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 during February 2005

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Centers for Disease Control and Prevention
Public Health Practice Program Office
Division of Laboratory Systems
Atlanta, Georgia 30341-3717

Report of the February 2005 Human Immunodeficiency Virus Type 1 (HIV-1) Ribonucleic Acid (RNA) Determinations Performance Evaluation Sample Testing Results Provided by Participant Laboratories in the Model Performance Evaluation Program (MPEP), Centers for Disease Control and Prevention (CDC)

Report of the February 2005 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 (MPEP), Centers for Disease Control and Prevention (CDC).

Report production

The production of this report was coordinated in CDC by:

Coordinating Center for Health Information and Services:…..	Edward J. Sondik, Ph.D., Blake Caldwell, M.D., M.P.H., Acting Directors
National Center for Health Marketing…	Steve L. Solomon, M.D., Acting Director
Division of Public Health Partnerships...	Robert Martin, M.P.H., Dr.P.H., Acting Director
Laboratory Practice Evaluation and Genomics Branch ……..	Devery Howerton, Ph.D., Chief
Model Performance Evaluation Program (MPEP)…….	Laurina O. Williams, Ph.D., M.P.H., G. David Cross, M.S. Co-Managers

---

Report content

The material in this report was developed and prepared by:

MPEP HIV-1 RNA Performance Evaluation…….	Suzette A. Park, M.S. HIV-1 RNA Project Coordinator

---

Contact Information

Questions about this report should be addressed to the Model Performance Evaluation Program by calling (770) 488-8097 or (770) 488-8130.

Table of Contents

Overview 4
Overview: Significant Findings 5
Donor Report 7
Donor Report: CDC HIV-1 RNA Testing Results 8
Laboratory Demographics and Methods 9
Kit Types Used by Participants 11
Summary of Results: Plasma MPEP Samples 12
Summary of Results: Simulated MPEP Samples 16
Quantitative and Qualitative Test Aggregate Results 18
Distribution of Qualitative Results by Sample 23
Discussion 25

TABLES

Table 1a. Results Summary (Donors 1, 2, 3 & 4)…….. 5

Table 1b. Results Summary (Donor 5 & 6)…… 5

Table 2. Donor Report…….. 7

Table 3. Donor Report: CDC HIV-1 RNA Testing Results……. 8

Table 4. Cumulative Frequencies of Test Results (Donors 1, 2, 3 & 4)…….. 12

Table 5. Test Kit Lower Limit Sensitivities Results by Test Kit (Donors 1, 2, 3 & 4)… 14

Table 6. Test Kit Lower Limit Sensitivities (Donors 1, 2, 3 & 4)……... 14

Table 7. Cumulative Frequencies of Test Results (Donor 5 & 6)…… 16

Table 8. Test Kit Lower Limit Sensitivities Results by Test Kit (Donors 5 & 6)…… 17

Table 9. HIV-1 RNA Determinations for Donor #1…… 19

Table 10A. HIV-1 RNA Determinations for Donor #2…… 19

Table 10B. HIV-1 RNA Determinations for Donor #2 Duplicate…… 20

Table 11. HIV-1 RNA Determinations for Donor #3…… 20

Table 12. HIV-1 RNA Determinations for Donor #4…… 21

Table 13. HIV-1 RNA Determinations for Donor #5 Simulated Sample……. 22

Table 14. HIV-1 RNA Determinations for Donor #6 Simulated Sample……. 22

FIGURES

Figure 1. Demographic Map: United States Participants……. 9

Figure 2. Types of Participant Laboratories…….. 10

Figure 3. Types of Test Kits…….. 11

Figure 4. Box Plot: Donor 1 and 6…… 23

Figure 5. Box Plot: Donor 2 and 5…… 24

Overview

Introduction

This report is an analysis of testing results reported by laboratories participating in the Centers for Disease Control and Prevention (CDC) Model Performance Evaluation Program (MPEP) for human immunodeficiency virus type 1 (HIV-1) ribonucleic acid (RNA) determinations performed using specimens sent on February 8, 2005.

Specimen panels

Each laboratory received a total of seven specimens. Five were plasma MPEP specimens obtained from individual donors (not pooled or diluted with plasma from other donors) and two were simulated samples. Overall, the shipment contained five HIV-1 antibody-positive and two HIV-1 antibody-negative samples.

- Before shipment, the CDC tested each donor with three viral RNA test kits approved by the Food and Drug Administration (FDA).

- One of the HIV-1 antibody-positive plasma specimens, Donor 2, was sent to the participant laboratories in duplicate. 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. The vial designations for this shipment were A1, A4, B1 and B2. These were the same samples sent out in the August 2004 shipment.

- **Two simulated samples**, Donor 5 and Donor 6 (vial designations A6, A7, B6, & B7) were included as a pilot test in this shipment to investigate their comparability with plasma MPEP specimens and their overall suitability as performance evaluation materials. These samples were prepared from highly purified infectious viruses, isolated from the plasma of infected individuals and rendered non-infectious while maintaining the integrity of the RNA. Donor 5 had a target value of 1,000 copies/ml and Donor 6 had a target value of 10,000 copies/ml.

- Not all laboratories received the same panel of specimens. Each laboratory received either Panel A or B.

Laboratory response

Of the 187 laboratories receiving specimen panels, 167 (89.3%) reported testing results.

- In general, the percentage of the laboratories reporting results has remained steady at about 89%-92% over the previous three shipments.

- The majority of the laboratories (115/167, 69%) reported their testing results using the online data entry system.

Note: We continue to encourage laboratories to use the online option as a method of streamlining the reporting process.

