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 2018Oct26

2018Oct26 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	B H	3.21[2], 2.99[3]	3.26[2], 3.10[3]	
B	D G	3.14[2], 2.98[3]	3.19[2], 3.04[3]	
D	C E	3.22[2], 3.00[3]	3.21[2], 3.13[3]	
	A 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.

All participants reported the correct final status for all samples in the 2018Oct26 HIV-VL panel.

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Final Report for Panel HIVVL 2018Oct26

Issued 2019-01-16

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 2018Oct26 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 2018Oct26 panel contained the following:
  - One negative sample sent in duplicate (A 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 duplicate (B,H) 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 (C, E) 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 (D, G) 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 2018Oct26 panel samples.				
Sample Identification	**Sample Type**	**Sample Subtype**	**Viral Load Consensus Mean[1]**	**Viral Load Mean Characterization by NLHRS**
B H	HIV-1	A/D	3.21[2], 2.99[3]	3.26[2], 3.10[3]
D G	HIV-1	B	3.14[2], 2.98[3]	3.19[2],3.04[3]
C E	HIV-1	D	3.22[2], 3.00[3]	3.21[2], 3.13[3]
A 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* – Seven different assays were used by the 18 participants (excluding the NLHRS) who returned results (Figure 1). Participant V04 switched to the new Roche Cobas 6800 platform.

- *Data entry* - The NLHRS Quality Assessment Program (QAP) used the web based Survey Monkey system to capture results. Participants were also asked to pilot a new NLHRS QAP website that will replace Survey Monkey in the future.

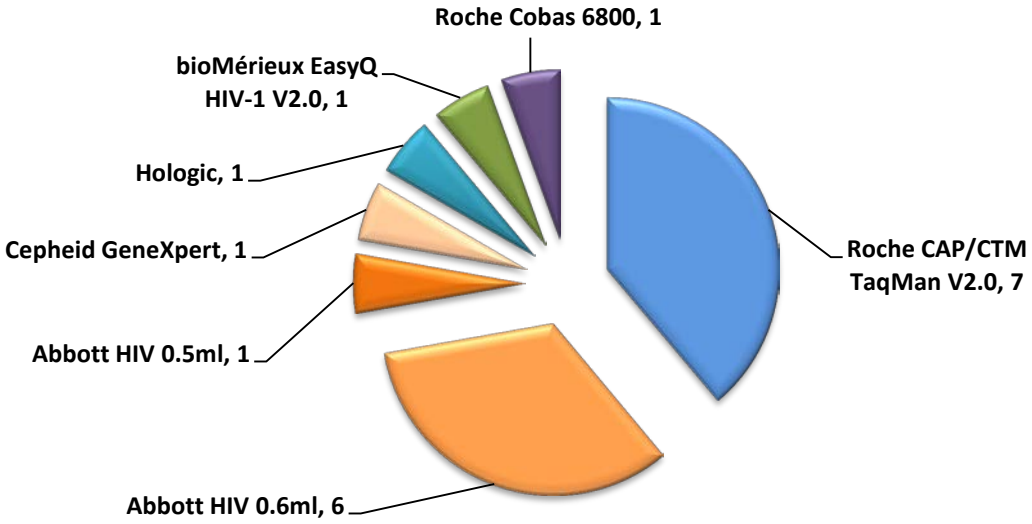

Figure 1: HIV-1 VL tests kits used by the participants for the NLHRS 2018Oct26 HIV-1 VL panel (excludes the NLHRS).

