INTRODUCTION

The Centers for Disease Control (CDC), National Center for Environmental Health's Division of Laboratory Sciences, through the Newborn Screening and Molecular Biology Branch, operates a multi-component quality assurance program for laboratories testing dried-blood spots (DBS) for human immunodeficiency virus type 1 (HIV) antibodies. This program is designed to help laboratories achieve excellent technical proficiency and maintain confidence in their performance. The materials also help those laboratories needing aid in method development and kit validation. A panel of proficiency testing (PT) specimens, representing a variety of HIV antibody reactivity is distributed quarterly to participating laboratories. Results are submitted and evaluated to monitor laboratory performance. Quality control (QC) materials are shipped semi-annually to all laboratories participating in the HIV PT program. The Newborn Screening Quality Assurance Program (NSQAP) is the only source for DBS HIV QC materials as manufacturers do not provide internal QC materials in their kits. Consultative services are always available for emerging concerns in laboratory quality assurance.

The HIV quality assurance program developed from a 1986 pilot project which demonstrated that residual DBS specimens collected from newborns for metabolic and inherited disease testing could be used to obtain the prevalence of HIV infection in child bearing women. As a result of this project, CDC developed and funded a national epidemiologic survey called the HIV Seroprevalence Survey Among Childbearing Women to monitor the seroprevalence among these women and to predict the perinatal transmission rate in new births[1]. Since NSQAP provided both DBS PT and QC materials to the state laboratories for newborn screening metabolic tests, the program was recruited to create and distribute QC and PT materials that could be used 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], their collection and use for HIV antibody testing has been adapted in many areas around the world. As of January 1, 2010, there are 80 total participants, 15 domestic laboratories and 65 foreign laboratories, who participate in CDC's Quality Assurance Program for Anti-HIV-1 in DBS.

METHODS

DBS materials prepared by NSQAP simulate newborn specimens and can be tested by many different assay systems, with minimal variance contributed by the

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manufactured specimens. An approved national standard exists for the collection of DBSs and the handling of the filter paper matrix.[3] DBS QC and PT specimens are certified for homogeneity, reproducibility, stability, and suitability for performance in enzyme immunoassay (EIA) and Western Blot kits from various sources before distribution to participants.

Bench-level DBS QC materials are prepared using heat-inactivated HIV positive-plasma or serum mixed with HIV negative-serum (non-heat inactivated) followed by 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 whole blood mixtures are spotted in 110 uL aliquots on Grade 903 filter paper cards (Whatman, Inc., Florham Park, NJ) 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 and then placed in low gas-permeable, plastic zip-closure bags containing desiccant packets. The DBS control 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.

We prepare DBS materials for PT in a manner similar to the preparation of the QC materials by using a single donor's serum without blending or pooling the serum. A library of various individual matrices is maintained for PT testing.

RESULTS

Each year we produce and certify more than 85,000 DBS for HIV QC and PT and distribute them to participating laboratories worldwide. We assess the analytical performance of immunochemical, EIA, and WB methods and monitor any noticeable problems or trends. If laboratories report analytical or specimen classification errors, they receive feedback to help them identify how the error occurred so future errors can be avoided.

The false-positive and false negative rates for domestic and foreign laboratories in 2009 are summarized in Table 1. Over the year there were four false negative results reported and five false positive results reported by foreign laboratories. The domestic false negative and false positive rate was zero.

Table 1. Summary of HIV-1 PT Errors by Domestic and Foreign Laboratories in 2009.

	Positive Specimens Assayed (N)	False-Negative Errors (%)	Negative Specimens Assayed (N)	False-Positive Errors (%)
Domestic Laboratories	141	0.0	94	0.0
Foreign Laboratories	510	0.78%	340	1.47%

Participating laboratories use a variety of anti-HIV-1 testing algorithms throughout the world.
Table 2 lists the combinations of HIV testing algorithms that laboratories reported for DBS PT specimens. Quarter 2, 2009 data was used for to create Table 2 because it had the largest number of laboratories reporting results for the quarter.

Table 2. HIV Testing Algorithms for DBS in Quarter 2, 2009.

Testing combination	Number of labs reporting
EIA/Western Blot	18
EIA Only	11
Agglutination/Western Blot	1
Agglutination Only	1
EIA/EIA	21
EIA//EIA/Western Blot	8
EIA/Agglutination/Western Blot	0

PT Material and Laboratory Performance

Tables 3 and 4 list the names of the screening and confirmatory methods used by participants in 2009 for the PT quarterly events. In 2009 there was only one EIA method, Genetic Systems rLAV EIA (BioRad) that was FDA approved and that carried a protocol for DBS. In late 2009, a new kit, Avioq HIV-1 microelisa System (Avioq Inc., Research Triangle Park, NC) was approved by FDA with claims for serum, plasma, oral fluid, and DBS. Table 2 shows the number of participants and the methods used over the course of the year. Several participants use EIA screening both as the primary method and as their secondary and confirmatory method.

Table 3. Primary and Secondary Screening Methods Used By Participants in 2009.

