National Laboratory for HIV Reference Services
National HIV and Retrovirology Laboratories
National Microbiology Laboratory
Public Health Agency of Canada

HIV Viral Load Quality Assessment Program

Summary for Panel HIVVL 2019Apr16

2019Apr1Apr16 HIV-1 VL Panel				
Subtype	**Panel Sample Pair**	**Viral Load Consensus Mean[1]**	**Viral Load Mean Characterization by the NLHRS**	**Labs Reporting Incorrect Status**
A/D	E G	3.21[2], 3.02[3]	3.26[2], 3.10[3]	• V28
B	C H	3.15[2], 3.00[3]	3.19[2], 3.04[3]	• V28
D	A D	3.19[2], 2.99[3]	3.21[2], 3.13[3]	• V28
	B F	TND	TND	

1. Mean consensus (Log10 cp/mL) calculated from results submitted by participants with outliers removed.
2. Based on Roche CAP/CTM v2.0 assay.
3. Based on Abbott RealTime HIV-1 0.6 mL assay.

V28
-Viral load results shown high deviation from the expected viral load results.

National Laboratory for HIV Reference Services
National HIV and Retrovirology Laboratories
National Microbiology Laboratory
Public Health Agency of Canada

HIV Viral Load Quality Assessment Program

Final Report for Panel HIVVL 2019Apr16

Issued 2019-August-02

Introduction

The NLHRS distributed the 2018Oct26 and 2019Apr16 panels on October 10th, 2018. This final report is specific to the 2018Oct26 only and is publicly available, however, the identity of participants is not disclosed. With the 2019Apr16 panel, we continued to look at the effect of HIV-1 non-B subtypes on the ability to quantitate HIV-1 viral loads across several platforms.

Panel Samples, HIV Test Kits, and Data Entry

- *Panel Composition* – The 2019Apr16 panel is the 2018Oct26 panel that is relabelled and contained the following:
  - One negative sample sent in duplicate (B and F); defibrinated human plasma.
  - One positive HIV-1 RNA sample (DLS-39, A/D recombinant subtype, Discovery Life Science) was diluted in defibrinated human plasma (Basemetrix 53, Seracare Life Sciences Inc.) aliquoted in duplicates (E,G) and stored at -80°C.
  - One positive HIV-1 RNA sample (DLS-17, subtype D, Discovery Life Science) diluted to approximately 1000 cp/mL in defibrinated human plasma (Basemetrix 53, Seracare Life Sciences Inc.) aliquoted in duplicates (A, D) and stored at -80°C
  - One positive HIV-1 RNA sample (VQA150000 RNA copy control, subtype B) diluted to approximately 1000 cp/mL in defibrinated human plasma (Basemetrix 53, Seracare Life Sciences Inc.) aliquoted in duplicates (C, H) and stored at -80°C.
  - The NLHRS characterized the positive panel members on both the Roche and Abbott platforms to assess the Log_{10} cp/mL value prior to panel send out (Table 1).

Table 1: Description of 2019Apr16 panel samples.				
Sample Identification	**Sample Type**	**Sample Subtype**	**Viral Load Consensus Mean[1]**	**Viral Load Mean Characterization by NLHRS**
E G	HIV-1	A/D	3.21[2], 3.02[3]	3.26[2], 3.10[3]
C H	HIV-1	B	3.15[2], 3.00[3]	3.19[2],3.04[3]
A D	HIV-1	D	3.19[2], 2.99[3]	3.21[2], 3.13[3]
B F	TND	-	TND	TND

1. *Mean consensus (Log10 cp/mL) calculated from results submitted by participants with outliers removed.*
2. *Based on Roche CAP/CTM v2.0 assay.*
3. *Based on Abbott RealTime HIV-1 0.6 mL assay.*

- *HIV Viral Load Test Kits* – Eight different assays were used by the 19 participants (excluding the NLHRS) who returned results (Figure 1). Participant V28 switched to the Roche Cobas 4800 platform.

- *Data entry* - The NLHRS Quality Assessment Program (QAP) switched from the web based Survey Monkey system to an in-house developed website for results entry in this panel

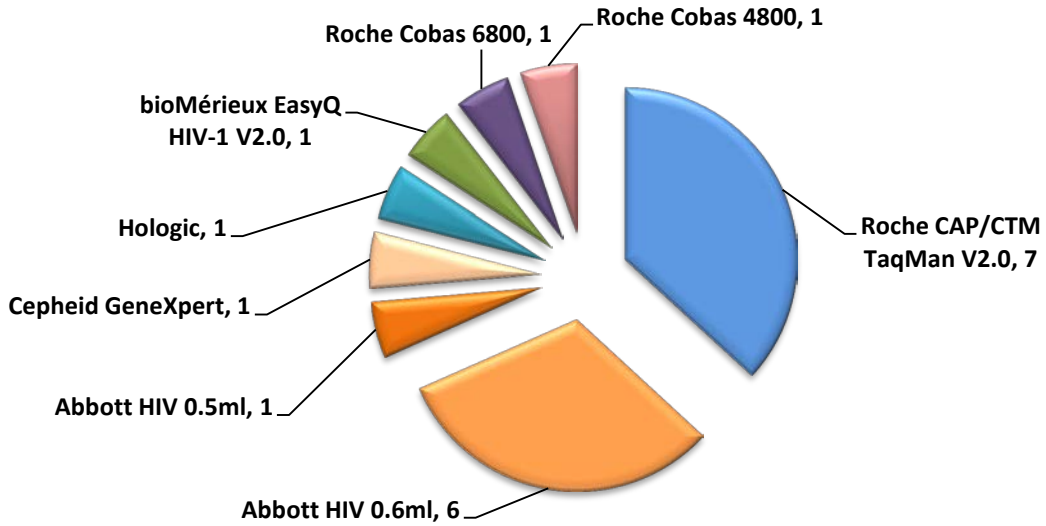

Figure 1: HIV-1 VL tests kits used by the participants for the NLHRS 2019Apr16 HIV-1 VL panel (excludes the NLHRS).

