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 2018Apr19

This panel focused on the impact of extended storage at -20°C and HIV-1 non-B subtype on quantitation.

2018Apr19 HIV-1 VL panel				
Storage Conditions	**Panel Sample Pair**	**Viral load Consensus mean[1]**	**Viral load mean characterization by the NLHRS**	**Labs Reporting Incorrect Final Status**
-20°C (35 days)	**B**	3.02[2], 2.93[3]	3.11[2], 3.11[3]	
	F			
-80°C	**A**	2.99[2],2.88[3]	3.06[2],3.07[3]	
	E			
-80°C (non-B)	**C**	3.26[2], 3.14[3]	3.29[2], 3.36[3]	
	H			
-80°C	**D**	TND	TND	
	G			

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 2018Apr19 HIV-1 VL panel.

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HIV Viral Load Quality Assessment Program

Final Report for Panel HIVVL 2018Apr19

Issued August-15-2018

Introduction

The NLHRS distributed the 2017Oct27 and the 2018Apr19 panels on Oct 11th 2017. This final report is specific to the 2018Apr19 only and is publicly available, however, the identity of participants is not disclosed. With the 2018Apr19 panel, we continued to look at the effect of extended storage at -20°C and the effect of HIV-1 non-B subtype on the ability to quantitate HIV-1 viral loads across several platforms.

Panel Samples, HIV Test Kits and Data Entry

1. *Panel Composition* – The 2018Apr19 panel is the 2017Oct27 HIV-VL panel that is relabelled (Table 1). It contained the following:
   - One negative sample sent in duplicate (D and G); defibrinated human plasma.
   - One positive sample HIV-1 RNA subtype B diluted to approximately 1000 copies/mL in defibrinated human plasma (Basemetrix 53, Seracare Life Sciences Inc.) and aliquoted for 4 identical samples (A,B, F, and E) to reduce the effect of variation due to preparation. Each pair was stored under different storage conditions (listed in Table 1).
   - 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.) and aliquoted in duplicates (C,H) and stored at -80°C
   - The NLHRS characterized the positive panel members on both the Roche and the Abbott platform to assess the $\log_{10}$ Cp/mL value for Sets 1, 2 and 3 with eight replicate prior to panel send out (Table 1).
     - Set 1 (B/F) was stored at -20°C for 35 days and then returned to -80°C.
     - Set 2 (A/E) was stored at the recommended temperature of -80°C.
     - Set 3 (C/H) was stored at the recommended temperature of -80°C.

Panel Samples, HIV Test Kits and Data Entry *(continued)*

Table 1: Description of panel 2018Apr19 samples					
Sample Identification	**Sample Type**	**Sample Subtype**	**Storage Conditions**	**Viral load Consensus mean[1]**	**Viral load characterization by NLHRS**
B F	HIV-1	B	-20°C (35 days)	3.02[2], 2.93[3]	3.11[2], 3.11[3]
A E	HIV-1	B	-80°C	2.99[2], 2.88[3]	3.06[2], 3.07[3]
C H	HIV-1	A/D	-80°C	3.26[2], 3.14[3]	3.29[2], 3.36[3]
D G	TND	-	-80°C	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*

2. *HIV Viral Load Test Kits* – 6 different assays were used by the 20 participants (excluding the NLHRS) who returned results (Figure 1).
3. *Data entry* - The NLHRS QAP used the web based Survey Monkey system to capture results.
4. *Submissions deadline* – April 19th, 2018.

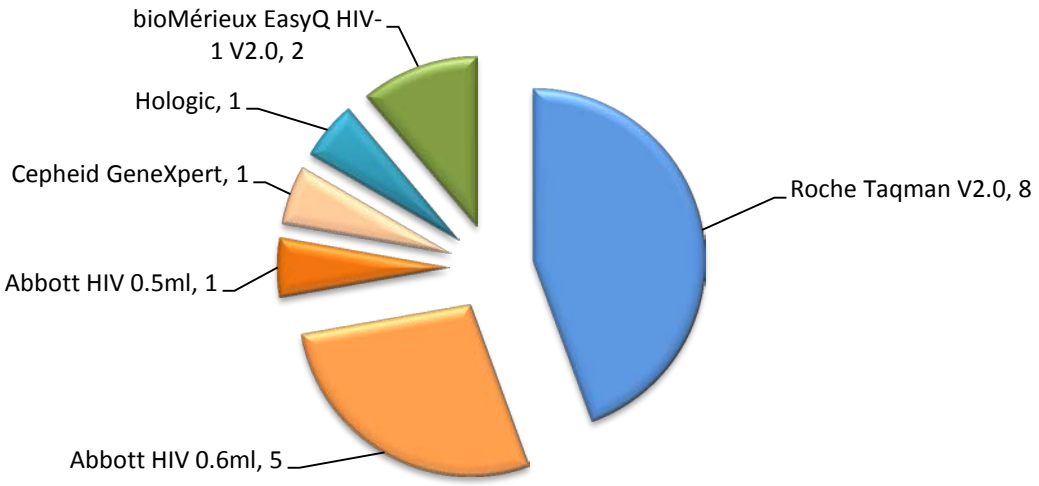

Figure 1: HIV-1 VL tests kits used by the participants for 2018Apr19 HIV-1 VL panel (excluding the NLHRS)

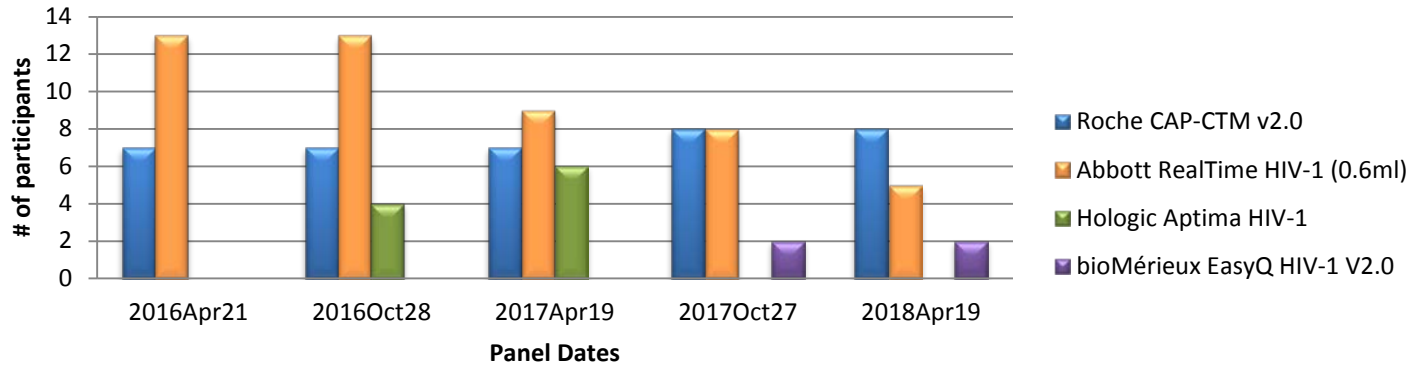

Figure 2: Distribution of HIV-1 assays (n>1) used by participants from 2016-2018 (excluding the NLHRS).

