DEPARTMENT OF HEALTH AND HUMAN SERVICES

Centers for Disease Control and Prevention Model Performance Evaluation Program

Human Immunodeficiency Virus Type 1 Ribonucleic Acid (RNA) Determinations

Report of Results for the Performance Evaluation Survey Conducted in February 2004

CENTERS FOR DISEASE™
CONTROL AND PREVENTION

PUBLIC HEALTH PRACTICE PROGRAM OFFICE
DIVISION OF LABORATORY SYSTEMS
ATLANTA, GEORGIA

Report of the February 2004 Human Immunodeficiency Virus Type I (HIV-1) Ribonucleic Acid (RNA) Performance Evaluation Sample Testing Results Provided by Participant Laboratories in the Model Performance Evaluation Program, Centers for Disease Control and Prevention (CDC).

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INTRODUCTION

This is an analysis of results reported to the Centers for Disease Control and Prevention (CDC) by laboratories participating in the Model Performance Evaluation Program (MPEP) after they performed human immunodeficiency virus type 1 (HIV-1) ribonucleic acid (RNA) determinations on performance evaluation samples shipped to them February 10, 2004 . Testing results were reported by 180 (91.4%) of the 197 laboratories who were sent sample panels. The online reporting option was utilized by 67.8% (122/180) of the laboratories that reported results. This represents an 11.6% increase as compared to the previous (August 2003) survey (56.2%). We continue to encourage laboratories to report results online as an option for streamlining the process.

METHODS AND MATERIALS

Samples used in the MPEP HIV-1 RNA determinations performance evaluation survey are plasma obtained from individual donors (not pooled or diluted with plasma from other donors) who are HIV-1 infected or uninfected. Before shipment, the CDC tested each donor with three viral RNA test kits approved by the Food and Drug Administration (FDA).

The tables on pages 4 and 5 provide information about the panels of donor sample vials which were shipped for this survey. Table 1 lists the panel and vial designations, the CDC donor numbers, CDC test results, donor HIV status, and a section where laboratorians can insert their test results to compare with the CDC test results. Table 2 lists the CDC panels for this shipment, the labeled vials contained in each panel, the CDC donor numbers, the CDC results (HIV-1 RNA detected or not detected) obtained for each donor by all three manufacturers' test kits, and the CDC interpretation of these results based on the manufacturers' criteria. For all of the HIV-1 infected donors, HIV-1 RNA was detected by all of the test kits used and the CDC interpretation for these donors was positive for HIV-1 RNA. The donors not infected with HIV-1 did not have HIV-1 RNA detected by any of the test kits, based upon the lower limits of the test kit sensitivities. Consistent with the detection criteria contained within the test kit manufacturers' inserts, these donors were interpreted by CDC as negative for HIV-1 RNA.

Centers for Disease Control and Prevention (CDC)
Model Performance Evaluation Program for
Human Immunodeficiency Virus Type 1 (HIV-1) Ribonucleic Acid (RNA) Testing

Table 1 **Panel and Vial Designations, CDC Donor Numbers, CDC HIV-1 RNA Test Results, and Donor HIV Status**

Panel Letter	Vial Label	CDC Donor Number	CDC Test Result[1]	Donor HIV Status	Laboratory Interpretation[2] and/or Results	
					Test Result	Interpretation
A	A1	1	Positive	Infected	_____	_____
	A2	5	Negative	Uninfected	_____	_____
	A3	4	Negative	Uninfected	_____	_____
	A4	2	Positive	Infected	_____	_____
	A5	1	Positive	Infected	_____	_____
B	B1	5	Negative	Uninfected	_____	_____
	B2	1	Positive	Infected	_____	_____
	B3	2	Positive	Infected	_____	_____
	B4	1	Positive	Infected	_____	_____
	B5	4	Negative	Uninfected	_____	_____

[1] The CDC result was obtained after pre-shipment testing with three manufactured kits for determining the presence of HIV-1 RNA. These kits are approved by the Food and Drug Administration (FDA). The CDC result is consistent with the manufacturer's criteria for interpretation of results.