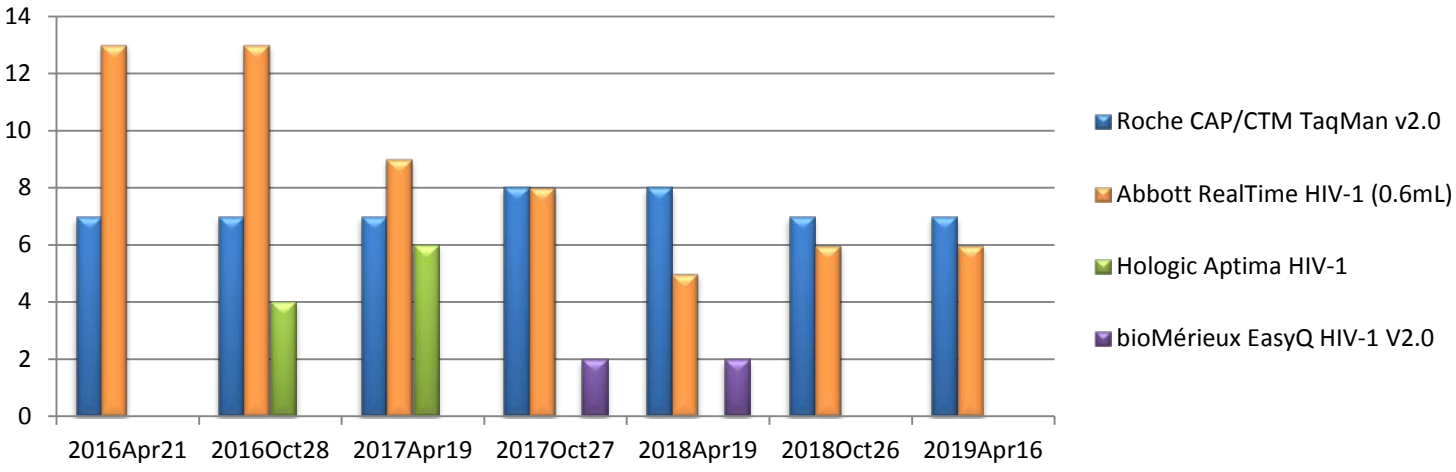

Figure 2: Distribution of HIV-1 assays (n > 1) used by participants from 2016-2019 (excludes the NLHRS).

Return rate

Results were returned from 95% of participants (19/20).

- One participant (V36) did not return results.
- Ten year average return rate is 90.6% (Figure 3).

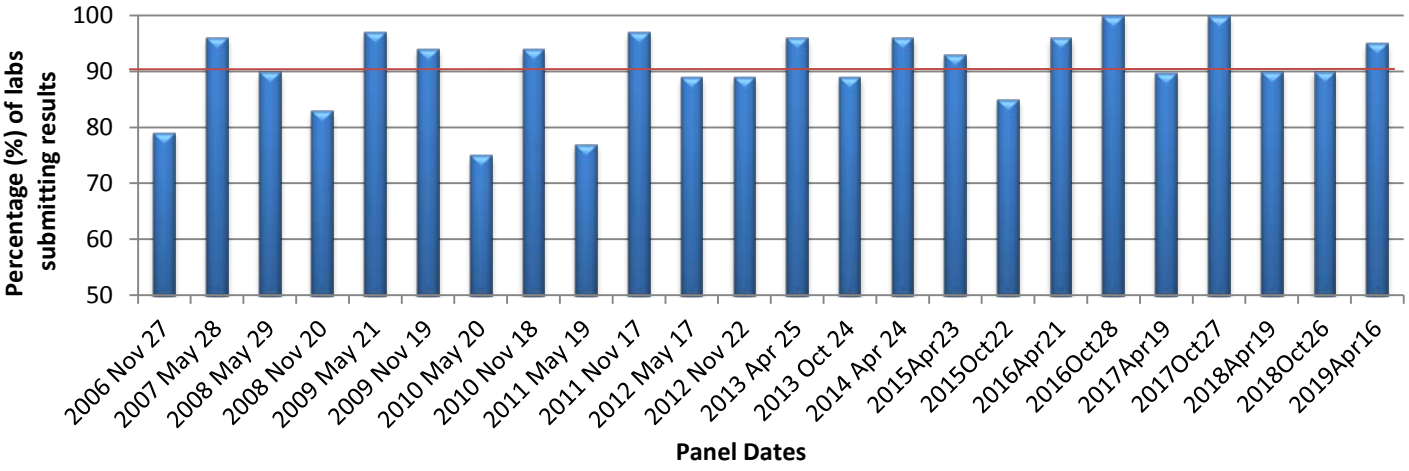

Figure 3: Historical participant return rate (2006 to 2019 inclusive).

Homogeneity and stability

- The homogeneity of the 2019Apr16 HIV-1 viral load panel was assessed by using the Roche assay peer group (n=8) and the Abbott assay peer group (n=7) results in the positive duplicate sample set

(E/G, C/H, A/D). All participants were able to detect HIV-1 RNA and the results were within ± 0.5 Log10 cp/mL of the group mean (Appendix 1). There is no indication of heterogeneity in the panel samples.

- The stability of the 2019Apr16 HIV-1 viral load panel was assessed by comparing the group mean generated by the participants in the positive duplicate sample sets with the results from the 2018Oct26 test event. The difference between both means does not exceed 0.5 Log10 cp/mL (Table 2).

Table 2: Stability testing for the 2019Apr16 panel.

	Sub A/D Group Mean (Log10 cp/mL)	Sub B Group Mean (Log10 cp/mL)	Sub D Group Mean (Log10 cp/mL)
Roche 2018Oct26	3.21	3.14	3.22
Roche 2019Apr16	3.21	3.15	3.19
Roche Difference in Means	0.00	-0.01	0.03
Abbott 0.6 mL 2018Oct26	2.99	2.98	3.00
Abbott 0.6mL 2019Apr16	3.02	3.00	2.99
Abbott Difference in Means	-0.03	-0.02	0.01

External QC and QA activities

1. *External quality control (QC) material* - Used in addition to controls provided in kits; allows users to detect technical problems and assay sensitivity from lot to lot.
   - Eight participants (44.4%, 8/18) reported using external QC material (Figure 4).