Return rate

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

- Two participants (V2 and V17) withdrew from the NLHRS HIV-1 VL QAP.
- One participant (V41) was not able to return results due to hardware failure on their instrument.
- One participant (V36) did not return results.
- Ten year average return rate of 90.7% (Figure 3).

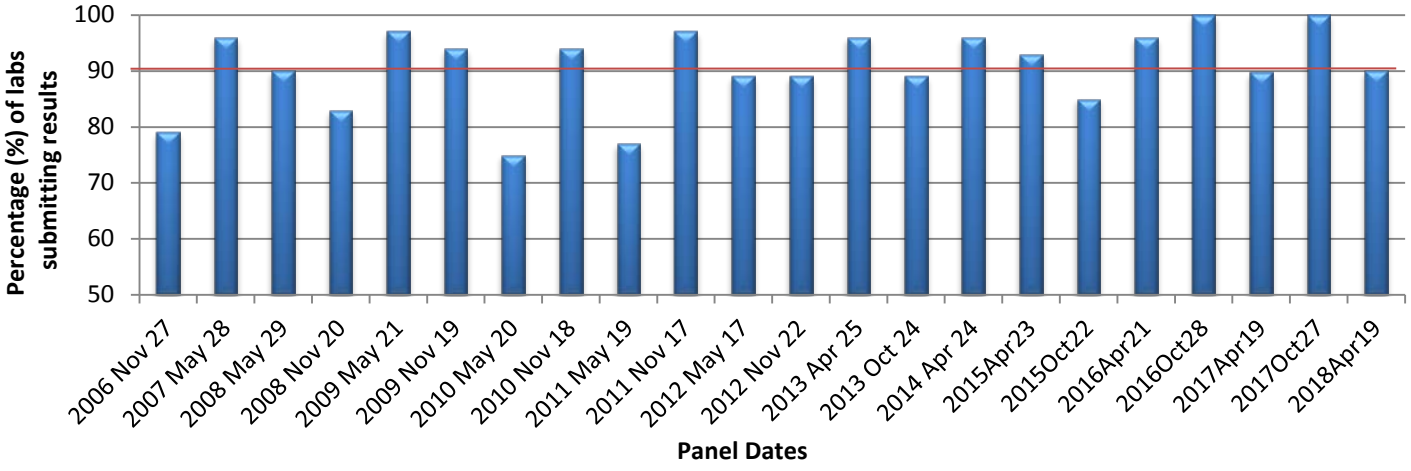

Figure 3: Percentage of HIV Viral Load Panel results submitted between 2006 and 2018

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.
   - 7 participants (38.9%, 7/18) reported using external QC material. Figure 4 illustrate the sources of the external control used.

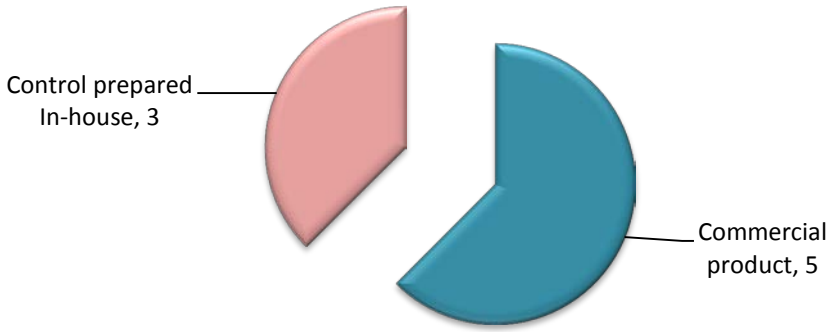

Figure 4: Distribution of HIV-1 viral load external control used by the participants (exclude the NLHRS).

2. *Quality Assurance (QA) programs* - Allows participants to evaluate their overall use of the assay and reporting of the results.
   - 15 participants (83.3%, 15/18) reported participation in other quality assurance programs (Figure 5).

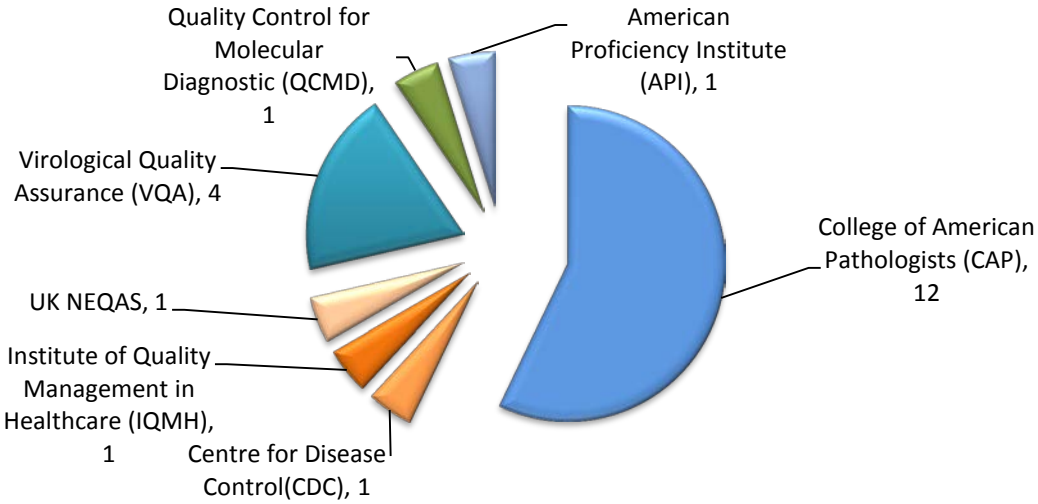

Figure 5: Distribution of other external quality assurance programs that the participants are enrolled in.