Continued on next page

Overview: Significant Findings

Table 1a: The following table summarizes the results grouped by test type for the **Plasma MPEP samples, donors 1, 2, 3, & 4.**

Results summary: Plasma MPEP samples

			Positive Donors		Negative Donors		Overall Performance
Method	**Total # of labs**	**Total # of results**	**Positive**	**False-negative**	**Negative**	**False-positive**	**(TP+TN/ total # results)[3]**
Quantitative[1]	159	824	491	3	318	12	98.2%
Qualitative[2]	8	40	24	0	16	0	100%
Total	167	864	518	3	334	12	98.3%

[1] Roche Amplicor HIV-1 Monitor, Bayer Versant HIV-1 RNA 3.0 Assay (bDNA), bioMérieux NucliSens® HIV-1 QT, bioMérieux NucliSens® EasyQ HIV-1 and In-house methods.
[2] Chiron Procleix method
[3] TP, true positives; TN, true negatives.

Table 1b: The following table summarizes the results grouped by test type for **Simulated samples, donors 5 and 6.**

Results summary, continued: Simulated Samples

			Positive Donors		Overall Performance
Method	**Total # of labs**	**Total # of results**	**Positive**	**False-negative**	**(TP+TN/ total # results)[3]**
Quantitative[1]	159	329	329	15	95.4%
Qualitative[2]	8	16	16	0	100%
Total	167	345	345	15	95.9%

[1] Roche Amplicor HIV-1 Monitor, Bayer Versant HIV-1 RNA 3.0 Assay (bDNA), bioMérieux NucliSens® HIV-1 QT, bioMérieux NucliSens® EasyQ HIV-1 and In-house methods.
[2] Chiron Procleix method
[3] TP, true positives; TN, true negatives.

False-negative results

The overall quality of testing performance for the Plasma MPEP samples as measured in this survey has improved compared to the previous shipment.

Plasma MPEP Samples

There were 0.6% (3/518) false-negative interpretations reported for Plasma MPEP samples for this shipment, compared to 3.5% (19/549) reported from the previous shipment.

Continued on next page

False-negative results (continued)

- Of the 3 false-negatives reported for Plasma MPEP samples, one was obtained using Roche's Amplicor HIV-1 Monitor® test, one using Bayer Versant® HIV-1 RNA 3.0 Assay (bDNA), and one using bioMerieux NucliSens EasyQ HIV-1.

Simulated Samples

There were 4.3% (15/345) false-negative interpretations reported for simulated donors 5 and 6 for this shipment.

- All fifteen of the false-negatives reported for simulated donors were associated with Donor 5 (1,000 copies/ml target).

- Of the 15 false-negatives reported for simulated donor 5, 13 were obtained using Roche's Amplicor HIV-1 Monitor® test, one using bioMerieux NucliSens EasyQ HIV-1, and one using bioMerieux NucliSens HIV-1 QT test.

False-positive results

The percentage of false-positive results, 3.5% (12/346), reported in this survey was similar to the previous survey false-positive rate of 3.3% (12/365).

- Of the 12 false-positives reported, six were associated with Donor 3 and six for Donor 4.

- Nine of the 12 false-positives were reported by laboratories using Bayer Versant HIV-1 RNA 3.0 Assay (bDNA). Of these nine, five had a lower limit sensitivity of 75 copies/ml. In the previous report, the majority of the false-positives were reported by laboratories using Roche's Amplicor HIV-1 Monitor® test with a lower limit sensitivity of 400 copies/ml.

Quality control

A total of 53.9% (90/167) of respondents indicated that they used external quality control materials.

Donor Report

Overview The Donor Report contains the specimen numbers and donor information for each performance evaluation specimen. Table 2, below, is provided for the participant laboratories to record and compare their results with CDC MPEP results for each performance evaluation specimen.

Table 2 Donor Identification for February 2005 Shipment Specimens

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	2	Positive	Infected	_____	_____
	A2	3	Negative	Uninfected	_____	_____
	A3	4	Negative	Uninfected	_____	_____
	A4	2	Positive	Infected	_____	_____
	A5	1	Positive	Infected	_____	_____
	*A6	5	Positive	Infected	_____	_____
	*A7	6	Positive	Infected	_____	_____
B	B1	2	Positive	Infected	_____	_____
	B2	2	Positive	Infected	_____	_____
	B3	3	Negative	Uninfected	_____	_____
	B4	1	Positive	Infected	_____	_____
	B5	4	Negative	Uninfected	_____	_____
	*B6	6	Positive	Infected	_____	_____
	*B7	5	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.

* Samples A6, A7, B6 and B7 were the simulated infected samples.

Continued on next page

Donor Report: CDC HIV-1 RNA Testing Results

Table 3 CDC HIV-1 RNA Testing Results for the February 8, 2005, Participant Laboratory Panel Samples

Panel Letter	Vial Label	CDC Donor Number	CDC Test Results[1]	Manufacturer Test Kit	CDC Interpretation[2]
A	A1, 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
	A2	3	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
	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
	A6	5	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
	A7	6	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, B2	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	3	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
	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
	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
	B6	6	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
	B7	5	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)