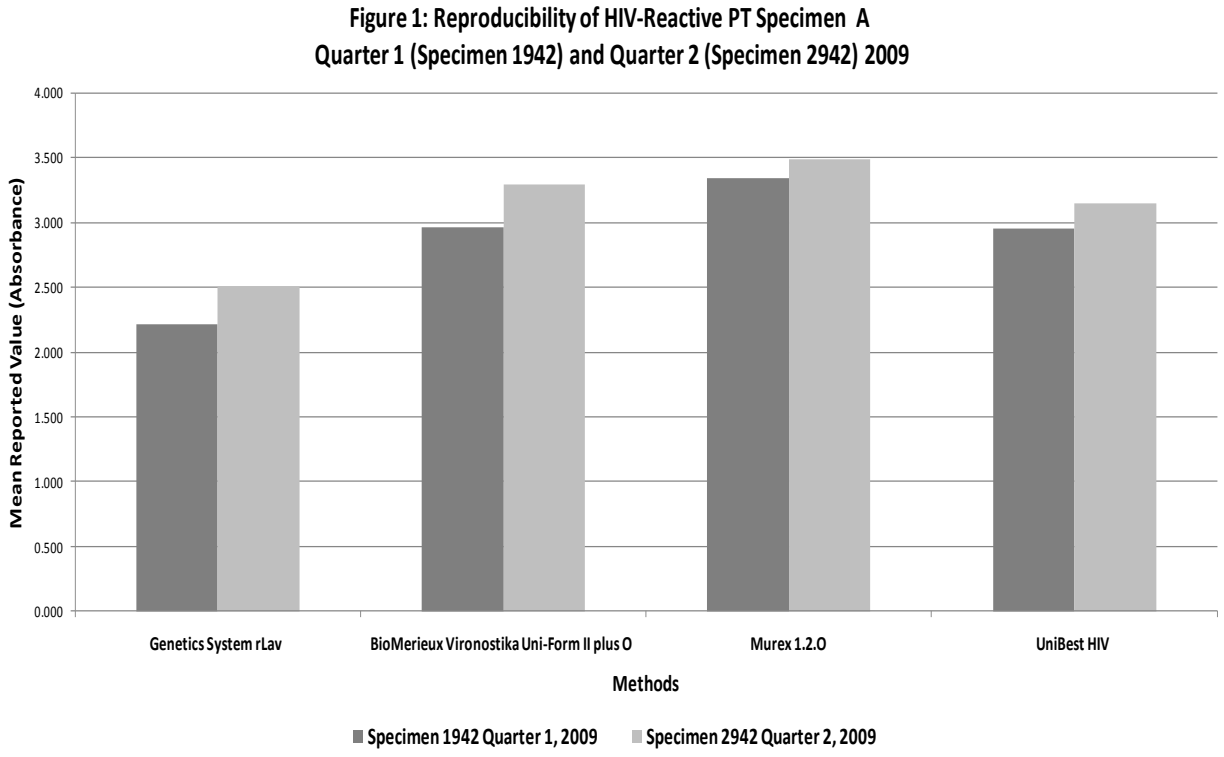
Method Code and Kit Source		# Participants
10	Fujirebio Serodia-HIV 1,2	3
11	In House	2
12	Other	9
15	***FDA Licensed for DBS*** - Genetic Systems rLAV EIA (Bio-Rad)	14
20	bioMerieux Vironostika UniForm II Ag/AB	3
21	bioMerieux Vironostika Uni-Form II plus O	6
22	Genescreen HIV 1/2 V2	4
23	Genescreen Plus HIV Ag/Ab (BioRad)	2
24	Murex HIV 1.2.0 (Abbott)	18
25	Murex HIV Ag/Ab Combination (Abbott)	4
26	Recombinant HIV 1/2, Russia	4
27	Tecnosuma (Cuba) UMELISA HIV 1+2	2
28	CombiBest Anti-HIV 1, 2 DCM, Russia	3
29	CombiBest 1/2, Ag/Ab, Russia	4
30	Anti-HIV Unif, Russia	9
31	Dade Behring Enzygnost Anti-HIV 1/2 Plus O	1
33	UniBest HIV 1,2 AB, Russia	10
34	Q-Preven HIV 1+2, DBS, Brazil	1
39	Genescreen Ultra HIV AG-AB (BioRad)	10

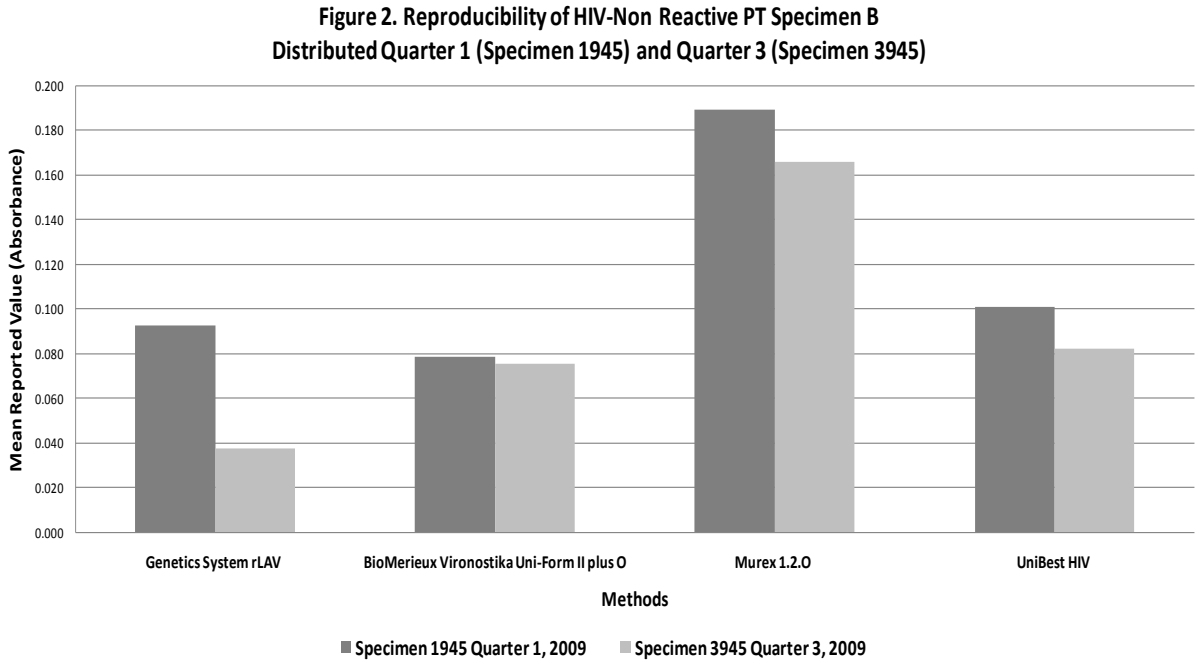
Table 4. Confirmatory Western Blot Methods Used By Participants 2009.

Method Code and Kit source		# Participants
12	Other	3
16	***FDA Licensed for DBS*** - Genetic Systems HIV-1 WB (Bio-Rad)	14
32	Cambridge Biotech HIV-1 WB Kit (Maxim)	3
35	OraSure HIV-1 WB Kit	1
36	New LAV Blot I (Bio-Rad)	3
37	Genelab diagnostics HIV 2.2 WB	3
38	ImmunoComb II HIV 1,2 CombFirm (Orgenics)	1

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 when comparing over-all-mean values between specimens, however, large variability's in absorbance are often seen within-methods.

Reproducibility of specimen A (reactive) EIA results between quarters is illustrated in Figure 1 and the reproducibility of specimen B (non-reactive) EIA results is shown in Figure 2.
The mean reported absorbance per method is shown in both Figure 1 and 2.





WB Method Performance with PT materials

In 2009, Specimen A (reactive) was distributed in quarter 1 and quarter 2. A summary of reported protein molecular weight bands for four methods is shown in Figure 3 and 4, respectively. Differences in methods between quarters are small and can be attributed to kit lot differences and the subjective nature of interpreting WB patterns. The Genetic Systems HIV-1 Western Blot (BioRad) also carries an FDA approved DBS testing protocol.

