

# Newborn Screening Quality Assurance Program

## 2005 ANNUAL SUMMARY REPORT

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### 2005 Annual Summary Report for Anti-HIV-1 in Dried-Blood Spots

#### INTRODUCTION

The Centers for Disease Control and Prevention (CDC), National Center for Environmental Health's (NCEH) Division of Laboratory Sciences, through the Newborn Screening Branch, operates a multi-component quality assurance (QA) 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 aid laboratories in method development and kit validation. A panel of proficiency testing (PT) specimens, representing a variety of HIV antibody reactivities, 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 DBS QC materials in their kits. Consultative services are always available for emerging concerns in laboratory QA.

The HIV QA 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. As of October 2006, 17 domestic laboratories and 55 foreign laboratories participate in CDC's Quality Assurance Program for Anti-HIV-1 in DBS.

#### METHODS

DBS materials produced at the CDC simulate newborn specimens and can be tested with many different assay systems, with minimal variance contributed by the manufactured specimens. An approved national standard exists for the collection of DBS and the handling of the 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 different 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



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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  $\mu$ L 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 QC materials are stored at  $-20^{\circ}$  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

performance was evaluated by the ability of participants to correctly identify the HIV reactivity of the blinded QC specimens. This challenge was voluntary and was not part of our graded quarterly PT program. To download a copy of this report, go to: [http://www.cdc.gov/labstandards/pdf/nsqap\\_HIVTitrationSummaryMay2006.pdf](http://www.cdc.gov/labstandards/pdf/nsqap_HIVTitrationSummaryMay2006.pdf)

## RESULTS

Each year we produce and certify more than 40,000 DBS for HIV QC 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. Table 1 gives the false-positive and false-negative rates for domestic and foreign laboratories in 2005.

Table 2 lists the combinations of HIV testing algorithms that laboratories reported for DBS PT specimens. Thirty-two (64%) of the participating laboratories reported using a combination of methods for testing DBS. Screening and confirmatory methods used by participants in 2005 are shown respectively in figures 1 and 2. Two EIA methods, the Genetic Systems rLAV (BioRad) and the bioMérieux

Table 1. 2005 Summary of HIV-1 Proficiency Testing Errors by Domestic and Foreign Laboratories

	Positive Specimens Assayed (N)	False-Negative Errors (%)	Negative Specimens Assayed (N)	False-Positive Errors (%)
Domestic Laboratories	182	0	123	0
Foreign Laboratories	316	0.9	199	3.0

donor's serum without blending or pooling the serum. A library of various individual matrices is maintained for PT testing.

Periodically we perform assessments of the analytical sensitivities of EIA and WB tests by using DBS blind-coded titration panels. In November 2005, a titration (dose-response) panel of HIV antibody-positive DBS specimens was sent to program participants. The panel was made up of 16 blind-coded specimens and included one each of negative, low-positive, and high-positive HIV QC materials. This testing challenge was provided to evaluate HIV DBS method performance and individual laboratory performance against a set of increasingly dilute HIV antibody-positive specimens. Laboratory

HIV-1 Microelisa System, carry protocols for testing DBS. The Genetic Systems HIV-1 Western Blot Kit (BioRad) also carries a DBS testing protocol.

### QC Material Performance –EIA

Figure 3 illustrates the overall performance of CDC QC materials with EIA methods. Error bars indicate 1 standard deviation. Figures 4-6 show the performance of three EIA kits with HIV-low positive QC materials, Lot 39 or Lot 65. Data were collected over four quarters in 2005. Kit lot numbers have been included and show that QC material values may shift over time with kit lot changes. Cutoff values and the 95% and 99% upper and lower control limits are also plotted for reference. Because of

**Table 2. HIV Testing Algorithms for DBS in 2005**

**Number of Participating Laboratories = 50**

**Number of Analytical Combinations Reported By Participants throughout the year:**

EIA/Western Blot	23
EIA Only	17
Agglutination/Western Blot	1
Agglutination Only	1
EIA/EIA	2
EIA/EIA/Western Blot	5
EIA/Agglutination/Western Blot	1

method performance differences, QC Low Positive Lot 39 was prepared specifically for the bioMérieux Vironostika HIV-1 microelisa system. Lot 39 was only distributed to users of this method.

