Newborn Screening Quality Assurance Program

Summary Report: Titration Curve for HIV Anti-HIV-1 in Dried-Blood Spots

Panel Distribution Date: November 15, 2005 May 2006

INTRODUCTION

During the fourth quarter of 2005, we distributed a panel of HIV antibody-positive dried-blood spot (DBS) specimens representing a titration (dose-response) curve. The panel was made up of 16 blind-coded specimens and included one each of negative, low-positive, and high-positive HIV quality control (QC) materials. From time to time, we have distributed titration panels to assess method performance. Testing the panel was voluntary and was not part of our quarterly Proficiency Testing (PT) program.

We distributed panels to 51 laboratories and received data reports from 34 participants (67%). Participants were evaluated solely on whether they correctly classified the HIV reactivity of the blind-coded QC materials and not on the titration panel. Participants conducting primary screening analysis reported 7 misclassifications of QC materials, those conducting secondary screening reported 3 misclassifications, and those conducting confirmatory analysis reported 3 misclassifications for a total of 13 errors (544 specimens tested) or a 2.4% error rate. Insight into laboratory performance can be gained by observing the ability of participants to correctly identify the blind-coded QC materials. Of the 10 screening misclassifications reported for both primary and secondary screening methods, 8 were false-negative results, where participants failed to identify as reactive either the low-positive or high-positive QC specimen(s). The other two were false-positive misclassifications, where the negative QC specimen was falsely identified as reactive. The confirmatory testing errors consisted of 2 false-positive misclassifications and 1 false-negative misclassification.

The titration panel consisted of 13 individual DBS pools that were prepared from serially diluted HIV antibody-positive human serum (heat inactivated). HIV-negative serum from a single donor was used as the diluent for each pool. Individual serum pools were mixed with washed red blood cells to reach a 50% hematocrit; the whole blood was spotted on Grade 903 filter paper (Whatman, Inc., Florham Park, NJ) and dried overnight. Table 1 provides the CDC assayed values for 2 enzyme immunoassay methods (EIA) and one Western Blot (WB) method. For all tables, the specimens are listed in the order from lowest to highest dilution. In Part 1 of the report, Table 2 shows the number of laboratories using each screening method/kit; Table 3 shows the expected results for the titration curve, as compared to the CDC assayed values, and the number of laboratories reporting reactive and non-reactive results; and Table 4 summarizes EIA absorbance ranges, means, and methods of analysis for four kits. Single EIA methods are grouped under "Other." Figure 1 shows the mean Optical Density (OD) for each DBS dilution plotted by method. A mean cutoff value (0.313) was calculated for all EIA primary screening methods and the line was added to the plot. Table 5 gives the summary of participant results for four primary EIA screening methods and the "Other" category.

In Part 2 of the report, Table 6 shows the number of laboratories using each confirmatory method/kit. Nineteen participants reported confirmatory results. Table 7 shows the expected WB results and the number of laboratories reporting reactive, non-reactive, or indeterminate results. Figure 2 shows the comparison of WB methods plotted by the percent of responses vs. the DBS titration curve for select dilutions. Because of space limitations and complexity, every other dilution in the 1:25 to 1:4000 series was plotted. Not all participants tested all HIV antibody-positive specimens by WB. Confirmatory testing was dependent on the sensitivity of the screening method used.
In Part 3 of the report, Table 8 shows the summary sta-

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tistics from the primary HIV EIA screening methods for the CDC DBS QC materials that were blinded and randomized into the 16-member panel. The data collected for the routine QC (unblinded) is also given for comparison, along with mean cutoff values and cutoff ranges for each method.