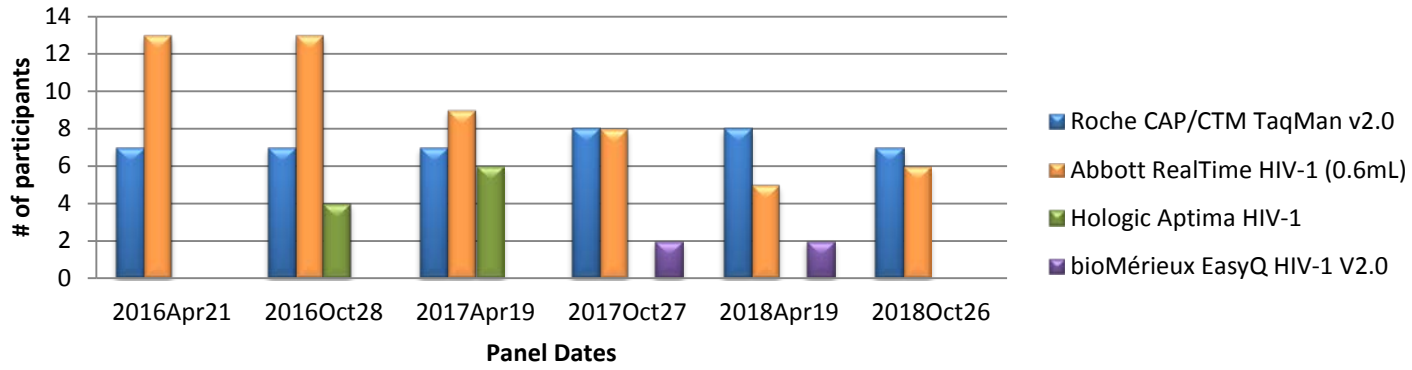

Figure 2: Distribution of HIV-1 assays (n > 1) used by participants from 2016-2017 (excludes the NLHRS).

Return rate

Results were returned from 90% of participants (18/20).

- One participant (V26) was not able to return results due to a customs delay.
- One participant (V36) did not return results.
- Ten year average return rate is 90.6% (Figure 3).

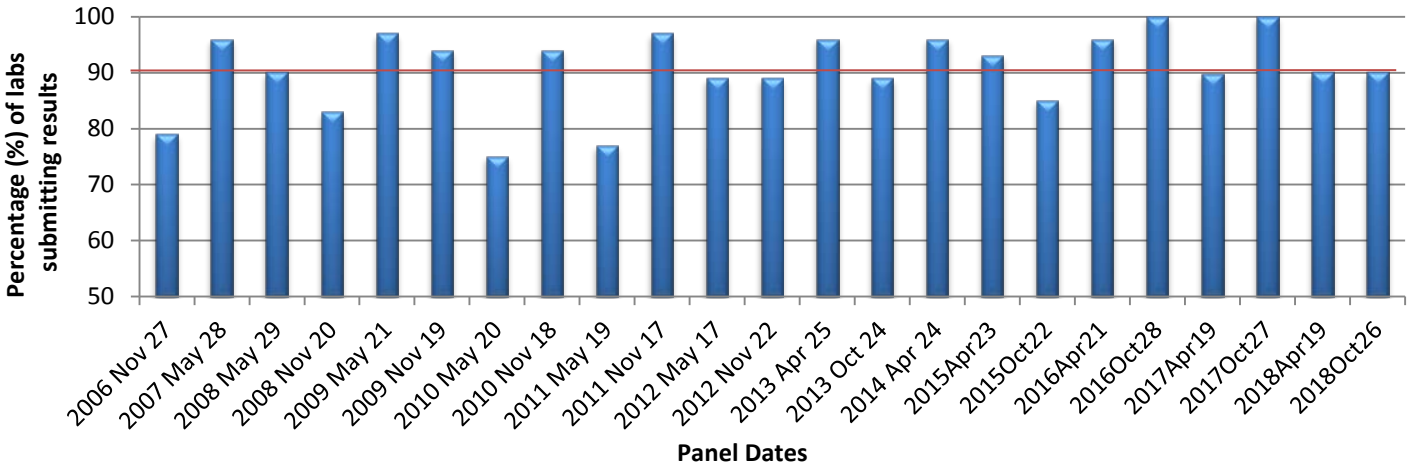

Figure 3: Historical participant return rate (2006 to 2018 inclusive).

Homogeneity and stability

- The homogeneity of the 2018Oct26 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 (B/H, C/E, D/G). 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 2018Oct26 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 obtained by the NLHRS for the characterization of the panel samples before storage and shipping. The difference between both means does not exceed 0.5 Log10 Cp/mL (Table 2).

Table 2: Stability testing for the 2018Oct26 panel.

	2018Oct26 Group Mean B/H (Log10 cp/mL)	2018Oct26 Group Mean C/E (Log10 cp/mL)	2018Oct26 Group Mean D/G (Log10 cp/mL)
Roche Peer Group	3.21	3.22	3.14
Roche Characterization Result	3.26	3.21	3.19
Roche Difference in Means	0.05	0.01	0.05
Abbott 0.6 mL Peer Group	2.99	3.00	2.98
Abbott Characterization Result	3.10	3.13	3.04
Abbott Difference in Means	0.11	0.13	0.06

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).

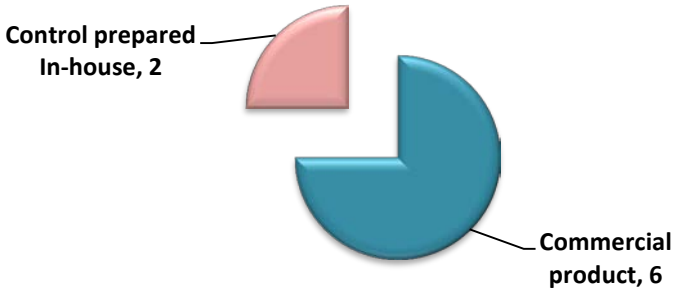

Figure 4: Source of external control used for the 2018Oct26 HIV-1 VL panel (excludes the NLHRS).