[2] Laboratory Interpretation space (to be completed by participant laboratory) provided to facilitate comparison of participant laboratory result with CDC result.

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Table 2 CDC HIV-1 RNA Testing Results for the February 10, 2004, Participant Laboratory Panel Samples

Panel Letter	Vial Label	CDC Donor Number	CDC Test Results[1]	Test Kit Manufacturer	Test Kit	CDC Interpretation[2]
A	A1, A5	1	HIV RNA detected	Roche	Amplicor HIV-1 Monitor®	Positive
			HIV RNA detected	bioMérieux	NucliSens® HIV-1 QT	Positive
			HIV RNA detected	Bayer	Versant® HIV-1 RNA 3.0 Assay (bDNA)	Positive
	A2	5	No HIV RNA detected	Roche	Amplicor HIV-1 Monitor®	Negative
			No HIV RNA detected	bioMérieux	NucliSens® HIV-1 QT	Negative
			No HIV RNA detected	Bayer	Versant® HIV-1 RNA 3.0 Assay (bDNA)	Negative
	A3	4	No HIV RNA detected	Roche	Amplicor HIV-1 Monitor®	Negative
			No HIV RNA detected	bioMérieux	NucliSens® HIV-1 QT	Negative
			No HIV RNA detected	Bayer	Versant® HIV-1 RNA 3.0 Assay (bDNA)	Negative
	A4	2	HIV RNA detected	Roche	Amplicor HIV-1 Monitor®	Positive
			HIV RNA detected	bioMérieux	NucliSens® HIV-1 QT	Positive
			HIV RNA detected	Bayer	Versant® HIV-1 RNA 3.0 Assay (bDNA)	Positive
B	B1	5	No HIV RNA detected	Roche	Amplicor HIV-1 Monitor®	Negative
			No HIV RNA detected	bioMérieux	NucliSens® HIV-1 QT	Negative
			No HIV RNA detected	Bayer	Versant® HIV-1 RNA 3.0 Assay (bDNA)	Negative
	B2, B4	1	HIV RNA detected	Roche	Amplicor HIV-1 Monitor®	Positive
			HIV RNA detected	bioMérieux	NucliSens® HIV-1 QT	Positive
			HIV RNA detected	Bayer	Versant® HIV-1 RNA 3.0 Assay (bDNA)	Positive
	B3	2	HIV RNA detected	Roche	Amplicor HIV-1 Monitor®	Positive
			HIV RNA detected	bioMérieux	NucliSens® HIV-1 QT	Positive
			HIV RNA detected	Bayer	Versant® HIV-1 RNA 3.0 Assay (bDNA)	Positive
	B5	4	No HIV RNA detected	Roche	Amplicor HIV-1 Monitor®	Negative
			No HIV RNA detected	bioMérieux	NucliSens® HIV-1 QT	Negative
			No HIV RNA detected	Bayer	Versant® HIV-1 RNA 3.0 Assay (bDNA)	Negative

[1]The CDC test results were obtained after pre-shipment testing with three manufactured kits for determining the presence of HIV-1 RNA. These kits are approved by the Food and Drug Administration (FDA).

[2]The CDC interpretation is consistent with the manufacturer's criteria for interpretation of results.
Positive = HIV-1 RNA detected; Negative = HIV-1 RNA not detected (based on lower limit of test kit sensitivity)

RESULTS

Laboratory Performance: Results Summary

The cumulative frequencies of quantitative and qualitative test results for all donor samples reported by laboratories are shown in Table 3. This table describes the final test interpretations (positive or negative for HIV-1 RNA) with respect to the donors' status (infected or uninfected) and includes the data for all test kits used. The first row presents results for donors who were HIV-1 infected and had detectable HIV-1 RNA. The "Percent Correct" for infected donors indicates the percentage of results that were positive for HIV-1 RNA in HIV-1 infected donors. The second row shows results for donors not infected with HIV-1 and where HIV-1 RNA was not detectable. In this case, "Percent Correct" indicates the percentage of results that had final interpretations of "not detected" for those samples from donors uninfected with HIV-1. The third row shows a summary of all results reported by participant laboratories; the "Percent Correct" for this row refers to the overall analytic accuracy, as a group, of all laboratories and all HIV-1 RNA test kits.

