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 2002

CENTERS FOR DISEASE™
CONTROL AND PREVENTION

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

Use of trade names is for identification only and does not constitute endorsement by the Department of Health and Human Services.

Report of the August 2002 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..... Edward L. Baker, M.D., M.P.H., Director
Division of Laboratory Systems..... Robert Martin, M.P.H., Dr.P.H., Director
Laboratory Practice Assessment Branch..... Devery Howerton, Ph.D., Chief

The content was developed by:

Model Performance Evaluation Program (MPEP)..... Laurina O. Williams, Ph.D., Co-Lead
..... G. David Cross, M.S., Co-Lead

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.

TABLE OF CONTENTS

Introduction……..... 3

Methods and Materials…….…….....3

Sample Identification and CDC Donor Testing Results..... 4-5

Results …….....……..... 6-13

Quantitative Test Results, Infected Donors and Uninfected Donors…… 6

Test Kit Lower Limit Sensitivities..... 7

Quantitative Test Aggregate Outcomes Reported…… 8- 10

Types of Laboratories Reporting Quantitative and/or Qualitative Results……..... 11

Types of Quantitative and Qualitative Test Kits Used by Laboratories…… 11 -12

Laboratory Performance..... 12

Use of Quality Control Materials……..... 13

Conclusion……..……..... 13

TABLES

Table 1. Panel and Vial Designations; Donor numbers and HIV status..... 4

Table 2. CDC HIV-1 RNA Panel Testing Results for the August 13, 2002 Survey..... 5

Table 3. Results Compiled for HIV-1 RNA Quantitative Determinations from the Three Infected Donors and from Two Uninfected Donors..... 6

Table 4. Test Kit Lower Limit Sensitivities..... 7

Table 5A. HIV-1 RNA Quantitative Determinations for Donor #2..... 8

Table 5B. HIV-1 RNA Quantitative Determinations for Donor #2 Duplicate..... 9

Table 6. HIV-1 RNA Quantitative Determinations for Donor #1..... 9

Table 7. HIV-1 RNA Quantitative Determinations for Donor #4..... 10

Table 8. HIV-1 RNA Quantitative Determinations for Donor #5..... 10

FIGURES*

Figure 1. Number of HIV-1 RNA Participant Laboratories, by Laboratory Type..... 11

Figure 2. Percentages of Laboratories Using the Various Types of Test Kits..... 12

* The "N=" on Figures 1 and 2 represents the number of laboratories that reported results. For some graphs, 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 2002 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 13, 2002. Testing results were reported by 185 (89.8%) of the 206 laboratories who were sent sample panels.

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 5 and 6 give 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, A3	2	Positive	Infected	_____	_____
	A2	4	Negative	Uninfected	_____	_____
	A4	1	Positive	Infected	_____	_____
	A5	5	Negative	Uninfected	_____	_____
B	B1	4	Negative	Uninfected	_____	_____
	B2, B4	2	Positive	Infected	_____	_____
	B3	5	Negative	Uninfected	_____	_____
	B5	1	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)
Model Performance Evaluation Program for
Human Immunodeficiency Virus Type 1 (HIV-1) Ribonucleic Acid (RNA) Testing

Table 2 CDC HIV-1 RNA Testing Results for the August 13, 2002 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, 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
	A2	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	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
	A5	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
B	B1	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
	B2, B4	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
	B3	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
	B5	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

[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

The cumulative frequencies of quantitative test results for all donor samples reported by laboratories are shown in Table 3. This table describes the final test interpretations (positive for HIV-1 RNA or negative for HIV-1 RNA) with respect to the donors' status (infected or uninfected) and includes the data for all of the different test kits used. The first row has the data 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 describes data for donors not infected with HIV-1 and in whose donor plasma HIV-1 RNA was not detectable. In this case, "Percent Correct" gives 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 of the 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.