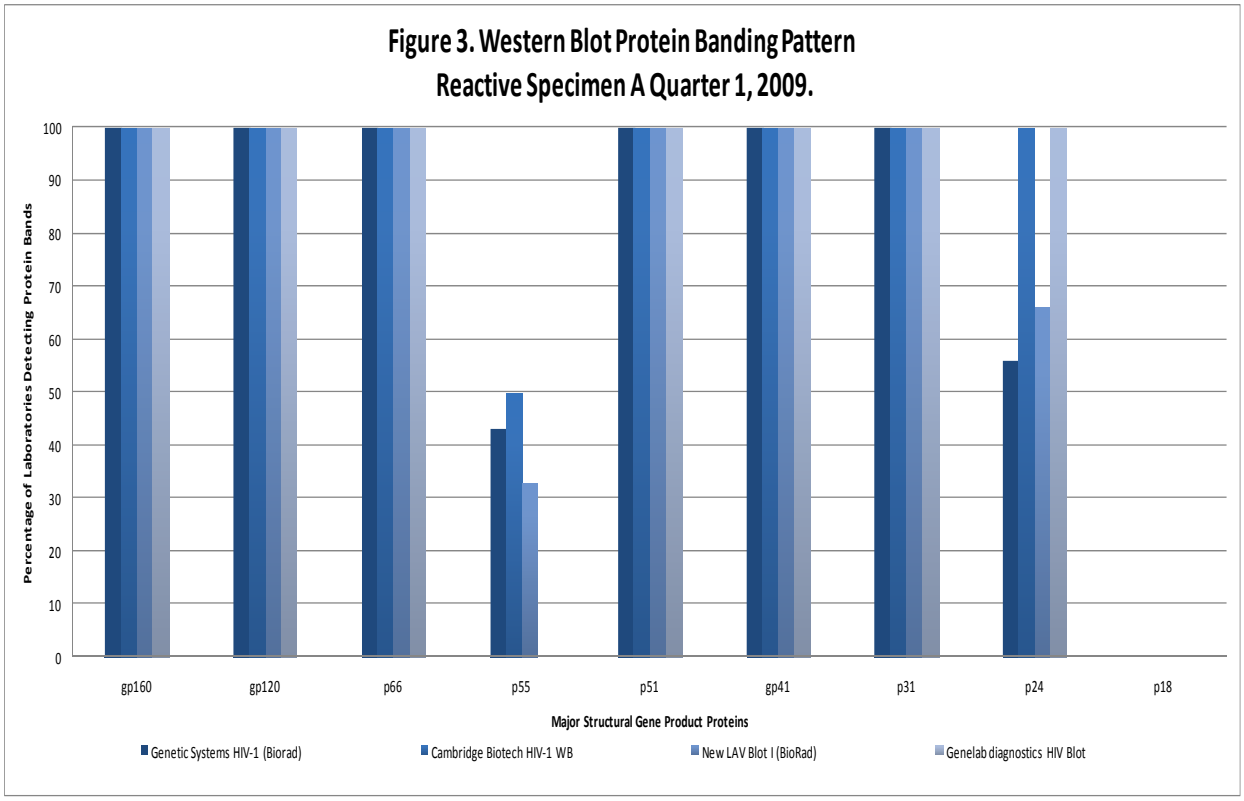


Figure 3. Western Blot Protein Banding Pattern
Reactive Specimen A Quarter 1, 2009.
Percentage of Laboratories Detecting Protein Bands
100
90
80
70
60
50
40
30
20
10
0
gp160
gp120
p66
p55
p51
gp41
p31
p24
p18
Major Structural Gene Product Proteins
Genetic Systems HIV-1 (Biorad)
Cambridge Biotech HIV-1 WB
New LAV Blot I (BioRad)
Genelab diagnostics HIV Blot

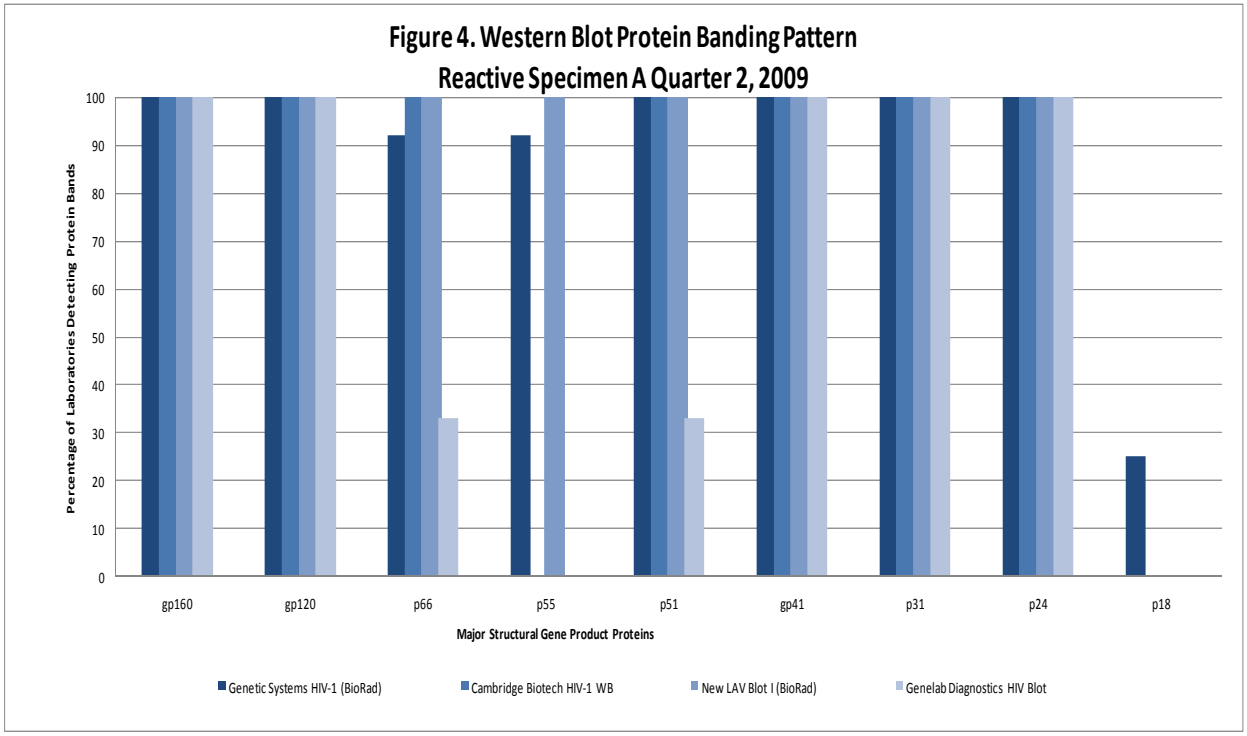


Figure 4. Western Blot Protein Banding Pattern
Reactive Specimen A Quarter 2, 2009
Percentage of Laboratories Detecting Protein Bands
100
90
80
70
60
50
40
30
20
10
0
gp160
gp120
p66
p55
p51
gp41
p31
p24
p18
Major Structural Gene Product Proteins
Genetic Systems HIV-1 (BioRad)
Cambridge Biotech HIV-1 WB
New LAV Blot I (BioRad)
Genelab Diagnostics HIV Blot

QC Material Performance–EIA

Figure 5 illustrates the overall performance of CDC QC materials used within each EIA method. Other and in-house methods that were not EIA specific were not used in this illustration.

The wide variability of measured absorbance units among kits for the CDC negative, low positive, and high positive control materials makes it difficult to certify an overall QC range to fit all methods; however we calculated the 95% control limits for each method using participants' reported results (see Table 5 comment below). Each laboratory must establish their own mean and acceptable range limits based on their method of choice.