Laboratories performed generally well in the testing of these performance evaluation samples. There were two false-positives (2/384, 0.5%) reported in the current survey, in contrast to no false-positive results reported in the previous performance survey (August 2003). Both of the false-positives were associated with Donor 5 and were obtained using Bayer Versant® HIV-1 RNA 3.0 Assay (bDNA); the Lower Limit Sensitivities (LLS) that were reported with these two results were 50 copies/mL and 75 copies/mL.

The percentage of false-negative results (1.9%, 11/575) reported in this survey was similar to that of the previous survey (1.6%, 9/551). Ten of the current false-negative results were associated with Donor 2 and the other result was reported for Donor 1 (also listed as Donor 1 Duplicate). It should be noted that Donor 2 comprised the "low-positive" samples, with a target value of approximately 1,000 RNA copies/ml, whereas Donor 1 had a target value of approximately 15,000 RNA copies/ml. Ten of these eleven false-negative results were obtained using Roche's Amplicor HIV-1 Monitor® test; nine of these reports specified using a Lower Limit Sensitivities (LLS) of 400 copies/mL (one report did not specify a LLS). The other result was obtained using bioMérieux NucliSens® HIV-1 QT; the reported LLS associated with this result was 160 copies/mL. The quantitative results for the "low-positive" (Donor 2) for the current survey had a statistically significant (p=0.06) increase in the number of false-negative results as compared to the previous survey. The current survey had 5.4% (10/184) false-negative quantitative results for Donor 2, whereas the August 2003 survey had 2.3%, (8/353) for challenge specimens from the same donor.

Table 3	Number of Results	Percent Correct	Percent False Negative	Percent False Positive
Infected Donor Samples	575	98.1% (564/575)	1.9% (11/575)	n/a
Uninfected Donor Samples	384	99.5% (382/384)	n/a	0.5% (2/384)
TOTAL RESULTS	959	98.6% (946/959)	***	***

Test Kit Lower Limit Sensitivities

There was variability in the lower limit sensitivities reported by the laboratories that used commercially manufactured quantitative HIV-1 RNA test kits. Table 4 displays the lower limit sensitivities reported by the participating laboratories, by type of test kit used. For each test kit, the percentage of the total reported results for each specified lower limit sensitivity is shown, and "n" is the number of sample result reports in this survey based on that test kit.

Table 4

Manufacturer Test Kit (n = number of reports)	Percent of Reports (n)	Lower Limit Sensitivity Used (copies/ml)
Roche Amplicor HIV-1 Monitor® (n = 629)	32% (200)	50
	1% (5)	200
	65% (409)	400
	2% (15)	not indicated
Bayer Versant® HIV-1 RNA 3.0 Assay (bDNA) (n= 245)	20% (50)	50
	78% (190)	75
	2% (5)	not indicated
bioMérieux NucliSens® HIV-1 QT (n= 35)	43% (15)	25
	29% (10)	160
	14% (5)	200
	14% (5)	250
In-House (n= 10)	50% (5)	100
	50% (5)	not indicated
bioMérieux Nuclisens® EasyQ HIV-1 (n=5)	100% (5)	25

Quantitative and Qualitative Test Aggregate Results

Tables 5 through 8 show the aggregate participant laboratories' testing results for each donor sample by test kit manufacturer. It should be noted that in this survey the plasma test samples were obtained from the same donors as in the previous survey (August 2003). The results columns provide the totals for the number of results reported detecting HIV-1 RNA and not detecting HIV-1 RNA. The lower limit sensitivities of the reported quantitative test kits ranged from 25 RNA copies/ml to 400 RNA copies/ml. For the quantitative results, the absolute minimum and maximum reported values of RNA copies/ml are given irrespective of the different kits' lower limit sensitivities. Also included for the quantitative results are the 25%, 50% (median) and 75% quartiles for those samples that had detectable RNA levels.