Participant's feedback

- 17 of 18 participants provided feedback for the 2018Apr19 HIV-1 VL survey. 15 liked the changes made to the survey but 2 did not (Figure 6).
- 7 participants preferred the current survey while 7 participants have identified areas of improvement for the next survey (Figure 7).

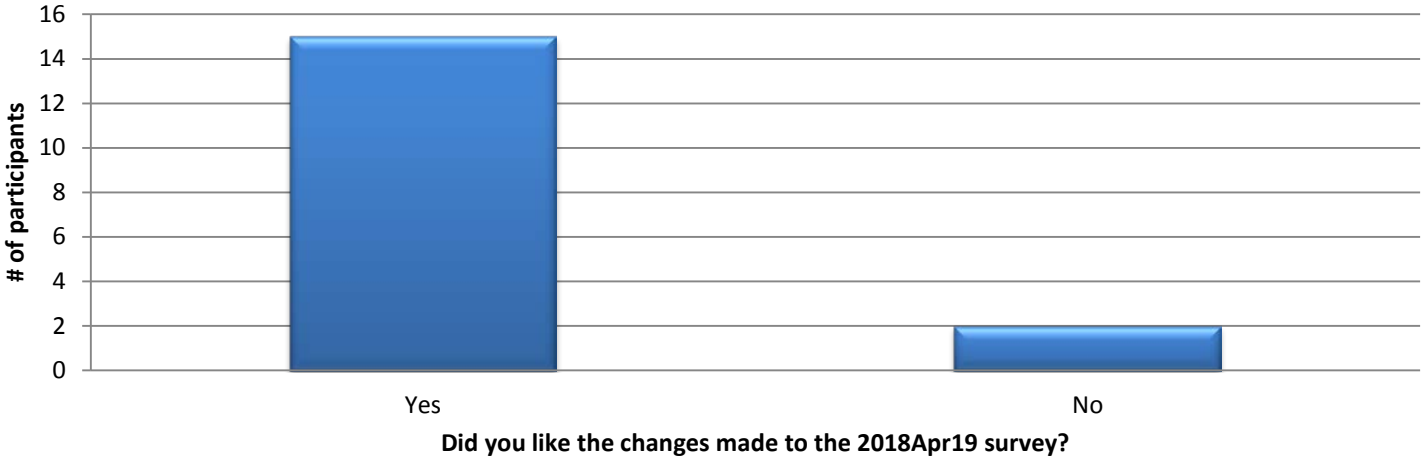

Figure 6: Participant's responses when ask if they liked the changes made to the 2018Apr19 survey.

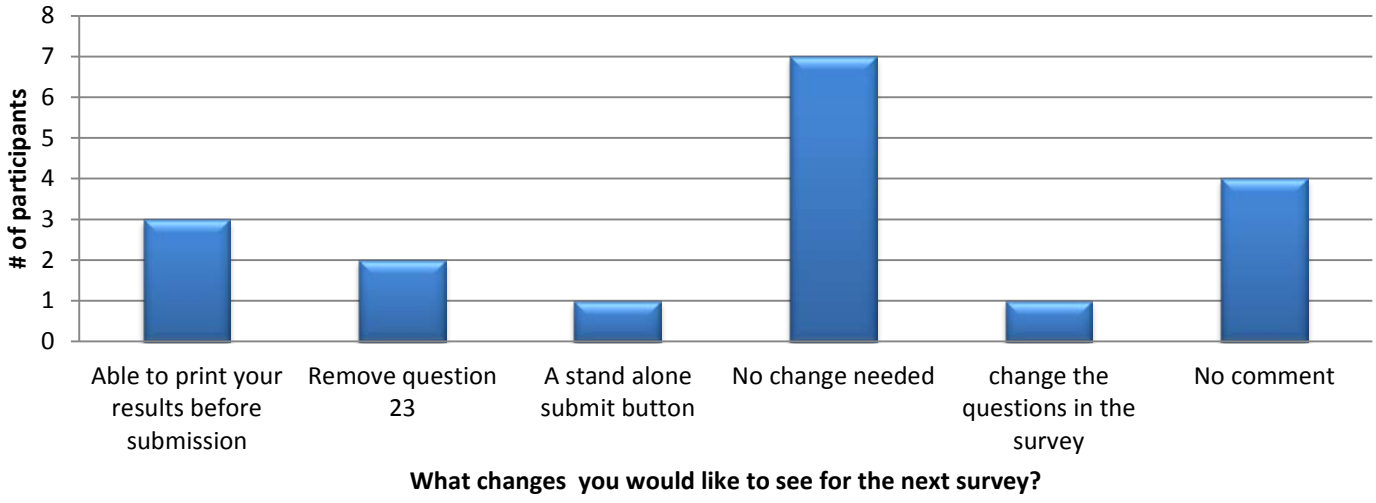

Figure 7: Participant's response to which area requires improvement in the NLHRS HIV-1 viral load survey.

Homogeneity and stability

- The homogeneity of the 2018Apr19 HIV-1 viral load panel is assessed by using the Roche assay peer group (n=9) results in the positive duplicate sample set (A/E, B/F, C/H). All participants were able to detect HIV-1 RNA and the results are within +/- 0.5 $\log_{10}$ Cp/mL of the group mean (Appendix 1). There is no indication of heterogeneity in the panel samples.
- The stability of the 2018Apr19 HIV-1 viral load panel is assessed by comparing the group mean generated by the participants in the positive duplicate sample set (A/E) to the 2017Oct27 group mean in the positive duplicate sample set (B/G). The difference between both means does not exceed 0.5 Log_{10} Cp/mL (Table 2). The panel is stable in -80°C storage between the 2 testing events (2017Oct27 and 2018Apr19).

Table 2: Stability testing of the 2018Apr19 HIV-1 viral load panel

LAB	2017Oct27 group mean for sample set (B,G), Log_{10} Cp/mL	2018Apr19 group mean for sample set (A,E), Log_{10} Cp/mL	Difference between both means, Log_{10} Cp/mL
Roche peer group	3.03	2.99	0.04
Abbott 0.6 mL peer group	3.03	2.88	0.15

Flags

1. Participant V10, did not submit their results as Log_{10} Cp/mL as instructed.
2. Participant, V21 and V28, did not submit their results in 2 decimal places as instructed.
3. Participant V36 did not return results.