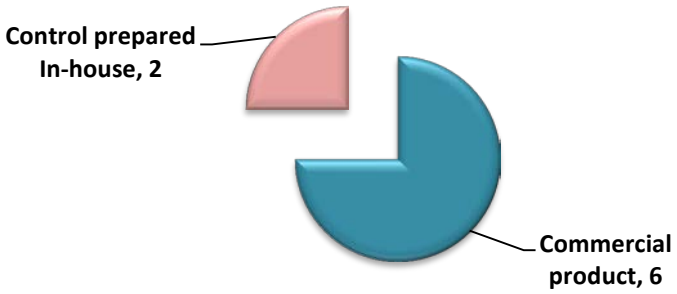

Figure 4: Source of external control used for the 2019Apr16 HIV-1 VL panel (excludes the NLHRS).

2. *Quality Assurance (QA) programs* - Allows participants to evaluate their overall use of the assay and reporting of the results.
   - Twelve participants (66.7%, 12/18) reported participation in other quality assurance programs (Figure 5).

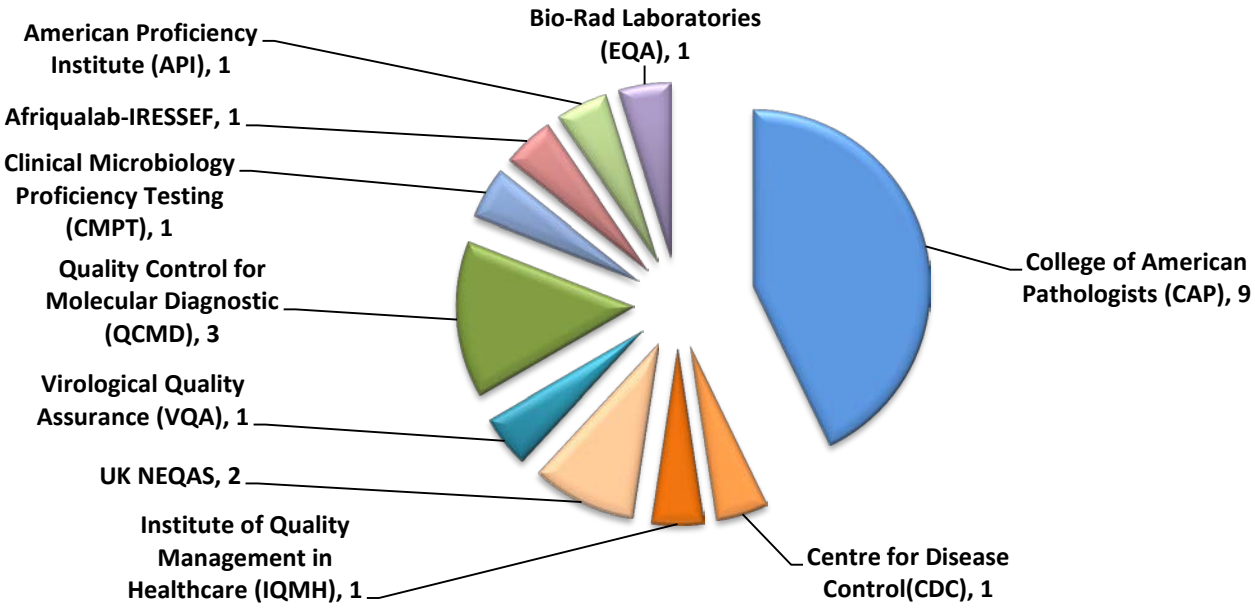

Figure 5: Distribution of external quality assurance programs which participants are enrolled in other than the NLHRS QAP.

Participants' feedback collected from this Survey

- Of the 20 participants, only 1 provided feedback in the new QAP website. This participant found the new website relatively easy to use and the format is similar to Survey Monkey.
- Suggestions for improvement collected in the new QAP website will be incorporated for the next survey.

Results

1. **Flags**
   - V28's viral load results deviated from the expected viral load results greater than 0.5 Log10 cp/mL.

2. **Statistical Analysis (General)**
   - Two outliers were detected and removed from analysis (Grubb's test).
   - All group comparisons were performed using the unpaired t test.
   - Since no significant differences ($p > 0.05$) were identified in the duplicate sets (A/D, C/H, E/G) between the Roche and Abbott users, their datasets were combined and analyzed together.
   - Analysis was not performed for peer groups of n=1 (Abbott 0.5mL, COBAS 6800, COBAS 4800, Hologic Aptima, bioMérieux EasyQ HIV-1 v2.0 and Cepheid GeneXpert II).
   - Negative samples were analyzed qualitatively.

3. **Group Analysis (Summary Statistics)** (Tables 3, 5A, 5E)
   - The duplicate panel samples were combined for the summary statistics (A/D, C/H, and E/G).

Inter-Lab Variation

- Difference between the minimum and maximum results for each sample within a peer group (the maximum value divided by the minimum).
  - Average of 1.13 log10 cp/mL for the Roche CAP/CTM v2, and 1.10 log10 Cp/mL for the Abbott RealTime (0.6mL) peer groups.

Reproducibility

- This is an important aspect of viral load testing; required to quantify changes in viral load.
- To assess intra-reproducibility, duplicates of the positive samples were included in the panel and the standard deviation of the sample duplicates are illustrated in Table 3.
- One participant (V26) has shown high standard deviation in sample duplicates E/G and A/D.

Table 3: Standard deviation (Log10 cp/mL) reported between duplicates from participants' results for the 2019Apr16 panel (excludes NLHRS).