Table 3	**Number of Results**	**Percent Correct**	**Percent False Negative**	**Percent False Positive**
Infected Donor Samples	549	98.5% (541/549)	1.5% (8/549)	n/a
Uninfected Donor Samples	366	97.3% (356/366)	n/a	2.7% (10/366)
TOTAL RESULTS	915	98.0% (897/915)	-----	-----

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, based on each type of test kit used. For each test kit, the percentage of the total reported results for each specified lower limit sensitivity is given. For each particular HIV-1 RNA test kit, "n" is the number of 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 = 625)	70% (435)	400
	24% (150)	50
	1% (5)	200
	5% (35)	not indicated
Bayer Versant® HIV-1 RNA 3.0 Assay (bDNA) (n = 230)	96% (220)	50
	2% (5)	510
	2% (5)	not indicated
bioMérieux NucliSens® HIV-1 QT (n = 45)	33% (15)	160
	11% (5)	40
	11% (5)	80
	11% (5)	100
	11% (5)	200
	11% (5)	400
	11% (5)	not indicated
In-House (n = 15)	67% (10)	100
	33% (5)	20

Quantitative Test Aggregate Outcomes

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 2002). The results columns provide the totals for results detecting HIV-1 RNA and not detecting HIV-1 RNA, as well as the median and range of values (number of RNA copies/ml sample) for the results that reported detectable RNA levels. The ranges of these results include the absolute minimum and maximum quantitatively calculated values as reported by type of test kit and irrespective of the different kits' lower limit sensitivities. The lower limit sensitivities of the reported test kits ranged from 20 RNA copies/ml to 510 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 5A shows the laboratory test results reported for CDC Donor 2. Table 5B has the information resulting from the duplicated specimen, Donor 2 Duplicate.

Table 5A: Results of the HIV-1 RNA Quantitative Determinations Reported by Participant Laboratories for the August 2002 Performance Evaluation Survey for CDC Donor #2

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**	**median**	**maximum**
Roche Amplicor HIV-1 Monitor®	122	3	413	895	2,972
Bayer Versant® HIV-1 RNA 3.0 Assay (bDNA)	46	0	596	955	1,700
bioMérieux NucliSens® HIV-1 QT	8	1	380	985	2,000
In House	3	0	430	790	900

Table 5B: Results of the HIV-1 RNA Quantitative Determinations Reported by Participant Laboratories for the August 2002 Performance Evaluation Survey for CDC Donor #2 Duplicate

Donor Status: HIV-1 Infected and HIV-1 RNA Detected
Panel Vial Labels: A3, B4

	No. of Results Detecting RNA	No. of Results Not Detecting RNA	Range of Quantitative Results Reported (RNA copies/ml)		
Test Kit			**minimum**	**median**	**maximum**
Roche Amplicor HIV-1 Monitor®	122	3	425	881	2,393
Bayer Versant® HIV-1 RNA 3.0 Assay (bDNA)	46	0	446	895	1,535
bioMérieux NucliSens® HIV-1 QT	9	0	300	950	1,600
In House	3	0	618	809	1,900

Table 6 shows the results reported for Donor 1, also an HIV-1 infected donor.

Table 6: Results of the HIV-1 RNA Quantitative Determinations Reported by Participant Laboratories for the August 2002 Performance Evaluation Survey for CDC Donor #1

Donor Status: HIV-1 Infected and HIV-1 RNA Detected
Panel Vial Labels: A4, B5

	No. of Results Detecting RNA	No. of Results Not Detecting RNA	Range of Quantitative Results Reported (RNA copies/ml)		
Test Kit			**minimum**	**median**	**maximum**
Roche Amplicor HIV-1 Monitor®	124	1	2,591	11,048	32,703
Bayer Versant® HIV-1 RNA 3.0 Assay (bDNA)	46	0	3,832	6,968	10,093
bioMérieux NucliSens® HIV-1 QT	9	0	14,000	19,000	26,000
In House	3	0	1,180	4,402	7,500

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 Quantitative Determinations Reported by Participant Laboratories for the August 2002 Performance Evaluation Survey for CDC Donor #4

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	median	maximum
Roche Amplicor HIV-1 Monitor®	3	122	400	494	805
Bayer Versant® HIV-1 RNA 3.0 Assay (bDNA)	1	45	50	50	50
bioMérieux NucliSens® HIV-1 QT	0	9	< kit sens.*	< kit sens.	< kit sens.
In House	1	2	39	39	39

Table 8: Results of the HIV-1 RNA Quantitative Determinations Reported by Participant Laboratories for the August 2002 Performance Evaluation Survey for CDC Donor #5

Donor Status: HIV-1 Uninfected and HIV-1 RNA Not Detected
Panel Vial Labels: A5, B3

Test Kit	No. of Results Detecting RNA	No. of Results Not Detecting RNA	Range of Quantitative Results Reported (RNA copies/ml)		
			minimum	median	maximum
Roche Amplicor HIV-1 Monitor®	3	122	667	741	814
Bayer Versant® HIV-1 RNA 3.0 Assay (bDNA)	2	44	50	69	88
bioMérieux NucliSens® HIV-1 QT	0	9	< kit sens.*	< kit sens.	< kit sens.
In House	0	3	< kit sens.	< kit sens.	< kit sens.