Results

1. **Statistical Analysis (General)**
   - No outlier was detected and removed from analysis (Grubb's test)
   - All group comparisons were done using the unpaired *t* test.
   - No significant differences were identified ($p > 0.05$) in duplicate sets; A/E, B/F, C/H between the Roche and Abbott users.
     - Data for each set was combined and analyzed together.
   - No analysis for peer groups of n=1 (Abbott 0.5mL, Hologic Aptima and Cepheid GeneXpertII)
   - Users of the bioMérieux EasyQ HIV-1 V2.0 were not included in the analysis due to limited users
   - Negative samples were analyzed qualitatively.

Results (continued)

2. **Group Analysis (Summary Statistics)** (Figure 7, Tables 7A, 7B)
   - o The duplicate panel samples were combined for the summary statistics (A/E, B/F, C/H).

Inter-Lab Variation

- o Difference between the minimum and maximum results for each sample within a peer group (the maximum value divided by minimum).
- o Average of 1.09 for the Roche CAP/CTM v2. and 1.23 for the Abbott RealTime (0.6mL).

Reproducibility

- o This is an important aspect of viral load testing, required to quantify changes in viral load.
- o To assess intra-reproducibility, duplicates of the positive samples were included in the panel.
- o All participants reported standard deviation (SD) of 0.33 or lower between duplicates (Table 3).

Table 3: Standard deviation (log_{10} Cp/mL) reported between duplicates from participant's results for the 2018Apr19 panel (excludes NLHRS).

LAB	Sample A and E	Sample B and F	Sample C and H
V01	0.06	0.08	0.10
V04	0.04	0.13	0.10
V05	0.02	0.15	0.03
V06	0.01	0.02	0.07
V07	0.01	0.05	0.04
V08	0.04	0.04	0.11
V10	0.11	0.01	0.04
V11	0.10	0.04	0.06
V13	0.18	0.08	0.33
V14	0.01	0.04	0.31
V21	0.07	0.14	0.07
V26	0.12	0.02	0.06
V27	0.04	0.01	0.03
V28	0.00	0.14	0.00
V29	0.03	0.18	0.06
V36	N/A	N/A	N/A
V37	0.01	0.04	0.08
V41	N/A	N/A	N/A
V48	0.03	0.04	0.05
V49	0.12	0.00	0.06