RESULTS AND DISCUSSION

HIV DBS EIA screening methods have varying sensitivities in their ability to detect total HIV antibodies from increasingly dilute DBS specimens. Two HIV screening EIAs have U.S. FDA-approved procedures for DBS: Genetic Systems rLAV EIA (Bio-Rad, Redmond, WA) and Vironostika HIV-1 Microelisa System (bioMériuex, Durham, NC). All other EIAs are serum/plasma tests that have been adapted or modified for the dried whole blood, filter paper matrix.
All but one method correctly identified the specimens as reactive up to a dilution of 1:200 (Tables 3 and 5). Assay sensitivity began to drop off for some methods at a dilution of 1:300. At the 1:2000 dilution, half of the participants identified the specimen as reactive and half identified it as non-reactive. Twenty six percent of participants identified the highest dilution, 1:4000, as reactive. Figure 1 shows the mean OD values from Table 4 plotted by method for the dilution series. All methods had sigmoidal-shaped curves. Method performance is also illustrated in Table 5, where the demarcation of reactivity for each method is shown. The bioMérieux Uni-Form II plus O EIA had the highest proportion of participants (50%) identifying specimen H-13 (1:4000) as reactive.

For HIV WB methods, the only kit with an approved protocol for DBS is the Genetic Systems HIV-1 WB (Bio-Rad, Redmond, WA). Nineteen participants reported WB results (Table 6). Some bands began to disappear at the 1:100 dilution, but enough bands were retained for the DBS specimens to be considered HIV reactive (Figure 2). As specimen dilution increased, the number of indeterminate and "not tested" results increased (Table 7). Five of 19 participants identified specimen H-13 (1:4000) as reactive or indeterminant.

The purpose of this exercise was to evaluate HIV DBS method performance and individual laboratory performance against a set of increasingly dilute HIV antibody- positive specimens. Laboratory performance was illustrated by the ability of participants to correctly identify the blinded negative, low-positive, and high-positive QC specimens. As part of our routine quality assurance program, we distribute these QC materials to participants two times per year. 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. Participants are familiar with the reactivities of these specimens. However, we observed 8 misclassification errors for the QC materials (Table 8) for primary screening methods. All errors except one were made by international laboratories. Misclassifications of the QC materials indicate laboratory errors and not necessarily method failures. Laboratory errors may be due to specimen mix-up, transcription errors, and low-quantitative values.

The NSQAP is designed to help screening laboratories achieve excellent technical proficiency and maintain confidence in their performance. We continually strive to produce certified DBS materials for HIV antibody testing and QC analysis. Our goal is to improve the quality of DBS HIV antibody testing for laboratories worldwide. Expanded challenges such as this titration panel of randomized, blinded specimens provide insight into method performance and the ability of laboratories to correctly identify blinded specimens. We will provide additional trials to challenge participants in the future.

DISCLAIMER

Use of trade names and commerical sources is for identification only and does not imply endorsement by the U.S. Department of Health and Human Services or the Assocation of Public Health Laboratories.

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Table 1. CDC assayed values for EIA and Western Blot (WB) methods grouped from lowest to highest serum dilution.

		Mean Absorbance (OD)		Western Blot (Genetic Systems HIV-1 WB)								
Specimen Number	HIV DBS Dilution	bioMérieux Vironostika HIV-1	Genetic Systems rLAV	gp160	gp120	p66	p55	p51	gpp41	p31	p24	p18
H-9	25	2.793	1.760	+	+	+	+	+	+	+	+	+
H-12	50	2.648	1.379	+	+	+	+	+	+	+	+	+
H-3	100	2.446	1.024	+	+	+	+	+	+	+	+	+
H-15	200	2.072	0.586	+	+	+	+	-	+	+	+	+
H-5	300	1.831	0.490	+	+	+	+	-	+	+	+	+
H-14	400	1.513	0.400	+	-	+	+	+	+	+	+	-
H-7	500	1.298	0.390	+	-	+	+	-	+	+	+	-
H-8	600	1.251	0.309	+	-	+	+	-	+	+	+	-
H-16	750	1.089	0.318	+	-	+	+	-	+	+	+	-
H-10	1000	1.182	0.231	+	-	+	+	-	+	+	+	-
H-4	1500	0.864	0.211	+	-	-	+	-	+	-	+	-
H-1	2000	0.655	0.192	+	-	-	+	-	+	+	+	-
H-13	4000	0.459	0.188	-	-	-	+	-	-	-	+	-
H-6	Negative QC	0.296	0.252	-	-	-	-	-	-	-	-	-
H-2	Low Positive QC	1.642	0.544	+	+	+	+	+	+	+	+	+
H-11	High Positive QC	2.382	1.315	+	+	+	+	+	+	+	+	+