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).

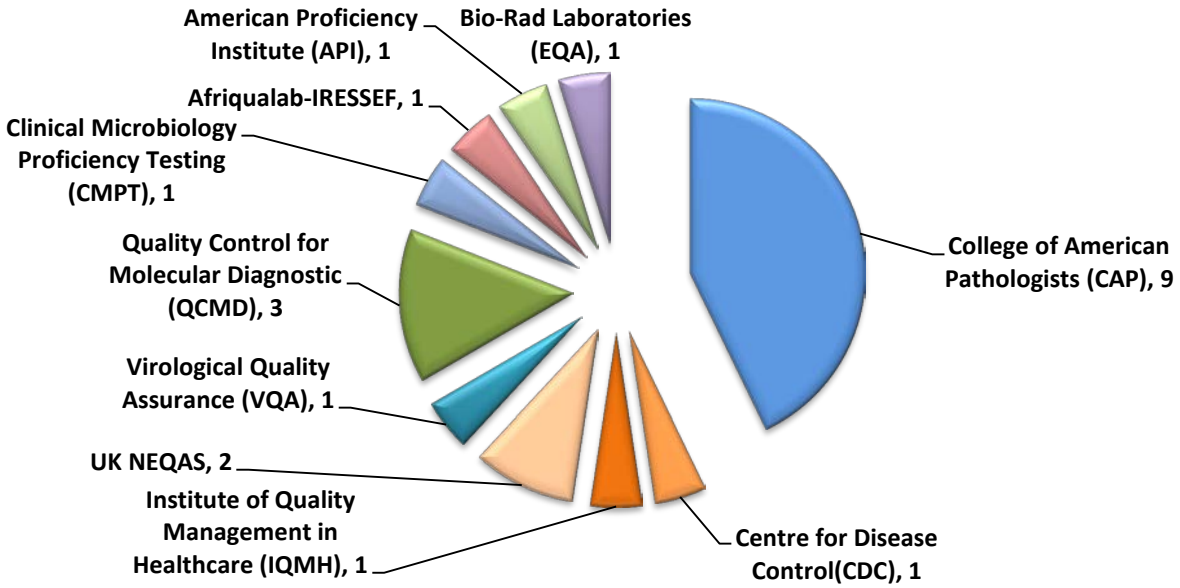

Figure 5: Distribution of external quality assurance programs which participants are enrolled in other than the NLHRS QAP.

Participants' feedback collected from Survey Monkey and the beta testing of the new QAP website

- Of the 18 participants, 16 provided feedback in Survey Monkey. Fourteen participants were satisfied with the changes made in Survey Monkey (Figure 6).
- 6 participants preferred the current survey while 5 participants have identified areas of improvement for the next survey (Figure 7).
- Of the 18 participants, 17 participated in the beta testing of the new NLHRS QAP website

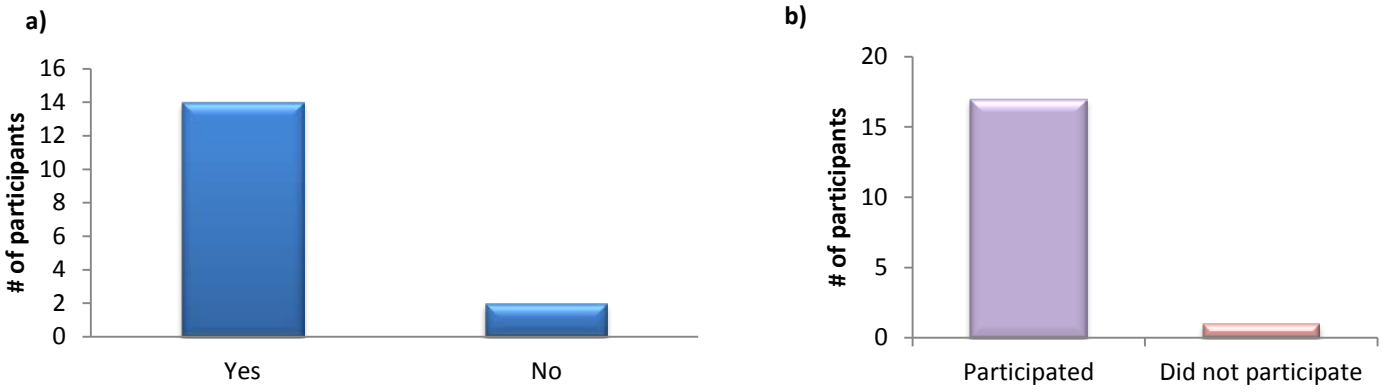

Figure 6: Number of participants' who a) liked the changes to Survey Monkey and b) used the new QAP website

