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 August 2003

CENTERS FOR DISEASE™
CONTROL AND PREVENTION

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

Report of the August 2003 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).

The production of this report was coordinated in CDC by:

Public Health Practice Program Office.....	Suzanne M. Smith, M.D., M.P.H., Acting Director
Division of Laboratory Systems.....	Robert Martin, M.P.H., Dr.P.H., Director
Laboratory Practice Evaluation and Genomics Branch....	Devery Howerton, Ph.D., Chief

The content was developed by:

Model Performance Evaluation Program (MPEP).....	Laurina O. Williams, Ph.D., M.P.H., Co-Manager
.....	G. David Cross, M.S., Co-Manager
MPEP HIV-1 RNA Performance Evaluation.....	Leigh Inge Vaughan, B.A. HIV-1 RNA Project Coordinator

Inquires should be directed to the Model Performance Evaluation Program by calling (770) 488-8095 or (770) 488-8130.

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* The "N=" on Figure 4 represents the number of laboratories that reported results. For this graph, laboratories reported results using more than one test; therefore, the number of results may exceed the actual number of laboratories providing reports. The number appearing directly above or within each bar represents a frequency of results only.

Analysis of the August 2003 Performance Evaluation
HIV-1 RNA Determinations (Viral Load) Results
Reported to the Centers for Disease Control and Prevention
by Laboratories Participating in the Model Performance Evaluation Program

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 August 12, 2003. Testing results were reported by 178 (90.4%) of the 197 laboratories who were sent sample panels. An online option was available to laboratories for recording their results. Of the laboratories that reported results, 56.2% (100/178) utilized the online option. We encourage laboratories to use online reporting, which will be available for all future shipments.

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	2	Positive	Infected	_____	_____
	A3	2	Positive	Infected	_____	_____
	A4	5	Negative	Uninfected	_____	_____
	A5	4	Negative	Uninfected	_____	_____
B	B1	2	Positive	Infected	_____	_____
	B2	4	Negative	Uninfected	_____	_____
	B3	1	Positive	Infected	_____	_____
	B4	5	Negative	Uninfected	_____	_____
	B5	2	Positive	Infected	_____	_____

[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 licensed 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.

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

Table 2 CDC HIV-1 RNA Testing Results for the August 12, 2003 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	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, A3	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
	A4	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
	A5	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
B	B1, B5	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
	B2	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
	B3	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
	B4	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

[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 licensed 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 each test kit 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 test kit results that detected 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 the different HIV-1 RNA test kits.

Laboratories performed generally well in the testing of these performance evaluation samples. There were no false-positive results reported in the current survey, in contrast to 7 false-positives (7/368,1.9%) reported in the previous performance survey (February 2003).

The percentage of false-negative results [1.6%, (9/551)] reported in this survey was slightly higher than that of the previous survey (0.9%, 5/552). Eight of the current false-negative results were associated with Donor 2 (also listed as Donor 2 Duplicate); the other result was reported for Donor 1. 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. Of the 9 false negatives, 5 were obtained using Roche's Amplicor HIV-1 Monitor® test, 2 using Bayer Versant® HIV-1 RNA 3.0 Assay (bDNA), and one each using bioMérieux NucliSens® HIV-1 QT and In-House tests.

Table 3	Number of Results	Percent Correct	Percent False Negative	Percent False Positive
Infected Donor Samples	551	98.4% (542/551)	1.6% (9/551)	n/a
Uninfected Donor Samples	364	100.0% (364/364)	n/a	0% (0/364)
TOTAL RESULTS	915	99.0% (906/915)	***	***

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 = 619)	28% (173)	50
	2% (10)	200
	67% (416)	400
	3% (20)	not indicated
Bayer Versant® HIV-1 RNA 3.0 Assay (bDNA) (n= 225)	18% (40)	50
	80% (180)	75
	2% (5)	452
bioMérieux NucliSens® HIV-1 QT (n= 35)	29% (10)	25
	43% (15)	160
	14% (5)	200
	14% (5)	250
In-House (n= 5)	100% (5)	20

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 different donors than in the previous survey (February 2003). The results columns provide the totals for detecting HIV-1 RNA and not detecting HIV-1 RNA. For the quantitative results, the median values and the 95% confidence intervals (CI) for the number of RNA copies/ml of sample are shown for those samples that had detectable RNA levels. Also included for the quantitative results are the absolute minimum and maximum calculated values as reported by type of test kit, irrespective of the different kits' lower limit sensitivities. The lower limit sensitivities of the reported quantitative test kits ranged from 20 RNA copies/ml to 452 RNA copies/ml.

