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 2017Oct27

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

2017Oct27 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)	**D** / **H**	2.99[2], 3.07[3]	3.11[2], 3.11[3]	
-80°C	**B** / **G**	3.03[2],3.03[3]	3.06[2],3.07[3]	
-80°C (non-B)	**A** / **E**	3.27[2], 3.30[3]	3.29[2], 3.36[3]	
-80°C	**C** / **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, Log
3. Based on Abbott RealTime HIV-1 0.6 ml assay

Incorrect test result:

All participants reported the correct final status for all samples in the 2017Oct27 HIV-1 VL panel.

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Final Report for Panel HIVVL 2017Oct27

Issued 12-December-2017

Introduction

The NLHRS distributed the 2017Oct27 panel and the 2018Apr19 panel on Oct 11th 2017. This final report is specific to the 2017Oct27 only and is publicly available, however, the identity of participants is not disclosed. The 2017Oct27 panel 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.

Panel Samples, HIV Test Kits and Data Entry

1. *Panel Composition* – Panel 2017Oct27 (Table 1) contained the following:
   - One negative sample sent in duplicate (C and F); 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 (B,D, G, and H) to reduce the effect of variation due to preparation. Each pair was stored under different storage conditions (listed in Table 1).
   - One positive sample HIV-1 RNA (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 (A,E) 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 test event (Table 1).
     - Set 1 (D/H) was stored at -20°C for 35 days and then returned to -80°C.
     - Set 2 (B/G) was stored at the recommended temperature of -80°.
     - Set 3 (A/E) was stored at the recommended temperature of -80°C.

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

Table 1: Description of panel 2017Oct27 samples					
Sample Identification	**Sample Type**	**Sample Subtype**	**Storage Conditions**	**Viral load Consensus mean[1]**	**Viral load characterization by NLHRS**
D H	HIV-1	B	-20°C (35 days)	2.99[2], 3.07[3]	3.11[2], 3.11[3]
B G	HIV-1	B	-80°C	3.03[2], 3.03[3]	3.06[2], 3.07[3]
A E	HIV-1	A/D	-80°C	3.27[2], 3.30[3]	3.29[2], 3.36[3]
C F	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* – 7 different assays were used by the 26 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* – October 27th, 2017.

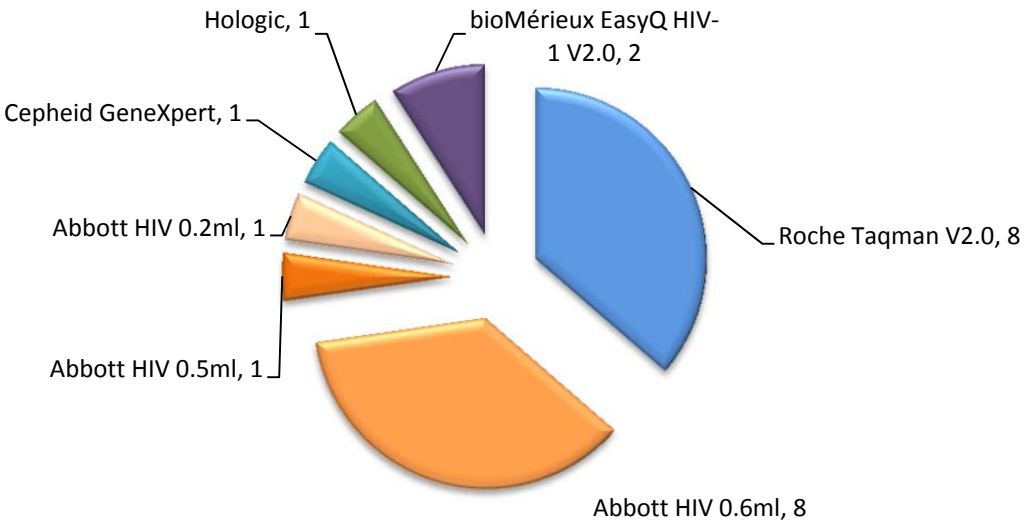

Figure 1: HIV-1 VL test kits used by the participants for 2017Oct27 HIV-1 VL panel (excluding the NLHRS)

