

# Newborn Screening Quality Assurance Program

2010 HIV ANNUAL SUMMARY REPORT

March 2011

## 2010 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<sup>1</sup>. 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<sup>2</sup>, their collection and use for HIV antibody testing has been adapted in many areas around the world. Over the span of 2010, NSQAP had as many as 80 participants in the Anti-HIV-1 program, 15 domestic laboratories and 65 foreign laboratories. Due to funding constraints in October 2010, we no longer support or serve the African and Central Asian HIV laboratories. Those laboratories were offered an opportunity to continue as participants in a serum-based proficiency testing program administered through the CDC Global Aids Program. Therefore, as of January 1, 2011, there are only 24 laboratories participating 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 manufactured specimens. An approved national standard exists for the collection of DBSs and the handling of the



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Table 1. Summary of HIV-1 PT Errors by Domestic and Foreign Laboratories in 2010.

	Positive Specimens Assayed (N)	False-Negative Errors (%)	Negative Specimens Assayed (N)	False-Positive Errors (%)
<b>Domestic Laboratories</b>	94	2.1%	101	0.0%
<b>Foreign Laboratories</b>	380	1.6%	345	1.4%

filter paper matrix.<sup>3</sup> DBS QC and PT specimens are certified for homogeneity, reproducibility, stability, and suitability for performance in enzyme immunoassay (EIA) and WB 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  $50\% \pm 1\%$ . 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.<sup>4-6</sup> 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  $-20^{\circ}\text{C}$  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.

Table 2. HIV Testing Algorithms for DBS in Quarter 2, 2010.

Testing combination	Number of labs reporting
EIA/WB	16
EIA Only	15
Agglutination/WB	1
Agglutination Only	1
EIA/EIA	33
EIA/EIA/WB	8
EIA/Agglutination/WB	1

## 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 2010 are summarized in Table 1. Over the year foreign laboratories reported six false negative and five false positive results. The domestic laboratories reported two false negative and no false positive results.

### Proficiency Testing

Laboratories participating in PT 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. The Quarter 2, 2010 data were used for to create Table 2 because it had the largest number of laboratories reporting results for the quarter.

### PT Material and Laboratory Performance

Tables 3 and 4 list the names of the screening and confirmatory methods used by participants in 2010 for the PT quarterly events. In 2010, there were two kits that were FDA approved and carried a protocol for DBS: Genetic Systems rLAV EIA (BioRad), and Avioq HIV-1 microelisa System (Avioq Inc., Research Triangle Park, NC). Table 3 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.

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

Table 3. Primary and Secondary Screening Methods Used by Participants in 2010.

Method	Code and Kit Source	# Participants
10	Fujirebio Serodia-HIV 1,2	2
11	In House	2
12	Other	7
15	<b>FDA Licensed for DBS</b> - Genetic Systems rLAV EIA (Bio-Rad)	10
20	bioMerieux Vironostika UniForm II Ag/AB	5
21	bioMerieux Vironostika Uni-Form II plus O	6
22	Genescreen HIV 1/2 V2	3
23	Genescreen Plus HIV Ag/Ab (BioRad)	2
24	Murex HIV 1.2.0 (Abbott)	22
25	Murex HIV Ag/Ab Combination (Abbott)	1
26	Recombinant HIV 1/2, Russia	6
27	Tecnosuma (Cuba) UMELISA HIV 1+2	3
30	Anti-HIV Unif, Russia	9
31	Dade Behring Enzygnost Anti-HIV 1/2 Plus O	1
33	UniBest HIV 1,2 AB, Russia	15
34	Q-Preven HIV 1+2, DBS, Brazil	1
39	Genescreen Ultra HIV AG-AB (BioRad)	16
40	<b>FDA Licensed for DBS</b> -Avioq HIV-1 Microelisa Systems	12
41	Bio-Rad HIV-1/2 plus O EIA	2

Table 4. Confirmatory WB Methods Used By Participants 2010.