For this performance survey shipment, Donor 2, an HIV-1 infected donor, was duplicated in each panel to provide the participant laboratories an opportunity to evaluate their intra-shipment reproducibility. For the samples designated Donor 2 and Donor 2 Duplicate, the material came from the same plasma but was sent to the laboratories as separate samples under different sample vial designations. Table 6A shows the laboratory test results reported for CDC Donor 2. Table 6B shows results for the duplicated specimen, Donor 2 Duplicate.

Table 5: Results of the HIV-1 RNA Determinations Reported by Participant Laboratories for the August 2003 Performance Evaluation Survey for CDC Donor #1

Donor Status: HIV-1 Infected and HIV-1 RNA Detected
Panel Vial Labels: A1, B3

	No. of Results Detecting RNA	No. of Results Not Detecting RNA	Range of Quantitative Results Reported (RNA copies/ml)		
Test Kit			**minimum**	**median (95% CI)**	**maximum**
Roche Amplicor HIV-1 Monitor	124	0	945	11,456 (10,203 - 12,710)	35,681
Bayer Versant HIV-1 RNA 3.0 Assay	45	0	4,970	7,279 (6,801 - 7,756)	12,352
bioMérieux NucliSens HIV-1 QT	7	0	2,000	7,300 (4,003 - 10,597)	12,000
In House	0	1	***	***	***
Chiron Procleix	6	0	n/a	n/a	n/a
Other	1	0	n/a	n/a	n/a

Table 6A: Results of the HIV-1 RNA Determinations Reported by Participant Laboratories for the August 2003 Performance Evaluation Survey for CDC Donor #2

Donor Status: HIV-1 Infected and HIV-1 RNA Detected
Panel Vial Labels: A2, B1

	No. of Results Detecting RNA	No. of Results Not Detecting RNA	Range of Quantitative Results Reported (RNA copies/ml)		
Test Kit			**minimum**	**median (95% CI)**	**maximum**
Roche Amplicor HIV-1 Monitor	122	2	290	965 (877 - 1,052)	2,650
Bayer Versant HIV-1 RNA 3.0 Assay	44	1	304	489 (450 - 527)	839
bioMérieux NucliSens HIV-1 QT	7	0	240	774 (409 - 1,139)	1,300
In House	1	0	***	***	***
Chiron Procleix	6	0	n/a	n/a	n/a
Other	1	0	n/a	n/a	n/a

Table 6B: Results of the HIV-1 RNA Determinations Reported by Participant Laboratories for the August 2003 Performance Evaluation Survey for CDC Donor #2 Duplicate

Donor Status: HIV-1 Infected and HIV-1 RNA Detected
Panel Vial Labels: A3, B5

	No. of Results Detecting RNA	No. of Results Not Detecting RNA	Range of Quantitative Results Reported (RNA copies/ml)		
Test Kit			**minimum**	**median (95% CI)**	**maximum**
Roche Amplicor HIV-1 Monitor	120	3	338	982 (878 - 1,086)	4,870
Bayer Versant HIV-1 RNA 3.0 Assay	44	1	181	457 (415 - 500)	798
bioMérieux NucliSens HIV-1 QT	6	1	210	1,792 (306 - 3,278)	4,300
In House	1	0	***	***	***
Chiron Procleix	6	0	n/a	n/a	n/a
Other	1	0	n/a	n/a	n/a

Tables 7 and 8 show the results reported for Donor 4 and Donor 5, respectively. These donors were both HIV-1 uninfected.