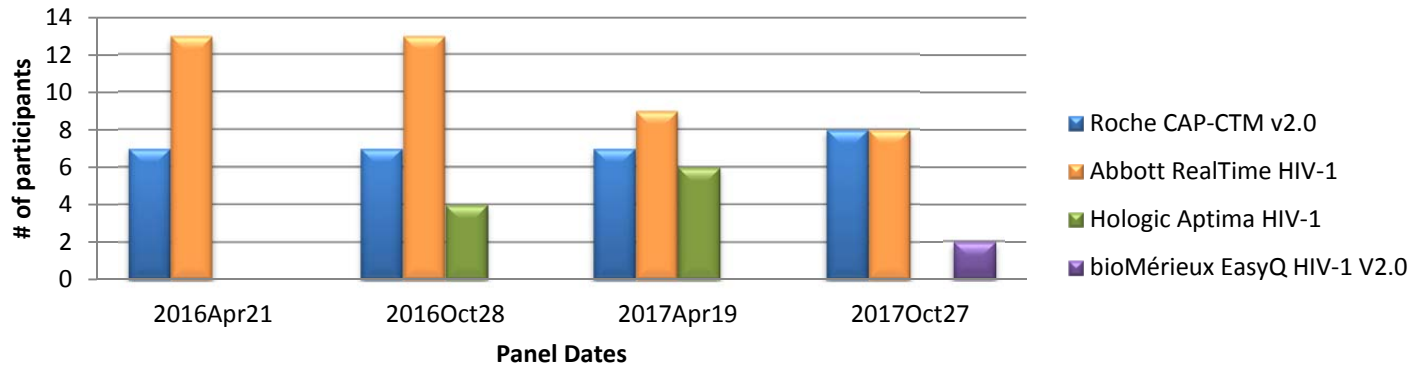

Figure 2: Distribution of HIV-1 assays (n>1) used by participants from 2016-2017 (excluding the NLHRS).

Return rate

Results were returned from 100% of participants (22/22).

- Six participants could not participate in the NLHRS QAP HIV-1 VL proficiency testing program for the time being, reducing the Hologic user group to one participant.
- One participant (V11) submitted results past the submission deadline (Oct 27, 2017)
- Ten year average return rate of 90.7% (Figure 3).

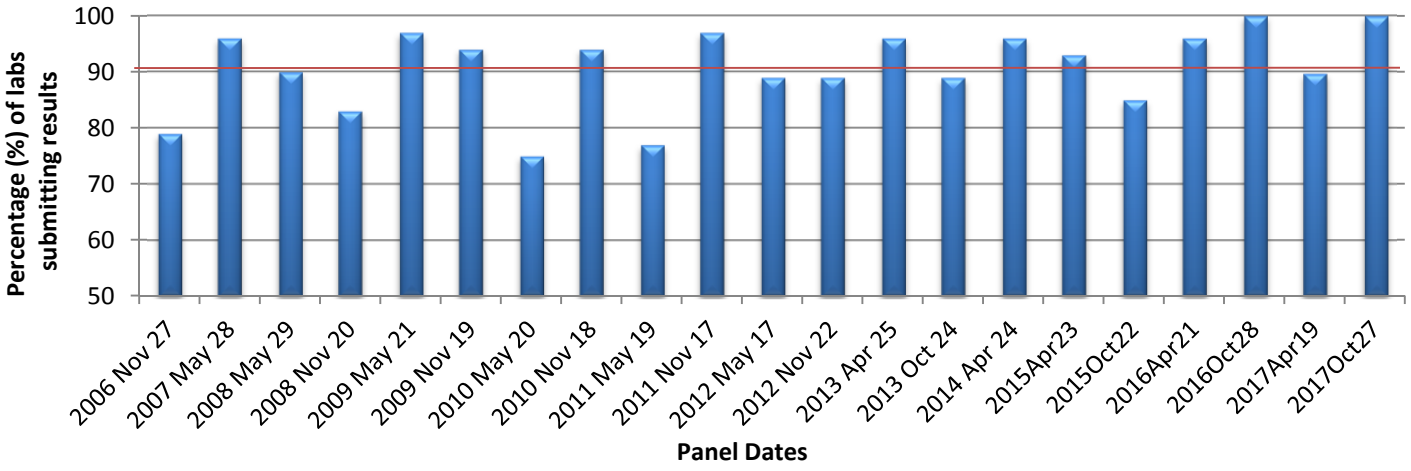

Figure 3: Percentage of HIV Viral Load Panel results submitted between 2006 and 2017

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.
   - 9 participants (40.9%, 9/22) reported using external QC material. The number of participants that reported using external quality control material in their assay remains the same as previous panels.

2. *Quality Assurance (QA) programs* - Allow participants to evaluate their overall use of the assay and reporting of the results.
   - 18 participants (81.8%, 18/22) reported participation in other quality assurance program (Figure 4).

