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 2017Apr19

This panel focused on the impact of extended storage at different temperatures on quantitation.

2017Apr19 HIV-1 VL panel			
Storage Conditions	**Panel Sample Pair**	**Viral load Consensus mean[1]**	**Labs Reporting Incorrect Final Status**
Room Temperature (1 week)	**B**	2.82[2], 2.84[3], 2.88[4]	
	E		
+37°C (26 hours)	**C**	2.85[2],2.92[3], 2.94[4]	
	H		
-80°C	**D**	2.84[2], 2.98[3], 3.03[4]	
	F		
-80°C	**A**	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
4. Based on Hologic Panther Aptima HIV-1 assay

Participants using the Abbott RealTime HIV-1 RNA PCR, Roche CAP/CTM HIV-1 Test v2.0 and bioMérieux EasyQ HIV-1 V2 continue to implement interpretive criteria that does not follow the kit inserts (please see page 3 of the final report).

Incorrect test result:

All participants reported the correct final status for all samples in the 2017Apr19 HIV-1 VL panel.

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HIV Viral Load Quality Assessment Program

Final Report for Panel HIVVL 2017Apr19

Issued 2017-06-27

Introduction

The NLHRS distributed the 2016Oct28 panel and the 2017Apr19 panel on Oct 12th 2016. This final report is specific to the 2017Apr19 only and is publicly available, however, the identity of participants is not disclosed.The 2017Apr19 panel continued to look at the effect of suboptimal storage on the ability to quantitate viral loads on an HIV-1 subtype B sample. It is noteworthy to mention a new user using the Cepheid GeneXpert II has joined the NLHRS QAP HIV-1 VL program.

Panel Samples, HIV Test Kits and Data Entry

1. *Panel Composition* – Panel 2017Apr19 (Table 1) contained the following:
   - One negative sample sent in duplicate (A 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 6 identical samples (B, C, D, E, F and H) to reduce the effect of variation due to preparation. Each pair was stored under different storage conditions (listed in table 1).
     - Set 1 (B/E) was stored at room temperature (RT) for 1 week and then returned to -80°C.
     - Set 2 (C/H) was stored +37°C for 26 hours and then returned to -80°C.
     - Set 3 (D/F) was stored at the recommended temperature of -80°C.

Table 1: Description of panel 2017Apr19 samples

Sample Identification	Sample Type	Sample Subtype	Storage Conditions	Viral load Consensus mean[1]
B E	HIV-1	B	Room Temperature (1 week)	2.82[2], 2.84[3], 2.88[4]
C H	HIV-1	B	+37°C (26 hours)	2.85[2], 2.92[3], 2.94[4]
D F	HIV-1	B	-80°C	2.84[2], 2.98[3], 3.03[4]
A G	TND	-	-80°C	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*
4. *Based on Hologic Panther Aptima HIV-1 assay*

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

2. *HIV Viral Load Test Kits* – 7 different assays were used by the 26 participants (excluding the NLHRS) who returned results (Figure 1). There is a shift in the number of participants that used the Roche CAP-CTM v2.0 assay and the number of participants that used the Abbott 0.6ml assay (Figure 2).
3. *Data entry* - The NLHRS Quality Assessment Program used the web based Survey Monkey system to capture results.
4. *Submissions deadline* – April 19th, 2017.