Table 7: Results of the HIV-1 RNA Determinations Reported by Participant Laboratories for the August Performance Evaluation Survey for CDC Donor #4

Donor Status: HIV-1 Uninfected and HIV-1 RNA Not Detected
Panel Vial Labels: A5, B2

	No. of Results Detecting RNA	No. of Results Not Detecting RNA	Range of Quantitative Results Reported (RNA copies/ml)		
Test Kit			**minimum**	**median (95% CI)**	**maximum**
Roche Amplicor HIV-1 Monitor	0	123	***	***	***
Bayer Versant HIV-1 RNA 3.0 Assay	0	45	***	***	***
bioMérieux NucliSens HIV-1 QT	0	7	***	***	***
In House	0	1	***	***	***
Chiron Procleix	0	6	n/a	n/a	n/a
Other	0	1	n/a	n/a	n/a

Table 8: Results of the HIV-1 RNA Determinations Reported by Participant Laboratories for the August 2003 Performance Evaluation Survey for CDC Donor #5

Donor Status: HIV-1 Uninfected and HIV-1 RNA Not Detected
Panel Vial Labels: A4, B4

	No. of Results Detecting RNA	No. of Results Not Detecting RNA	Range of Quantitative Results Reported (RNA copies/ml)		
Test Kit			**minimum**	**median (95% CI)**	**maximum**
Roche Amplicor HIV-1 Monitor	0	121	***	***	***
Bayer Versant HIV-1 RNA 3.0 Assay	0	45	***	***	***
bioMérieux NucliSens HIV-1 QT	0	7	***	***	***
In House	0	1	***	***	***
Chiron Procleix	0	6	n/a	n/a	n/a
Other	0	1	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 figure 2 depicts the results for donor 2 and donor 2 duplicate.

Fig. 1

Histogram of HIV-1 RNA Quantitative Determinations for Donor 1 in the August 2003 Performance Evaluation Survey

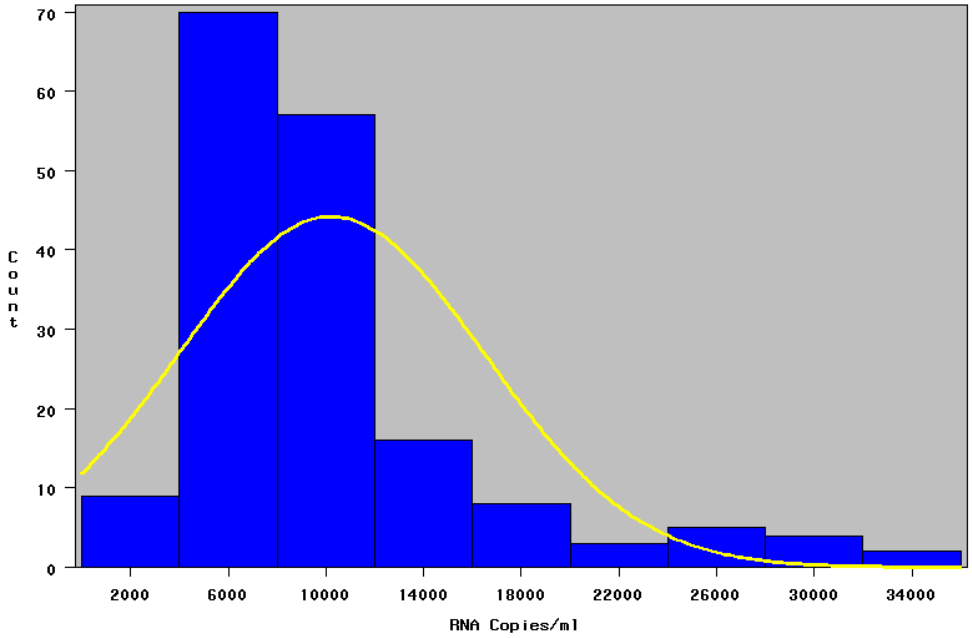
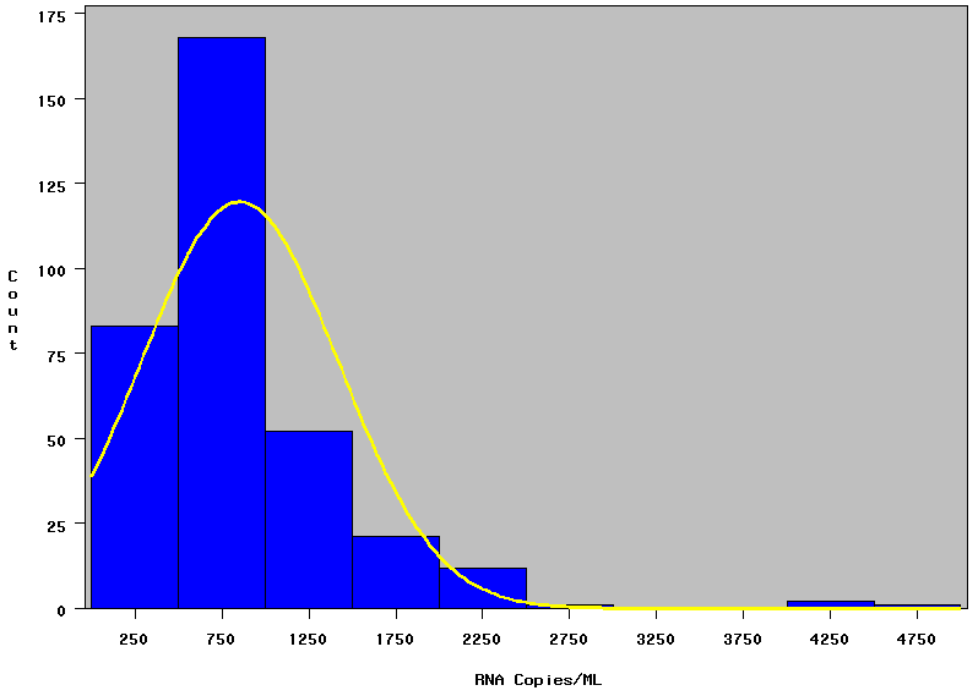