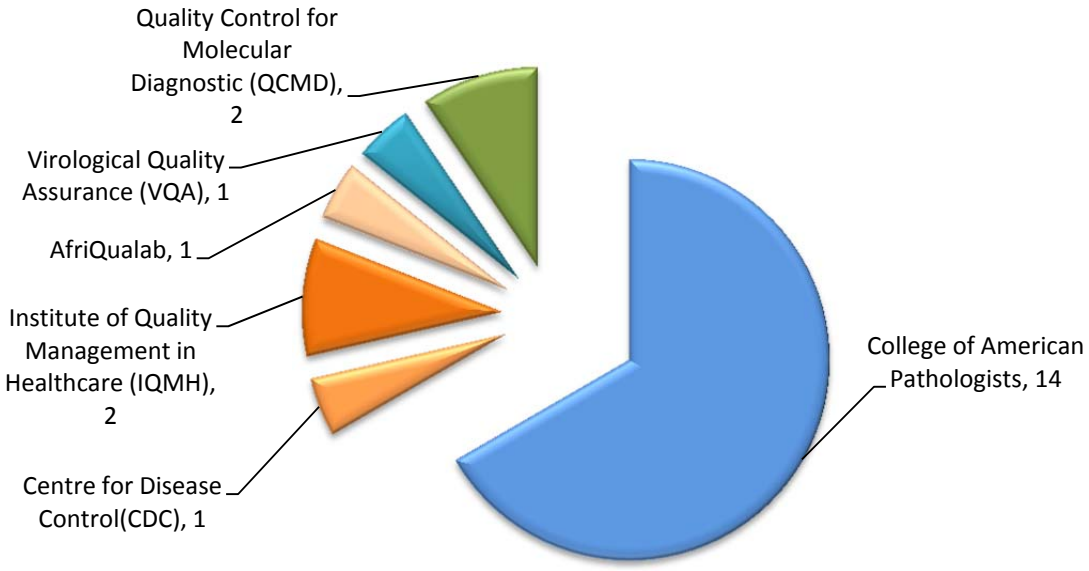

Figure 4: Distribution of other external quality assurance programs that the participants are enrolled in.

Participant's feedback

- The participants were asked to give their feedback and suggestions on how the NLHRS as a proficiency testing provider for HIV-1 Viral load could improve.
- 19 of 22 participants provided feedback and found the NLHRS HIV-1 viral load quality assurance program to be satisfactory. 3 participants did not provide feedback (Figure 5).
- 9 of 18 participants provided no comment when asked which area the NLHRS could improve upon while 3 participants see no need for improvement.
- 6 of 18 participants did provide suggestions on how the NLHRS could improve as a proficiency testing provider (Figure 6)

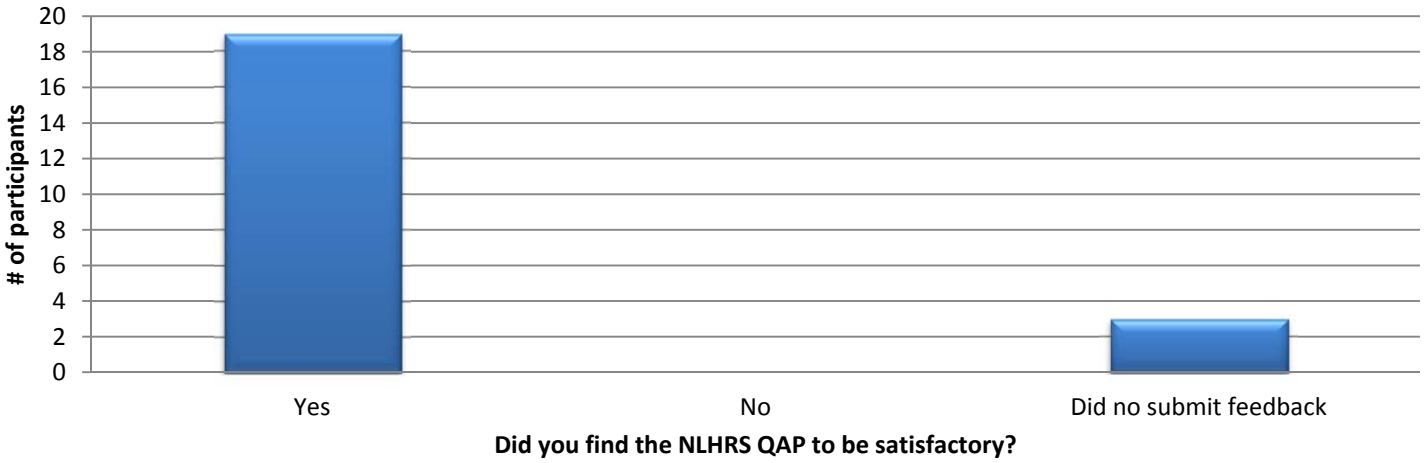

Figure 5: Participant's responses when ask if they find the NLHRS QAP to be satisfactory.