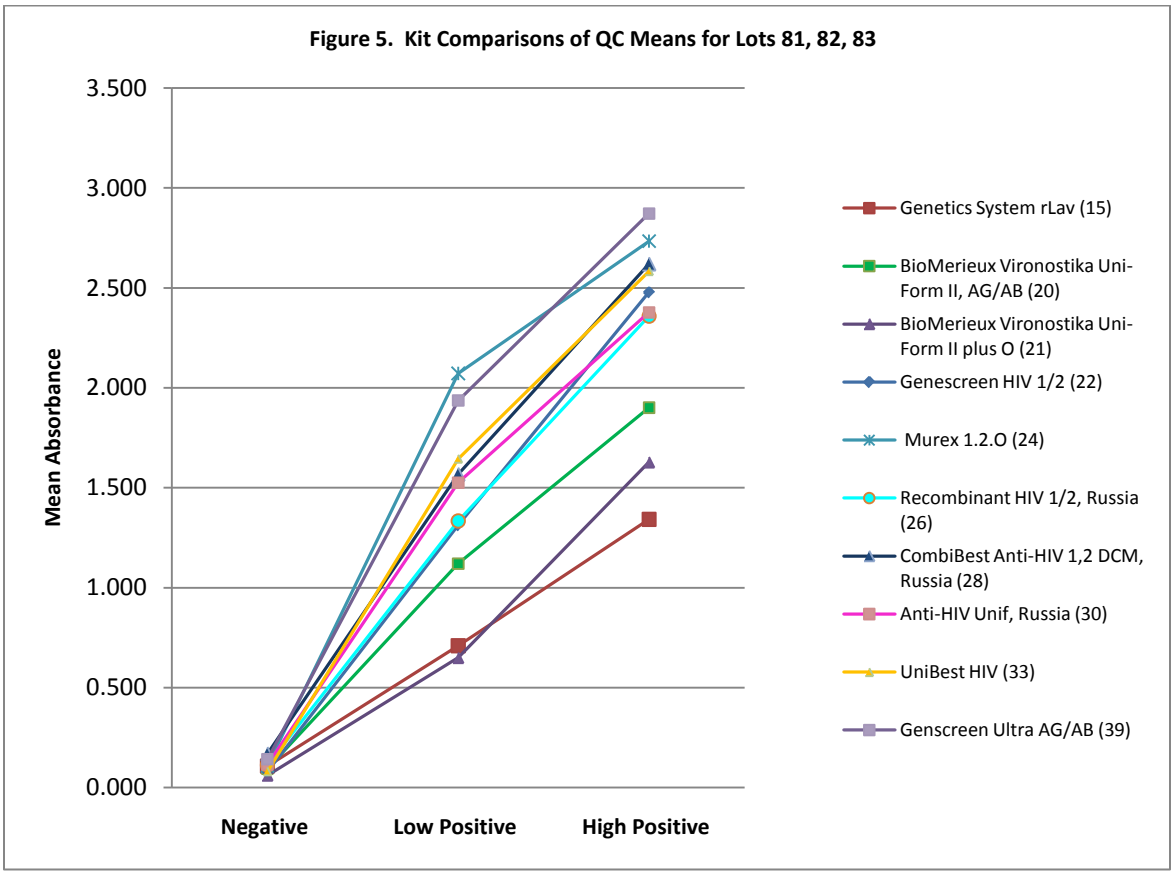
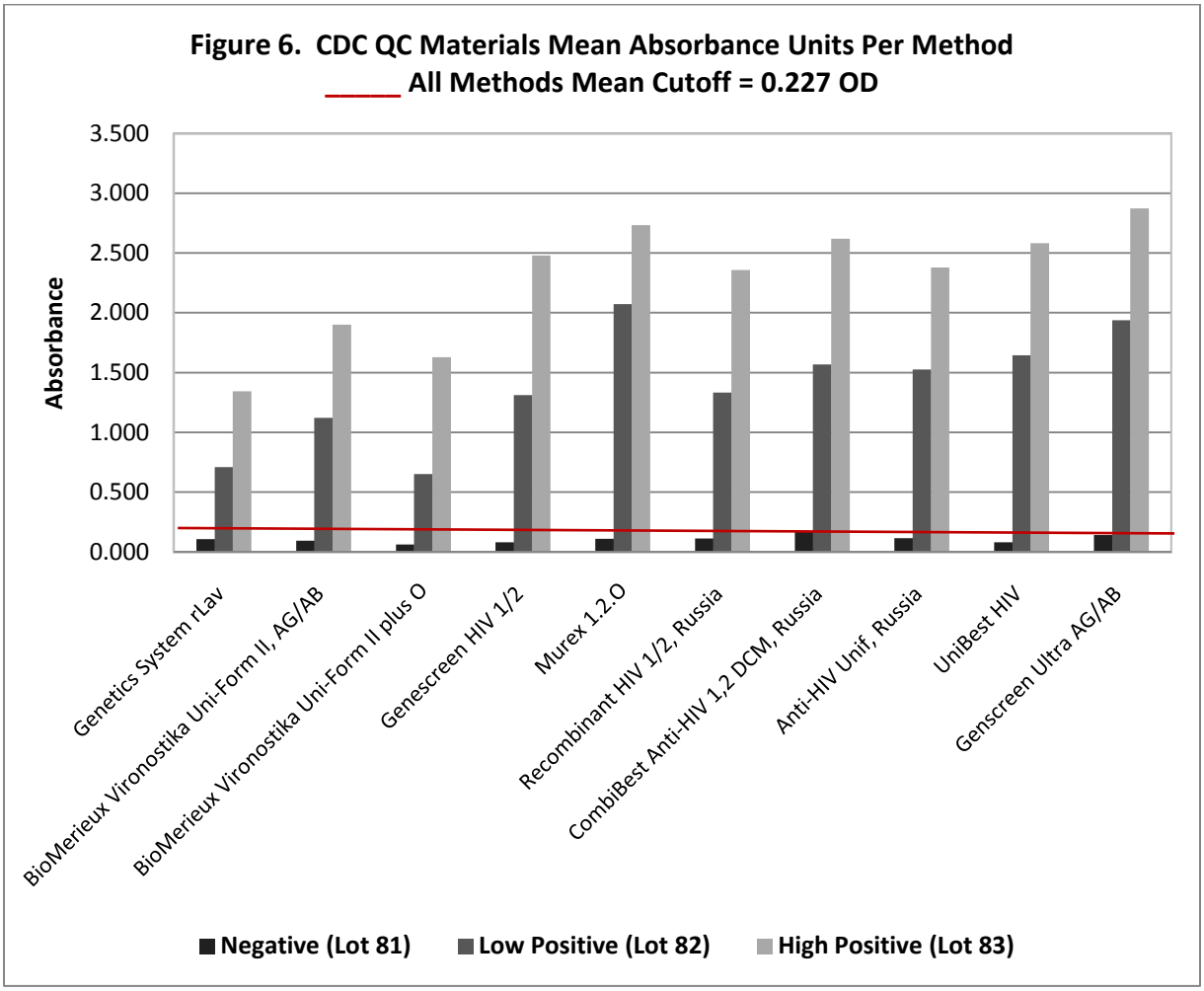


Table 5 gives the mean, standard deviation, min and max values for the CDC QC materials by each of the EIA methods most used.

Table 5. Summary of reported data by method for CDC QC Lots 81, 82, and 83.