Method	Code and Kit Source	# Participants
16	<b>FDA Licensed for DBS</b> - Genetic Systems HIV-1 WB (Bio-Rad)	12
32	Cambridge Biotech HIV-1 WB Kit (Maxim)	4
35	OraSure HIV-1 WB Kit	1
36	New LAV Blot I (Bio-Rad)	5
37	Genelab Diagnostics HIV 2.2 WB	2
42	MP Diagnostics HIV Blot 2.2	2
12	Other	1

values between specimens, however, a large amount of variability in absorbance is often seen within methods. Reproducibility of specimen A (reactive) EIA methods between quarters is illustrated in Figure 1 and the reproducibility of specimen B (non-reactive) EIA methods is shown in Figure 2.

#### WB Method Performance with PT materials

In 2010, Specimen A (reactive) was distributed in quarter 2 and quarter 3. A summary of reported protein molecular weight bands for three methods is shown in Figures 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.

#### QC Material Performance – EIA

Tables 5a and 5b illustrate the overall performance of CDC QC materials used with each EIA method for lots series 81-83 and 91-91, respectively. In-house and Other methods 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. Each laboratory must establish their own mean and acceptable range limits based on their method of choice.

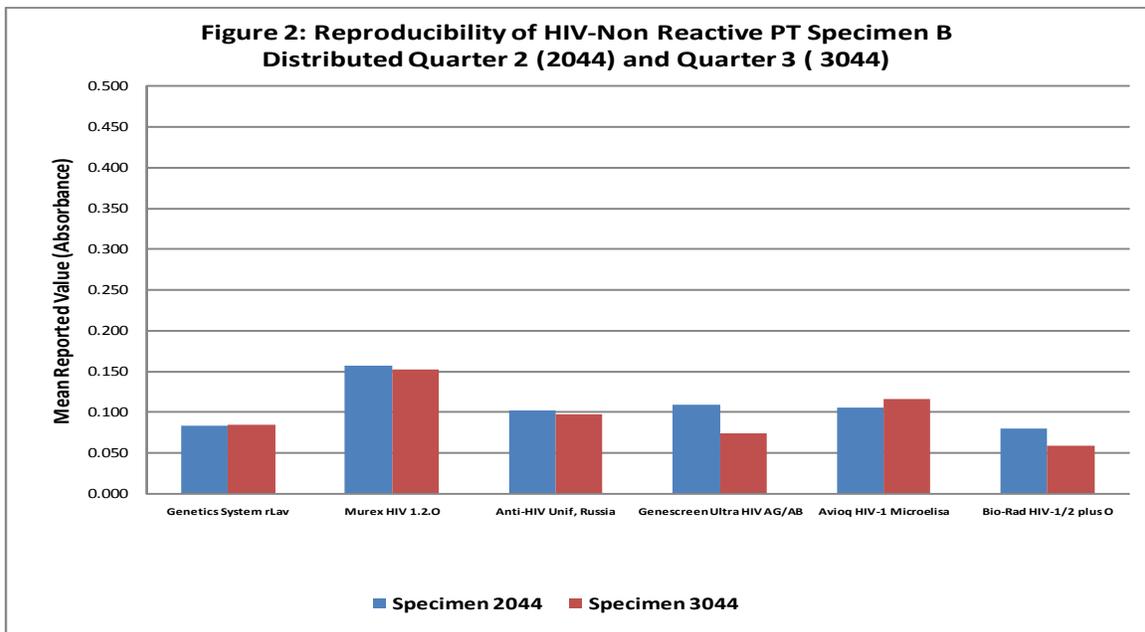
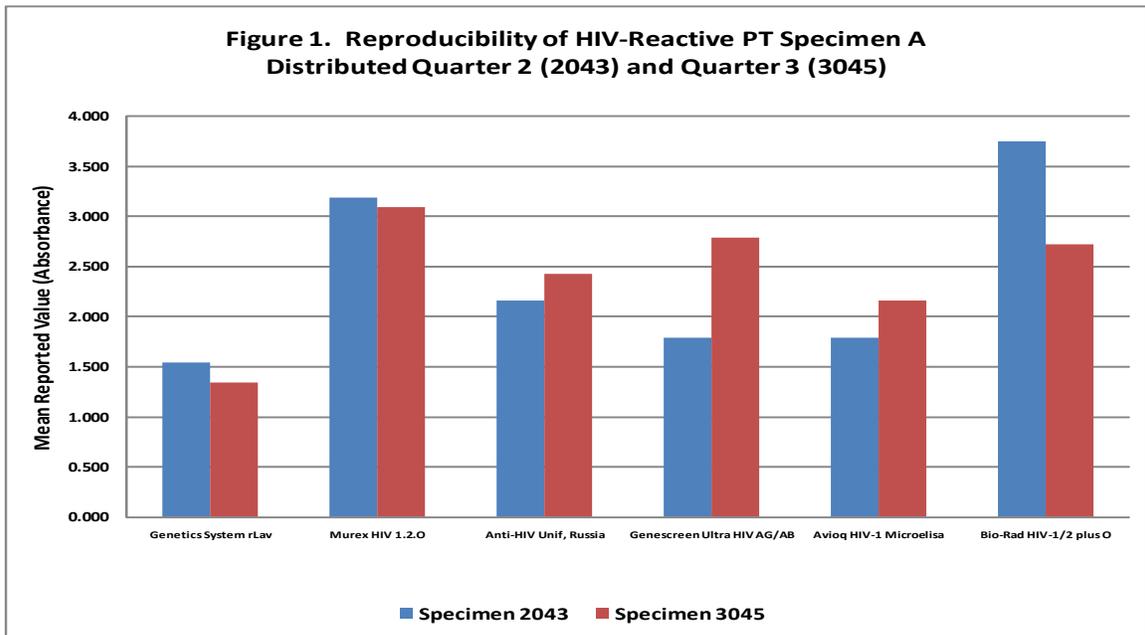
Figure 5 shows the mean of the CDC QC Lots 81-83 materials for each of the most commonly reported EIA methods during quarters 1 and 2 of 2010. Figure 6 shows CDC QC Lots 91-93 for quarters 3 and 4 of 2010. The