For this performance survey shipment, samples from Donor 1, an HIV-1 infected donor, were duplicated in each panel to provide the participant laboratories an opportunity to evaluate their intra-shipment reproducibility. For the samples designated Donor 1 and Donor 1 Duplicate, the material came from the same plasma but was sent to the laboratories as separate samples under different sample vial designations. Table 5A shows the laboratory test results reported for CDC Donor 1. Table 5B shows results for the duplicated specimen, Donor 1 Duplicate.

Table 5A: Results of the HIV-1 RNA Determinations Reported by Participant Laboratories for Donor #1

Donor Status: HIV-1 Infected and HIV-1 RNA Detected
Panel Vial Labels: A1, B2

	No. of Results Detecting RNA	No. of Results Not Detecting RNA	Range of Quantitative Results Reported (RNA copies/ml)				
Test Kit			**minimum**	**maximum**	**25% Quartile**	**Median (50%)**	**75% Quartile**
Roche Amplicor HIV-1 Monitor	126	0	1580	68000	5162	**6950**	9880
Bayer Versant HIV-1 RNA 3.0 Assay	49	0	3171	9959	5476	**6223**	7424
bioMérieux NucliSens HIV-1 QT	7	0	4200	8900	5900	**7300**	8300
Chiron Procleix	7	0	-	-	-	**-**	-
In House	2	0	3850	4900	3850	**4375**	4900
bioMérieux Nuclisens EasyQ HIV-1	1	0	320	320	-	**320**	-

Table 5B: Results of the HIV-1 RNA Determinations Reported by Participant Laboratories for Donor #1 Duplicate

Donor Status: HIV-1 Infected and HIV-1 RNA Detected
Panel Vial Labels: A5, B4

Test Kit	No. of Results Detecting RNA	No. of Results Not Detecting RNA	Range of Quantitative Results Reported (RNA copies/ml)				
			minimum	maximum	25% Quartile	Median (50%)	75% Quartile
Roche Amplicor HIV-1 Monitor	125	1	1029	62400	5145	**6985**	9445
Bayer Versant HIV-1 RNA 3.0 Assay	49	0	3015	9197	5326	**6162**	6897
bioMérieux NucliSens HIV-1 QT	7	0	2900	8700	3300	**4500**	5700
Chiron Procleix	7	0	-	-	-	**-**	-
In House	2	0	3400	3646	3400	**3523**	3646
bioMérieux Nuclisens EasyQ HIV-1	1	0	590	590	-	**590**	-

Table 6: Results of the HIV-1 RNA Determinations Reported by Participant Laboratories for Donor #2

Donor Status: HIV-1 Infected and HIV-1 RNA Detected
Panel Vial Labels: A4, B3

Test Kit	No. of Results Detecting RNA	No. of Results Not Detecting RNA	Range of Quantitative Results Reported (RNA copies/ml)				
			minimum	maximum	25% Quartile	Median (50%)	75% Quartile
Roche Amplicor HIV-1 Monitor	116	9	61	5860	531	**695**	1090
Bayer Versant HIV-1 RNA 3.0 Assay	49	0	156	1173	345	**437**	528
bioMérieux NucliSens HIV-1 QT	6	1	180	5600	220	**753**	1400
Chiron Procleix	7	0	-	-	-	**-**	-
In House	2	0	569	1900	569	**1235**	1900
bioMérieux Nuclisens EasyQ HIV-1	1	0	1000	1000	-	**1000**	-

Table 7: Results of the HIV-1 RNA Determinations Reported by Participant Laboratories for Donor #4

Donor Status: HIV-1 Uninfected and HIV-1 RNA Not Detected
Panel Vial Labels: A3, B5

Test Kit	No. of Results Detecting RNA	No. of Results Not Detecting RNA	Range of Quantitative Results Reported (RNA copies/ml)				
			minimum	maximum	25% Quartile	Median (50%)	75% Quartile
Roche Amplicor HIV-1 Monitor	0	126	n/a	n/a	n/a	**n/a**	n/a
Bayer Versant HIV-1 RNA 3.0 Assay	0	49	n/a	n/a	n/a	**n/a**	n/a
bioMérieux NucliSens HIV-1 QT	0	7	n/a	n/a	n/a	**n/a**	n/a
Chiron Procleix	0	7	n/a	n/a	n/a	**n/a**	n/a
In House	0	2	n/a	n/a	n/a	**n/a**	n/a
bioMérieux Nuclisens EasyQ HIV-1	0	1	n/a	n/a	n/a	**n/a**	n/a