PART 1. SCREENING RESULTS

Table 2.	Primary screening	Screening Methods
Kit Source	Total Participants	Total Participants
Genetic Systems rLAV EIA (Bio-Rad)	6	0
bioMérieux Vironostika HIV-l Microelisa System	9	0
bioMérieux Vironostika Uni-Form II *plus* 0	6	0
Fujirebio Serodia-HIV	1	0
Abbott Murex HIV 1+2 Gacelisa	1	0
Murex HIV 1.2.0	4	0
Tecnosuma (Cuba) UMELISA HIV 1+2	1	0
Other	6	1
In House	0	1
Total	34	2

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Summary Statistics

Table 3. Number of Labs Reporting Screening Results

Specimen Number	HIV Dilution	Expected Result	Reactive	Non-reactive	No Interpretation
H-9	25	Reactive	34	0	0
H-12	50	Reactive	34	0	0
H-3	100	Reactive	34	0	0
H-15	200	Reactive	33	1	0
H-5	300	Reactive	29	5	0
H-14	400	Reactive	29	5	0
H-7	500	Reactive	28	6	0
H-8	600	Reactive	27	7	0
H-16	750	Reactive	23	11	0
H-10	1000	Reactive	23	11	0
H-4	1500	Reactive	18	16	0
H-1	2000	Reactive	17	17	0
H-13	4000	Reactive or Non-Reactive	9	25	0
H-6	Negative QC	Non-reactive	1	32	0
H-2	Low Positive QC	Reactive	29	6	0
H-11	High Positive QC	Reactive	33	1	0

Table 4. Reported Ranges and Means (OD) by Method for the HIV DBS Titration Panel. Specimens are listed from lowest to highest serum dilution. Specimens H-6, H-2, and H-11 represent HIV-negative, low-positive, and high-positive QC materials, respectively.

	Genetic Systems rLAV		bioMérieux Vironostika HIV-1		bioMérieux Vironostika Uniform II *plus* O		Abbott Murex 1.2.0.		Other (EIA)	
Specimen Number	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
H-9	1.270-3.000	1.919	1.823-3.000	2.430	1.823-3.000	2.430	3.680-9.952	5.383	1.117-3.879	2.765
H-12	0.686-2.165	1.400	1.464-3.000	2.370	1.464-3.000	2.370	3.900-9.955	5.480	0.884-3.353	2.158
H-3	0.597-1.402	1.052	1.204-3.000	2.325	1.220-3.313	2.442	2.879-9.958	5.434	0.673-2.434	1.475
H-15	0.317-0.767	0.563	0.391-2.430	1.753	0.391-2.430	1.753	2.498-3.898	3.334	0.353-1.482	0.806
H-5	0.209-0.574	0.438	0.668-2.517	1.663	0.668-2.517	1.663	1.822-3.739	2.830	0.138-1.281	0.598
H-14	0.144-0.472	0.340	0.643-2.333	1.383	0.643-2.333	1.383	1.676-3.754	2.414	0.249-0.799	0.478
H-7	0.120-0.422	0.301	0.668-2.023	1.191	0.668-2.023	1.191	1.345-3.662	2.117	0.198-0.683	0.383
H-8	0.132-0.437	0.279	0.435-2.010	1.114	0.435-2.010	1.114	1.068-3.458	1.878	0.161-0.547	0.325
H-16	0.074-0.313	0.211	0.470-1.616	1.071	0.136-2.021	0.729	0.972-3.384	1.825	0.134-0.423	0.270
H-10	0.066-0.287	0.198	0.393-1.298	0.842	0.393-1.298	0.824	0.794-3.043	1.606	0.117-0.341	0.239
H-4	0.061-0.226	0.165	0.379-1.109	0.677	0.379-1.109	0.678	0.174-2.387	1.053	0.085-1.478	0.333
H-1	0.052-0.246	0.157	0.335-0.785	0.532	0.089-0.583	0.299	0.144-1.943	0.876	0.135-0.222	0.176
H-13	0.044-0.190	0.117	0.195-0.575	0.373	0.195-0.575	0.373	0.132-0.985	0.447	0.045-0.204	0.125
H-6	0.037-0.154	0.109	0.042-0.325	0.241	0.042-0.325	0.241	0.054-0.204	0.097	0.070-0.533	0.193
H-2	0.318-0.633	0.527	0.759-2.333	1.551	0.070-1.453	0.608	1.105-3.303	1.721	0.260-1.133	0.471
H-11	0.721-1.713	1.259	1.223-2.914	2.120	1.223-2.914	2.120	3.300-3.900	3.632	0.609-2.587	1.284