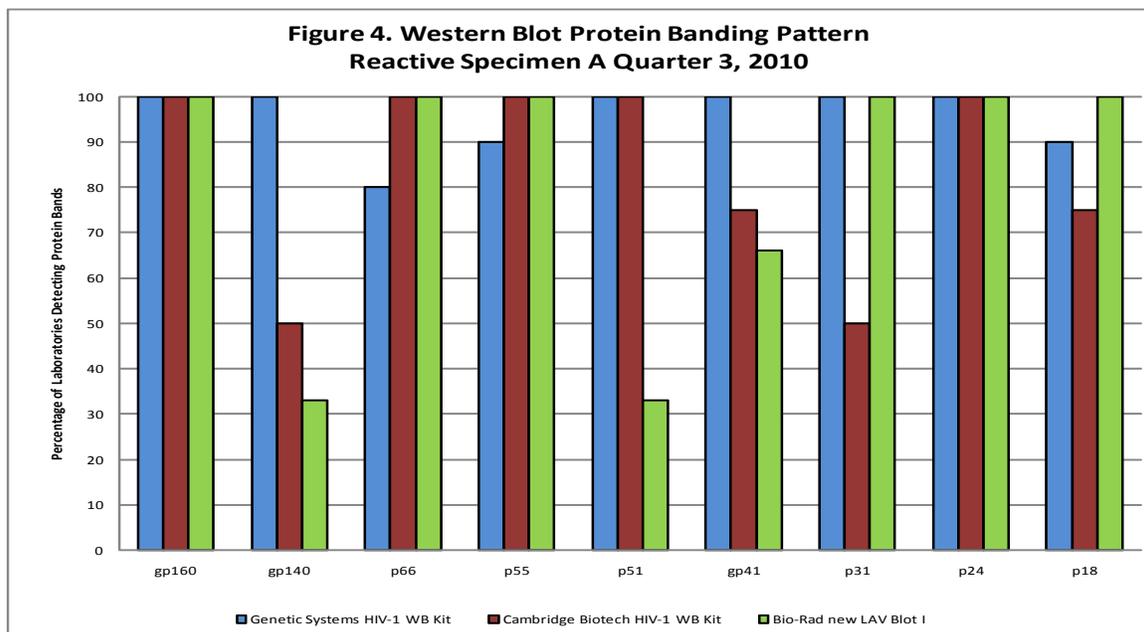
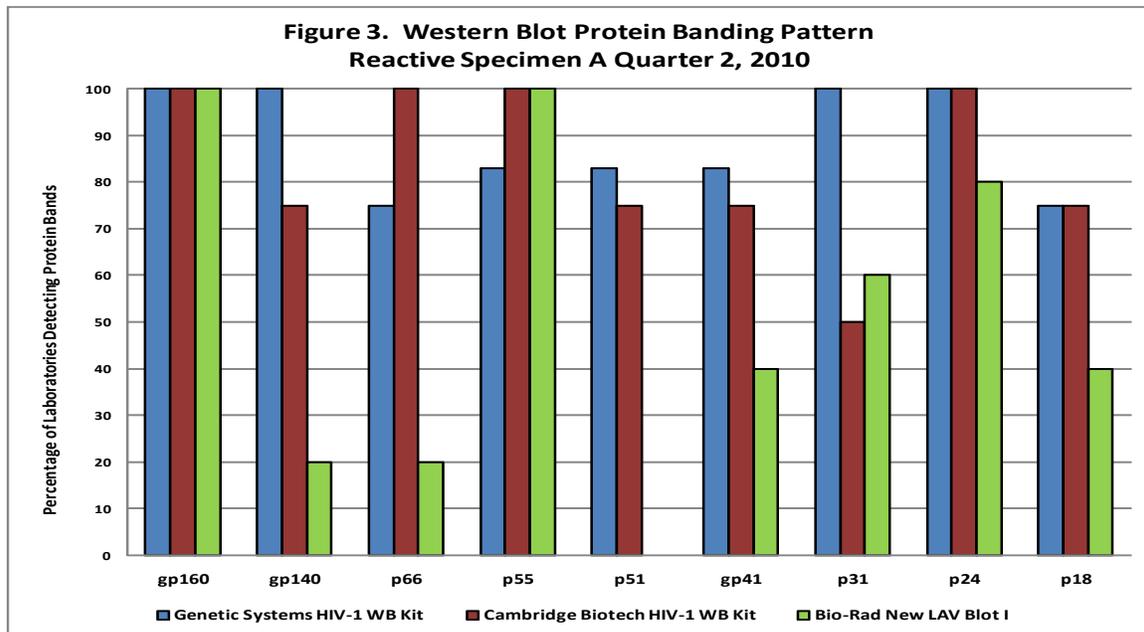
mean cutoff of all methods, represented by the dotted line, clearly shows that our QC materials perform as expected with all methods reported by participants.