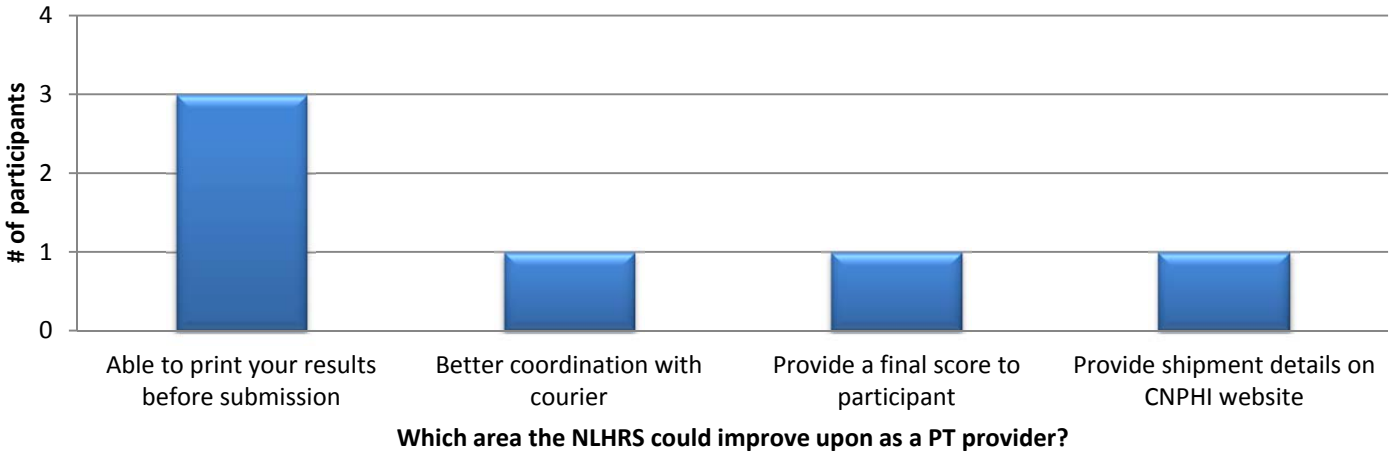

Figure 6: Participant's suggestions for improvement for the NLHRS HIV-1 viral load quality assurance program.

Homogeneity and stability

- The homogeneity and stability of the 2017Oct27 HIV-1 viral load panel was assessed by comparing the participant's viral load results with the viral load results obtained by the NLHRS for the characterization of the panel samples.
- Similary there was no indication of heterogeneity in the 2017Oct27 panel as all participants were able to detect HIV-1 in the positive duplicate sample set (A/E, B/G, D/H) within +/- 0.5 $\log_{10}$ cp/ml of the group mean (Appendix 1).
- There was no indication of instability in the positive duplicate sample set (A/E, B/G, D/H) as the group mean generated by the participants did not show a 0.5 $\log_{10}$ difference when compared to viral load test result obtained by the NLHRS one month prior to the panel send out (Table 1).

Flags

1. Participant, V10, did not submit their results as Log_{10} cp/ml as instructed.
2. Participant, V11, did not submit their results by the submission deadline.
3. Participant, V21 and V28, did not submit their results in 2 decimal places as instructed.

Results

1. **Statistical Analysis (General)**
   - An 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; D/H, B/G, A/E between the Roche and Abbott users.
     - Data for each set was combined and analyzed together.
   - A significant difference was identified ($p < 0.05$) in duplicate sets; B/G for the bioMérieux EasyQ HIV-1 V2.0
     - Data for each set was not combined and analyzed together.
   - No analysis for peer groups of n=1 (Abbott 0.2mL, 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 5A, 5B)
   - o The duplicate panel samples were combined for the summary statistics (D/H, B/G, A/E).

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 1.13 for the Roche CAP/CTM v2. and 1.12 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 0.28 or lower between duplicates (Table 2).

Table 2: Standard deviation (log_{10} cp/ml) reported between duplicates from participant's results for the 2017Oct27 panel (excludes NLHRS).

LAB	Sample A and E	Sample B and G	Sample D and H
V01	0.04	0.08	0.04
V02	0.03	0.07	0.05
V04	0.07	0.21	0.02
V05	0.05	0.03	0.26
V06	0.06	0.07	0.03
V07	0.02	0.04	0.01
V08	0.04	0.06	0.00
V10	0.06	0.02	0.00
V11	0.08	0.11	0.13
V13	0.07	0.15	0.04
V14	0.04	0.06	0.01
V17	0.01	0.01	0.08
V21	0.00	0.07	0.07
V26	0.18	0.10	0.10
V27	0.00	0.07	0.07
V28	0.21	0.14	0.28
V29	0.05	0.01	0.16
V36	0.12	0.17	0.08
V37	0.04	0.04	0.01
V48	0.01	0.07	0.02
V49	0.01	0.00	0.03