Lab	Sample E and G	Sample C and H	Sample A and D
V01	0.03	0.12	0.11
V04	0.03	0.05	0.06
V05	0.20	0.14	0.01
V06	0.05	0.06	0.18
V07	0.08	0.12	0.12
V08	0.08	0.04	0.06
V10	0.04	0.05	0.10
V11	0.08	0.01	0.19
V13	0.00	0.06	0.01
V14	0.02	0.03	0.11
V21	0.00	0.14	N/A
V26	0.42	0.12	0.30
V27	0.03	0.04	0.04
V28	0.18	0.06	0.06
V29	0.05	0.05	0.06
V37	N/A	0.03	0.17
V41	0.14	0.04	0.06
V48	0.04	0.07	0.06
V49	0.04	0.08	0.02

4. **Effect of Different Subtypes** (Figure 6 and 7)

Non-B subtype (Samples B, C, E, H)

- There was a significant difference in the viral load results for recombinant subtype A/D between the Roche and Abbott peer groups (p-value < 0.0001).

- There was a significant difference in the viral load results for subtype D between the Roche and Abbott peer groups (p-value < 0.0001).

Subtype B (Samples D, G)

- There was a significant difference in the viral load results for subtype B between the Roche and Abbott peer groups (p-value = 0.0004).