#### 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 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. 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 for their algorithm (Table 2). In 2010, two HIV EIA and one WB method had protocols specifically for DBS. These products are primarily available in North America. Laboratories outside of North America have adapted serum-based methods to the filter paper DBS matrix (Tables 3 and 4) with good results.





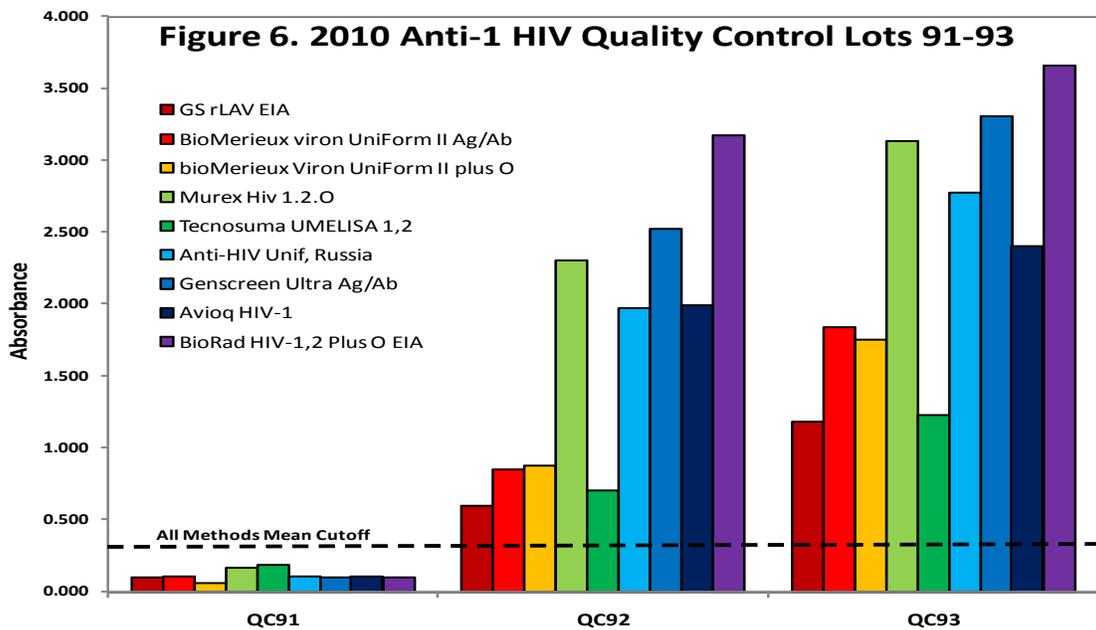
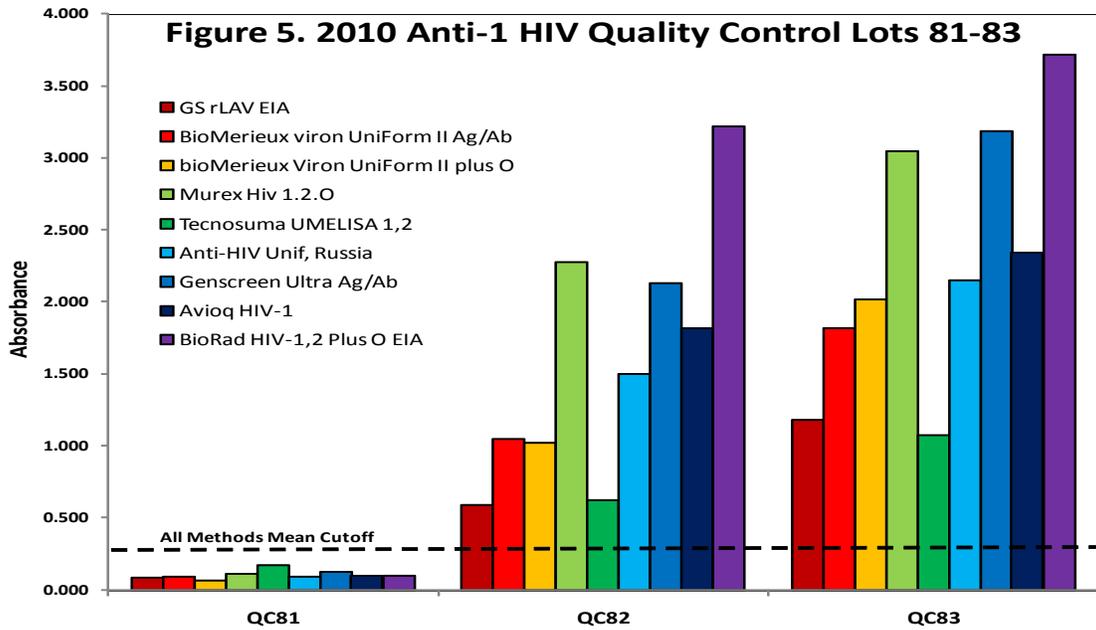


Table 5a. Statistics for Lots 81-83 per kit.