Table 8: Results of the HIV-1 RNA Determinations Reported by Participant Laboratories for the Donor #5

Donor Status: HIV-1 Uninfected and HIV-1 RNA Not Detected
Panel Vial Labels: A2, B1

Test Kit	No. of Results Detecting RNA	No. of Results Not Detecting RNA	Range of Quantitative Results Reported (RNA copies/ml)				
			minimum	maximum	25% Quartile	Median (50%)	75% Quartile
Roche Amplicor HIV-1 Monitor	0	126	n/a	n/a	n/a	**n/a**	n/a
Bayer Versant HIV-1 RNA 3.0 Assay	2	47	96	193	96	**145**	193
bioMérieux NucliSens HIV-1 QT	0	7	n/a	n/a	n/a	**n/a**	n/a
Chiron Procleix	0	7	n/a	n/a	n/a	**n/a**	n/a
In House	0	2	n/a	n/a	n/a	**n/a**	n/a
bioMérieux Nuclisens EasyQ HIV-1	0	1	n/a	n/a	n/a	**n/a**	n/a

Histograms depicting the aggregate results of RNA copies/ml for the two HIV-positive donors are shown in Figures 1 and 2 below. Figure 1 depicts the quantitative results for Donor 1 and Donor 1 Duplicate, and Figure 2 depicts the results for Donor 2.