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Summary Statistics

Table 5. Summary of primary EIA screening* results grouped from lowest to highest serum dilution.

Method Specimen Number/ dilution	Genetic Systems rLAV		bioMérieux Vironostika HIV-1		bioMérieux Uni-Form II *plus O*		Abbott Murex HIV 1.2.0		Other**	
	Reactive	Non-reactive	Reactive	Non-reactive	Reactive	Non-reactive	Reactive	Non-reactive	Reactive	Non-reactive
H-9/ 1:25	6	0	9	0	6	0	4	0	9	0
H-12/1:50	6	0	9	0	6	0	4	0	9	0
H-3/1:100	6	0	9	0	6	0	4	0	9	0
H-15/1:200	6	0	9	0	5	1	4	0	9	0
H-5/1:300	4	2	9	0	4	2	3	1	8	1
H-14/1:400	4	2	9	0	5	1	3	1	8	1
H-7/1:500	4	2	9	0	4	2	3	1	8	1
H-8/1:600	4	2	8	1	4	2	3	1	8	1
H-16/1:750	1	5	8	1	4	2	3	1	7	2
H-10/1:1000	1	5	9	0	4	2	3	1	6	3
H-4/1:1500	0	6	8	1	4	2	3	1	3	6
H-1/1:2000	0	6	8	1	4	2	3	1	2	7
H-13/1:4000	0	6	2	7	3	3	3	1	1	8
H-6 Neg QC	0	6	0	9	0	6	0	4	1	8
H-2 LP QC	5	1	9	0	3	3	3	1	7	2
H-11 HPQC	6	0	9	0	5	1	4	0	9	0

*Secondary screening results are not included.
**Other includes single methods grouped together so as to not identify any one laboratory.
Neg: Negative; LP: Low Positive; HP: High Positive.

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PART 2. CONFIRMATORY

Table 6. Confirmatory Methods

Kit Source	Number of Participants
Genetic Systems HIV-1 Western Blot (Bio-Rad)	12
Bio-Rad New LA V Blot I	2
OraSure HIV-1 Western Blot Kit	3
Genelab Diagnostics HIV 2.2 Western Blot	1
Cambridge biotech HIV-1 Western Blot Kit (Calypte)	1
Other	0
Total	19

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PART 2. Continued

Table 7. Number of Labs Reporting Western Blot results.