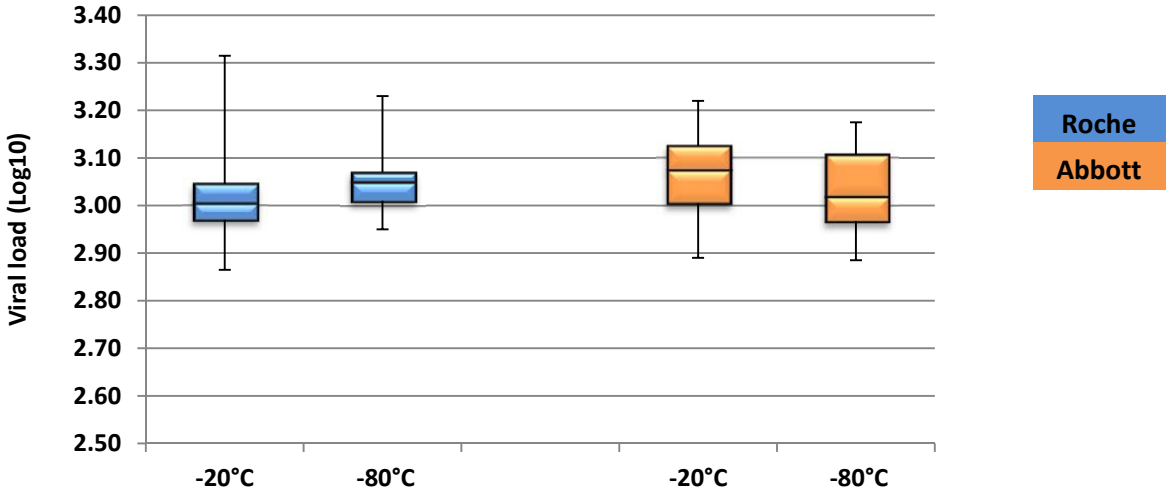

Figure 7: Effect of sample storage temperature, 2017Oct27 HIV-1 VL panel

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

   <u>Storage at -20°C for 35 days</u> (Samples D, H)

   - *Abbott RealTime 0.6mL (n=9)* - Participant results (including the NLHRS) showed no statistical difference between storage at -20°C for 35 days compared to -80°C (p=0.2623). 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= 0.1875). This is consistent with what was observed in previous panels

4. **Effect of non-B HIV-1 subtype (**Table 1 and Table 5A, 5B)

   <u>Non-B subytpe</u> (Samples A, E)

   - The NLHRS was not able to determine if there was any significance between a non B HIV-1 subtype and a B HIV-1 subtype because samples (A,E) viral load results were not comparable to samples (B, G) for a fair group comparison.
   - The group mean results from the Abbott and Roche users peer group indicates both platform are able to quantitate non-B HIV-1 subtype.

5. **Individual Analysis (Participant Statistics)** (Figures 8, 9, 10 and Tables 5A, 5B, 5C,5D,5E,5F,5G)

   - 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.

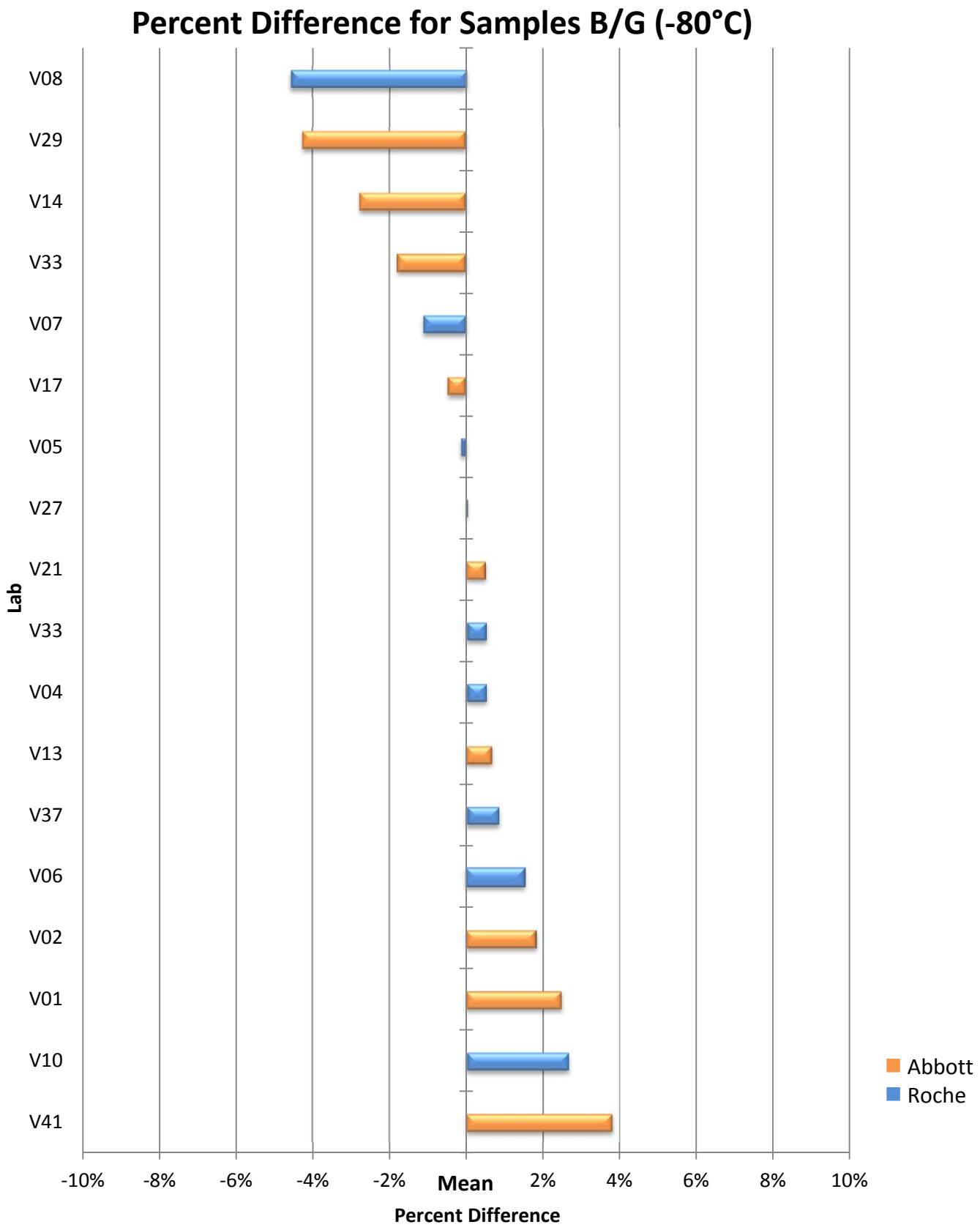

Figure 8: Percent Difference from the Peer Group Mean of B/G.