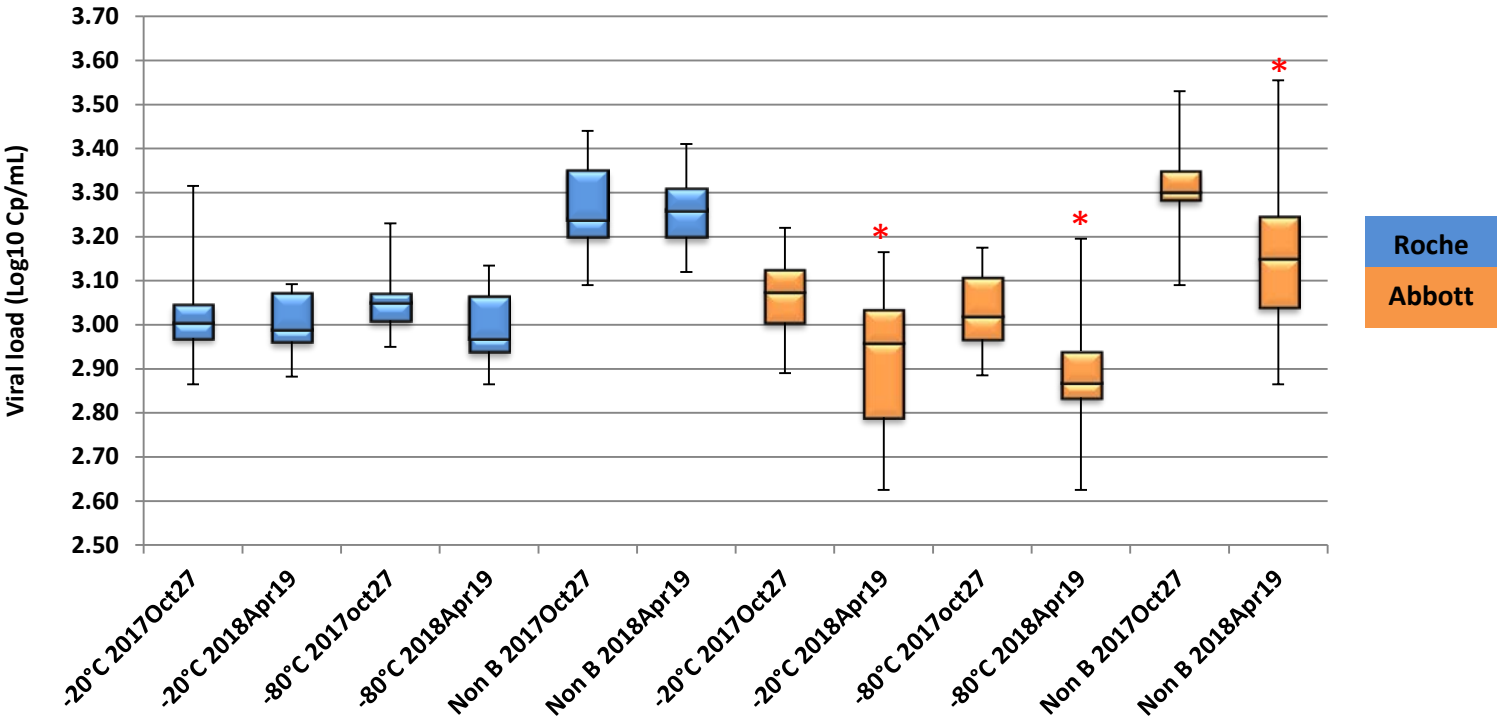

Figure 7: Comparison of the 2017Oct27 and 2018Apr19 HIV-1 Viral load results

* *Difference between maximum and minimum is > 0.5 Log_{10}*

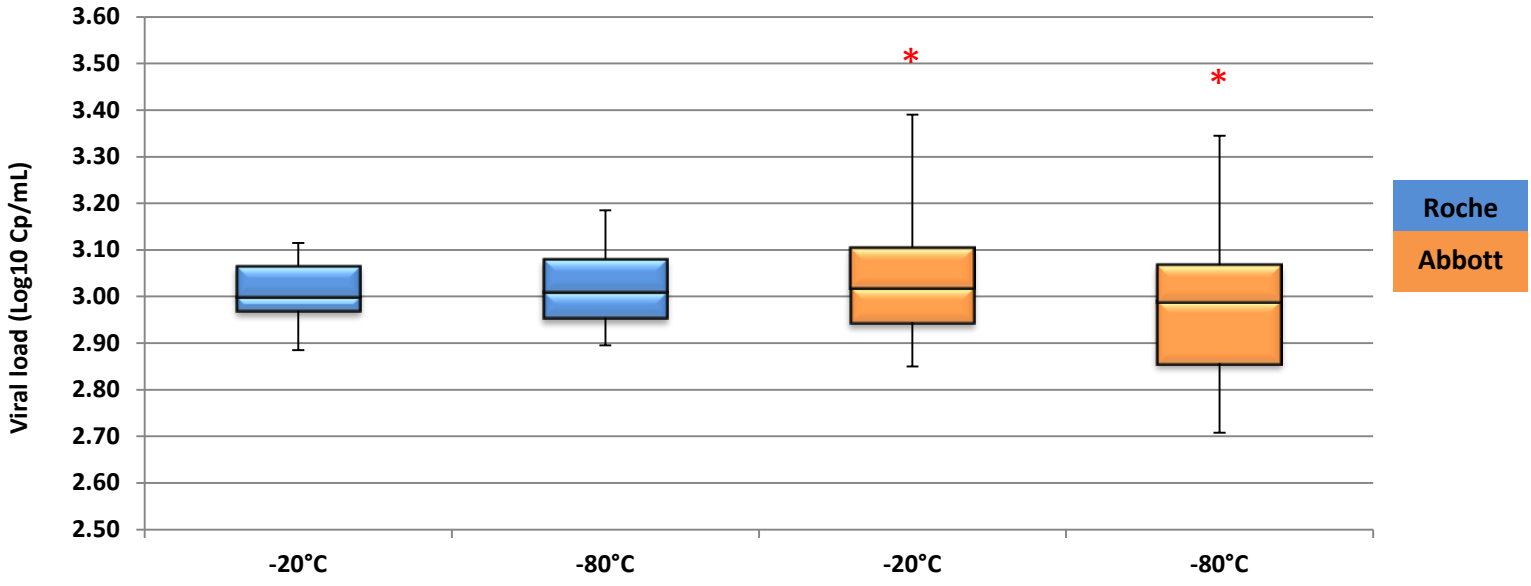

Figure 8: The combined results of the 2017Oct27 and 2018Apr19 HIV-1 VL panel at different storage temperature.

* *A statistical difference between the 2017Oct27 and 2018Apr19 HIV-1 VL results,* ($p<0.05$)

Table 4: Comparison of the mean viral load of the 2018Apr19 panel between the major and minor peer group			
LAB	**Sample A/E**	**Sample B/F**	**Sample C/H**
Roche peer group	2.99	3.02	3.26
Abbott 0.6 mL peer group	2.88	2.93	3.14
V11	2.90	2.85	3.10
V26	2.81	2.84	3.10
V28	2.10	2.20	3.10
V48	3.02	3.02	3.24
V49	3.10	3.15	3.26

3. **Effect of Suboptimal Storage** (Figure 7 and Figure 8)

   Storage at -20°C for 35 days (Samples B, F)

   - *Abbott RealTime 0.6mL (n=6)* - Participant results (including the NLHRS) showed no statistical difference between storage at -20°C for 35 days compared to -80°C (p=0.4776). This is consistent to what was observed in previous panels.
   - *Roche CAP/CTM v2.0 (n=9)* - Participant results (including the NLHRS) showed no statistical difference between storage at -20°C for 35 days compared to -80°C (p= 0.3618). This is consistent with what was observed in previous panels
   - The results from participants that had participated in both panels are combined and analyzed. There is no significant difference in the viral results between sample stored at -20°C and -80°C.

4. **Effect of non-B HIV-1 subtype (**Table 1 and Table 7A, 7B)

   Non-B subtype (Samples C, H)

   - The NLHRS was not able to determine if there was any significance between a HIV-1 non-B subtype and a HIV-1 B subtype since viral load results for samples C and H were not comparable to samples A and E. This would not have been a fair group comparison.
   - The group mean results from the Abbott and Roche users peer group indicates both platforms are able to quantitate non-B HIV-1 subtype.

5. **Individual Analysis (Participant Statistics)** (Figures 9, 10, 11 and Tables 7A, 7B, 7C,7D,7E,7F,7G)
   - This is the difference from the mean of the peer group for each sample expressed as a percentage.
   - The percent difference (%D) was calculated for each storage condition for each lab
   - The +/- 2 and 3 standard deviation from the mean of the peer group is expressed as a percentage as well.