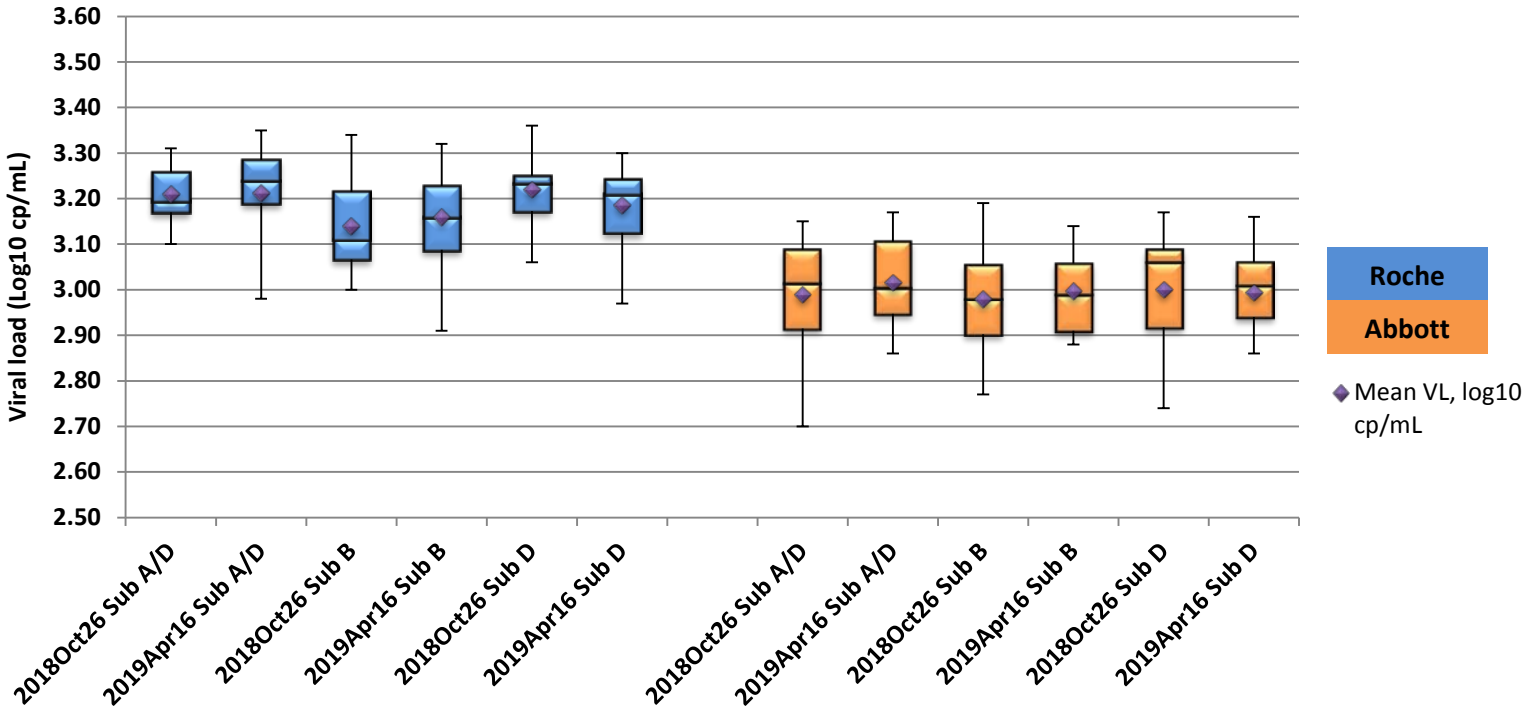

Figure 6: Comparison of the 2018Oct26 and the 2019Apr16 HIV-1 VL panel

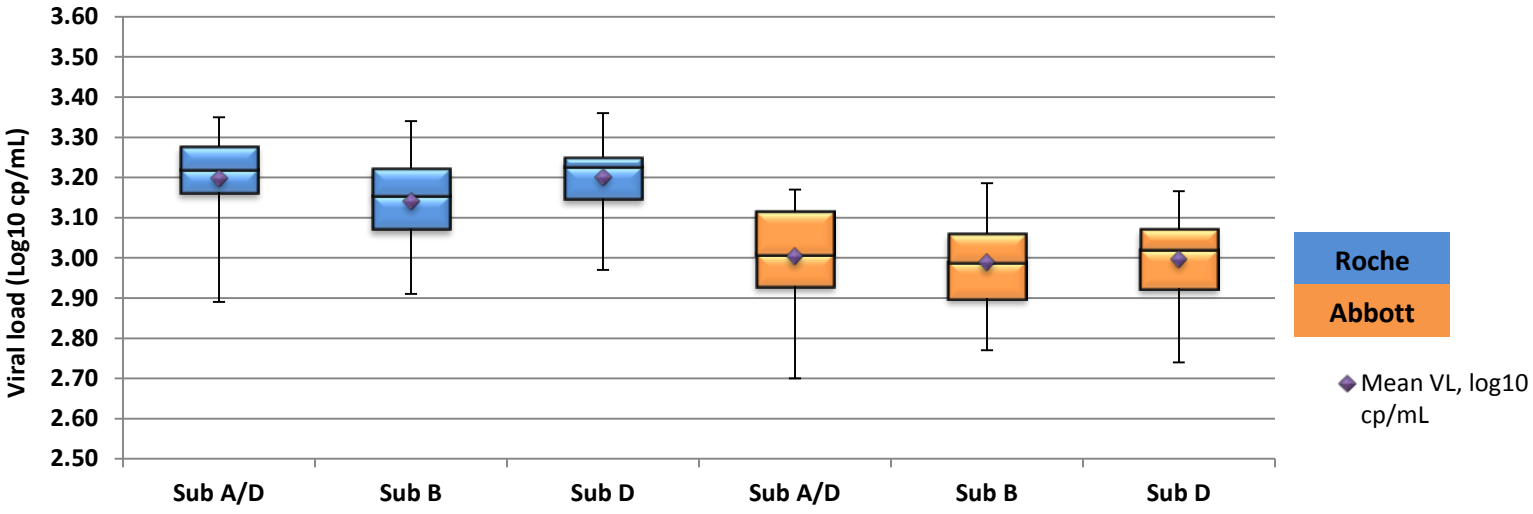

Figure 7: The combined results of the 2018Oct26 and the 2019Apr16 HIV-1 VL panel

5. *Comparison between the major peer group and other users group* (Table 4)
   - This is to provide a comparison of the results from individual lab in a small peer group (n=<2) with the major peer groups, the Roche and Abbott 0.6mL users.
   - The results from the Cepheid GeneXpertII, Hologic Aptima HIV-1, COBAS 6800, bioMerieux BV NucliSens EASYQ HIV-1 and the Abbott 0.5 mL users are comparable to the Roche and Abbott 0.6mL peer group.
   - A proper and fair comparison between the different peer groups would require more users of the GeneXpertII, Hologic Aptima, Abbott 0.5 mL, COBAS 6800, COBAS 4800 and the bioMerieux BV NucliSens EASYQ HIV-1 platforms.

Table 4: Comparison of the mean viral load of the 2019Apr16 panel between the major and minor peer groups.

Lab	Sample E/G	Sample C/H	Sample A/D
Roche Peer Group	3.19	3.15	3.21
Abbott 0.6 mL Peer Group	3.02	3.00	2.99
V04	3.08	3.18	3.09
V11	2.83	2.80	2.88
V26	3.34	2.76	3.26
V28	2.15	2.19	2.07
V48	3.08	3.02	2.86
V49	3.06	2.97	3.09

6. **Individual Analysis (Participant Statistics)** (Figures 8, 9, 10, and Tables 5A, 5B, 5C,5D, 5E, 5F, 5G, 5H)
   - The percent difference (% D), the difference from the mean for each peer group, was calculated for each participant per sample pair.