	Genetics System rLav			
	CDC QC Lot 81	CDC QC Lot 82	CDC QC Lot 83	Cutoff
MEAN	0.107	0.709	1.343	0.276
STD	0.052	0.225	0.396	0.018
MINIMUM	0.025	0.302	0.645	0.208
MAXIMUM	0.274	1.282	2.610	0.317
	BioMerieux Vironostika Uni-Form II, AG/AB			
	CDC QC Lot 81	CDC QC Lot 82	CDC QC Lot 83	Cutoff
MEAN	0.095	1.120	1.901	0.187
STD	0.014	0.820	1.046	0.012
MINIMUM	0.074	0.231	0.819	0.177
MAXIMUM	**0.122**	**2.296**	**3.225**	0.209
	BioMerieux Vironostika Uni-Form II plus O			
	CDC QC Lot 81	CDC QC Lot 82	CDC QC Lot 83	Cutoff
MEAN	0.062	0.650	1.628	0.195
STD	0.038	0.249	0.588	0.086
MINIMUM	0.026	0.342	0.504	0.129
MAXIMUM	0.190	1.160	2.392	0.376
	Genescreen HIV 1/2			
	CDC QC Lot 81	CDC QC Lot 82	CDC QC Lot 83	Cutoff
MEAN	0.081	1.313	2.478	0.154
STD	0.050	0.734	0.714	0.049
MINIMUM	0.026	0.331	1.034	0.088
MAXIMUM	0.198	2.863	3.312	0.288
	Murex 1.2.O			
	CDC QC Lot 81	CDC QC Lot 82	CDC QC Lot 83	Cutoff
MEAN	0.109	2.072	2.734	0.304
STD	0.053	0.955	0.866	0.031
MINIMUM	0.023	0.445	0.807	0.210
MAXIMUM	0.225	4.000	4.000	0.352
	Recombinant HIV 1/2, Russia			
	CDC QC Lot 81	CDC QC Lot 82	CDC QC Lot 83	Cutoff
MEAN	0.112	1.334	2.358	0.245
STD	0.076	0.892	0.956	0.035
MINIMUM	0.010	0.411	0.906	0.208
MAXIMUM	**0.281**	**2.752**	**3.875**	0.296
	CombiBest Anti-HIV 1,2 DCM, Russia			
	CDC QC Lot 81	CDC QC Lot 82	CDC QC Lot 83	Cutoff
MEAN	0.170	1.568	2.620	0.219
STD	0.060	1.087	1.231	0.048
MINIMUM	0.039	0.488	0.750	0.151
MAXIMUM	**0.264**	**3.532**	**4.000**	0.270
	Anti-HIV Unif, Russia			
	CDC QC Lot 81	CDC QC Lot 82	CDC QC Lot 83	Cutoff
MEAN	0.116	1.526	2.377	0.222
STD	0.057	0.727	0.872	0.023
MINIMUM	0.003	0.499	0.791	0.202
MAXIMUM	0.244	3.289	4.000	0.264
	UniBest HIV (33)			
	CDC QC Lot 81	CDC QC Lot 82	CDC QC Lot 83	Cutoff
MEAN	0.081	1.645	2.583	0.182
STD	0.041	0.810	0.615	0.022
MINIMUM	0.023	0.655	1.390	0.156
MAXIMUM	0.214	3.622	3.797	0.217
	Genscreen Ultra AG/AB			
	Negative Lot 81	Low Positive Lot 82	High Positive Lot 83	Cutoff
MEAN	0.142	1.936	2.872	0.286
STD	0.055	0.695	0.593	0.029
MINIMUM	0.074	0.696	1.452	0.229
MAXIMUM	0.299	3.087	3.820	0.323

Figure 6 shows the mean of the CDC QC materials for each most commonly reported methods during 2009. The mean cutoff of all methods, represented by the red line, clearly shows that our QC materials perform as expected with all methods reported by participants.



The following charts (Figures 7-15) show the individual method performance over four quarters of PT data in 2009. Each point represents a participant's reported value.

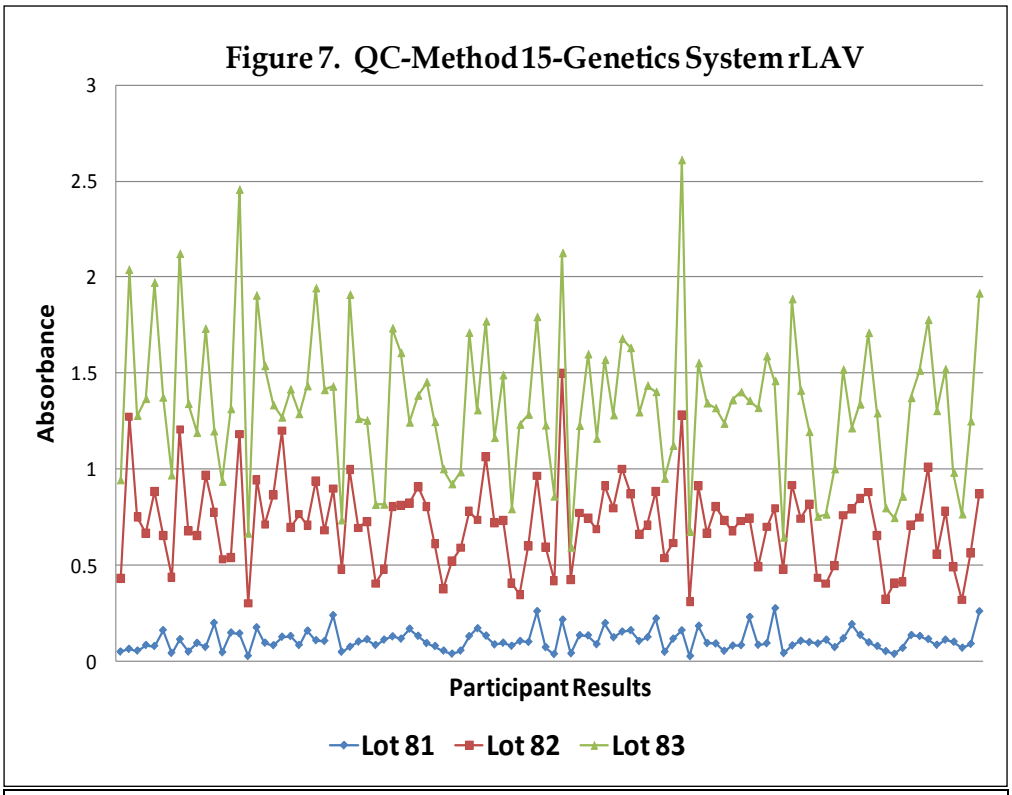


Figure 7. QC-Method 15-Genetics System rLAV
Absorbance
3
2.5
2
1.5
1
0.5
0
Participant Results
Lot 81
Lot 82
Lot 83