Fig. 2

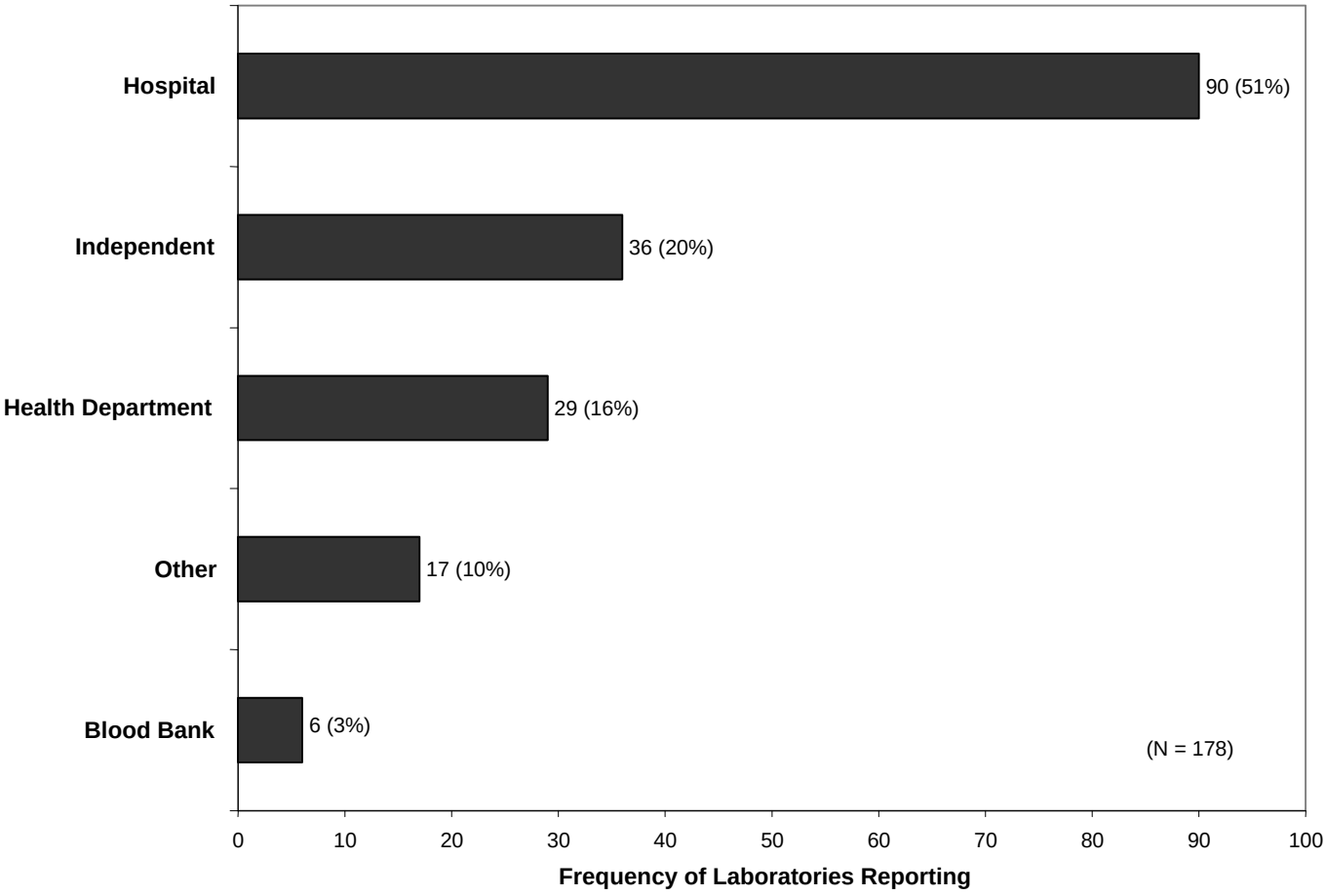
Histogram of HIV-1 RNA Quantitative Determinations for Donor 2 and Donor 2 Duplicate in the August 2003 Performance Evaluation Survey



Types of Laboratories Performing HIV-1 RNA Determinations

Figure 3 shows the type of laboratory reporting quantitative or qualitative HIV-1 RNA results. Among the 178 reporting laboratories, each type is listed by decreasing frequency.

Fig. 3 Types of Laboratories Performing HIV-1 RNA Determinations in the August 2003 Performance Evaluation Survey