### PT Material and Laboratory Performance

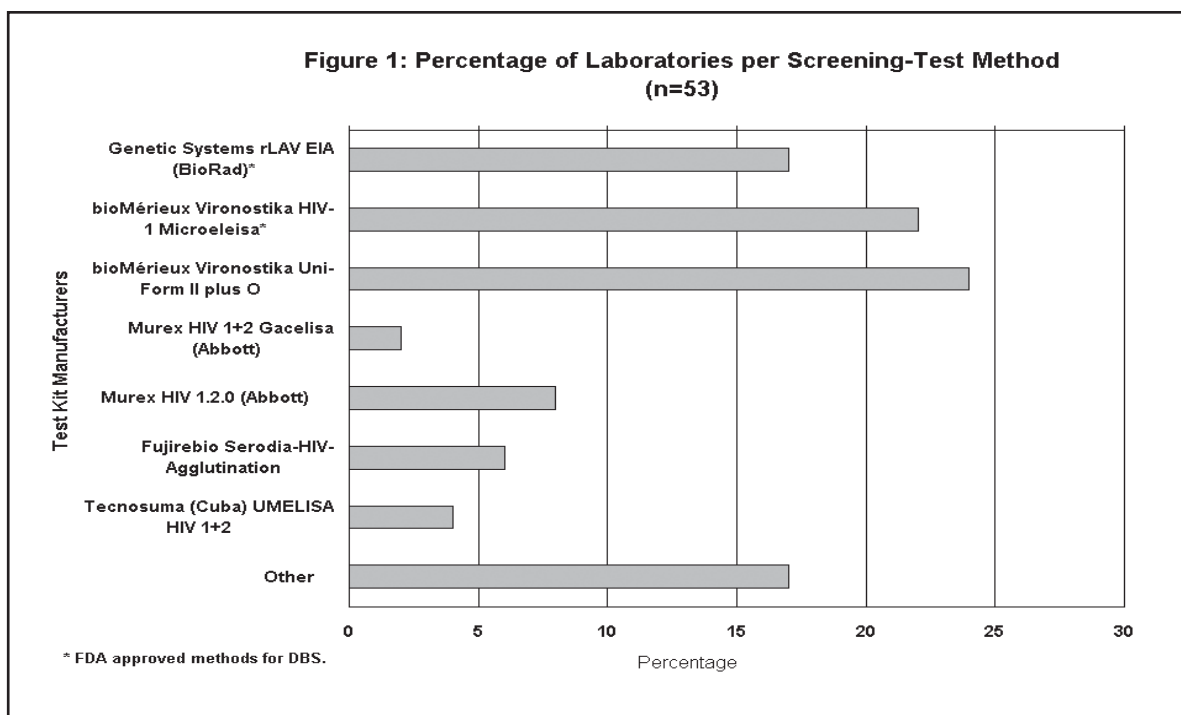
Figures 7 and 8 illustrate the reproducibility of EIA results for HIV-reactive and HIV-non-reactive specimens sent during two quarters of 2005. Mean values for three methods are shown and error bars represent the minimum and maximum values reported for each method. Overall means between quarters are very close; however, a large amount of variability exists as shown by the range of minimum and maximum responses received.