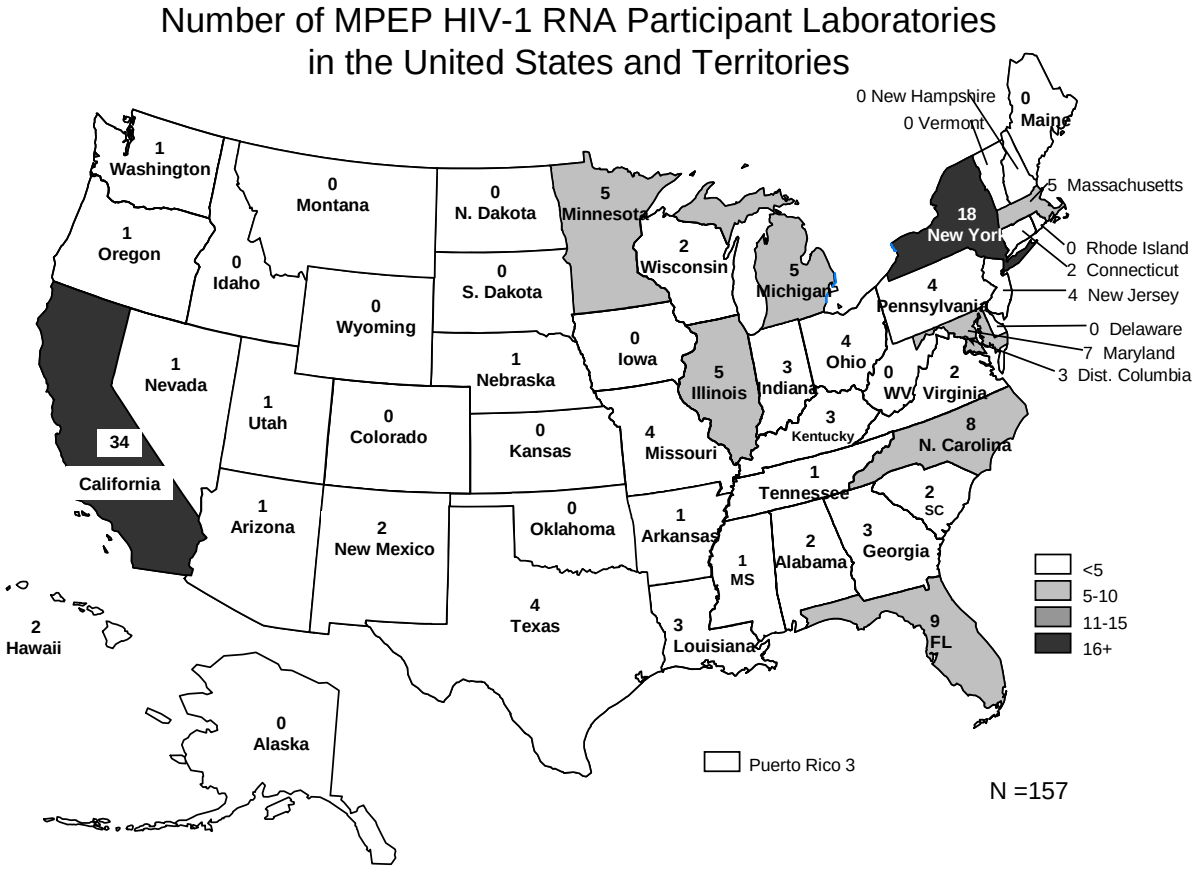
Laboratory Demographics and Methods

Overview

A total of 167 different laboratories submitted results. Of these:

- the 157 United States and U.S. associated laboratories are depicted in ***Figure 1.***
- 10 testing sites were Canadian laboratories.
- ***Figure 2*** shows the primary classification of laboratories reporting quantitative or qualitative HIV-1 RNA results.
    - Hospital laboratories predominated.