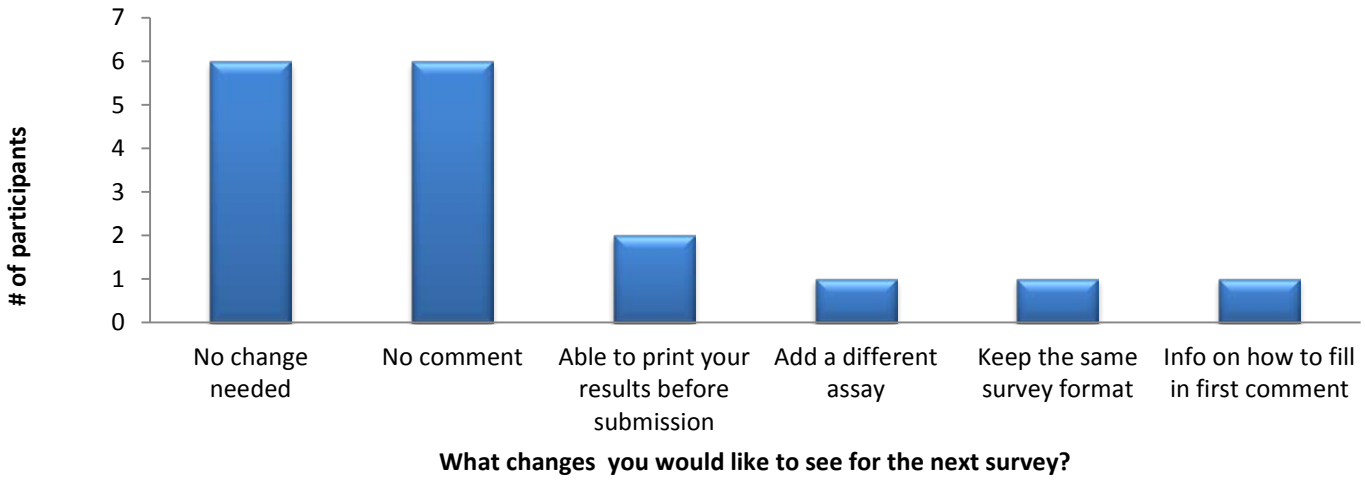

Figure 7: Participants' responses to which area requires improvement in the NLHRS HIV-1 viral load survey.

Results

1. **Flags**
   - Participant V08, did not submit their results as Log10 cp/ml
   - Participant V28, did not submit their results with 2 decimal places

2. **Statistical Analysis (General)**
   - One outlier was 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 (B/H, C/E, D/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, 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 (B/H, D/G, and C/E).

   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 1.09 log10 Cp/mL for the Roche CAP/CTM v2, and 1.15 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.
- All participants reported standard deviation (SD) of 0.28 Log10 Cp/mLor lower between duplicates (Table 3).

Table 3: Standard deviation (Log10 cp/mL) reported between duplicates from participants' results for the 2018Oct26 panel (excludes NLHRS).

Lab	Sample B and H	Sample C and E	Sample D and G
V01	0.12	0.24	0.04
V04	0.06	0.02	0.02
V05	0.05	0.02	0.03
V06	N/A	0.04	0.06
V07	0.10	0.04	0.01
V08	0.03	0.01	0.04
V10	0.04	0.06	0.08
V11	0.03	0.03	0.02
V13	0.13	0.03	0.01
V14	0.09	0.02	0.01
V21	0.14	0.07	0.00
V27	0.06	0.01	0.06
V28	0.07	0.14	0.28
V29	0.04	0.01	0.03
V37	N/A	0.08	0.15
V41	0.09	0.14	0.10
V48	0.01	0.01	0.05
V49	0.03	0.01	0.06

4. **Effect of Different Subtypes** (Figure 8)

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).