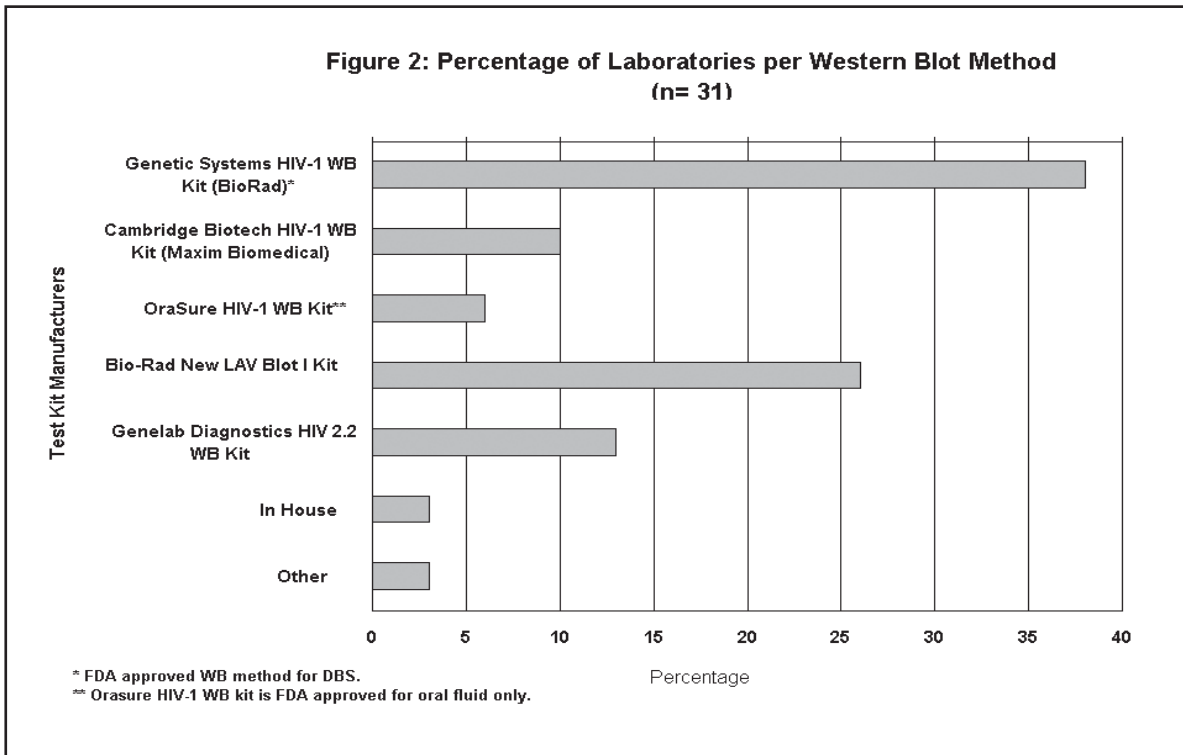
### WB Method Performance with PT materials

In 2005, Specimen A was distributed in quarter 1 and quarter 4. A summary of the reported protein molecular weight bands for two methods is shown in Figure 9. Differences in methods between quarters can be attributed to kit lot differences and the subjective nature of interpreting WB patterns.