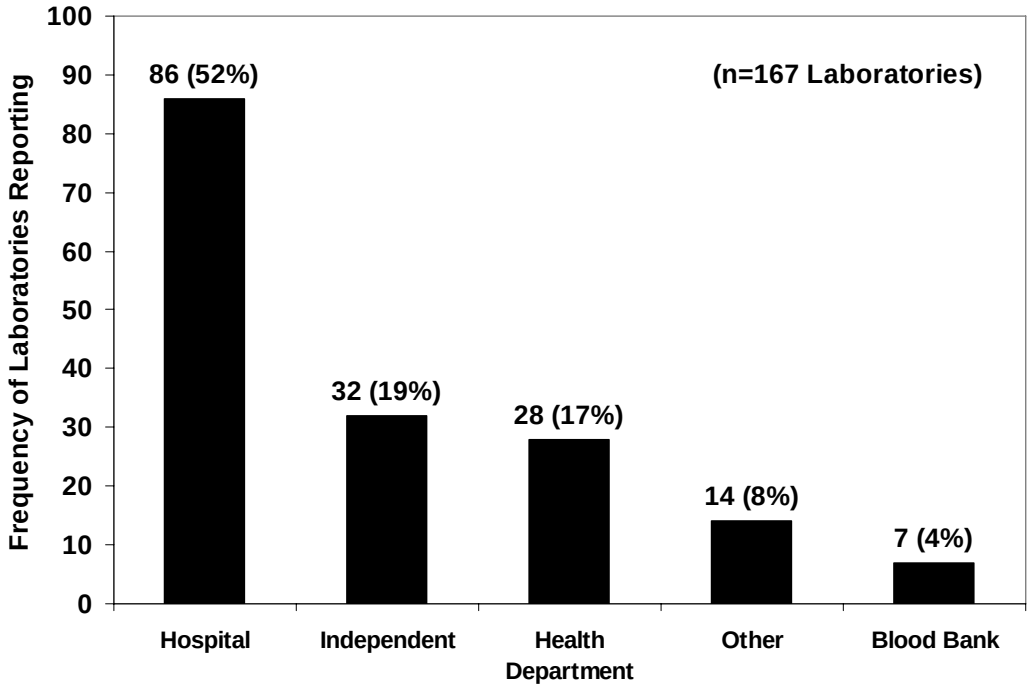
Figure 1



Continued on next page

Figure 2: Types of Participant Laboratories

Test methods by laboratory type



Kit Types Used by Participants

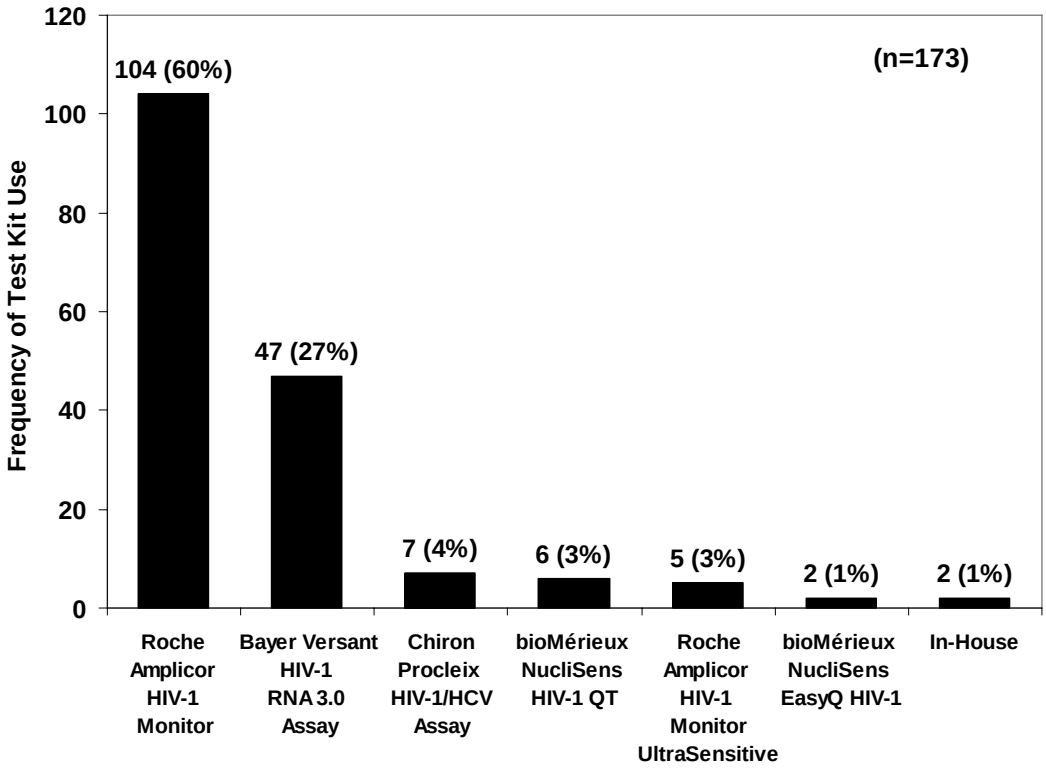
Overview

This section describes the types of test kits used by laboratories performing viral RNA quantitative and qualitative determinations in this survey.

- Roche's Amplicor HIV-1 Monitor® test kit was used most frequently (104/173, 60%) in reporting results.
- Seven of the eight 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™ HIV-1/HCV Assay.

Note: The "n=" on Figure 3 represents the number of reported results. For this graph, some laboratories used more than one test kit, therefore, the number of results reported (n =173) exceeds the number of laboratories reporting results (n =167).

Figure 3 Types of Test Kits Used to Perform HIV-1 RNA Determinations



Summary of Results: Plasma MPEP Samples

Overview

There were 12 false-positives (12/346, 3.5%) reported in the current survey, similar to the 12 false-positive (12/365, 3.3%) results reported in the previous performance survey (August 2004). The percentage of false-negative results (0.6%, 3/518) reported for plasma MPEP donors 1, 2, 3 and 4 in this survey was less than that of the previous survey (3.5%, 19/549).

Summary of participant results

Table 4 contains the cumulative frequencies of quantitative and qualitative test results for all donor samples reported by participants. 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.

Table 4:

Cumulative frequencies of test results

	Number of Results	Percent Correct	Percent False Negative	Percent False Positive
Infected Donor Samples	518	99.4% (515/518)	0.6% (3/518)	n/a
Uninfected Donor Samples	346	96.7% (334/346)	n/a	3.5% (12/346)
TOTAL RESULTS	864	98.3% (849/864)	***	***

Continued on next page

Test kit lower limit sensitivities

Table 5 shows the false-positive and false-negative results categorized by kit type and lower limit sensitivities.

Table 6 shows the variability in the lower limit sensitivities reported by the laboratories using commercially manufactured quantitative HIV-1 RNA test kits. The lower limit sensitivities of the reported quantitative kits ranged from 25 RNA copies/ml to 400 copies/ml. 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 results reported using that test kit.

False Negative Results

Three false-negative results were reported using the following test kits and LLS:

- One reported using Roche's Amplicor HIV-1 Monitor® test; the reported LLS associated with this result was 50 copies/mL, and
- One reported using Bayer Versant HIV-1 RNA 3.0 Assay; the reported LLS associated with this result was 50 copies/mL, and
- One reported using bioMérieux NucliSens® EasyQ HIV-1; the reported LLS associated with this result was 357 copies/mL.

False Positive Results

Twelve false-positives results were reported using the following test kits and LLS:

- Nine of the twelve false positive results were obtained using Bayer Versant HIV-1 RNA 3.0 Assay; the reported LLS associated with five results was 75 copies/ml, the reported LLS with two was 50 copies/mL, and two of the results had missing LLS.
- Three false positive results were reported using Roche's Amplicor HIV-1 Monitor® test; the reported LLS associated with two results was 400 copies/mL, and one result reported an associated LLS of 50 copies/mL.