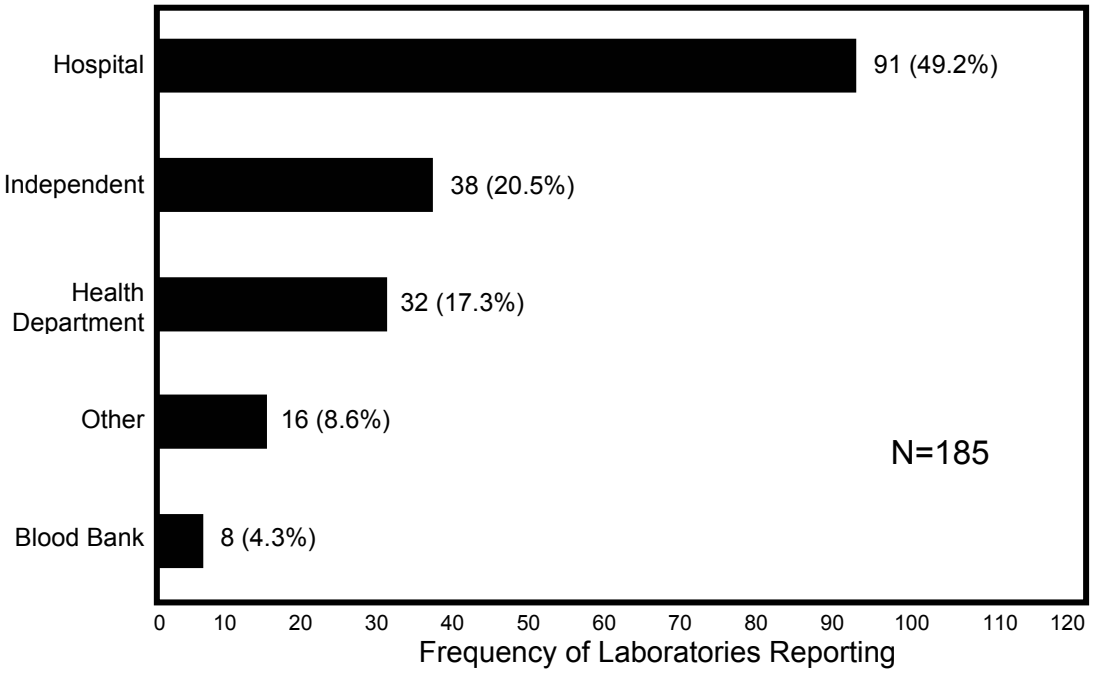
*** = less than test kit lower limit sensitivity**

Types of Laboratories Performing HIV-1 RNA Determinations

Figure 1 (below) shows the types of laboratories reporting quantitative or qualitative HIV-1 RNA results. Among the 185 laboratories reporting results, each laboratory type is listed by decreasing frequency.

Fig. 1

Types of Laboratories Performing HIV-1 RNA Determinations* in the August 2002 Performance Evaluation Survey