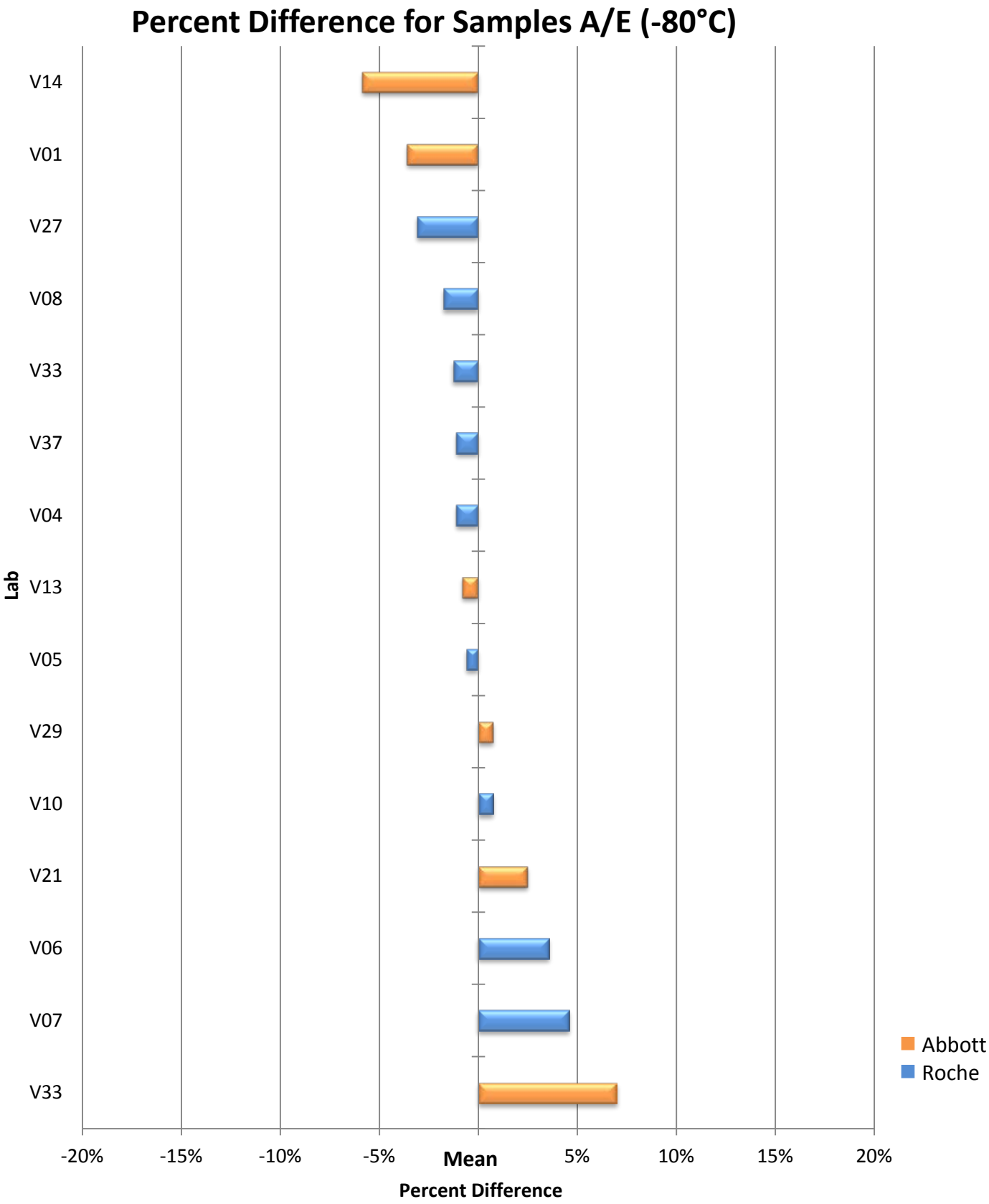

Figure 9: Percent Difference from the Peer Group Mean of A/E.

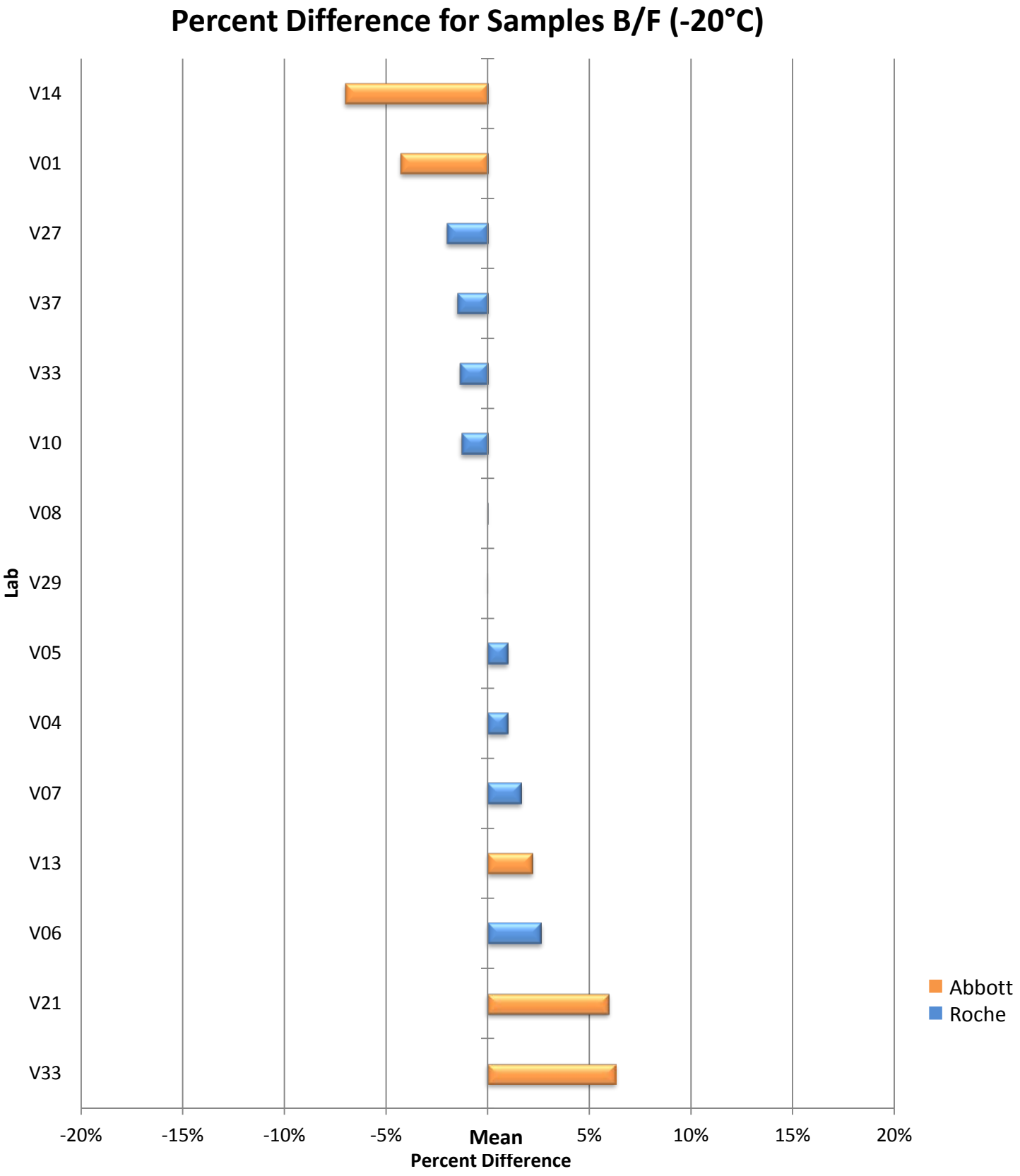

Figure 10: Percent Difference from the Peer Group Mean of B/F.