Continued on next page

Table 5:

LLS Results by Kit Manufacturer (Plasma MPEP samples)

Manufacturer	Total # of Results	FP*	LLS‡ for FP # of Results	LLS for FP LLS	FN†	LLS for FN # of Results	LLS for FN LLS
Roche Amplicor HIV-1 Monitor	514	3 (%0.6)	1 2	50 400	1 (0.2%)	1	50
Bayer Versant HIV-1 RNA 3.0 Assay (bDNA)	235	9 (3.8%)	2 5 2	50 75 missing	1 (0.4%)	1	50
bioMérieux NucliSens HIV-1 QT	30	0			0		
bioMérieux NucliSens® EasyQ HIV-1	10	0			1 (10%)	1	357
Roche Amplicor HIV-1 Monitor UltraSensitive	15	0			0		
In House	5	0			0		
Other	15	0			0		
Total	824	12 (1.5%)			3 (0.4%)		

*FP, False-positives †FN, False-negatives ‡LLS, Lower Limit Sensitivity Used (copies/ml)

Table 6 (For plasma donors)

Manufacturer Test Kit (n = number of reports)	Lower Limit Sensitivity Used (copies/ml)	Percent of Reports (n) for each kit type
Roche Amplicor HIV-1 Monitor® (n = 520)	40	1% (5)
	50	33% (170)
	400	61% (315)
	not indicated	6% (30)
Bayer Versant® HIV-1 RNA 3.0 Assay (bDNA) (n= 235)	50	11%(25)
	75	85% (200)
	not indicated	4 % (10)
bioMérieux NucliSens® HIV-1 QT (n= 30)	25	33% (10)
	160	33% (10)
	200	17% (5)
	250	17%(5)
bioMérieux NucliSens® EasyQ HIV-1 (n=10)	25	50% (5)
	357	50%(5)
Roche Amplicor HIV-1 Monitor® UltraSensitive (n=25)	50	100% (25)
In-House (n= 5)	30	100% (5)

Continued on next page

Summary of Results: Plasma MPEP Samples, Continued

Results by donor

Of the 12 false-positive quantitative results reported (12/330, 3.6%), six were associated with Donor 3 and six with Donor 4. Out of the 3 false-negatives reported for donors 1 and 2, one each was associated with Donors 1, 2, and 2 duplicate.

Donor 1 (HIV-1 infected, high-positive)	1 false-negative
Donor 2 (HIV-1 infected, low-positive)	1 false-negative
Donor 2 Duplicate (HIV-1 infected, low-positive)	1 false-negative
Donor 3 (HIV-1 negative)	6 false-positives
Donor 4 (HIV-1 negative)	6 false-positives

Summary of Results: Simulated MPEP Samples

Overview

The percentage of false-negative results for Simulated donors 5 and 6 was 4.3% (15/345). Simulated Donor 5 comprised the "low-positive" samples, with a target value of approximately 1,000 RNA copies/ml, whereas Simulated Donor 6 had a target value of approximately 10,000 RNA copies/ml. All false-negative results were reported for Donor 5.

Summary of participant results

Table 7 contains the cumulative frequencies of quantitative and qualitative test results for Simulated donor samples reported by laboratories.

Table 7:

Cumulative frequencies of test results: donors 5 & 6

	Number of Results	Percent Correct	Percent False Negative	Percent False Positive
Infected Donor Samples	345	95.7% (330/345)	4.3% (15/345)*	n/a
Uninfected Donor Samples	n/a	n/a	n/a	n/a
TOTAL RESULTS	345	95.7% (330/345)	***	***

*All false-negatives were reported for Donor 5.

LLS results by kit manufacturer simulated donors 5 & 6

Table 8 shows the variability in the lower limit sensitivities reported by the laboratories using commercially manufactured quantitative HIV-1 RNA test kits for the Simulated Donors 5 and 6.

False Negative Results and Lower Limit Sensitivity (LLS)

Of the 15 false-negative results reported:

- Thirteen were reported using Roche's Amplicor HIV-1 Monitor® test; eleven of these reports specified using a Lower Limit Sensitivity (LLS) of 400 copies/mL and one specified using a Lower Limit Sensitivity (LLS) of 50 copies/mL (one report did not specify a LLS).
- One reported using bioMérieux NucliSens® EasyQ HIV-1; the reported LLS associated with this result was 357 copies/mL, and
- One reported using bioMérieux NucliSens® HIV-1 QT; the reported LLS associated with this result was 250 copies/mL.

Continued on next page

Table 8:

LLS results by kit manufacturer simulated donors 5 & 6

			LLS for FN	
Manufacturer	**Total # of Results**	**FN[†]**	**# of Results**	**LLS**
Roche Amplicor HIV-1 Monitor	206	13 (6.3%)	1 11 1	50 400 missing
Bayer Versant HIV-1 RNA 3.0 Assay (bDNA)	94	0		
bioMérieux NucliSens HIV-1 QT	12	1 (8.3%)	1	250
bioMérieux NucliSens® EasyQ HIV-1	4	1 (25%)	1	357
Roche Amplicor HIV-1 Monitor UltraSensitive	6	0		
In House	2	0		
Other	5	0		
Total	329	15 (4.6%)		

[*]FP, False-positives [†]FN, False-negatives [‡]LLS, Lower Limit Sensitivity Used (copies/ml)

Continued on next page

Quantitative and Qualitative Test Aggregate Results

Aggregate test results

Tables 9 through 12 show the aggregate participant laboratories' testing results for each Plasma MPEP donor sample by test kit manufacturer.

Tables 13 and 14 show the aggregate participant laboratories' testing results for each simulated donor sample by test kit manufacturer.

Description: Tables 9-14

Result columns provide the totals for the number of results reported detecting HIV-1 RNA and not detecting HIV-1 RNA.

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.

Description: Table 10A and 10B, Duplicate sample

Table 10A shows the laboratory test results reported for Donor 2 and table 10B shows results for the duplicated specimen, Donor 2 Duplicate.

- 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. These were the same samples that were shipped during the previous (August 2004) shipment.