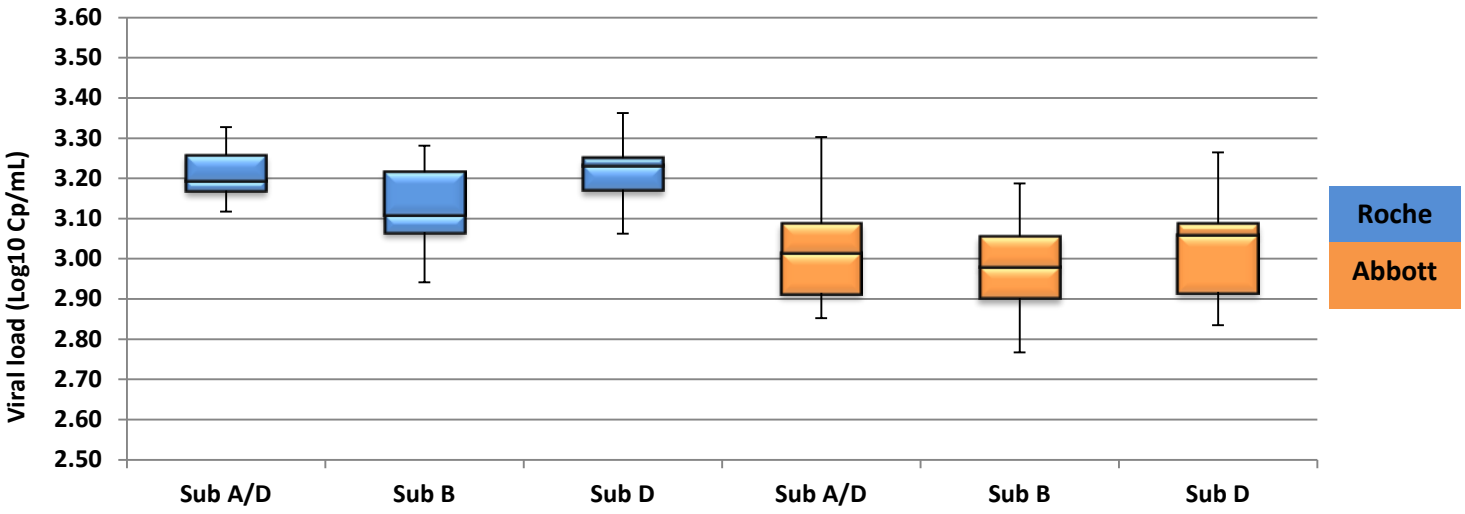

Figure 8: The results of the different subtypes analyzed in the 2018Oct26 HIV-1 VL panel

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 and the bioMerieux BV NucliSens EASYQ HIV-1 platforms.

Table 4: Comparison of the mean viral load of the 2018Oct26 panel between the major and minor peer groups.

Lab	Sample B/H	Sample D/G	Sample C/E
Roche Peer Group	3.21	3.14	3.22
Abbott 0.6 mL Peer Group	2.99	2.98	3.00
V04	2.97	3.03	3.05
V11	3.07	3.08	3.10
V28	2.95	2.60	3.00
V48	3.16	3.17	3.00
V49	3.10	3.05	3.16

6. **Individual Analysis (Participant Statistics)** (Figures 9, 10, 11, and Tables 5A, 5B, 5C,5D,5E,5F,5G)
   - The percent difference (% D), the difference from the mean for each peer group, was calculated for each participant per sample pair.