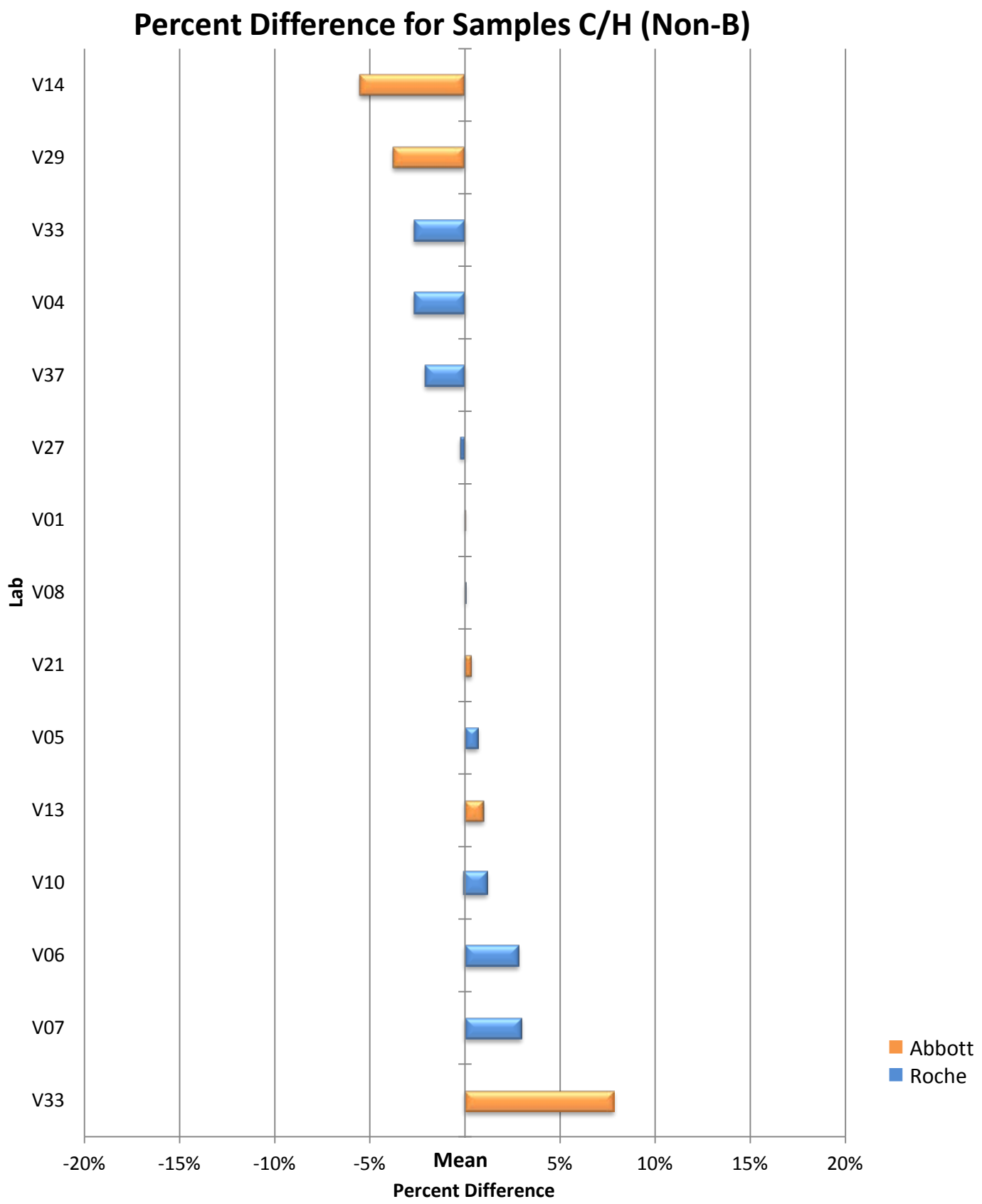

Figure 11: Percent Difference from the Peer Group Mean of C/H.

Table 5: Statistical comparison of results for Roche CAP/CTM v2.0 and Abbott RealTime 0.6mL for samples stored at -20°C (2013-2018 NLHRS panels)				
Sample	**Storage Temperature vs -80C**	**Assay**	**Panel**	**p-value**
Subtype B	-20°C for 35 days	Abbott RealTime 0.6mL	2018Apr19	**0.4776**
			2017Oct27	**0.2623**
		Roche CAP/CTm v2.0	2018Apr19	**0.3618**
			2017Oct27	**0.1875**
Subtype B	-20°C for 13 months	Abbott RealTime 0.6mL	2015Oct22	**0.0243**
			2015Apr23	**0.1927**
		Roche CAP/CTM v2.0	2015Oct22	**0.1262**
			2015Apr23	**0.9328**
	-20°C for 8 months	Abbott RealTime 0.6mL	2015Oct22	**0.0469**
			2015Apr23	**0.0217**
		Roche CAP/CTM v2.0	2015Oct22	**0.1550**
			2015Apr23	**0.2400**
	-20°C for 35 days	Abbott RealTime 0.6mL	2014Oct23	**0.0600**
			2014Apr24	**0.9628**
		Roche CAP/CTM v2.0	2014Oct23	**0.8970**
			2014Apr24	**0.5628**
Subtype C	-20°C for 6 days	Abbott RealTime 0.6mL	2013*	**0.0076**
		Roche CAP/CTM v2.0	2013Oct24	**0.4019**
			2013Apr25	**0.6202**

** Combined the 2013Apr25 and 2013Oct24 panel results, no significant statistical difference (p > 0.2)*

Conclusion

1. *Effect of Temperature*
   - Over the course of the last 5 years, we challenged 3 commercial viral load platforms with sub-optimal storage temperatures.
   - Outlined below in Table 6 is the summary of the storage temperatures at -20°C for each platform.

Table 6. Impact of sample stored at -20°C on HIV-1 quantitation on Abbott RealTime HIV-1 0.6mL, Roche CAP/CTM v2.0 and Hologic Panther Aptima HIV-1 observed in the NLHRS QAP HIV-1 VL testing program from 2013-2018	
Platforms	**-20°C(at various storage time)**
Abbott RealTime HIV-1 0.6 mL	Not Significant[1]
Roche CAP/CTM v2.0	Not Significant
Hologic Panther Aptima HIV-1	No data

1. *The results from the surveys is indicative there is no effect on HIV-1 quantitation for storage at -20°C*

   - Confounding factors such as kit lots used, duration of sub-optimal temperature and different technologists performing the assay must be taken into account.

Conclusion (continued)

2. *Effect of non-B subtype on quantitation of HIV-1.*
    - The results from this panel indicated all platforms were able to quantitate non-B HIV-1 subtype without any advantages over one another.
    - The NLHRS will continue to investigate for the effect of non-B subtype on quantitation of HIV-1 in the future

3. *Performance of the participants in each peer group.*
    - All participants returned the expected results.
    - V14's viral load results were lower in the duplicate sets among the Abbott user peer group. This trend was observed in the previous panel and it is indicative of a potential systematic error.
    - V28's results in all three sample groups (A/E, B/F, C/H) were comparably lower to the same sample group in the previous panel
    - The NLHRS will continue to monitor this trend and will be interacting with individual laboratories to identify the potential systematic error.