Continued on next page

Table 9 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: 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	103	1	460	63700	6300	**7300**	10271
Bayer Versant HIV-1 RNA 3.0 Assay	47	0	939	10199	4961	**5409**	6313
bioMérieux NucliSens HIV-1 QT	6	0	3800	8000	4400	**5650**	7850
Chiron Procleix	7	0	n/a	n/a	n/a	**n/a**	n/a
bioMérieux NucliSens EasyQ HIV-1	2	0	7500	8600	7500	**8050**	8600
Roche Amplicor HIV-1 Monitor UltraSensitive	5	0	4685	9779	8680	**9347**	9568
In House	2	0	15807	15807	n/a	**n/a**	n/a

Table 10A 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: A1, 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	104	0	388	4288	751	**982**	1313
Bayer Versant HIV-1 RNA 3.0 Assay	47	0	689	2303	934	**1080**	1335
bioMérieux NucliSens HIV-1 QT	6	0	130	1600	620	**1080**	1500
Chiron Procleix	7	0	n/a	n/a	n/a	**n/a**	n/a
bioMérieux NucliSens EasyQ HIV-1	1	1	230	430	230	**330**	430
In House	2	0	2135	2135	n/a	**n/a**	n/a
Roche Amplicor HIV-1 Monitor UltraSensitive	5	0	740	1050	847	**913**	1050

Continued on next page

Table 10B Results of the HIV-1 RNA Determinations Reported by Participant Laboratories for Donor #2 Duplicate
Donor Status: HIV-1 Infected and HIV-1 RNA Detected
Panel Vial Labels: A4, B2

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	103	0	453	18000	863	**1090**	1500
Bayer Versant HIV-1 RNA 3.0 Assay	46	1	599	1858	899	**1095**	1246
bioMérieux NucliSens HIV-1 QT	6	0	270	1700	430	**905**	1100
Chiron Procleix	7	0	n/a	n/a	n/a	**n/a**	n/a
bioMérieux NucliSens EasyQ HIV-1	2	0	710	1400	710	**1055**	1400
In House	2	0	2386	2386	n/a	**n/a**	n/a
Roche Amplicor HIV-1 Monitor UltraSensitive	5	0	761	1089	928	**956**	974

Table 11 Results of the HIV-1 RNA Determinations Reported by Participant Laboratories for Donor #3
Donor Status: HIV-1 Uninfected and HIV-1 RNA Not Detected
Panel Vial Labels: A2, 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	2	102	50	1710	50	**880**	1710
Bayer Versant HIV-1 RNA 3.0 Assay	4	43	76	110	79	**95**	109
bioMérieux NucliSens HIV-1 QT	0	6	n/a	n/a	n/a	**n/a**	n/a
Chiron Procleix	0	7	n/a	n/a	n/a	**n/a**	n/a
bioMérieux NucliSens EasyQ HIV-1	0	2	n/a	n/a	n/a	**n/a**	n/a
In House	0	2	n/a	n/a	n/a	**n/a**	n/a
Roche Amplicor HIV-1 Monitor UltraSensitive	0	5	n/a	n/a	n/a	**n/a**	n/a

Continued on next page

Table 12 Results of the HIV-1 RNA Determinations Reported by Participant Laboratories for the 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	1	103	50	50	n/a	**n/a**	n/a
Bayer Versant HIV-1 RNA 3.0 Assay	5	42	53	5487	81	**121**	468
bioMérieux NucliSens HIV-1 QT	0	6	n/a	n/a	n/a	**n/a**	n/a
Chiron Procleix	0	7	n/a	n/a	n/a	**n/a**	n/a
bioMérieux NucliSens EasyQ HIV-1	0	2	n/a	n/a	n/a	**n/a**	n/a
In House	0	2	n/a	n/a	n/a	**n/a**	n/a
Roche Amplicor HIV-1 Monitor UltraSensitive	0	5	n/a	n/a	n/a	**n/a**	n/a

Continued on next page

Table 13: Simulated samples

Results of the HIV-1 RNA Determinations Reported by Participant Laboratories for the Donor #5
Donor Status: HIV-1 Infected and HIV-1 RNA Detected
Panel Vial Labels: A6, B7

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	91	13	135	8210	505	**626**	864
Bayer Versant HIV-1 RNA 3.0 Assay	47	0	221	7076	952	**669**	752
bioMérieux NucliSens HIV-1 QT	5	1	300	1300	330	**360**	600
Chiron Procleix	7	0	n/a	n/a	n/a	**n/a**	n/a
bioMérieux NucliSens EasyQ HIV-1	1	1	53	510	53	**282**	510
In House	2	0	820	820	n/a	**n/a**	n/a
Roche Amplicor HIV-1 Monitor UltraSensitive	5	0	453	618	479	**581**	618
Other	3	0	453	1139	453	**581**	1139