<b>2010 Anti-HIV-1 Quality Control Lots 81-83</b>				
<b>Genetics System rLav</b>				
	<b>CDC QC Lot 81</b>	<b>CDC QC Lot 82</b>	<b>CDC QC Lot 83</b>	<b>Cutoff</b>
<b>MEAN</b>	0.084	0.591	1.180	0.272
<b>Min</b>	0.032	0.320	0.733	0.261
<b>Max</b>	0.196	0.954	1.730	0.286
<b>BioMerieux Vironostika Uni-Form II, AG/AB</b>				
	<b>CDC QC Lot 81</b>	<b>CDC QC Lot 82</b>	<b>CDC QC Lot 83</b>	<b>Cutoff</b>
<b>MEAN</b>	0.089	1.050	1.818	0.191
<b>Min</b>	0.042	0.314	0.892	0.164
<b>Max</b>	0.130	2.502	3.266	0.234
<b>BioMerieux Vironostika Uni-Form II plus O</b>				
	<b>CDC QC Lot 81</b>	<b>CDC QC Lot 82</b>	<b>CDC QC Lot 83</b>	<b>Cutoff</b>
<b>MEAN</b>	0.062	1.019	2.013	0.156
<b>Min</b>	0.009	0.151	0.496	0.135
<b>Max</b>	0.181	4.000	4.000	0.459
<b>Murex 1.2.O</b>				
	<b>CDC QC Lot 81</b>	<b>CDC QC Lot 82</b>	<b>CDC QC Lot 83</b>	<b>Cutoff</b>
<b>MEAN</b>	0.109	2.272	3.048	0.311
<b>Min</b>	0.005	0.669	1.394	0.205
<b>Max</b>	0.234	4.000	4.154	0.446
<b>Tecnosuma UMELISA 1,2</b>				
	<b>CDC QC Lot 81</b>	<b>CDC QC Lot 82</b>	<b>CDC QC Lot 83</b>	<b>Cutoff</b>
<b>MEAN</b>	0.170	0.623	1.070	0.345
<b>Min</b>	0.109	0.356	0.583	0.202
<b>Max</b>	0.261	0.879	1.720	0.459
<b>Anti-HIV Unif, Russia</b>				
	<b>CDC QC Lot 81</b>	<b>CDC QC Lot 82</b>	<b>CDC QC Lot 83</b>	<b>Cutoff</b>
<b>MEAN</b>	0.091	1.496	2.146	0.242
<b>Min</b>	0.004	0.387	1.047	0.202
<b>Max</b>	0.267	3.140	3.200	0.329
<b>Genscreen Ultra AG/AB</b>				
	<b>CDC QC Lot 81</b>	<b>CDC QC Lot 82</b>	<b>CDC QC Lot 83</b>	<b>Cutoff</b>
<b>MEAN</b>	0.123	2.129	3.186	0.280
<b>Min</b>	0.059	0.700	1.742	0.247
<b>Max</b>	0.237	3.668	4.000	0.369
<b>Avioq HIV-1</b>				
	<b>CDC QC Lot 81</b>	<b>CDC QC Lot 82</b>	<b>CDC QC Lot 83</b>	<b>Cutoff</b>
<b>MEAN</b>	0.097	1.816	2.341	0.373
<b>Min</b>	0.072	1.426	2.056	0.349
<b>Max</b>	0.140	2.546	3.000	0.417
<b>BioRad HIV-1,2 Plus O EIA</b>				
	<b>CDC QC Lot 81</b>	<b>CDC QC Lot 82</b>	<b>CDC QC Lot 83</b>	<b>Cutoff</b>
<b>MEAN</b>	0.094	3.216	3.718	0.300
<b>Min</b>	0.065	1.710	3.245	0.289
<b>Max</b>	0.135	4.000	4.000	0.318

Table 5b. Statistics for Lots 91-93 per kit.