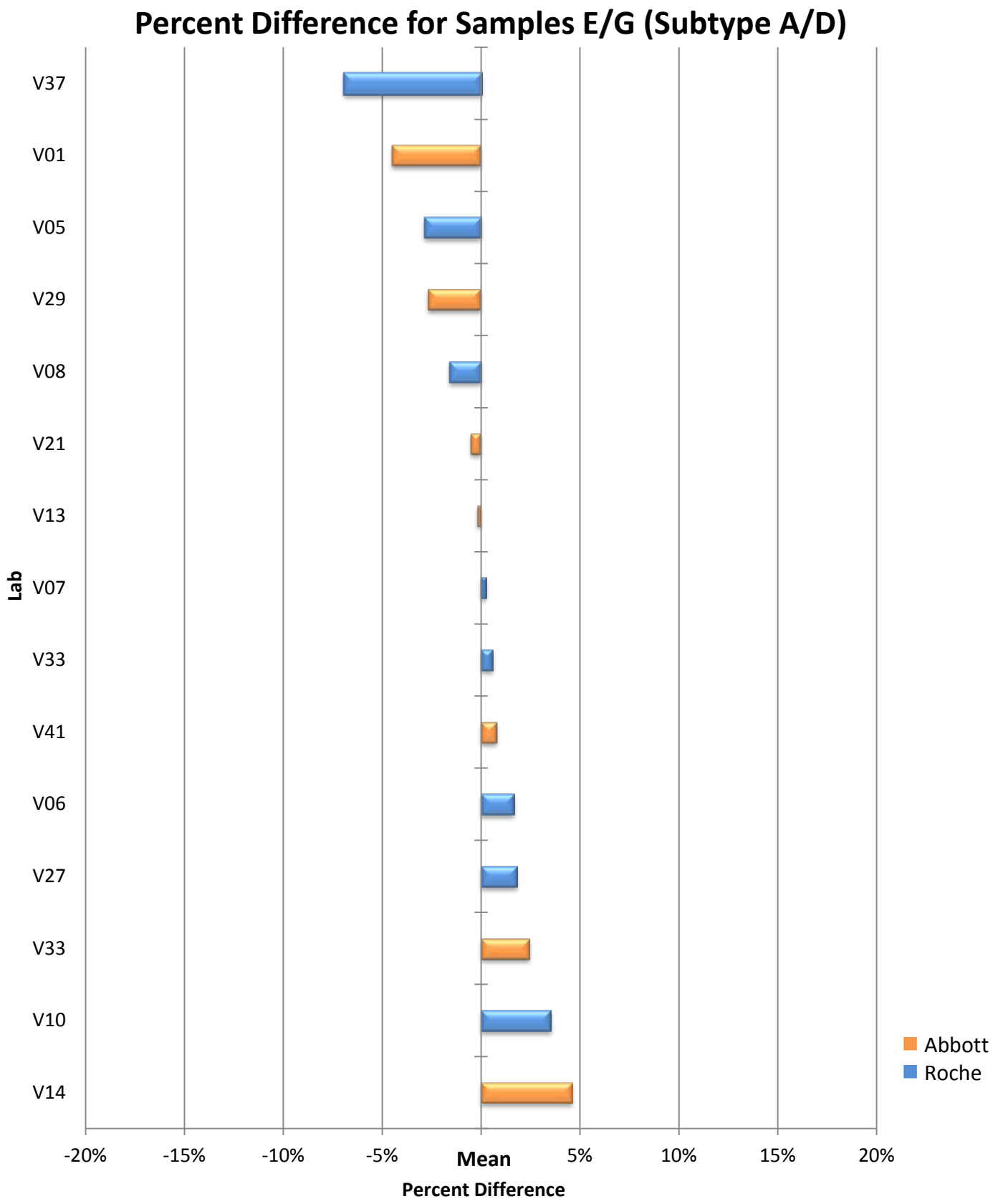

Figure 8: Percent difference from the peer group mean for E/G.

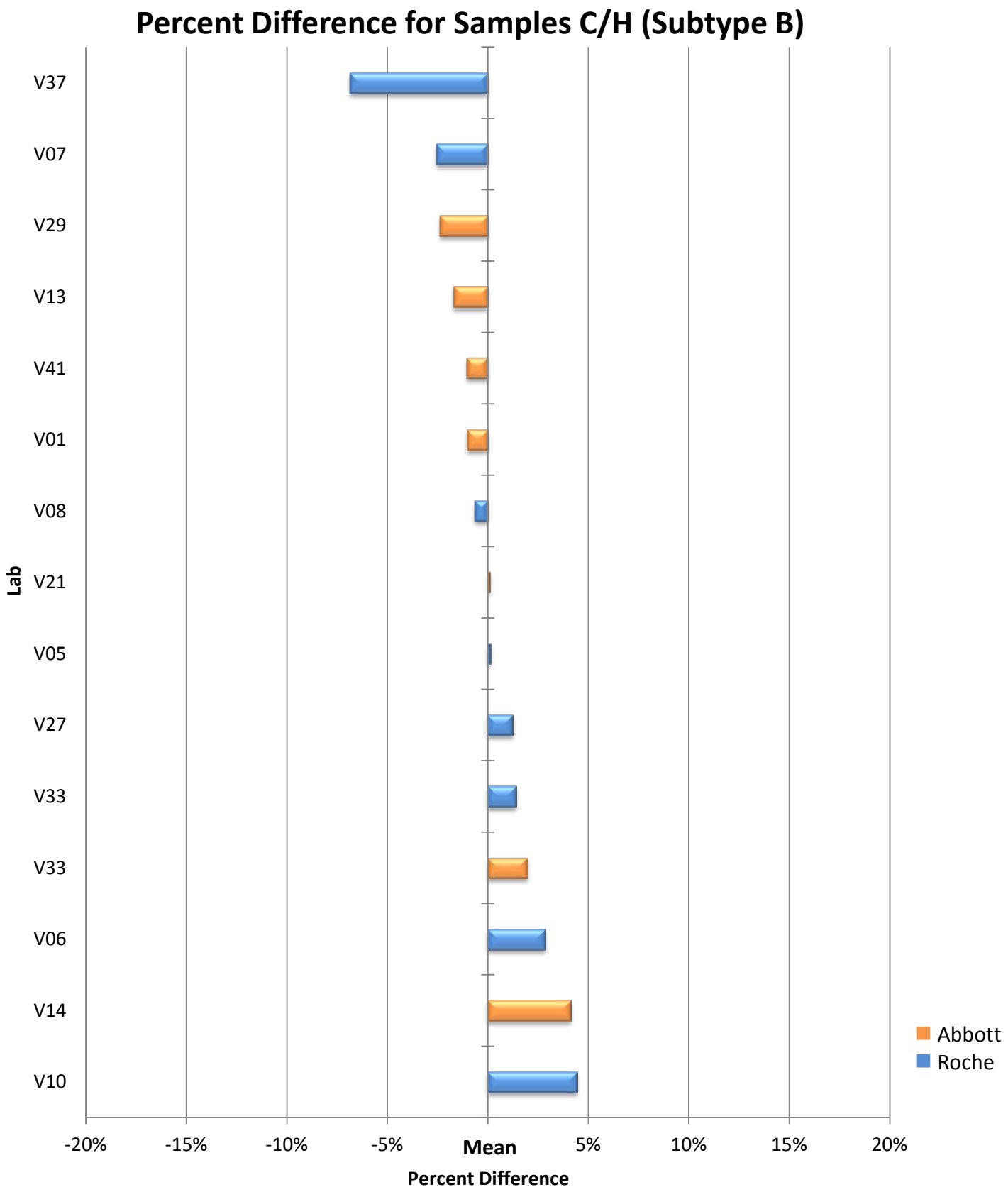

Figure 9: Percent difference from the peer group mean for C/H.