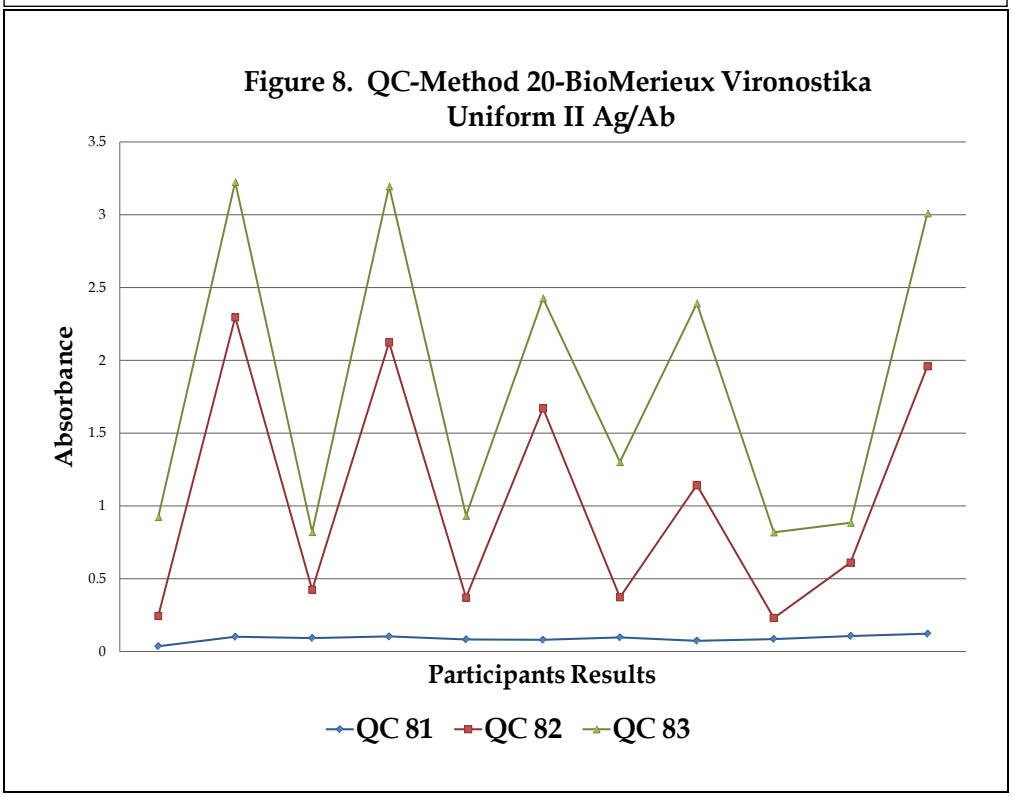


Figure 8. QC-Method 20-BioMerieux Vironostika Uniform II Ag/Ab
Absorbance
3.5
3
2.5
2
1.5
1
0.5
0
Participants Results
QC 81
QC 82
QC 83

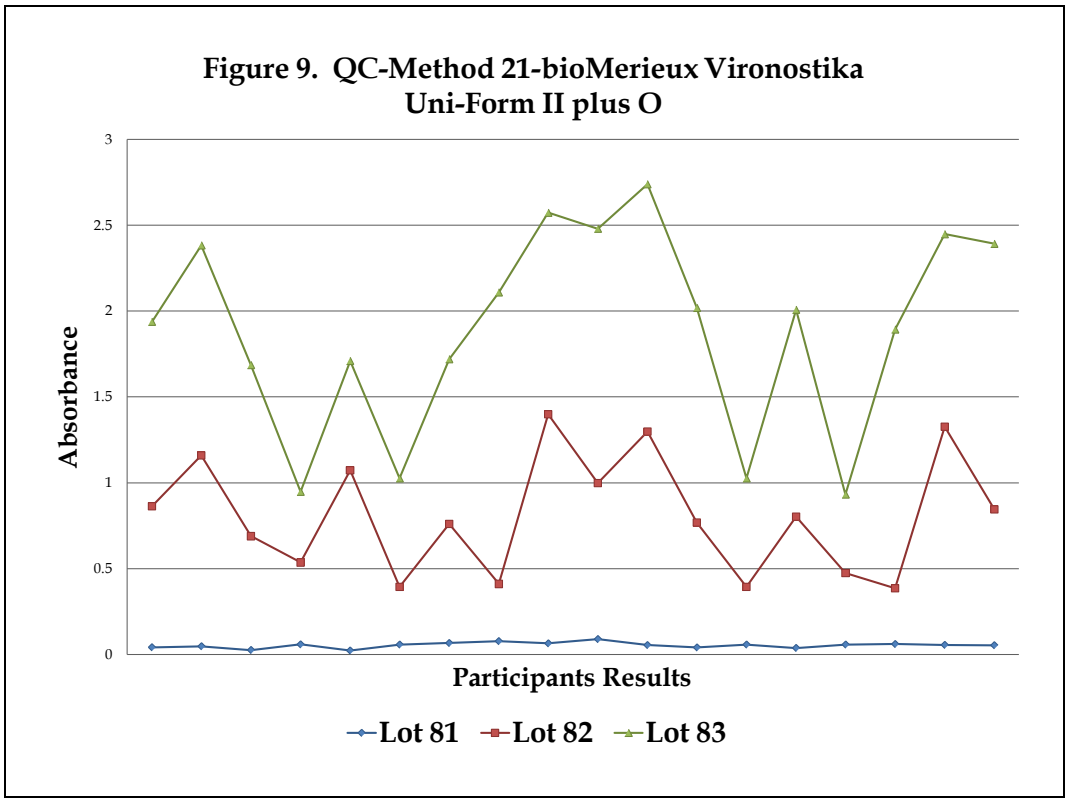


Figure 9. QC-Method 21-bioMerieux Vironostika Uni-Form II plus O
Absorbance
3
2.5
2
1.5
1
0.5
0
Participants Results
Lot 81
Lot 82
Lot 83