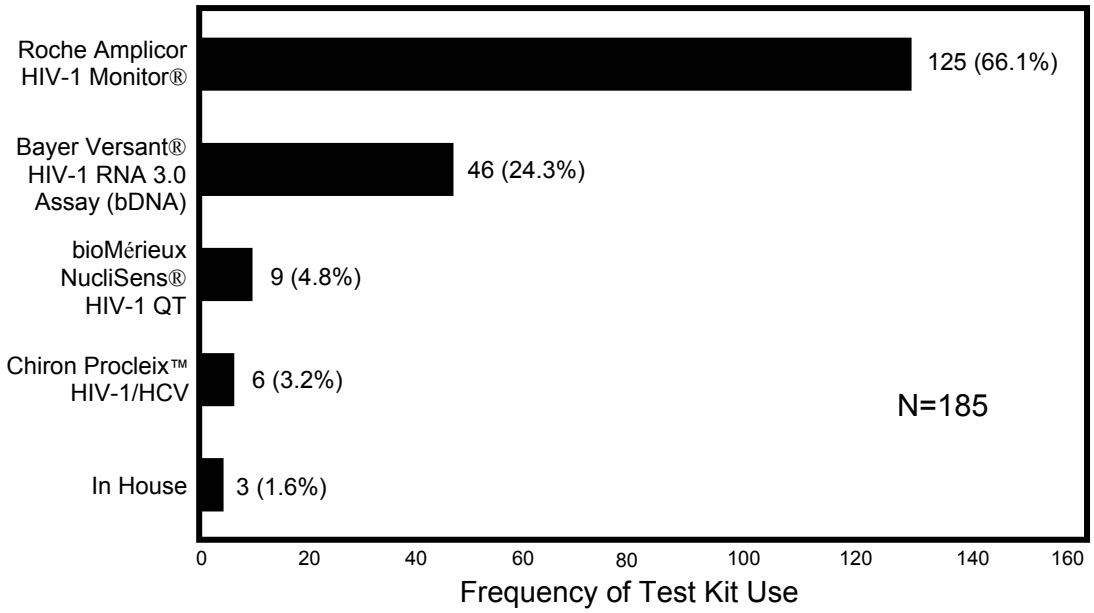
***Qualitative and Quantitative**

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 2 and are listed by decreasing frequency. Please note that some laboratories used more than one test kit, which explains why in this figure the total number of tests reported exceeds the number of laboratories reporting results (N=185). 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, 66.1%), in reporting results. Six of the eight participating Blood Bank laboratories used the FDA approved 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 six test results (3.2%) were the only qualitative test results reported in this survey.

Fig. 2

Types of Test Kits Used to Perform HIV-1 RNA Determinations* in the August 2002 Performance Evaluation Survey



***Qualitative and Quantitative**

Laboratory Performance

Laboratories performed generally well in the testing of these performance evaluation samples. The percentage of false-positive quantitative results, 2.7% (10/366), was increased compared with the percentage of false-positive results, 1.4% (5/361) reported in the previous (February 2002) performance survey. However, it should be noted that different donors were used in the two surveys. The 10 false-positive results in the current survey were reported for both Donor 4 and Donor 5; six results were obtained using the Roche's Amplicor HIV-1 Monitor® test, three results were obtained using Bayer's Versant® HIV-1 RNA 3.0 Assay (bDNA), and the other false-positive result occurred using a kit manufactured in-house.

The percentage of false-negative quantitative results (1.5%, 8/549) reported in this performance survey was similar to that of the previous survey (1.1%, 6/540). Seven 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. Seven of the eight false-negatives were obtained using Roche's Amplicor HIV-1 Monitor® test for either Donor 1 or Donor 2, and the one other result was obtained using bioMérieux's NucliSens® HIV-1 QT kit.

The six laboratories that submitted reports indicating the use of qualitative tests had no false-positive or false-negative results.

Use of Quality Control Testing Material

Information was collected on the use of external quality control (QC) samples in addition to the controls contained in the test kits. Depending on the manufactured test kit used, internal kit control samples are one of the following types: positive and negative test controls, test standards, or test calibrators. These internal controls are used to validate a test run and to quantify HIV-1 RNA copies/ml. These kit controls may not validate the analytic testing process, which may include testing problems related to pipetting, inadequate incubation conditions, inadequate washing, or variability in kit lot sensitivity. The use of external QC materials is summarized below.

Of the 185 laboratories that reported results in the current survey:

- 93% (172/185) provided information on external QC samples
  - 51.2% (88/172) of those providing external QC information indicated that they used external QC samples, and of these labs:
    - 53.4% (47/88) used commercial material
    - 39.8% (35/88) used In-House material
    - 6.8% (6/88) used both commercial and In-House external QC material
  - 48.8% (84/172) did not use external QC samples
- 7% (13/185) of all reporting laboratories gave no external QC information

CONCLUSION

The results of this performance evaluation shipment for quantitative HIV-1 RNA determinations showed an increase in the relative number of false-positive results when compared with the previous performance survey, while the number of false-negative results remained approximately the same. This comparison should take into account the fact that different donor samples were tested in the previous survey. While there is continued variability of results within a 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 quantitative results in this performance survey was 98.0%.

Qualitative results for the Procleix^{TM} HIV-1/HCV Assay, marketed by Chiron, were included in the analysis for the first time in this performance survey. The analytic performance of these (n=6) reported tests was 100%.