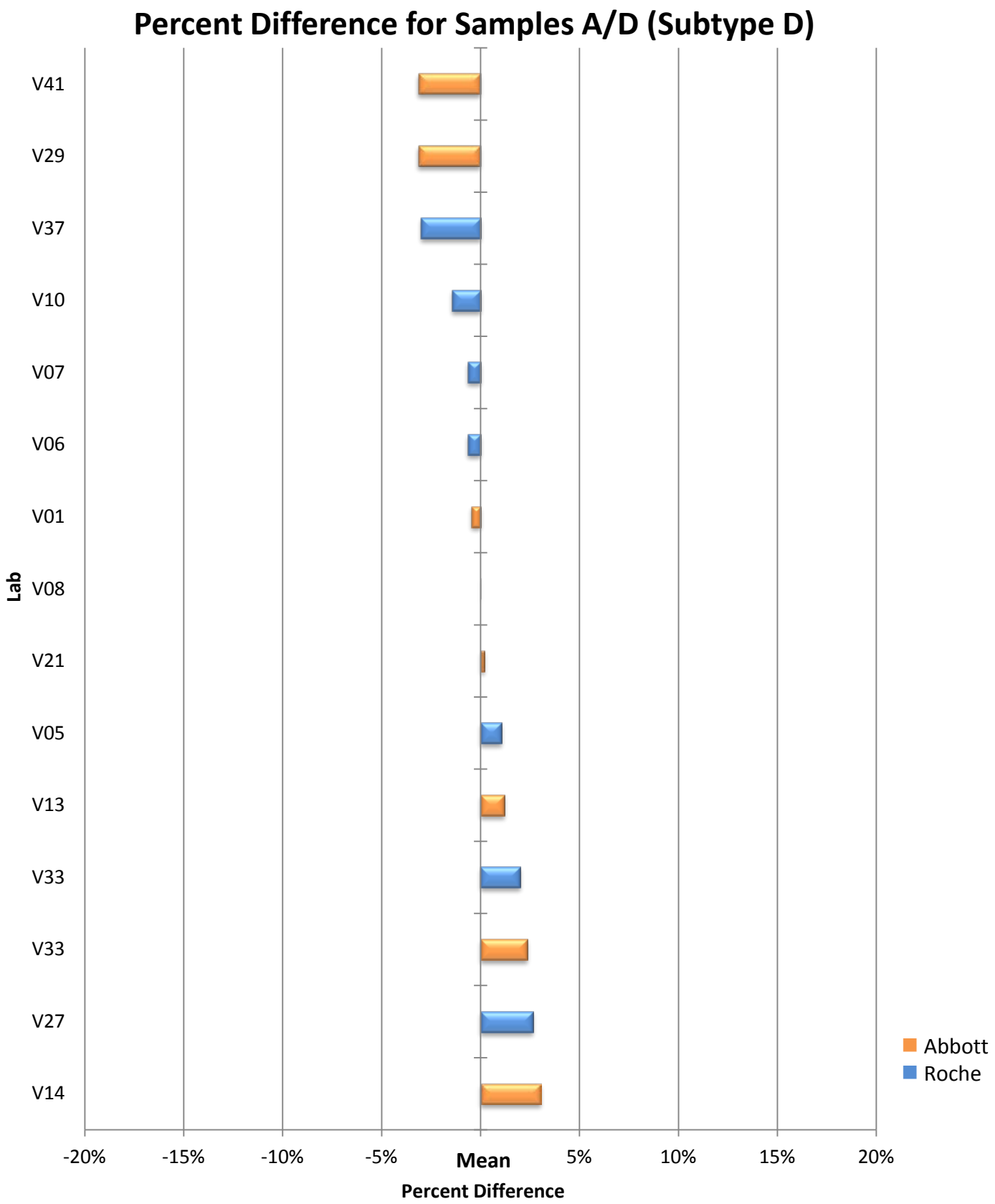

Figure 10: Percent difference from the peer group mean for A/D.

Conclusion

1. *Effect of non-B subtype on quantitation of HIV-1.*
   - The results from this panel indicate there was a difference between the Abbott and the Roche peer groups when comparing the viral load results between the different subtypes. However, this difference may be attributed to the performance characteristic between the two platforms as there is a difference in the results in the subtype B sample as well. Other confounding factors: a) different technologist performing the assay, b) kit lots used, would need to be considered.

2. *V28 aberrant results*
   - This participant has been contacted to determine the likely cause of the high deviation from the expected viral load results

3. *High standard deviation between sample duplicate*
   - Ensure adequate mixing of the samples before testing
   - Using calibrated pipet to load the samples to your instrument

We value each laboratory's participation in these QA panels and your suggestions for improvement. The NLHRS is committed to improving all aspects of the HIV-1 viral load proficiency testing program in order to provide quality proficiency testing to our participants.

If you have any comments or concern please contact us at:

phac.nlhrs.qap-peg.lnsrv.aspc@canada.ca

Thank you for your participation in the NLHRS Quality Assurance Program

John Ho
Quality Assurance Program Coordinator
National Laboratory for HIV Reference Services
Public Health Agency of Canada
Tel: (204) 789-6522

Dr. John Kim
Laboratory Chief
National Laboratory for HIV Reference Services
Public Health Agency of Canada
Tel: (204) 789-6527

Appendix 1: Test Results

Legend: **Incorrect Result** **Outliers Removed**

Table 5A: Roche CAP/CTM TaqMan v2.0 Results (Log10 HIV RNA cp/mL)

Lab ID #	Sample Code								Sample Prep/PCR Kit Lot	Exp. Date
	E	G	C	H	A	D	B	F		
V05	2.98	3.26	3.25	3.05	3.23	3.21			E05473	2019-12-31
V06	3.23	3.30	3.28	3.19	3.29	3.04			E05473	2019-12-31
V07	3.28	3.16	3.15	2.98	3.08	3.25			E05473	2019-12-31
V08	3.10	3.22	3.15	3.10	3.14	3.23			E05473	2019-12-31
V10	3.35	3.30	3.25	3.32	3.07	3.21			E05473	2019-12-31
V27	3.25	3.29	3.21	3.16	3.24	3.30			E05473	2019-12-31
V33	3.22	3.24	3.16	3.22	3.29	3.21			E05473	2019-12-31
V37	2.72	2.99	2.95	2.91	3.21	2.97			E11965	2020-02-29
Mean	3.21		3.15		3.19					
Minimum	2.98		2.91		2.97					
Median	3.24		3.16		3.21					
Maximum	3.35		3.32		3.30					
% CV	3.42		3.80		3.06					
SD	0.11		0.12		0.10					
Inter-lab Variation	1.12		1.14		1.11					
Measurement of Uncertainty	0.43		0.43		0.43					

Table 5B: Hologic Panther Aptima HIV-1 Results (Log10 HIV RNA cp/mL)