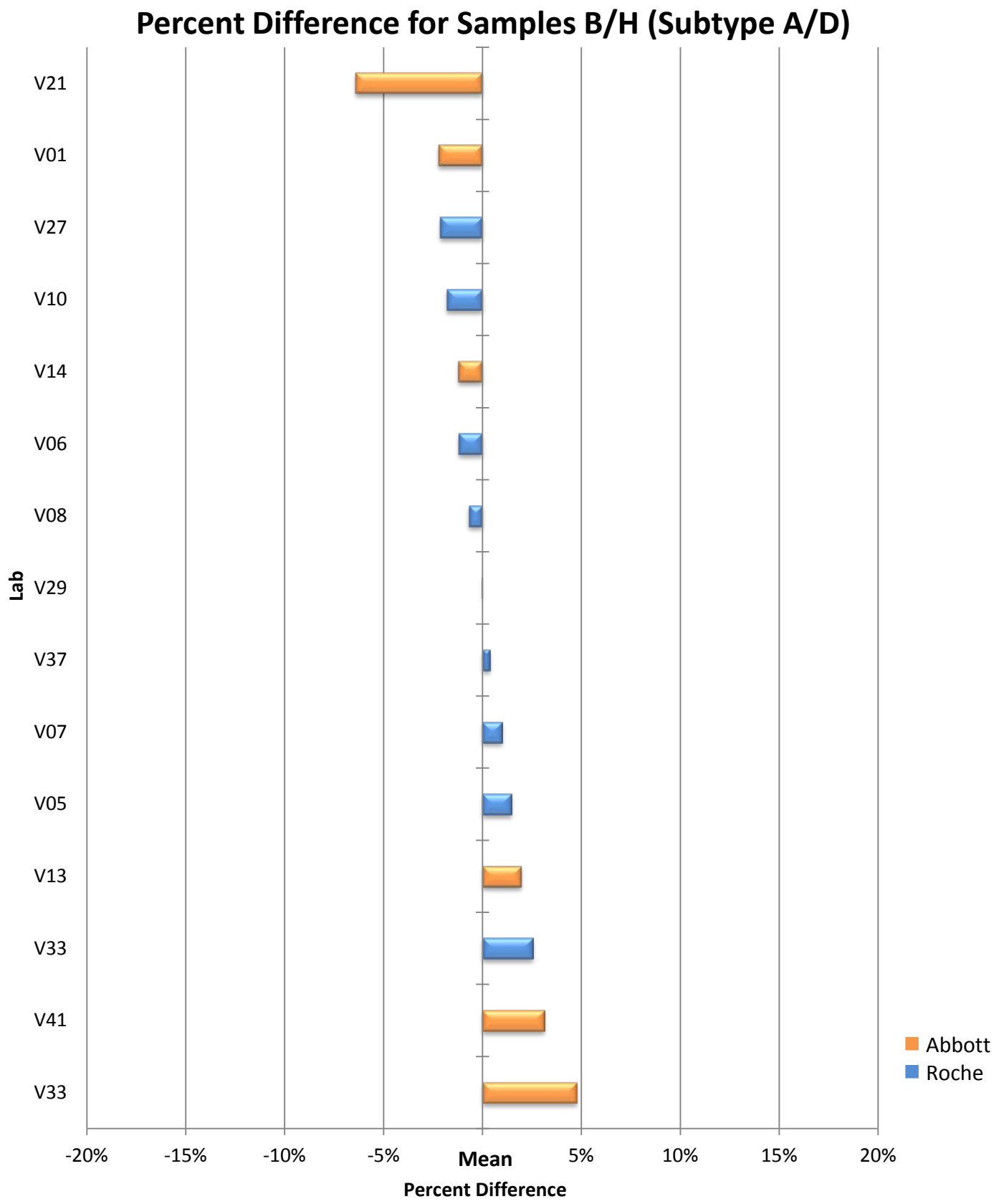

Figure 9: Percent difference from the peer group mean for B/H.