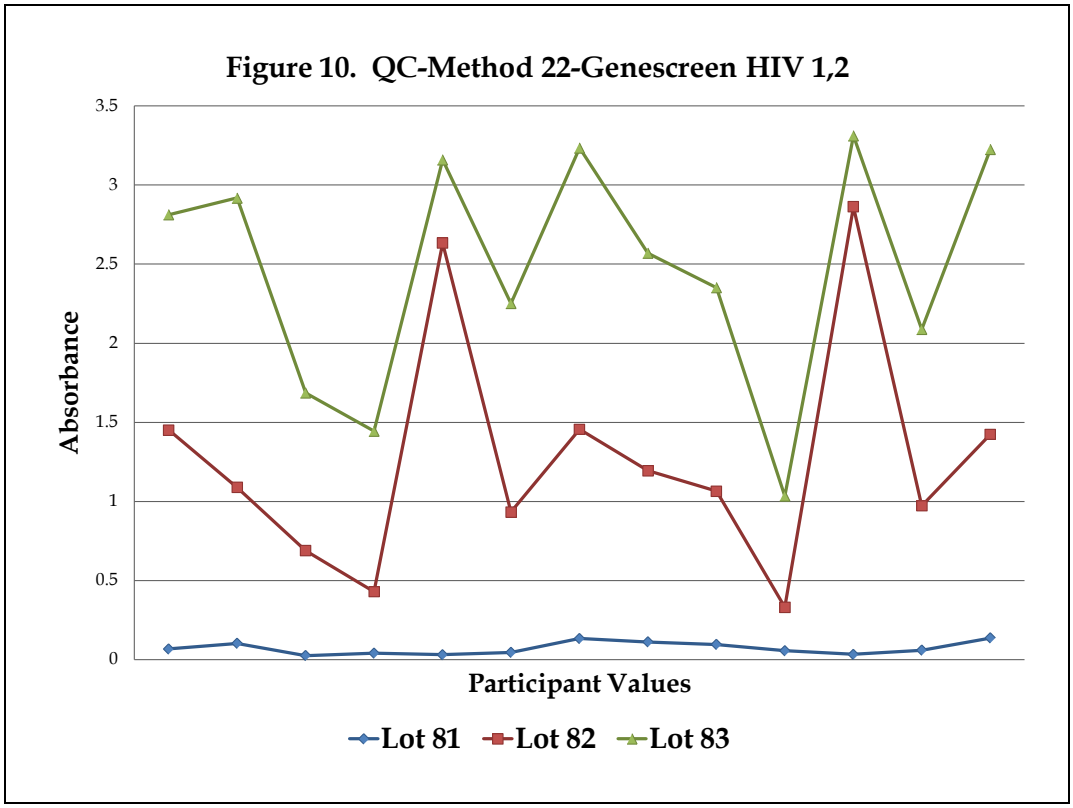


Figure 10. QC-Method 22-Genescreen HIV 1,2
Absorbance
3.5
3
2.5
2
1.5
1
0.5
0
Participant Values
Lot 81
Lot 82
Lot 83

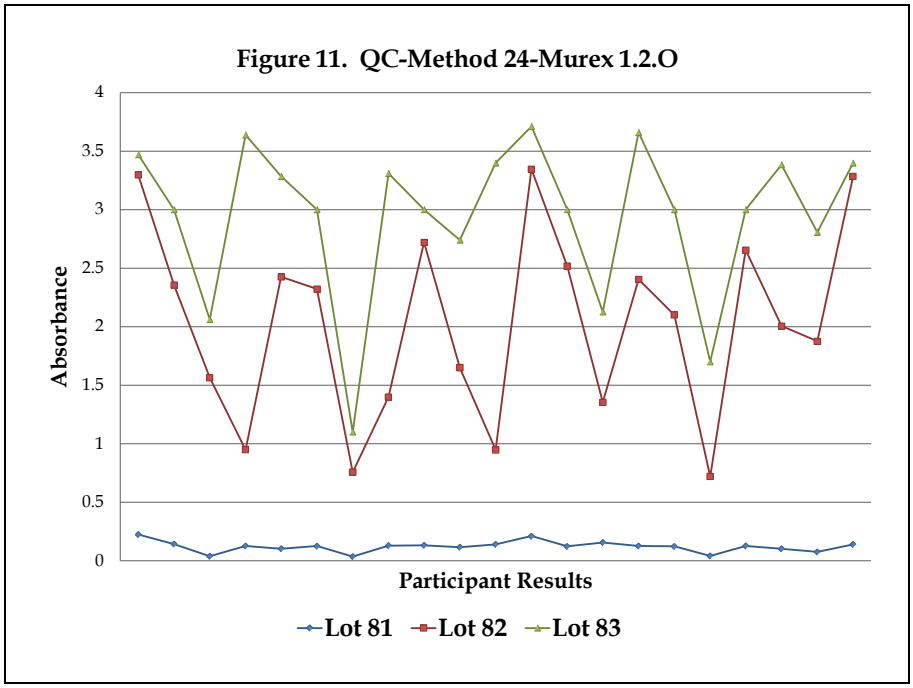


Figure 11. QC-Method 24-Murex 1.2.O
Absorbance
4
3.5
3
2.5
2
1.5
1
0.5
0
Participant Results
Lot 81
Lot 82
Lot 83

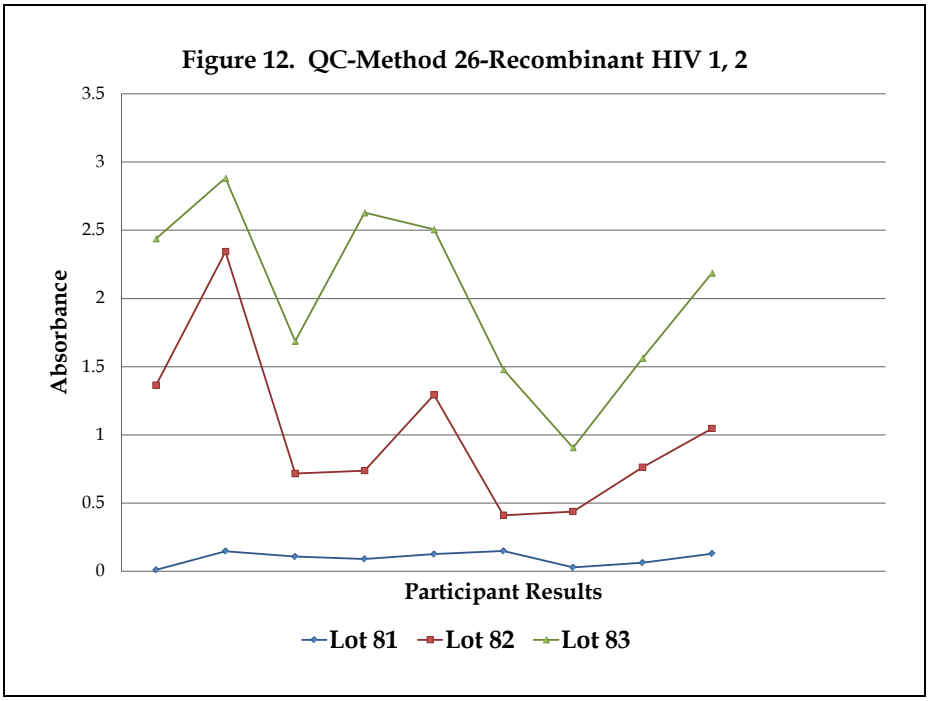


Figure 12. QC-Method 26-Recombinant HIV 1, 2
Absorbance
3.5
3
2.5
2
1.5
1
0.5
0
Participant Results
Lot 81
Lot 82
Lot 83