Fig. 1 **Histogram of HIV-1 RNA Quantitative Determinations for Donor 1**

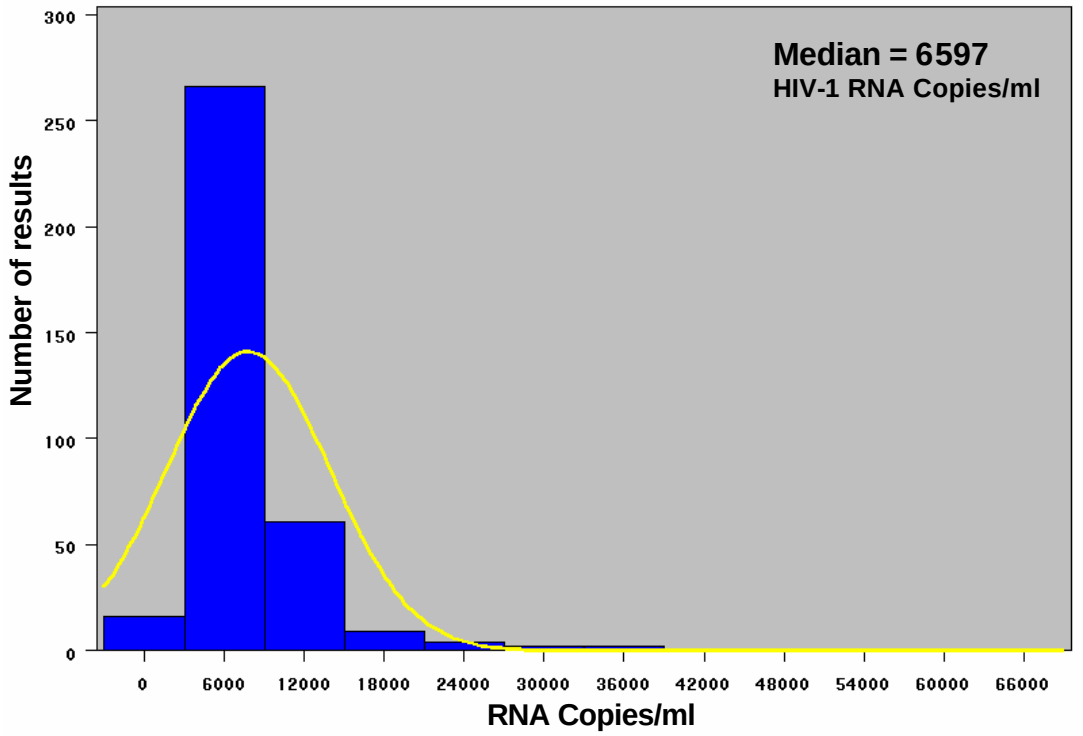
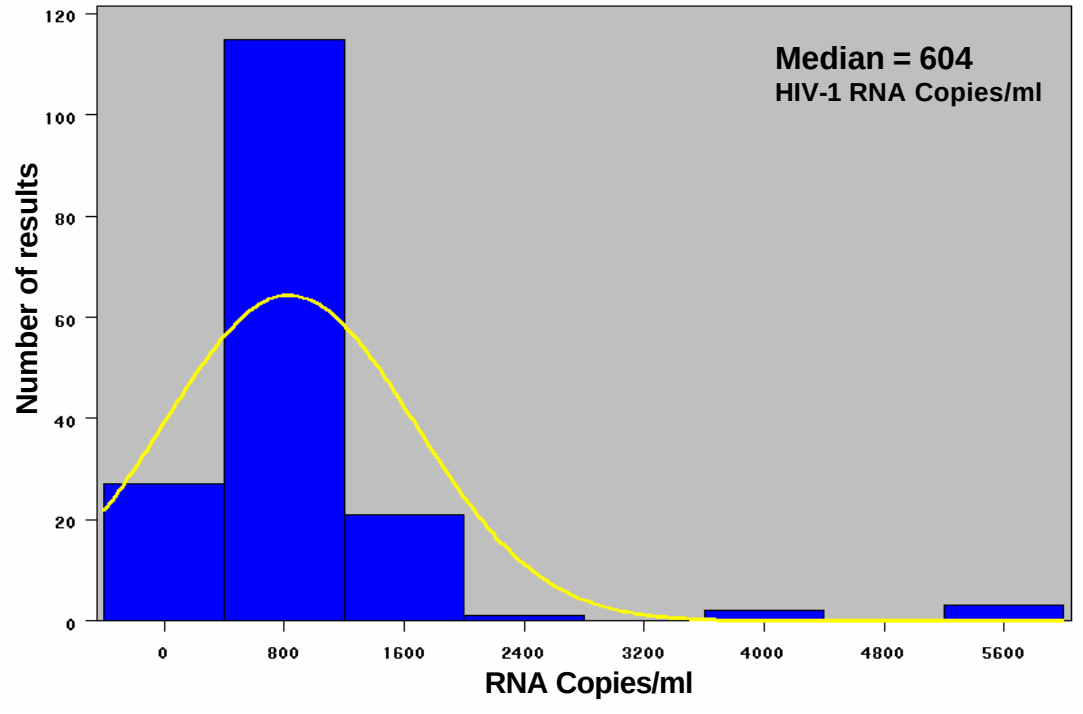