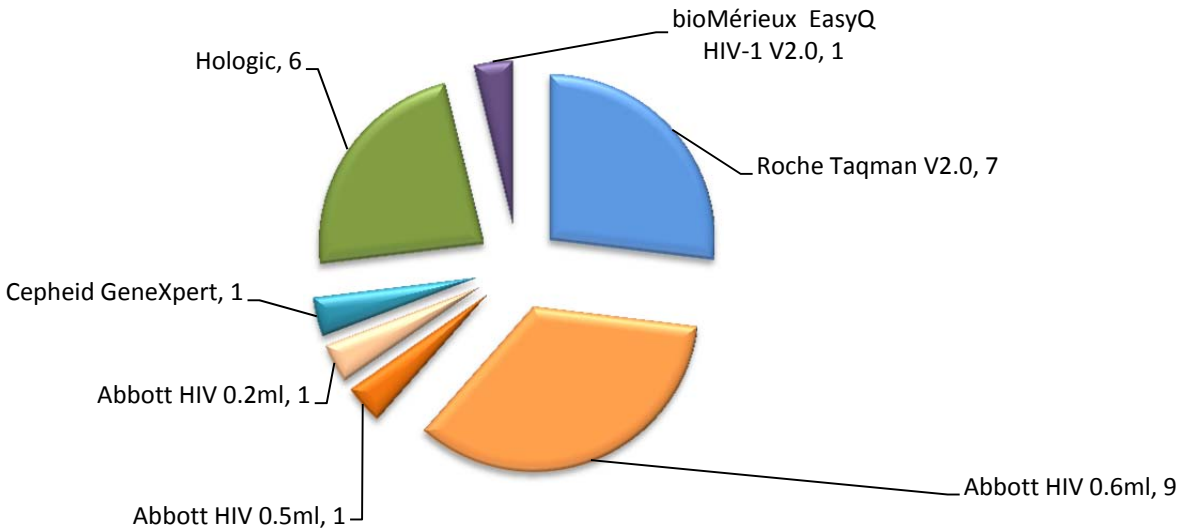

Figure 1: HIV-1 VL test kits used by the participants for 2017Apr19 HIV-1 VL (excluding the NLHRS)

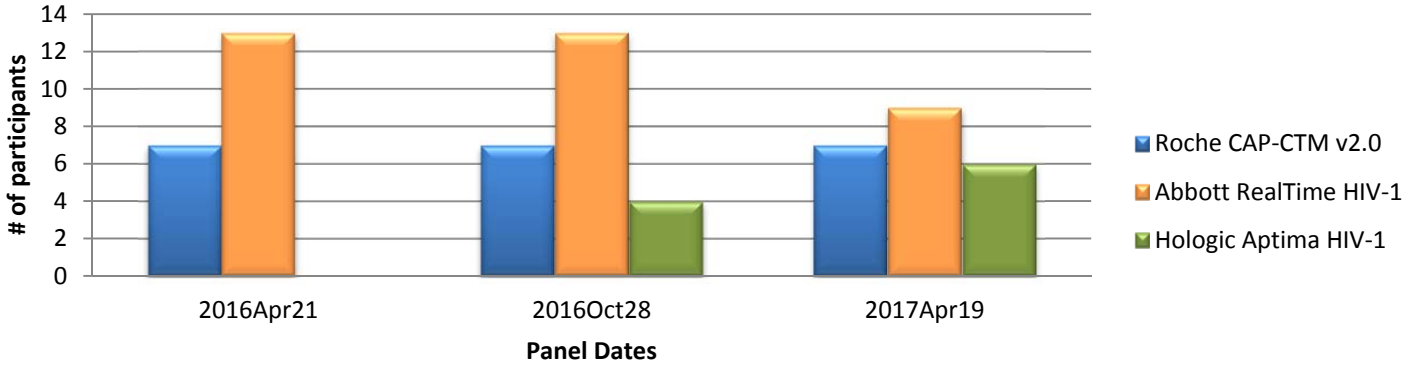

Figure 2: Distribution of HIV-1 assays (n>1) used by participants from 2016-2017 (excluding the NLHRS).

Return rate

Results were returned from 89.7% of participants (26/29).

- Two participants (V03 and V12) withdrew from the NLHRS QAP HIV-1 VL proficiency testing program
- Two participants (V25 and V37) did not submit results.
- Four participants(V36, V45, V46 and V51) submitted results past the submission deadline.
- One participant (V44) was unable to submit results due to instrumental error.
- Ten year average return rate of 90.2% (Figure 3).