4. *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 group, the Roche and Abbott 0.6mL users
    - The results from the Cepheid GeneXpertII, Hologic Aptima HIV-1 and the Abbott 0.5 mL users are comparable to the Roche and Abbott 0.6mL peer group.
    - The HIV-1 viral load results of the bioMerieux BV NucliSens EASYQ HIV-1 users were lower when compared to the Roche and Abbott 0.6mL. This was also observed in the 2017Oct27 panel.
    - A proper and fair comparison between the different peer groups would require more users of the GeneXpertII, Hologic Aptima, Abbott 0.5 mL and the bioMerieux BV NucliSens EASYQ HIV-1 platform

Conclusion (continued)

5. There is a large variation in the viral load results in the Abbott user group for all three samples. This lack of precision did not occur in the 2017Oct27 panel. Compared with the results submitted for the 2017Oct27 panel, the results from the 2018Apr19 panel is noticeably lower. This attributed to a statistical difference between the 2017Oct27 and the 2018Apr19 results. Unlike the Abbott peer group, the results from the Roche peer group are precise and there are no statistical differences between the 2 panels. This suggests something specific to the Abbott reagents used in the 2018Apr19 panel might be the cause of this imprecision. The NLHRS will investigate this further.

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

If you would like to make an appeal, please submit your concerns to:

phac.nlhrslnsrv.aspc@canada.ca

We value each laboratory's participation in these QA panels therefore we are taking into consideration suggestions to improve the method of data entry and reporting.

Thank you for your participation in the NLHRS Quality Assurance Program

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

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

Appendix 1: Test Results

Legend: **Incorrect result** **Outliers Removed**

Table 7A Roche CAP/CTM v2.0 Test Results (Log_{10} HIV RNA Copies/mL)

Lab ID #	Sample Code								Kit lot	Exp. date
	A	E	B	F	C	H	D	G		
V04	2.93	2.99	3.14	2.95	3.10	3.24	<LDL	<LDL	Y10651	2018-11-30
V05	2.99	2.96	2.94	3.15	3.26	3.30			Y16242	2019-04-30
V06	3.11	3.09	3.11	3.08	3.40	3.30			X57399	2018-08-31
V07	3.14	3.12	3.10	3.03	3.33	3.38			Y16242	2019-04-30
V08	2.91	2.97	3.04	2.99	3.34	3.18			Y16436	2019-06-30
V10	2.94	3.09	2.99	2.97	3.32	3.27			Y17461	2019-06-30
V27	2.93	2.87	2.95	2.96	3.27	3.23			Y17461	2019-06-30
V33	2.94	2.97	2.97	2.98	3.15	3.19			Y16436	2019-06-30
V37	2.95	2.97	3.00	2.94	3.13	3.25			X55545	2018-06-30
Mean	2.99		3.02		3.26					
Minimum	2.87		2.94		3.10					
Median	2.97		2.99		3.26					
Maximum	3.14		3.15		3.40					
% CV	2.70		2.35		2.58					
SD	0.08		0.07		0.08					
Inter-lab variation	1.10		1.07		1.09					
Measurement of Uncertainty	0.43		0.43		0.43					

Table 7B Abbott RealTime Results (0.6mL) (Log_{10} HIV RNA Copies/mL)

Lab ID #	Sample Code								Kit lot	Exp. date
	A	E	B	F	C	H	D	G		
V01	2.73	2.82	2.86	2.74	3.07	3.21			481998	2019-04-13
V13	2.85	2.86	2.96	3.02	2.95	3.39			481998	2019-04-13
V14	2.58	2.84	2.66	2.78	3.20	2.73			481998	2019-04-13
V21	3.00	2.90	3.20	3.00	3.20	3.10			483394	2019-05-28
V29	2.92	2.88	3.05	2.80	3.06	2.98			480537	2018-09-10
V33	3.15	3.01	3.11	Error	3.35	3.42			480291	2018-11-04
V41	N/A	N/A	N/A	N/A	N/A	N/A			N/A	N/A
Mean	2.88		2.93		3.14					
Minimum	2.58		2.66		2.73					
Median	2.87		2.96		3.15					
Maximum	3.15		3.20		3.42					
% CV	4.97		5.79		6.37					
SD	0.14		0.17		0.20					
Inter-lab variation	1.22		1.20		1.25					
Measurement of Uncertainty	0.14		0.14		0.14					

Appendix 1: Test Results

Legend: **Incorrect result** **Outliers Removed**

Table 7C Hologic Panther Aptima HIV-1 (Log_{10} HIV RNA Copies/mL)										
Lab ID #	Sample Code								Kit lot	Exp. date
	A	E	B	F	C	H	D	G		
V48	3.04	2.97	2.99	3.04	3.24	3.27			185343	2018-04-15

Table 7D Abbott RealTime (0.2mL) Results (Log_{10} HIV RNA Copies/mL)										
Lab ID #	Sample Code								Kit lot	Exp. date
	A	E	B	F	C	H	D	G		
V36	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	*Not provided*	

Table 7E Abbott RealTime (0.5mL) Results (Log_{10} HIV RNA Copies/mL)										
Lab ID #	Sample Code								Kit lot	Exp. date
	A	E	B	F	C	H	D	G		
V11	2.83	2.97	2.87	2.82	3.14	3.05			*481998*	*2019-04-13*

Table 7F Cepheid GeneXpert Results (Log_{10} HIV RNA Copies/mL)										
Lab ID #	Sample Code								Kit lot	Exp. date
	A	E	B	F	C	H	D	G		
V49	3.01	3.18	3.12	3.15	3.47	3.39			*1000080419*	*2018-07-29*

Table 7G bioMerieux BV NucliSens EASYQ HIV-1 Results (Log_{10} HIV RNA Copies/mL)										
Lab ID #	Sample Code								Kit lot	Exp. date
	A	E	B	F	C	H	D	G		
V26	2.99	2.62	3.04	2.64	3.68	3.47			*17051501*	*2018-04-28*
V28	2.10	2.10	2.30	2.10	3.10	3.10			*17062701*	*2018-06-28*

Appendix 2: 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.