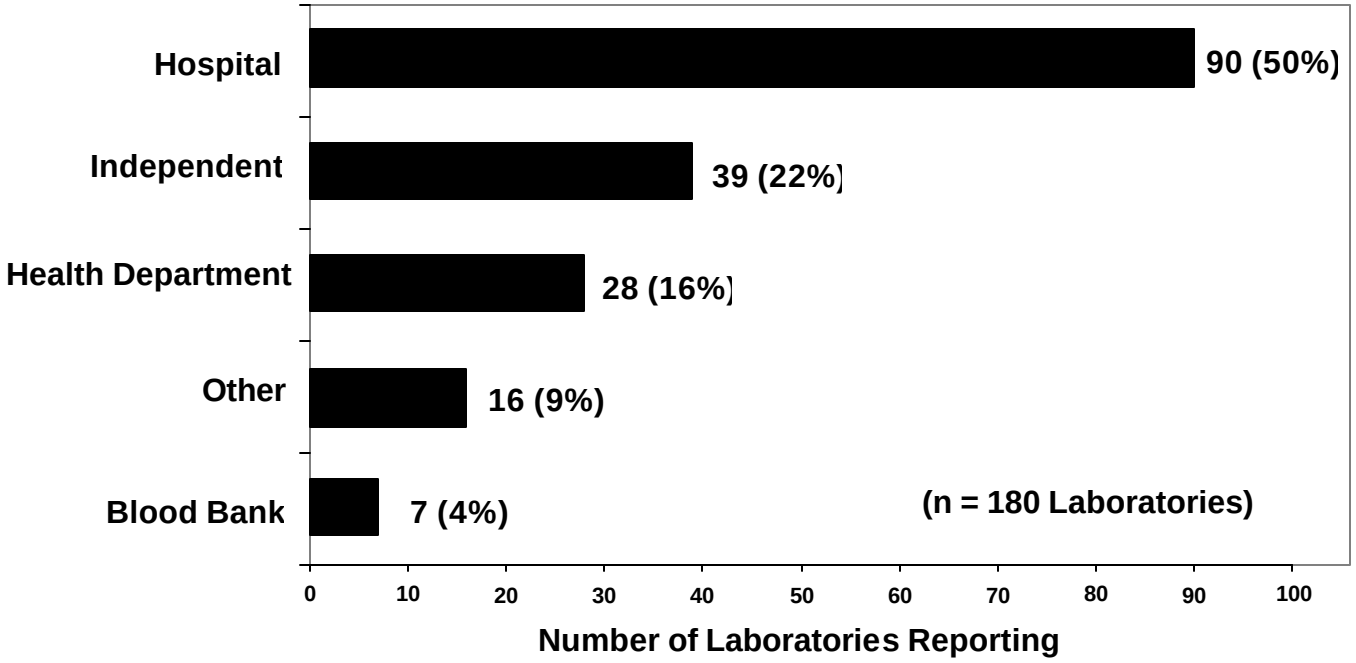
Fig. 2 **Histogram of HIV-1 RNA Quantitative Determinations for Donor 2**



Types of Laboratories Reporting HIV-1 RNA Determinations

Figure 3 shows the types of laboratories reporting quantitative or qualitative HIV-1 RNA results.

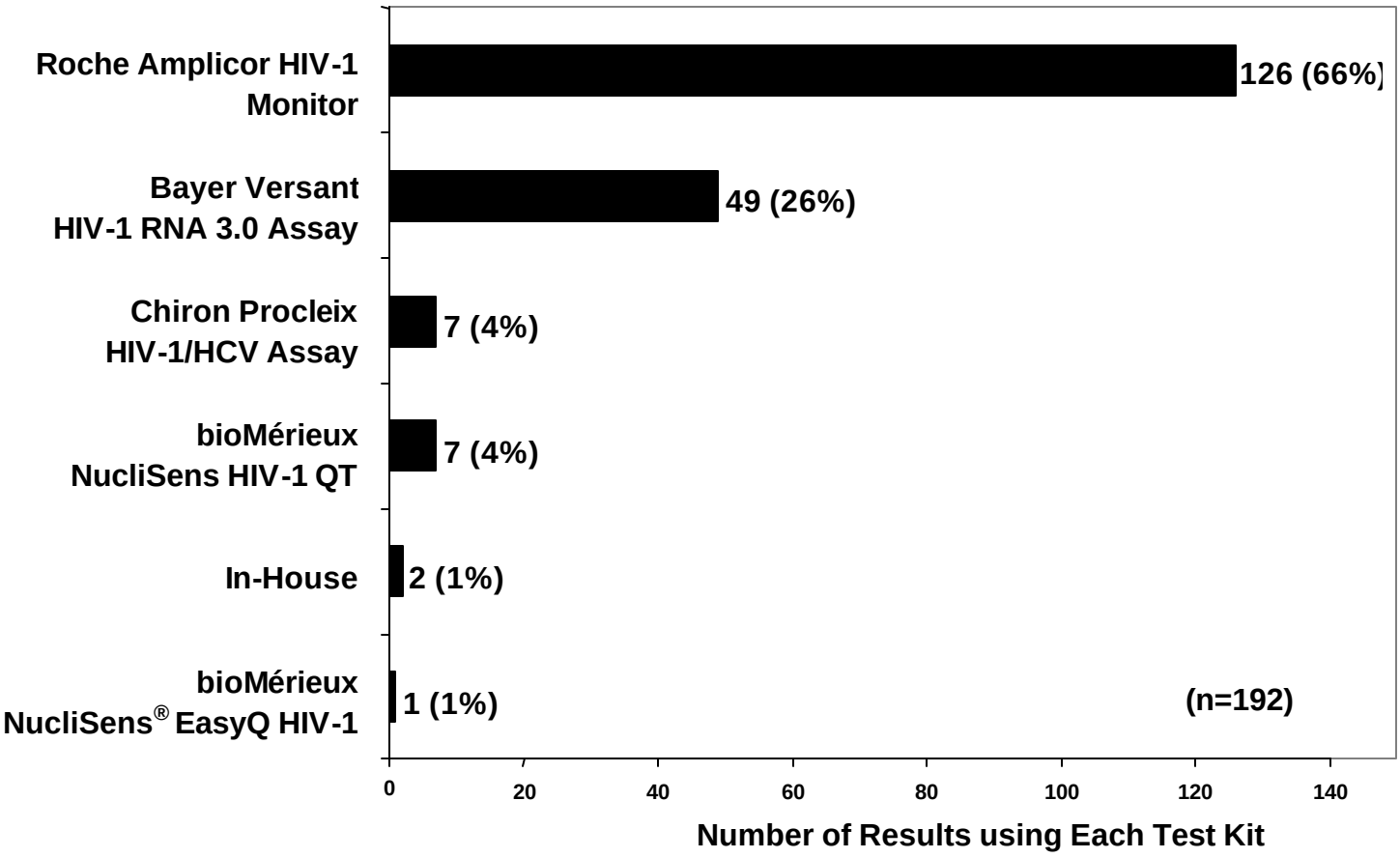
Fig. 3 **Types of Laboratories Performing HIV-1 RNA Determinations in the February 2004 Performance Evaluation Survey**