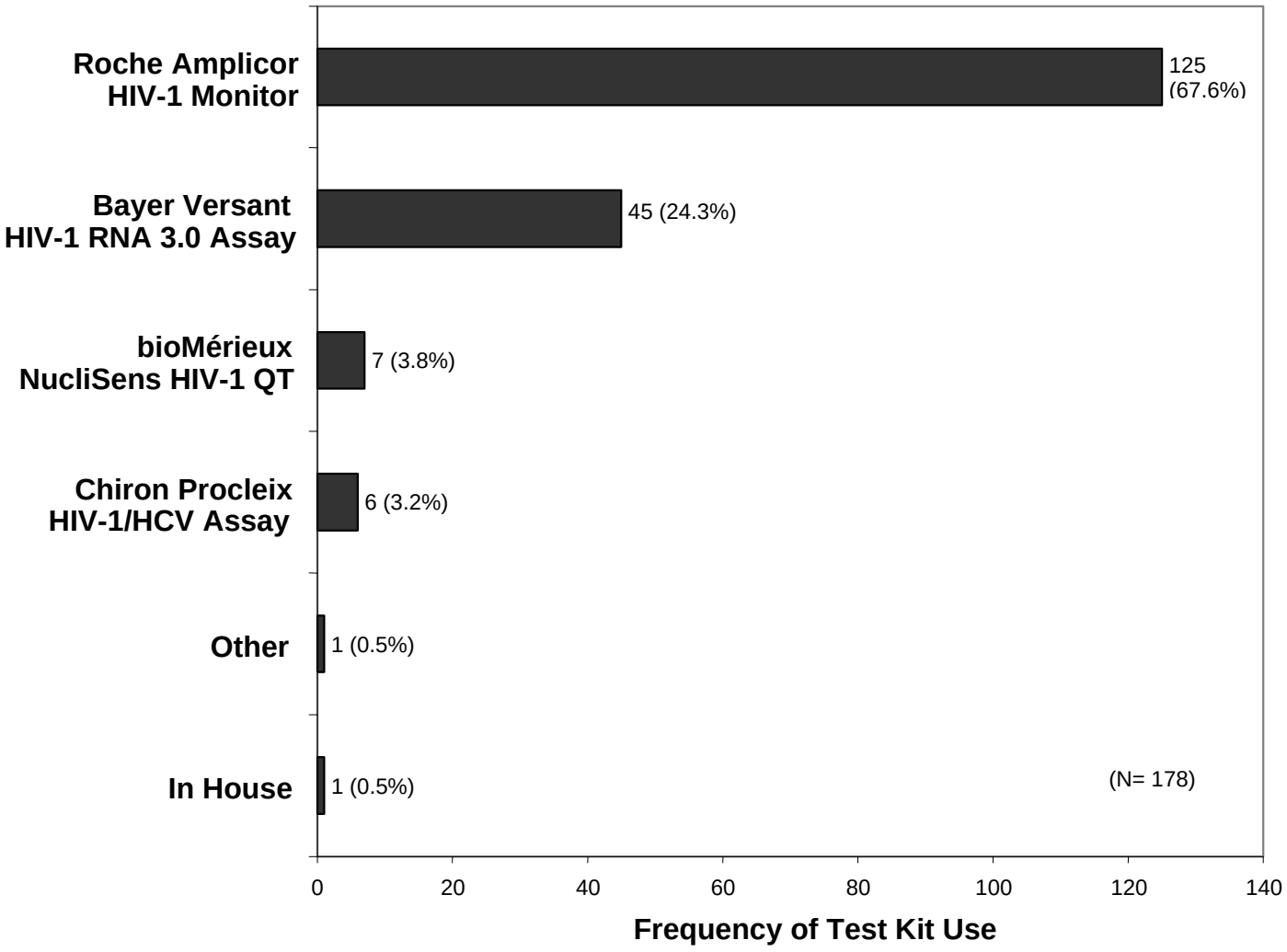
Types of Test Kits Used by Laboratories

The type of test kit used by laboratories performing viral RNA quantitative and qualitative determinations are shown in Figure 4 and are listed by decreasing frequency. Please note that some laboratories used more than one test kit, which explains why the total number of tests reported (n =185) exceeds the number of laboratories reporting results (N =178). The three most frequently used quantitative commercial kits, Roche's Amplicor HIV-1 Monitor®, Bayer's Versant® HIV-1 RNA 3.0 Assay (bDNA), and bioMérieux's NucliSens® HIV-1 QT, have been approved by

the FDA. Of the quantitative kits, the Roche's Amplicor HIV-1 Monitor® test kit was used most frequently (125, 67.6%) in reporting results. Six of the seven participating laboratories that reported using qualitative RNA testing procedures used the HIV-1/HCV assay, which was developed by Gen-Probe and is marketed by Chiron under the name of Procleix^{TM} HIV-1/HCV Assay. One lab reported results using a nonspecified "Other" commercial qualitative 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 August 2003 Performance Evaluation Survey



Use of External Quality Control (QC) Testing Material

Of the 178 laboratories that reported results in the current survey:

- 97.2% (173/178) provided information on external QC samples
  - 53.2% (92/173) of those providing external QC information indicated that they used external QC samples, and of these labs 91/92 (98.9%) specified the source of their external QC material:
    - 57.1% (52/91) used commercial material
    - 40.7% (37/91) used In-House material
    - 2.2% (2/91) used both commercial and In-House external QC material
  - 46.8% (81/173) did not use external QC samples
- 2.8% (5/178) 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 results, when compared with the previous performance survey, remained approximately the same; no false-negative results were reported for the current survey. While there is continued variability of results within kit manufacturer and between 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 99.0%.