### 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

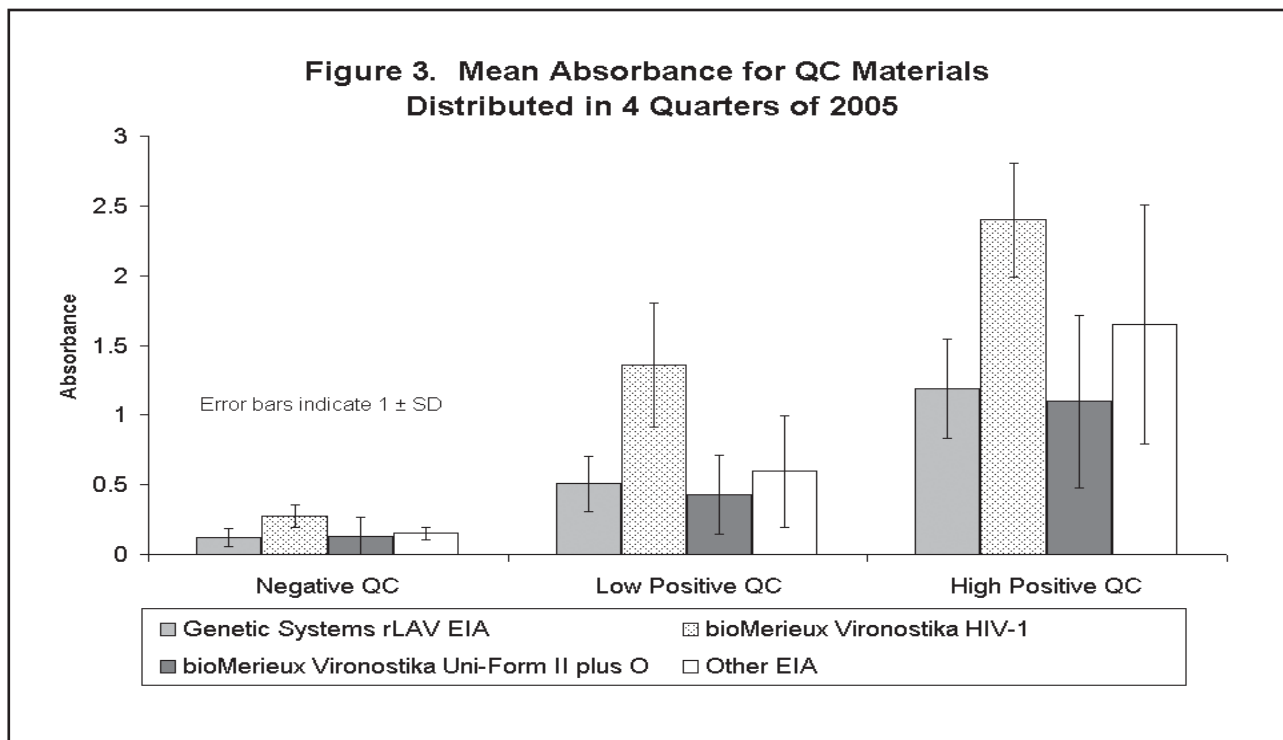




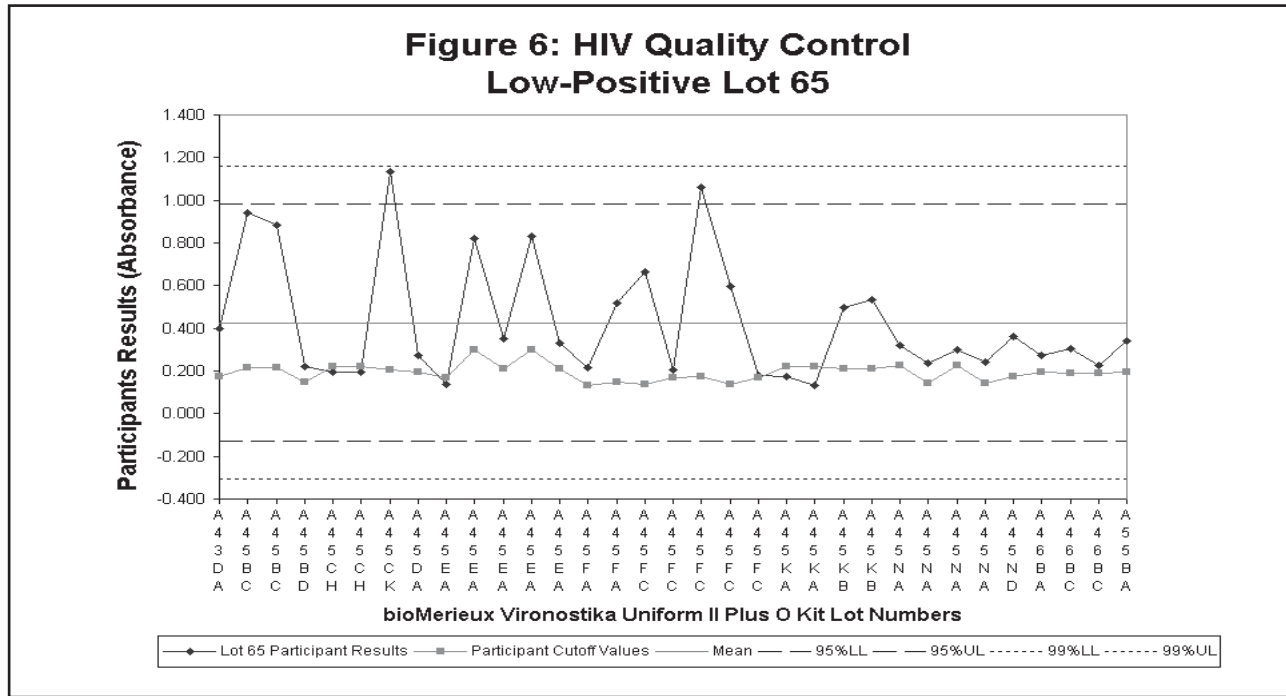
provide DBS QC materials. Three levels of QC materials are distributed to participants to cover a range of HIV reactivities. By tracking the performance of the CDC QC materials, laboratories can monitor their methods and identify shifts in values over time. Materials can also be used for method evaluation 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). Two HIV EIA methods and one WB method have protocols specifically for DBS. These products are primarily available in North America. Laboratories outside of North America have adapted serum or plasma-based methods for the filter paper matrix (Figures 1 and 2) with good results.