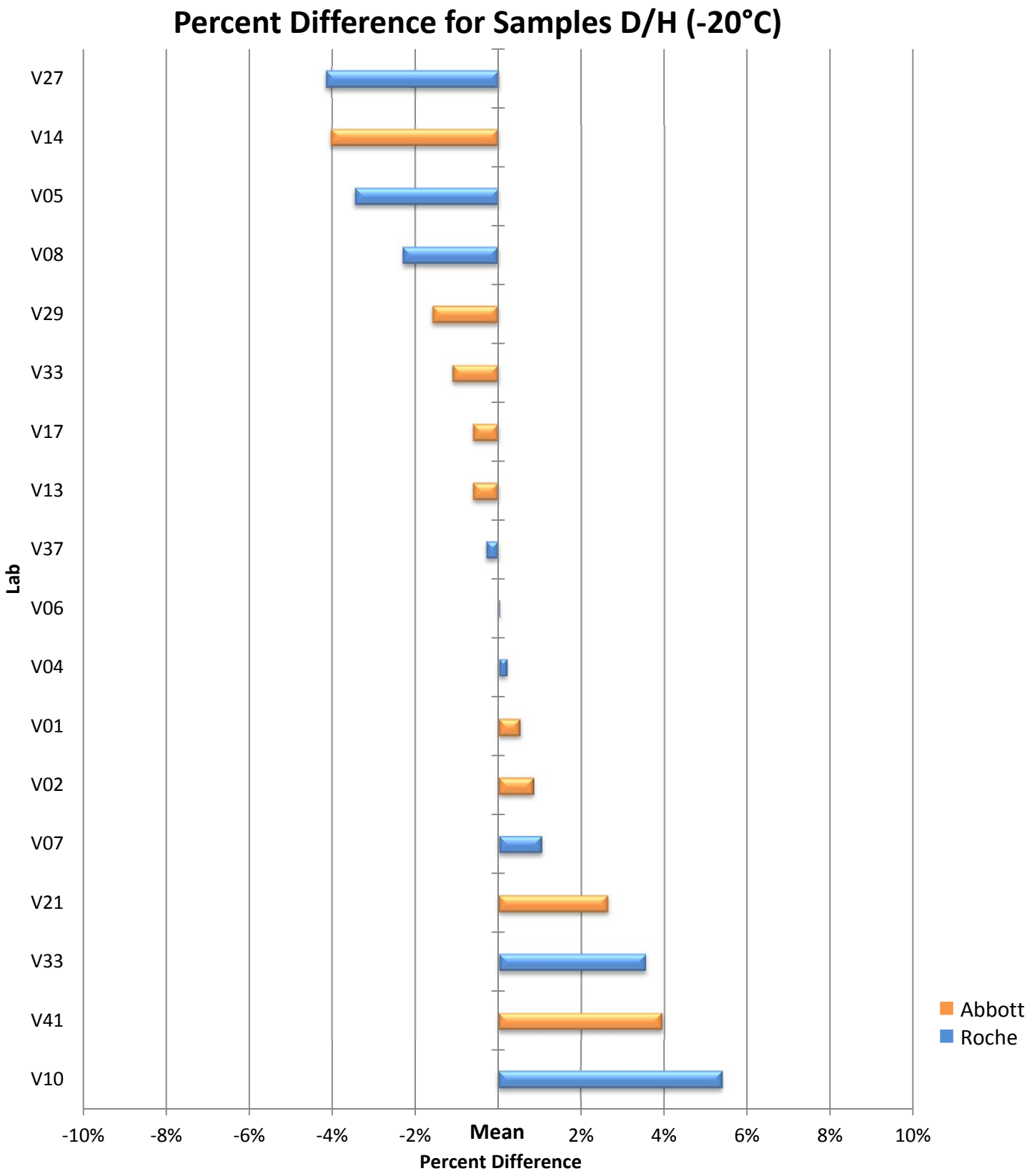

Figure 9: Percent Difference from the Peer Group Mean of D/H.