<b>2010 Anti-HIV-1 Quality Control Lots 91-93</b>				
<b>Genetics System rLav</b>				
	<b>CDC QC Lot 91</b>	<b>CDC QC Lot 92</b>	<b>CDC QC Lot 93</b>	<b>Cutoff</b>
<b>MEAN</b>	0.099	0.595	1.181	0.269
<b>Min</b>	0.026	0.305	0.632	0.262
<b>Max</b>	0.163	0.952	1.717	0.283
<b>BioMerieux Vironostika Uni-Form II, AG/AB</b>				
	<b>CDC QC Lot 91</b>	<b>CDC QC Lot 92</b>	<b>CDC QC Lot 93</b>	<b>Cutoff</b>
<b>MEAN</b>	0.105	0.850	1.837	0.222
<b>Min</b>	0.064	0.430	1.232	0.189
<b>Max</b>	0.134	1.935	3.061	0.270
<b>BioMerieux Vironostika Uni-Form II plus O</b>				
	<b>CDC QC Lot 91</b>	<b>CDC QC Lot 92</b>	<b>CDC QC Lot 93</b>	<b>Cutoff</b>
<b>MEAN</b>	0.059	0.878	1.753	0.167
<b>Min</b>	0.014	0.180	0.495	0.133
<b>Max</b>	0.082	1.447	2.437	0.272
<b>Murex 1.2.O</b>				
	<b>CDC QC Lot 91</b>	<b>CDC QC Lot 92</b>	<b>CDC QC Lot 93</b>	<b>Cutoff</b>
<b>MEAN</b>	0.167	2.300	3.132	0.352
<b>Min</b>	0.073	1.273	2.096	0.278
<b>Max</b>	0.323	3.509	3.768	0.403
<b>Tecnosuma UMELISA 1,2</b>				
	<b>CDC QC Lot 91</b>	<b>CDC QC Lot 92</b>	<b>CDC QC Lot 93</b>	<b>Cutoff</b>
<b>MEAN</b>	0.181	0.702	1.228	0.332
<b>Min</b>	0.101	0.386	0.647	0.154
<b>Max</b>	0.240	1.114	1.970	0.426
<b>Anti-HIV Unif, Russia</b>				
	<b>CDC QC Lot 91</b>	<b>CDC QC Lot 92</b>	<b>CDC QC Lot 93</b>	<b>Cutoff</b>
<b>MEAN</b>	0.101	1.968	2.773	0.231
<b>Min</b>	0.035	0.516	2.074	0.154
<b>Max</b>	0.158	2.894	4.000	0.277
<b>Genscreen Ultra AG/AB</b>				
	<b>CDC QC Lot 91</b>	<b>CDC QC Lot 92</b>	<b>CDC QC Lot 93</b>	<b>Cutoff</b>
<b>MEAN</b>	0.099	2.524	3.304	0.269
<b>Min</b>	0.035	1.503	2.426	0.237
<b>Max</b>	0.165	3.878	3.867	0.366
<b>Avioq HIV-1</b>				
	<b>CDC QC Lot 91</b>	<b>CDC QC Lot 92</b>	<b>CDC QC Lot 93</b>	<b>Cutoff</b>
<b>MEAN</b>	0.108	1.994	2.405	0.379
<b>Min</b>	0.073	1.295	2.074	0.291
<b>Max</b>	0.184	3.000	3.000	0.495
<b>BioRad HIV-1,2 Plus O EIA</b>				
	<b>CDC QC Lot 91</b>	<b>CDC QC Lot 92</b>	<b>CDC QC Lot 93</b>	<b>Cutoff</b>
<b>MEAN</b>	0.101	3.171	3.654	0.282
<b>Min</b>	0.064	1.450	2.924	0.272
<b>Max</b>	0.130	4.000	4.000	0.288

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