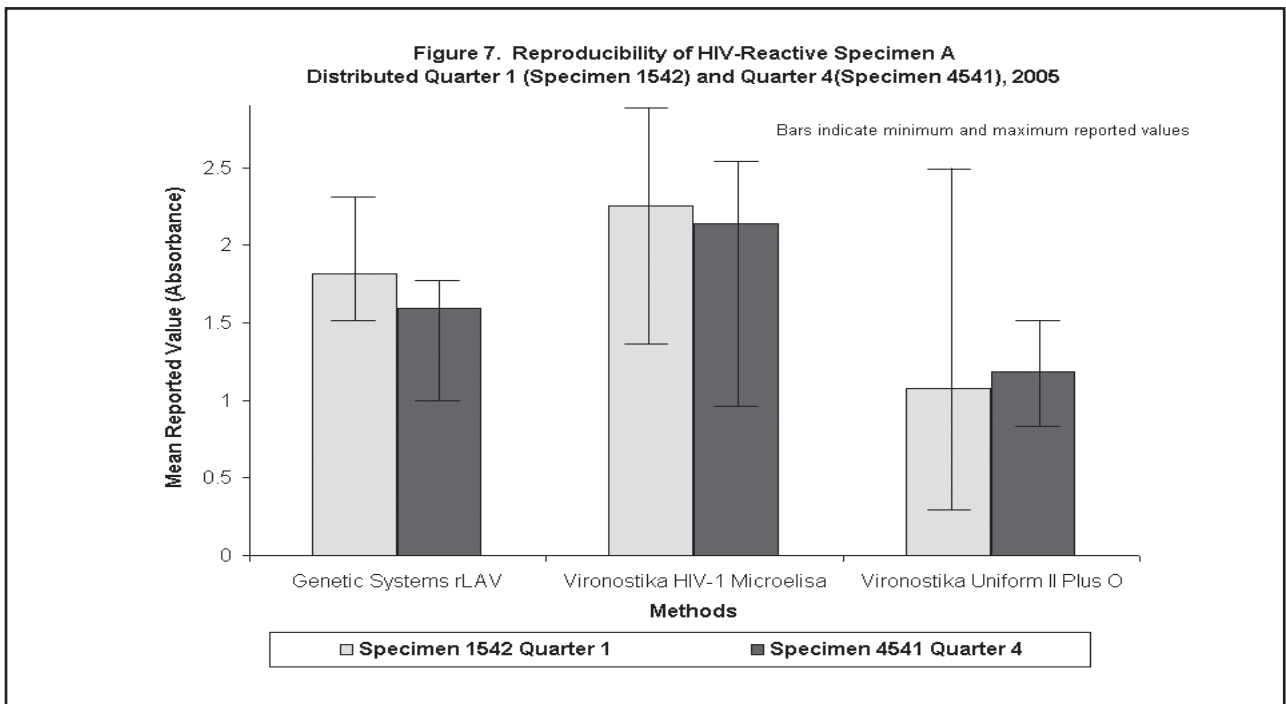


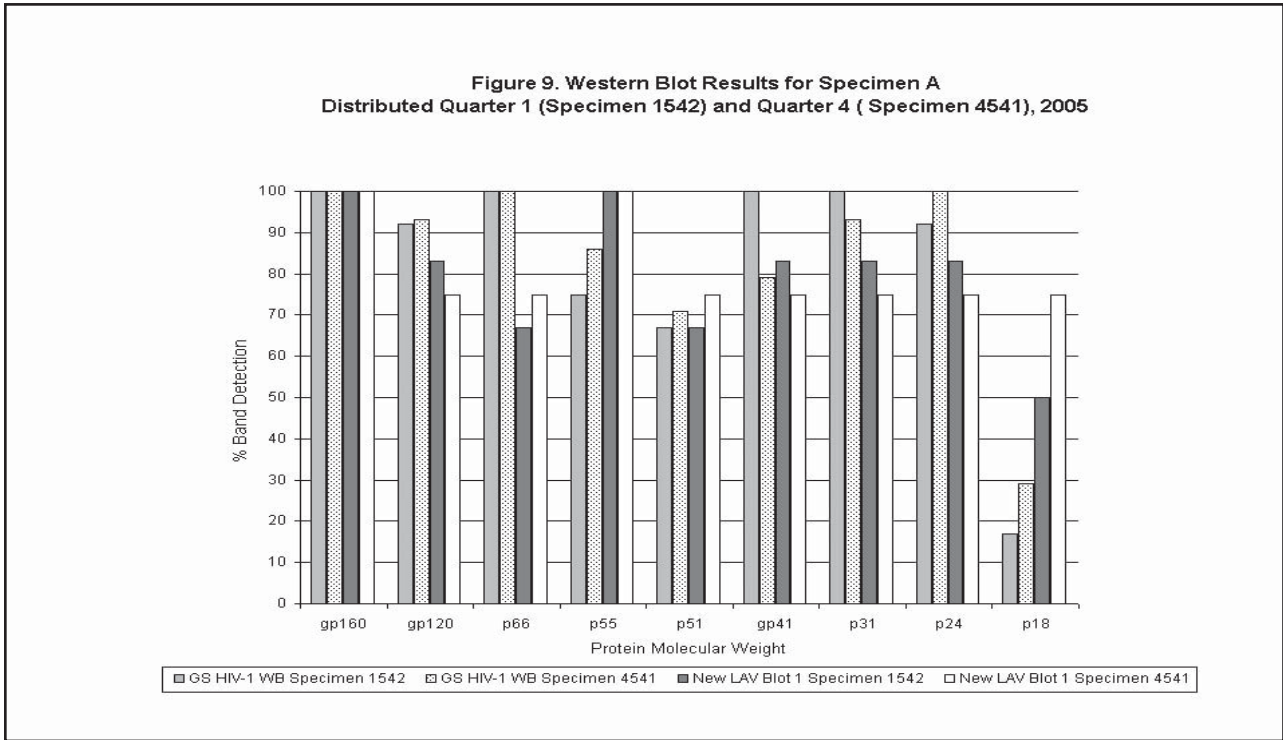
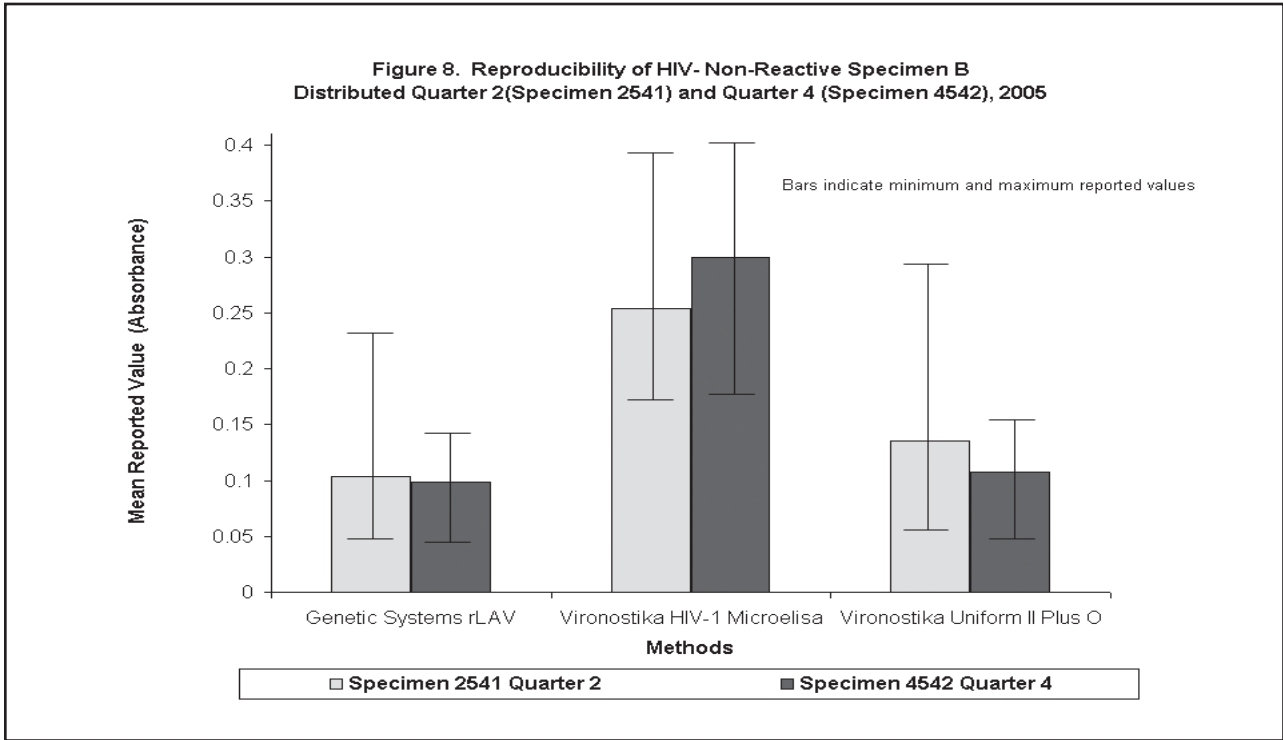




The performance of the CDC HIV QC materials during 2005 is shown in Figures 3-6. A large amount of variability within and between methods can be seen in Figure 3. The performance for three individual methods with low-positive QC lots 65 and 39 were tracked over the year (Figures 4-6). Each method had multiple kit lots in use. Shifts in reported means were observed with some kit lots, and in one case large swings in mean values were observed for the low-positive control (Figure 6). The HIV DBS QC materials prepared and distributed by CDC provide laboratories with a means to track the continuity of performance of their methods.

The reproducibility of proficiency testing materials tested in quarters of 2005 was good (Figures 7 and 8), however within-method variability was large as illustrated by the reported minimum and maximum values for each method by quarter. Within-method variability was also seen for two Western Blot kits that tested the same specimen in two quarters (Figure 9). Even though there was heterogeneity of results among HIV EIA and WB methods for PT DBS materials, the majority of laboratories correctly identified HIV-positive and HIV-negative specimens.





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