Table 14: Simulated samples

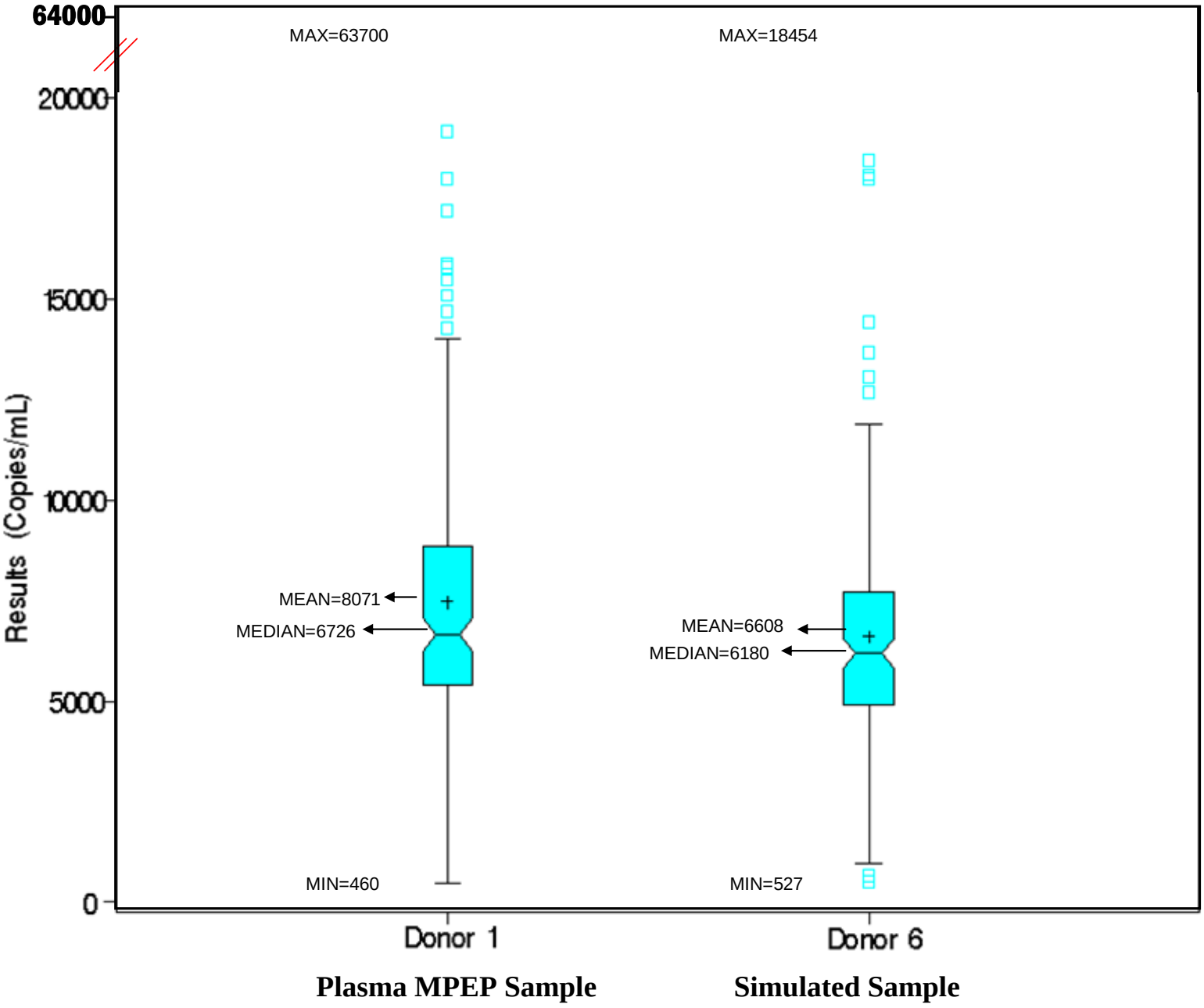
Results of the HIV-1 RNA Determinations Reported by Participant Laboratories for the Donor #6
Donor Status: HIV-1 Infected and HIV-1 RNA Detected
Panel Vial Labels: A7, B6

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	104	0	527	18454	4620	**5940**	8060
Bayer Versant HIV-1 RNA 3.0 Assay	47	0	676	13679	5565	**6289**	7709
bioMérieux NucliSens HIV-1 QT	6	0	2950	18000	5750	**6650**	9600
Chiron Procleix	7	0	n/a	n/a	n/a	**n/a**	n/a
bioMérieux NucliSens EasyQ HIV-1	2	0	3700	7900	3700	**5800**	7900
In House	2	0	6593	6593	n/a	**n/a**	n/a
Roche Amplicor HIV-1 Monitor UltraSensitive	4	0	4592	7218	5506	**6630**	7029

Distribution of Qualitative Results by Sample

Figure 4: Box Plot – Donor 1 and 6

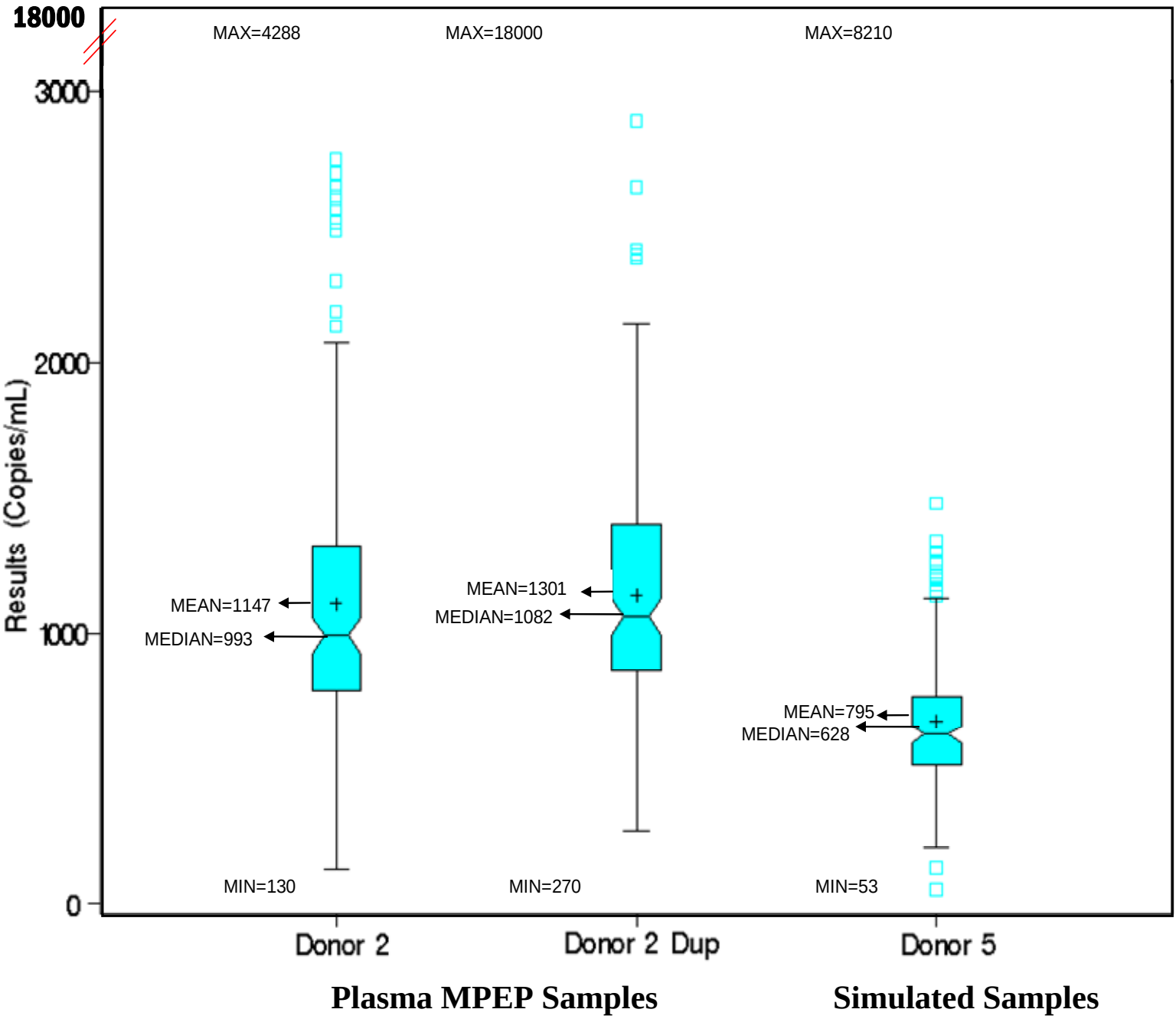
In this box plot, Donor 1 was a plasma sample and Donor 6 was the simulated sample; both had target values of 10,000 copies/ml. Results for both samples showed similar medians and distributions around 6,000 copies/ml.