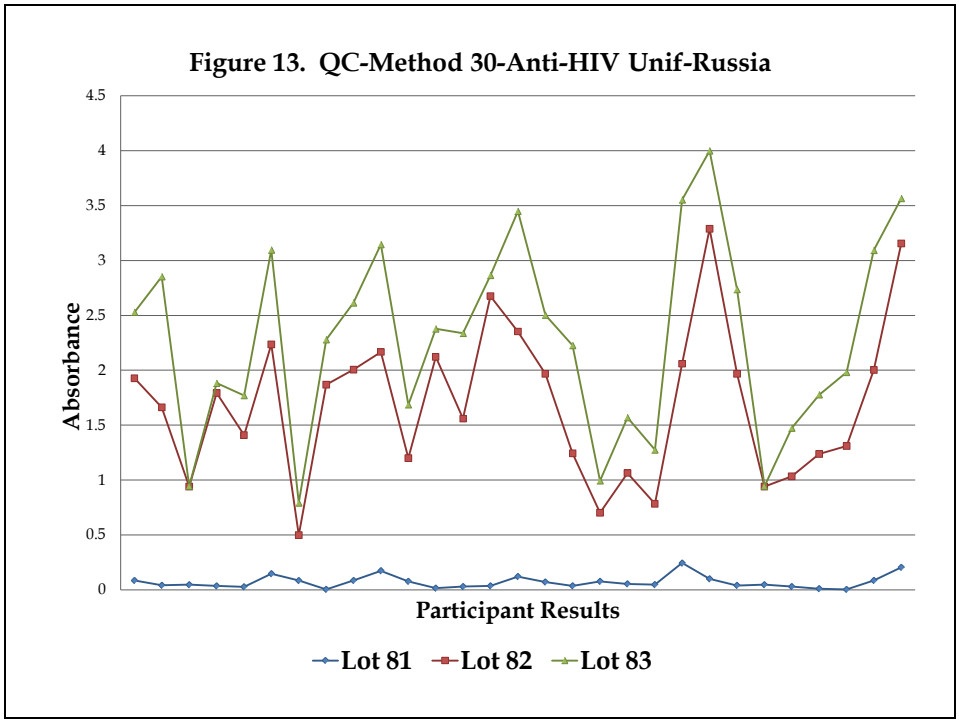


Figure 13. QC-Method 30-Anti-HIV Unif-Russia
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Absorbance
Participant Results
Lot 81
Lot 82
Lot 83

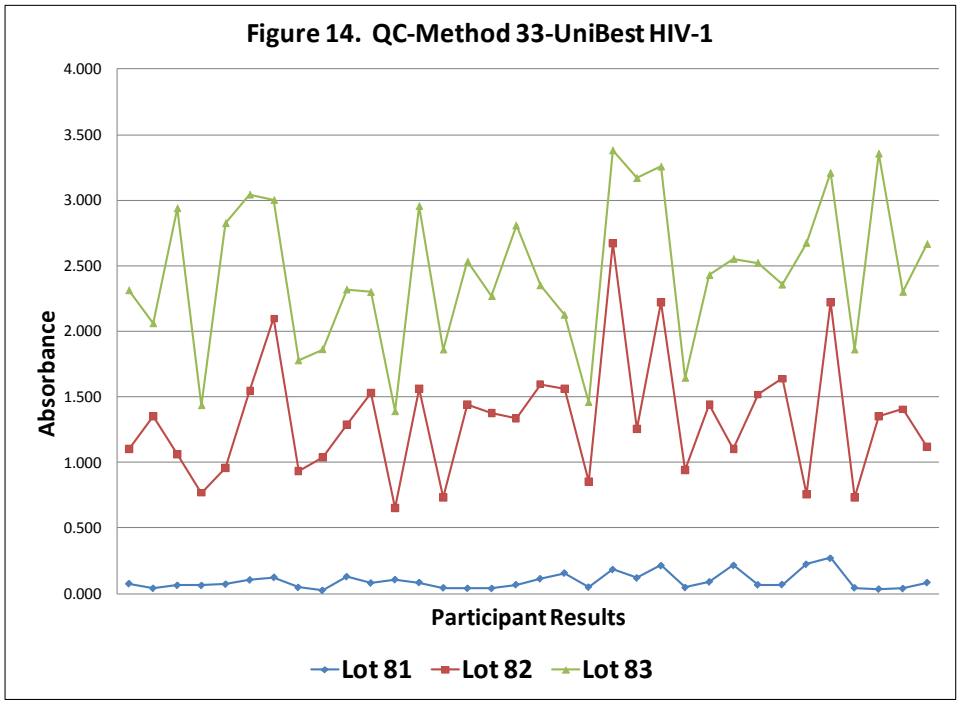
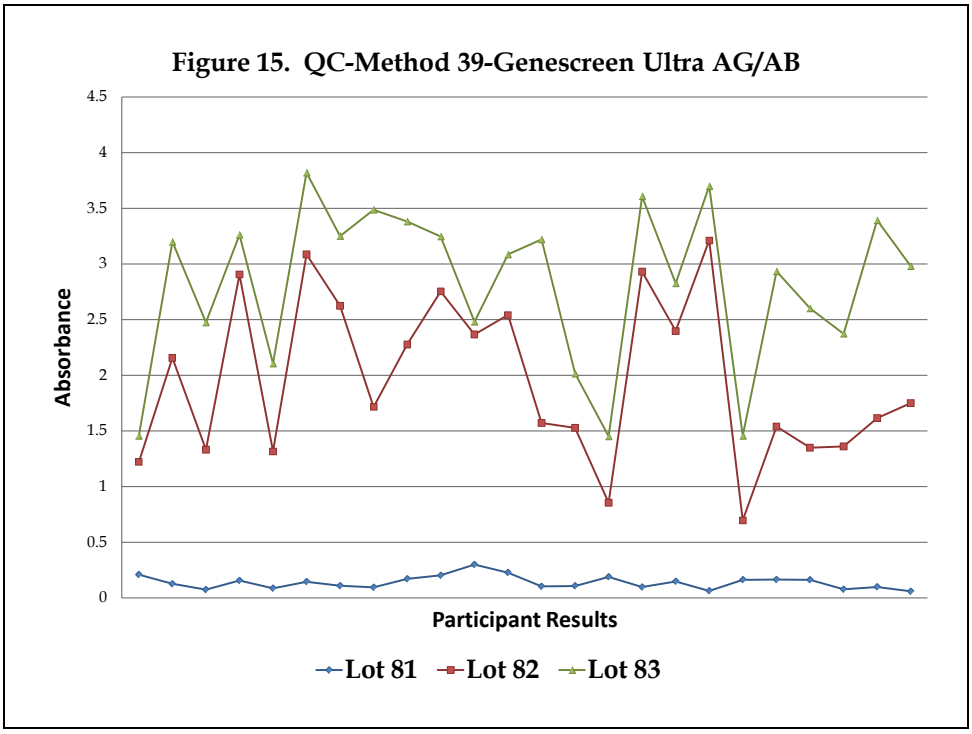


Figure 14. QC-Method 33-UniBest HIV-1
4.000
3.500
3.000
2.500
2.000
1.500
1.000
0.500
0.000
Absorbance
Participant Results
Lot 81
Lot 82
Lot 83



DISCUSSION

NSQAP provides unique materials for laboratories testing DBS for HIV antibodies.
The materials provide a level of confidence for testing methods that do not provide DBS QC materials. 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. Materials can also be used for method evaluation and validation.
Results from laboratories using the same method can vary widely. Despite the within-method variability, the majority of laboratories correctly identified PT specimens as non-reactive or HIV- reactive (Table 1). Testing algorithms for DBS also vary widely, with most labs using a combination of EIA and WB testing as for their algorithm (Table 2). In 2009, one HIV enzyme immunoassay and one Western Blot method had protocols specifically for dried blood spots. These products are primarily available in North America. Laboratories outside of North America have adapted serum-based methods to the filter paper dried blood spot matrix (Tables 3 and 4) with good results.

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