Specimen Number	Reciprocal Titer	Expected Result	Reactive	Non-Reactive	Indeterminant	Not Tested
H-9	25	Reactive	19	0	0	0
H-12	50	Reactive	19	0	0	0
H-3	100	Reactive	19	0	0	0
H-15	200	Reactive	19	0	0	0
H-5*	300	Reactive	16	0	0	2
H-14	400	Reactive	15	0	3	1
H-7*	500	Reactive	12	0	4	2
H-8	600	Reactive	12	1	4	2
H-16	750	Reactive	10	1	4	4
H-10	1000	Reactive	9	2	4	4
H-4	1500	Reactive	6	3	4	6
H-1	2000	Reactive	7	1	4	7
H-13	4000	Reactive	2	4	3	10
H-6	Negative QC	Non-reactive	1	6	1	11
H-2	Low Positive QC	Reactive	16	0	2	1
H-11	High Positive QC	Reactive	19	0	0	0

*No result given or result could not be confirmed.

Figure 1. Mean DBS Titration Curves for HIV EIA Screening Methods

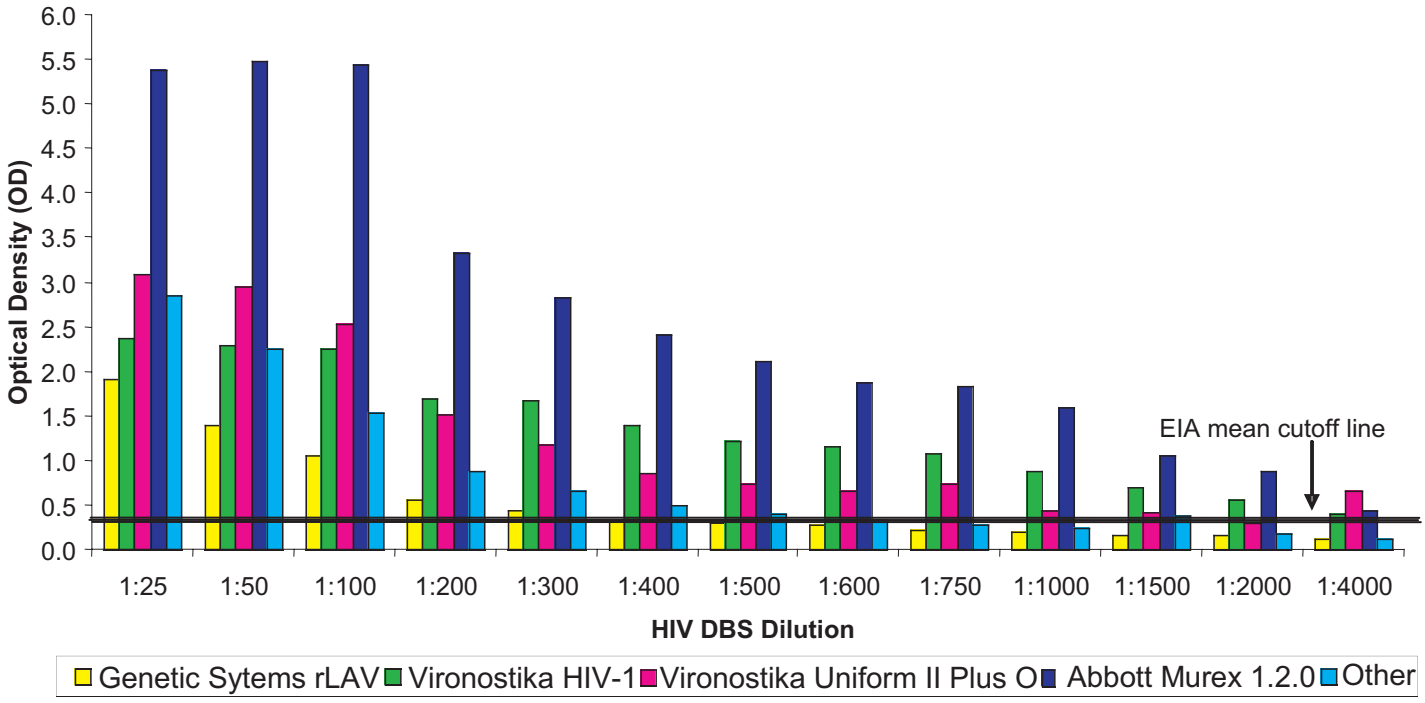
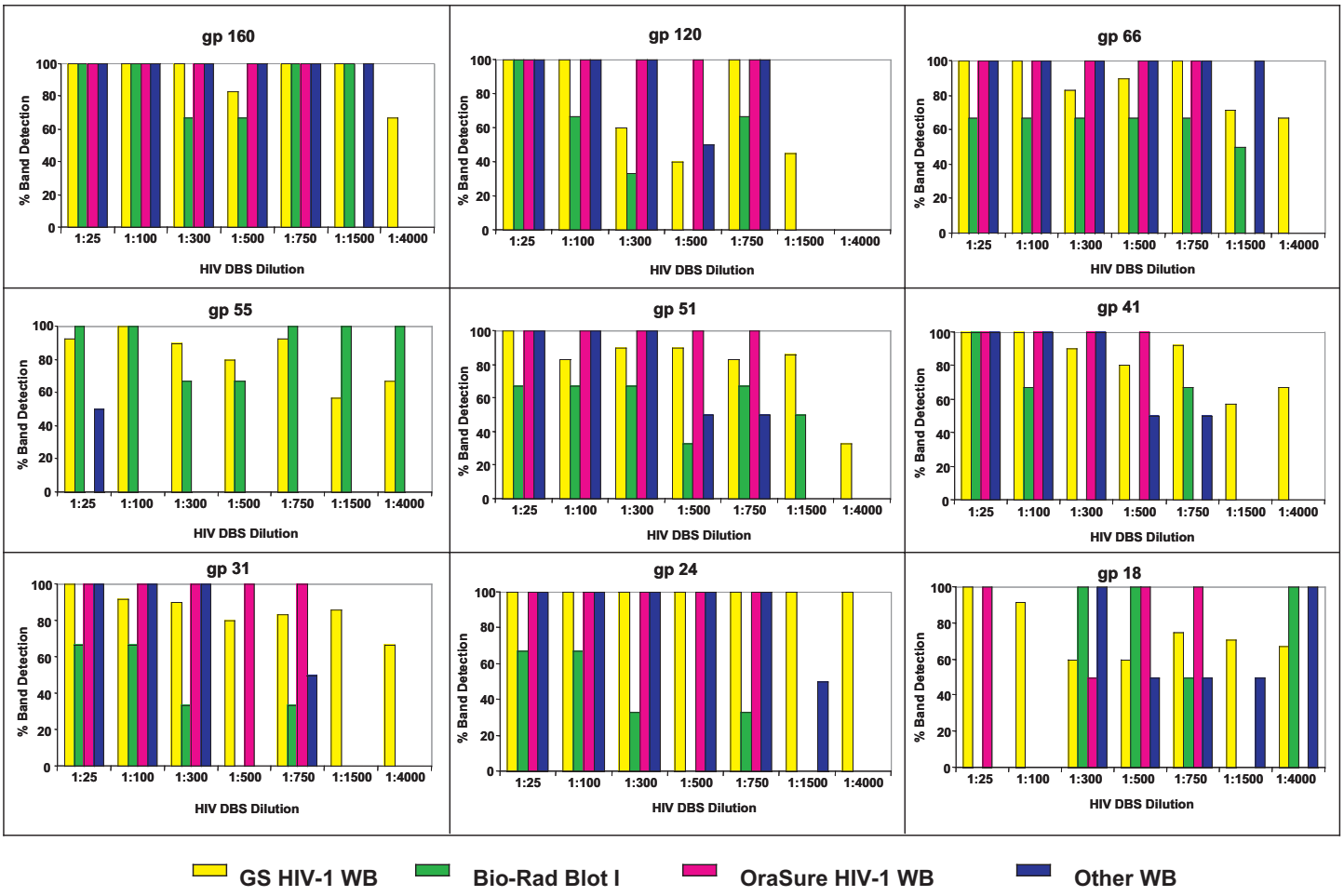


Figure 2. Comparison of Titration Curve Data By Method: Western Blot



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