Types of Test Kits Used by Laboratories

The types of test kits used by laboratories performing viral RNA quantitative and qualitative determinations are shown in Figure 4. Please note that some laboratories used more than one test kit, which explains why the total number of tests reported (n =192) exceeds the number of laboratories reporting results (n =180). Of the quantitative kits, the Roche's Amplicor HIV-1 Monitor® test kit was used most frequently (126, 66%) in reporting results. The seven participating laboratories that reported using qualitative RNA testing procedures all used the HIV-1/HCV assay, which was developed by Gen-Probe and is marketed by Chiron under the name of Procleix™ HIV-1/HCV Assay. These seven laboratories (3.9%) provided the only qualitative test results reported in this survey.

Fig. 4* **Types of Test Kits Used to Perform HIV-1 RNA Determinations in the February 2004 Performance Evaluation Survey**



*The "n=" on Figure 4 represents the number of reported results. For this graph, laboratories reported results using more than one test; therefore, the number of results exceed the actual number of laboratories providing reports (n=180). The number appearing next to each bar represents the number and percentage of results.

Use of External Quality Control (QC) Testing Material

Of the 180 laboratories reporting results in this survey:

- 98.3% (177/180) provided information on external QC materials
  - 50.8% (90/177) indicated that they used external QC materials. The source of their external QC materials are as follows:
    - 58.9% (53/90) used commercial material
    - 37.8% (34/90) used In-House material
    - 3.3% (3/90) used both commercial and In-House external QC material
  - 49.2% (87/177) did not use external QC samples
- 1.7% (3/180) of all reporting laboratories gave no external QC information

CONCLUSION

The results of this performance evaluation shipment for quantitative and qualitative HIV-1 RNA determinations showed that the relative number of false-positive and false-negative results, when compared with the previous performance survey, remained approximately the same. While there is continued variability of results within laboratories using the same kit manufacturer and among laboratories using different kit manufacturers across all performance surveys, a comparison of the results reported for the duplicate donor in this performance survey showed good reproducibility within the results reported for each kit manufacturer. The overall analytic performance of the results in this MPEP survey was 98.6%.