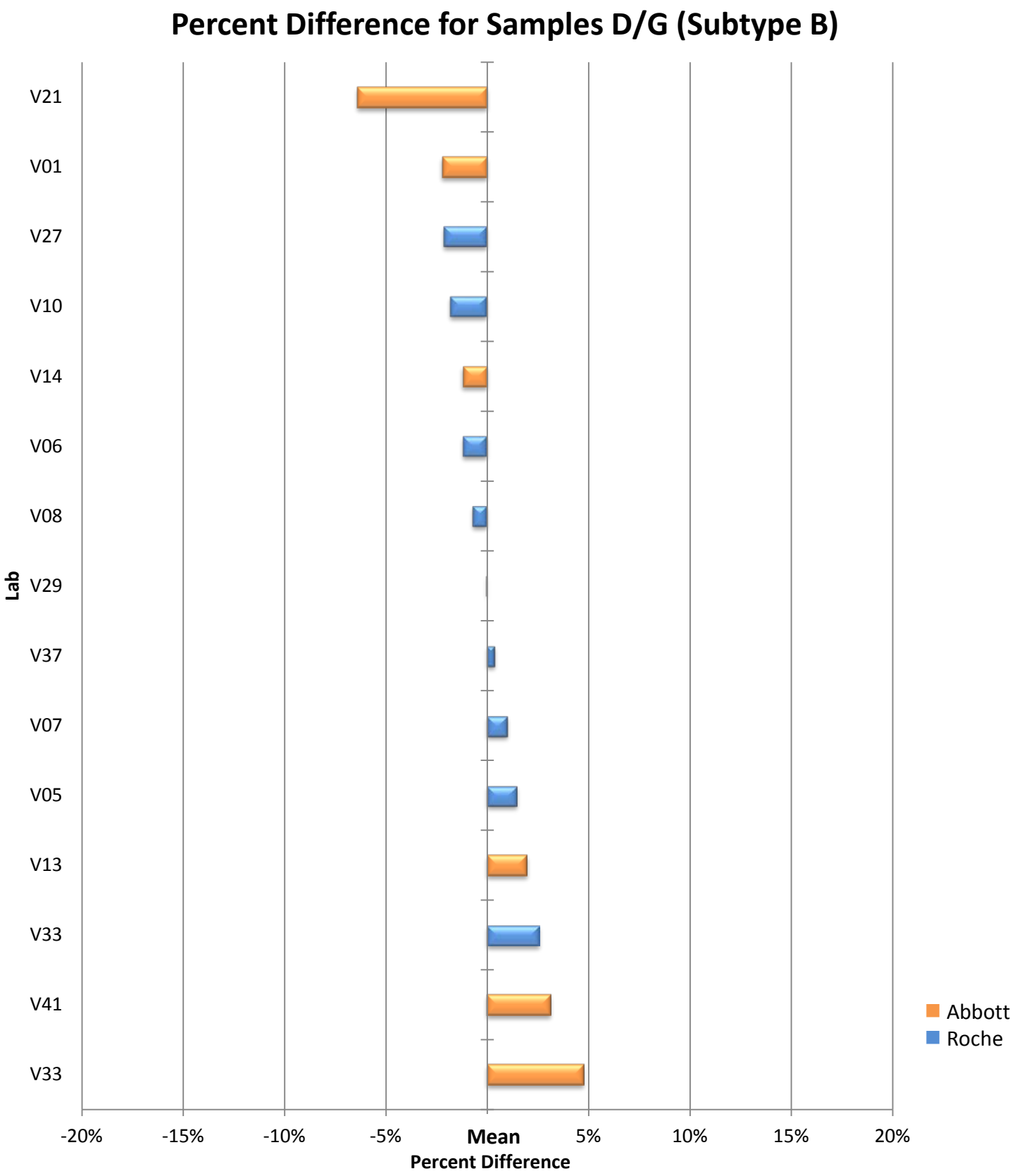

Figure 10: Percent difference from the peer group mean for D/G.