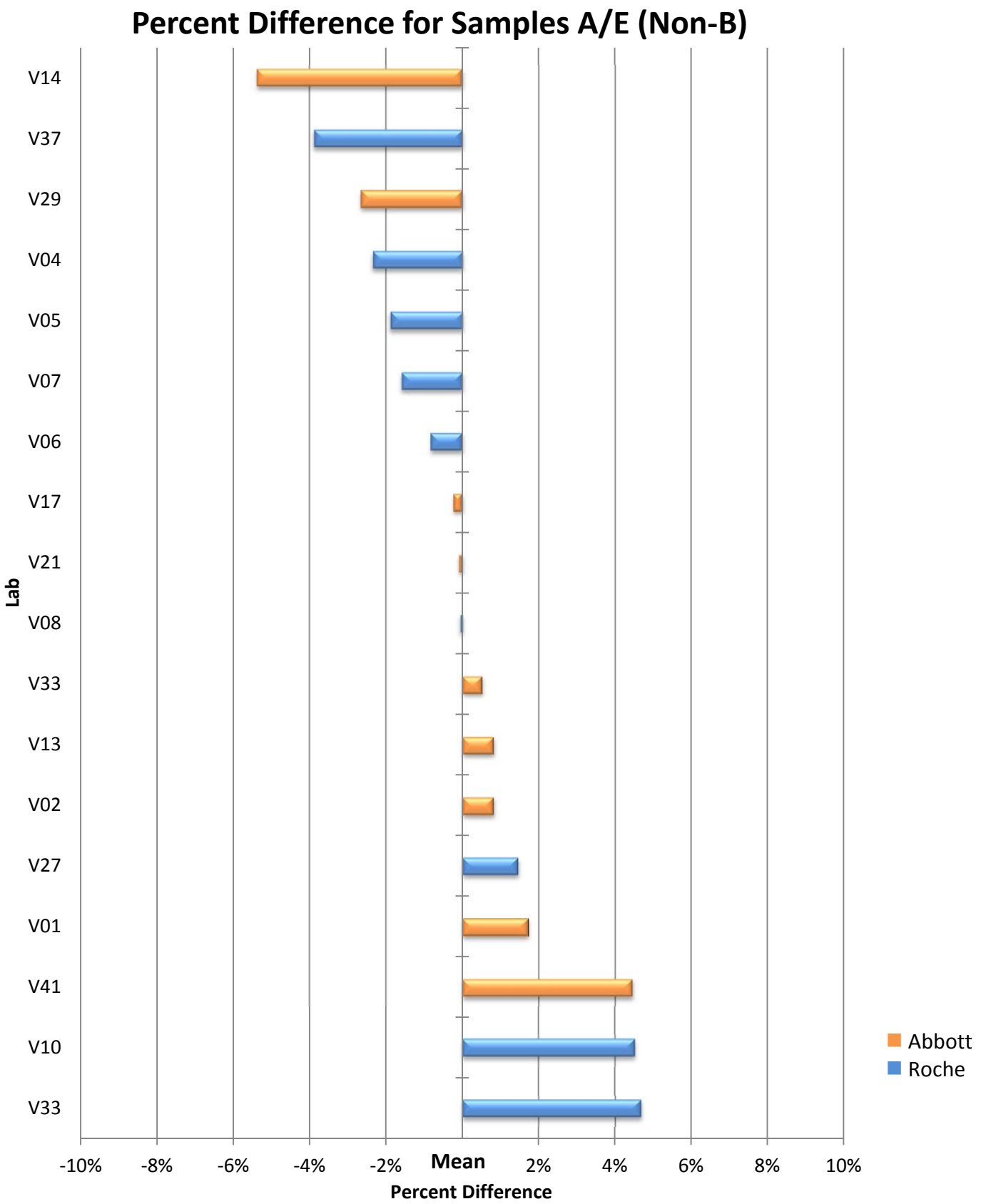

Figure 10: Percent Difference from the Peer Group Mean of A/E.

Table 3: Statistical comparison of results for Roche CAP/CTM v2.0 and Abbott RealTime 0.6ml for samples stored at -20°C (2013-2017 NLHRS panels)				
Sample	**Storage Temperature vs -80C**	**Assay**	**Panel**	**p-value**
Subtype B	-20°C for 35 days	Abbott RealTime 0.6ml	2017Oct27	**0.2623**
		Roche CAP/CTm v2.0	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 4 is the summary of the storage temperatures at -20°C for each platform

Table 4. 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 2013-2017	
Platforms	**-20°C(at various storage time)**
Abbott RealTime HIV-1 0.6 ml	Inconclusive[1]
Roche CAP/CTM v2.0	Not Significant
Hologic Panther Aptima HIV-1	No data

1. *The results from the surveys were not able to provide a definitive conclusion on the effect of 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.

3. *Performance of the participants in each peer group.*
    - All participants were able to return the correct results.
    - V14 and V29's viral load results were noticeably lower in the duplicate sets among the Abbott user peer group and vice versa for V41's viral load results.
    - V10's viral load results were noticeably higher in all duplicate sets among the Roche users peer group.
    - The NLHRS will continue to monitor this trend and will be interacting with individual laboratories to identify ways to minimize this variation

4. The NLHRS has observed that the viral load results in samples B/G and D/H duplicates from the bioMérieux EasyQ HIV-1 V2.0 users were noticeably lower when compared to the Abbott and Roche user peer groups but not in the sample A/E duplicate, a non-B HIV-1 subtype. The NLHRS will continue to monitor this effect.