Continued on next page

Figure 5: Box plot – Donor 2 and 5

In this box plot, Donor 2 and Donor 2 Duplicate were the plasma samples and Donor 5 was the simulated sample; all samples had target values of 1,000 copies/ml. The median values and distributions were similar for Donor 2 and Donor 2 Duplicate. Donor 5, had a lower median and a different distribution.



Discussion

Overall performance for plasma MPEP samples overtime

The overall performance in this survey was 98.3% for the Plasma MPEP samples, donors 1, 2, 3 & 4, representing an overall improvement compared with the last shipment. Overall performance for the last 4 surveys was as follows:

Shipment Date	Overall Performance
August 2003	99.0%
February 2004	98.6%
August 2004	96.6%
February 2005	98.3%

False-negative rate overtime

The false-negative rate for this shipment was 0.6%, well below the average, 2.3%, of the false-negative rates in the previous three shipments.

Shipment Date	% False-negative
August 2003	1.6%
February 2004	1.9%
August 2004	3.5%
February 2005	0.6%

- There were a total of 3 false-negative interpretations reported for HIV-1 RNA positive samples Donor 1, Donor 2, and Donor 2 duplicate in this shipment.
    - One laboratory reported a false-negative interpretation for Donor 1 (10,000 copies/ml).
    - Two laboratories reported false-negative interpretations for Donor 2 and Donor 2 duplicate (identical "low-positive" samples with a target value of 1,000 copies/ml).

Continued on next page

False-positive rate overtime

The rate of false-positive interpretations was 3.5%, similar to that in the last shipment (3.3%).

- There were a total of 12 false-positive interpretations reported for HIV-1 RNA negative samples Donor 3 and Donor 4 in this shipment.
    - There were 6 reported false-positives results on both negative donors 3 and 4 respectively.
    - Three laboratories reported false-positive interpretations for both Donor 3 and Donor 4.

Positive Donor 2 and Donor 2 duplicate results

In order to compare reproducibility, the same samples that were sent out in the August 2004 shipment were assigned different vial designations and sent out in duplicate again for the February 2005 shipment. The results improved for both Donor 2 and Donor 2 duplicate.

In the August 2004 shipment for Donor 2 there were 7/183 (3.8%) false-negative results compared to 1/173 (0.6%) for the same sample in the February 2005 shipment

Donor 2 only Comparison			Results		% false-negative
Panel Shipment	**Total # of labs**	**Total # of results**	**Positive**	**False-negative**	
August 2004	175	183	176	7	3.8%
February 2005	167	173	172	1	0.6%

For Donor 2 duplicate (the identical sample) there were 11/183 (6.0%) false-negative results for the August 2004 shipment compared to 1/172 (0.6%) for the February 2005 shipment.

Donor 2 Duplicate Comparison			Results		% false-negative
Panel Shipment	**Total # of labs**	**Total # of results**	**Positive**	**False-negative**	
August 2004	175	183	172	11	6.0%
February 2005	167	172	171	1	0.6%

Continued on next page

Simulated samples performance

Simulated samples, Donor 5 and Donor 6 (vial designations A6, A7, B6, & B7) were included in this shipment to investigate their comparability with Plasma MPEP donor samples.

- The distribution of values reported for donor 6 was comparable to that of the Plasma MPEP sample, donor 1 of similar copy number (target value of 10,000 copies/ml). There were no false-negative results reported for this sample.

- Fifteen false-negative interpretations (4.3%) were reported for Donor 5 (target value 1,000 copies/ml). The mean, median and distribution of values reported for this sample were much lower than values reported for the Plasma MPEP samples with the same target concentrations. Since the entire distribution was lower, many values may have fallen below the positive cut-off range for some test systems.

External Quality Control (QC)

Of the 167 laboratories reporting results in this survey:

- 99.4% (166/167) provided information on external QC materials

  - 45.5% (76/167) reported they did not use external QC samples

  - 49.7% (83/167) indicated that they used external QC materials. The source of their external QC materials are as follows:

Commercial Material	67.1% (51/76)
In-House material	46.1% (35/76)
Both Commercial and In-House Material	1.3% (4/76)