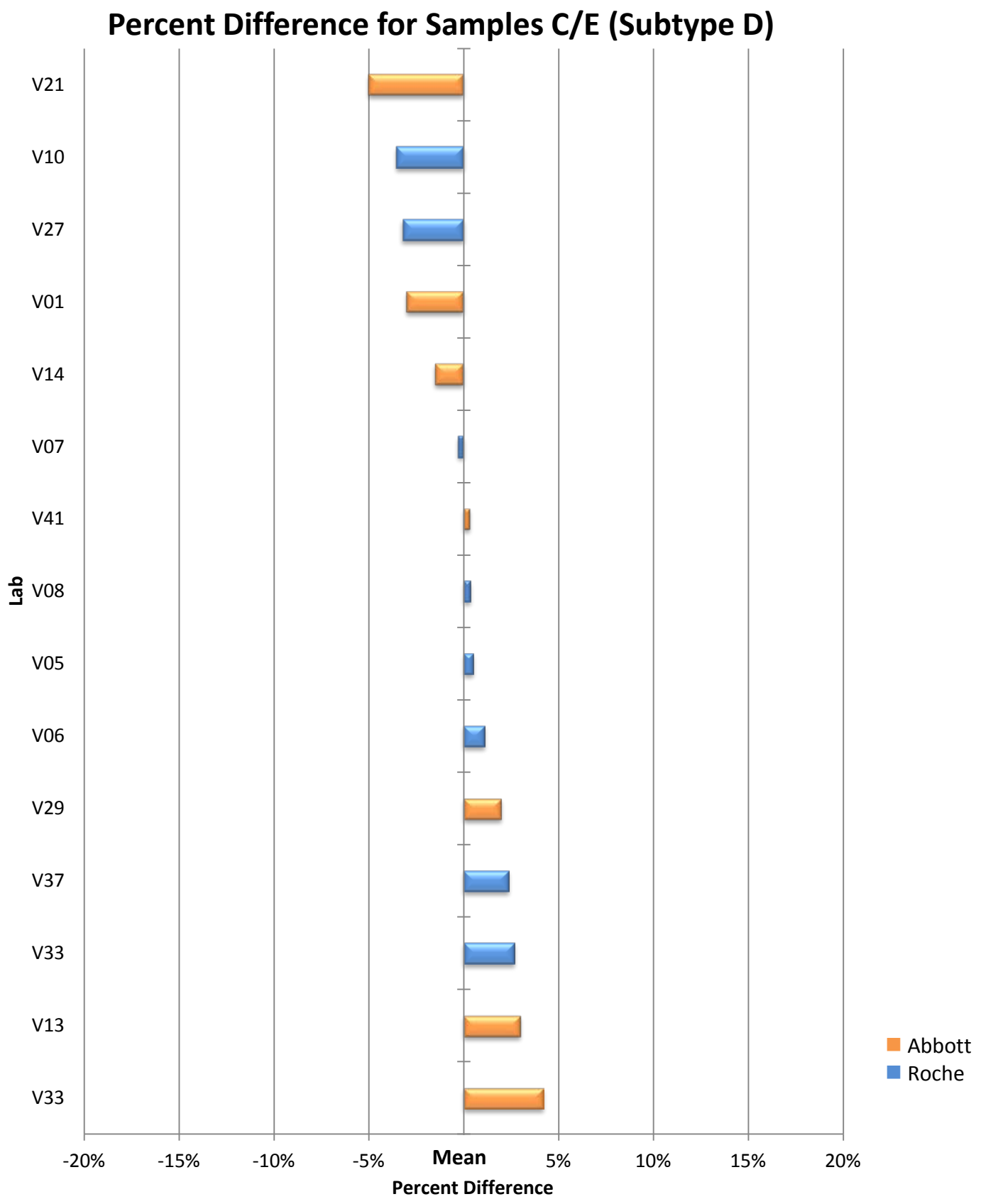

Figure 11: Percent difference from the peer group mean for C/E.

Conclusion

1. *Effect of non-B subtype on quantitation of HIV-1.*
    - The results from this panel indicate there is a difference between the Abbott and the Roche peer groups when compare the viral load results between the different subtype.

We would like to express our gratitude to those that participated in the beta testing of the new NLHRS QAP website. Your feedback will be used to finalize the submission website before it is fully implemented.

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:

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Thank you for your participation in the NLHRS Quality Assurance Program

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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
	B	H	C	E	D	G	A	F		
V05	3.22	3.29	3.25	3.22	3.23	3.19	< LDL	< LDL	Y16436	2019-06-30
V06	3.17	Error	3.28	3.23	3.34	3.25			Y16436	2019-06-30
V07	3.17	3.31	3.24	3.18	3.10	3.09			Y16436	2019-06-30
V08	3.16	3.21	3.24	3.23	3.08	3.03			E05473	2019-12-31
V10	3.18	3.12	3.06	3.15	3.12	3.01			E05473	2019-12-31
V27	3.10	3.18	3.11	3.12	3.00	3.08			E05473	2019-12-31
V33	3.27	3.31	3.36	3.25	3.24	3.21			Y17461	2019-06-30
V37	3.22	2.89	3.24	3.35	3.21	3.00			Y23998	2019-10-31
Mean	3.21		3.22		3.14					
Minimum	3.10		3.06		3.00					
Median	3.19		3.23		3.11					
Maximum	3.31		3.36		3.34					
% CV	2.18		2.66		3.20					
SD	0.07		0.09		0.10					
Inter-lab Variation	1.07		1.10		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
	B	H	C	E	D	G	A	F		
V48	3.15	3.17	3.01	2.99	3.20	3.13			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
	B	H	C	E	D	G	A	F		
V04	2.93	3.01	3.03	3.06	3.01	3.04			Y19586	2019-03-31

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
	B	H	C	E	D	G	A	F		
V28	3.00	2.90	3.10	2.90	2.30	2.70			17121501 18042302	2018-11-28 2019-08-28

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
	B	H	C	E	D	G	A	F		
V01	2.84	3.01	2.74	3.08	2.95	2.89			11621871 483548	2019-04-30 2019-05-28
V13	3.14	2.96	3.07	3.11	3.08	3.06			11726091 481998	2019-09-30 2019-04-13
V14	2.89	3.02	2.97	2.94	2.99	2.97			11621871 483548	2019-04-30 2019-05-28
V21	2.70	2.90	2.90	2.80	2.90	2.90			11726091 483548	2019-09-30 2019-05-28
V29	3.02	2.96	3.07	3.05	3.04	3.00			11614451 485508	2019-04-30 2019-07-04
V33	3.11	3.15	3.09	3.17	3.09	3.19			11718981 483548	2019-08-31 2019-05-28
V41	3.15	3.02	3.11	2.91	2.91	2.77			11726091 485976	2019-09-30 2019-09-28
Mean	2.99		3.00		2.98					
Minimum	2.70		2.74		2.74					
Median	2.99		3.01		3.01					
Maximum	3.15		3.11		3.11					
% CV	4.21		4.15		2.96					
SD	0.12		0.12		0.09					
Inter-lab Variation	1.17		1.14		1.14					
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
	B	H	C	E	D	G	A	F		
V49	3.12	3.08	3.17	3.15	3.09	3.01			1000096095	2019-03-10

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

Lab ID #	Sample Code								Sample Prep/PCR Kit Lot	Exp. Date
	B	H	C	E	D	G	A	F		
V11	3.09	3.05	3.08	3.12	3.09	3.06			11621871 483548	2019-04-13 2019-05-28

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.