5. The NLHRS was pleased to hear from our participants of their opinion on the NLHRS HIV-1 viral load proficiency testing program. The NLHRS is committed to improve the HIV-1 viral load proficiency testing program based on the suggestions provided by our participants.

If you would like to make an appeal, please submit your concerns to: nlhrs-lnsrv@phac-aspc.gc.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

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Appendix 1: Test Results

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

Table 5A Roche CAP/CTM v2.0 Test Results (Log_{10} HIV RNA Copies/mL)

Lab ID #	Sample Code								Kit lot	Exp. date
	A	E	B	G	D	H	C	F		
V04	3.24	3.14	3.05	2.75	2.98	3.01	<LDL	<LDL	X14181	2018-06-30
V05	3.24	3.17	3.05	3.01	3.07	2.70			X05405	2018-03-31
V06	3.20	3.28	3.13	3.03	3.01	2.97			X05405	2018-03-31
V07	3.23	3.20	2.97	3.03	3.03	3.01			X57399	2018-08-31
V08	3.29	3.24	2.94	2.85	2.92	2.92			X14181	2018-06-30
V10	3.46	3.37	3.10	3.13	3.15	3.15			X57399	2018-08-31
V27	3.21	3.42	3.07	3.00	3.00	2.73			X57399	2018-08-31
V33	3.43	3.41	3.05	3.05	3.05	3.14			X57399	2018-08-31
V37	3.17	3.11	3.03	3.09	2.97	2.99			X55545	2018-06-30
Mean	3.27		3.03		2.99					
Minimum	3.11		2.85		2.70					
Median	3.19		3.05		2.01					
Maximum	3.46		3.13		3.15					
% CV	3.27		2.28		4.05					
SD	0.11		0.07		0.12					
Inter-lab variation	1.11		1.10		1.17					
Measurement of Uncertainty	0.43		0.43		0.43					

Table 5B Abbott RealTime Results (0.6mL) (Log_{10} HIV RNA Copies/mL)

Lab ID #	Sample Code								Kit lot	Exp. date
	A	E	B	G	D	H	C	F		
V01	3.39	3.33	3.05	3.17	3.11	3.06			471862	2018-12-01
V02	3.31	3.35	3.14	3.04	3.06	3.13			471862	2018-12-01
V13	3.38	3.28	3.16	2.95	3.08	3.02			472630	2017-11-02
V14	3.10	3.15	2.91	2.99	2.95	2.94			471561	2018-01-12
V17	3.30	3.29	3.03	3.01	3.11	2.99			471862	2018-01-12
V21	3.30	3.30	3.00	3.10	3.20	3.10			472630	2017-11-02
V29	3.25	3.18	2.91	2.90	2.91	3.13			479049	2018-04-03
V33	3.30	3.34	3.00	2.96	3.00	3.07			472630	2017-11-02
V41	3.54	3.36	3.19	3.11	3.24	3.14			478665	2018-12-05
Mean	3.30		3.03		3.07					
Minimum	3.10		2.90		2.91					
Median	3.30		3.02		3.08					
Maximum	3.54		3.19		3.24					
% CV	2.94		3.06		2.88					
SD	0.10		0.09		0.09					
Inter-lab variation	1.14		1.10		1.11					
Measurement of Uncertainty	0.14		0.14		0.14					

Appendix 1: Test Results

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

Table 5C Hologic Panther Aptima HIV-1 (Log_{10} HIV RNA Copies/mL)										
Lab ID #	Sample Code								Kit lot	Exp. date
	A	E	B	G	D	H	C	F		
V48	3.47	3.46	3.09	3.19	3.12	3.15			188567	2018-04-15

Table 5D Abbott RealTime (0.2mL) Results (Log_{10} HIV RNA Copies/mL)										
Lab ID #	Sample Code								Kit lot	Exp. date
	A	E	B	G	D	H	C	F		
V36	3.25	3.08	2.88	3.12	3.17	3.06			*Not provided*	

Table 5E Abbott RealTime (0.5mL) Results (Log_{10} HIV RNA Copies/mL)										
Lab ID #	Sample Code								Kit lot	Exp. date
	A	E	B	G	D	H	C	F		
V11	3.14	3.26	2.97	2.82	3.03	2.85			*471561*	*2018-01-12*

Table 5F Cepheid GeneXpert Results (Log_{10} HIV RNA Copies/mL)										
Lab ID #	Sample Code								Kit lot	Exp. date
	A	E	B	G	D	H	C	F		
V49	3.40	3.41	3.05	3.05	3.10	3.05			*1000070457*	*2018-06-03*

Table 5G bioMerieux BV NucliSens EASYQ HIV-1 Results (Log_{10} HIV RNA Copies/mL)										
Lab ID #	Sample Code								Kit lot	Exp. date
	A	E	B	G	D	H	C	F		
V26	3.49	3.23	2.56	2.70	2.57	2.71			*17051501*	*2018-04-28*
V28	3.30	3.00	2.50	2.70	2.90	2.50			*16081001*	*2018-11-28*

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.