Lab ID #	Sample Code								Sample Prep/PCR Kit Lot	Exp. Date
	E	G	C	H	A	D	B	F		
V48	3.10	3.05	2.97	3.07	2.82	2.90			241884	2020-01-15

Table 5C: Roche COBAS 6800 Results (Log10 HIV RNA cp/mL)

Lab ID #	Sample Code								Sample Prep/PCR Kit Lot	Exp. date
	E	G	C	H	A	D	B	F		
V04	3.10	3.06	3.14	3.21	3.01	3.05			E14088	2019-11-30

Table 5D: bioMerieux BV NucliSens EASYQ HIV-1 Results (Log10 HIV RNA cp/mL)

Lab ID #	Sample Code								Sample Prep/PCR Kit Lot	Exp. date
	E	G	C	H	A	D	B	F		
V26	3.64	3.04	2.84	2.67	3.04	3.47			18061501 18072301	2019-05-28 2019-12-28

Appendix 1: Test Results

Legend: **Incorrect Result** **Outliers Removed**

Table 5E: Abbott RealTime Results (0.6mL) (Log10 HIV RNA cp/mL)

Lab ID #	Sample Code								Sample Prep/PCR Kit Lot	Exp. Date
	E	G	C	H	A	D	B	F		
V01	2.90	2.86	3.05	2.88	2.90	3.06			11818001 488758	2019-12-31 2019-12-25
V13	3.01	3.01	2.90	2.99	3.04	3.02			11818001 487985	2019-12-31 2019-11-21
V14	3.14	3.17	3.14	3.10	3.16	3.01			11818001 487985	2019-12-31 2019-11-21
V21	3.00	3.00	2.90	3.10	2.60	3.00			11818001 487985	2019-12-31 2019-11-21
V29	2.97	2.90	2.96	2.89	2.86	2.94			11836131 492720	2019-12-31 2020-03-24
V33	3.12	3.06	3.05	3.06	3.07	3.06			11818001 487985	2019-12-31 2019-11-21
V41	2.94	3.14	2.94	2.99	2.94	2.86			11849131 486856	2019-12-31 2019-10-13
Mean	3.02		3.00		2.99					
Minimum	2.86		2.88		2.86					
Median	3.01		2.99		3.01					
Maximum	3.17		3.14		3.16					
% CV	3.28		2.93		2.98					
SD	0.10		0.09		0.09					
Inter-lab Variation	1.11		1.09		1.10					
Measurement of Uncertainty	0.14		0.14		0.14					

Table 5F: Cepheid GeneXpert Results (Log10 HIV RNA cp/mL)

Lab ID #	Sample Code								Sample Prep/PCR Kit Lot	Exp. Date
	E	G	C	H	A	D	B	F		
V49	3.03	3.09	3.03	2.91	3.10	3.07			1000103363	2019-04-28

Table 5G: Abbott RealTime (0.5mL) Results (Log10 HIV RNA cp/mL)

Lab ID #	Sample Code								Sample Prep/PCR Kit Lot	Exp. Date
	E	G	C	H	A	D	B	F		
V11	2.88	2.77	2.80	2.79	3.01	2.74			11818001 487985	2019-12-31 2019-11-21

Table 5H: Roche COBAS 4800 Results (Log10 HIV RNA cp/mL)

Lab ID #	Sample Code								Sample Prep/PCR Kit Lot	Exp. date
	E	G	C	H	A	D	B	F		
V28	2.02	2.27	2.23	2.14	2.11	2.03			E16371 E17128	2019-11-01 2019-10-01

Appendix 2: Troubleshooting

Troubleshooting; common causes of outlying and/or aberrant results in Serology and Molecular Laboratories.

Type of Error	Possible Cause(s)	Pre-Analytical	Analytical	Post- Analytical
Sample mix-up	Can occur during specimen reception or testing. May result in outlying/aberrant results for one or all samples mixed-up.	✓	✓	
Transcription	• Incorrect test ordering by physician	✓		
	• Incorrect shipment address	✓		
	• Selecting the wrong assay for data entry	✓		
	• Interchanging results for two or more specimens			✓
	• Entering incorrect results			✓
	• Entering values in the incorrect field (e.g., OD as S/Co)			✓
	• Entering values in the incorrect unit (e.g., IU/mL instead of $\log_{10}$ copies/mL)			✓
	• Using a comma instead of a dot to denote a decimal point			✓
	• Selecting the incorrect assay interpretation or analyte			✓
	• Failure to recommend follow-up testing where necessary			✓
	It is recommended all results that are manually transcribed or entered electronically be checked by a second individual to avoid transcription errors.			
Outlying and/or Aberrant Results (random error)	Sporadic test results identified as outlying and/or aberrant can be classified as random events. Possible causes of random error include:			
	• Incorrect sample storage/shipping conditions	✓	✓	
	• Incorrect test method	✓	✓	
	• Insufficient mixing of sample, especially following freezing		✓	
	• Poor pipetting		✓	
	• Ineffective or inconsistent washing		✓	
	• Transcription errors	✓		✓
	• Cross-contamination or carryover	✓	✓	
	• Presence of inhibitors to PCR		✓	
Outlying and/or Aberrant Results (systematic error)	A series of test results identified as outlying and/or aberrant may be due to a systematic problem. Systematic problems may be due to:			
	• Reagents contaminated, expired, or subject to batch variation		✓	
	• Instrument error or malfunction		✓	
	• Insufficient washing		✓	
	• Incorrect wavelength used to read the assay result		✓	
	• Cycling times too long/short or temperature too high/low		✓	
	• Incubation time too long/short or temperature too high/low		✓	
	• Insufficient mixing/centrifuging before testing		✓	
	• Incorrect storage of test kits and/or reagents	✓		
	• Contamination of master-mix, extraction areas or equipment		✓	
	• Ineffective extraction process		✓	
	• Degradation of master-mix components		✓	
	• Suboptimal primer design (in-house assays)		✓	

This table was modified from a report produced by the National Reference Laboratory (NRL), Melbourne, Australia.