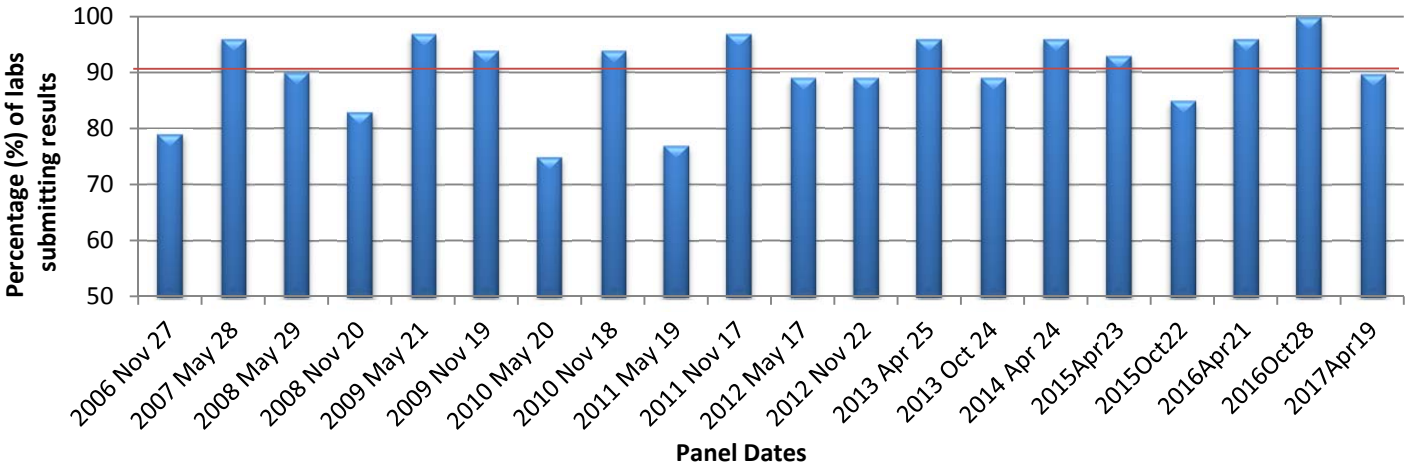

Figure 3: Percentage of HIV Viral Load Panel results submitted between 2006 and 2017

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.
   - Ten participants (38.5%, 10/26) reported using external QC material, a slight increased from the last survey.

2. *Quality Assurance (QA) programs* - Allow participants to evaluate their overall use of the assay and reporting of the results. One participant provided no response.
   - Sixteen participants (61.5%, 16/26) reported participation in QA programs other than the NLHRS panels, a slight decrease from the last survey.

Flags

1. Starting with this panel and onward, the NLHRS will no longer flag participants (V06, V13, and V26) that implement interpretative criteria different from the kit insert for negative samples (A,G) on the Abbott RealTime HIV-1 RNA PCR, Roche CAP/CTM HIV-1 Test v2.0, and bioMérieux EasyQ HIV-1 V2.0

Table 2: Kit Insert Recommendations			
Sample	**Reported Result**	**Viral Load**	**Reported Interpretation**
Negative/Non Reactive *"There is **no evidence of RNA**"*	Target not detected	n/a	Not detected
Below the Limit of Detection *"There is **evidence of RNA** but it is below the limit of detection and not quantifiable"*	< LDL	<LDL	Detected but < LDL
Positive	Detected	Value	Detected

Table 3: Participant Interpretive Criteria for Negative Samples			
Sample	**Reported Result**	**Viral Load**	**Reported Interpretation**
Negative	Target not detected	**< LDL**	Target not detected

2. Labs submitted results past the submission deadline, April 19, 2017

V36, V45, V46 and V51 submitted their results after the submission deadline

Results

1. **Statistical Analysis (General)**
   - An outlier was detected and removed from analysis (Grubb's test)
   - All group comparisons is done using the Unpaired *t* test.
   - No significant difference ($p > 0.05$) between duplicate sets; C/H, D/F, B/E
     - Data for each set was combined and analyzed together.
   - No analysis for peer groups of n=1 (Abbott 0.2mL, Abbott 0.5ml,bioMérieux EasyQ HIV-1 V2.0, and Cepheid GeneXpertII)
   - Users of the Hologic Panther Apitma HIV-1 Quant are included in the anaylsis even though they are a small group (n=6)
   - Negative samples are analyzed qualitatively.

2. **Group Analysis (Summary Statistics)** (Figure 4, Tables 6A, 6B, 6C)
   - o The duplicate panel samples were combined for the summary statistics (C/H, D/F, B/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 1.19 for the Roche CAP/CTM v2., 1.15 for the Abbott RealTime (0.6mL) and 1.09 for Hologic Panther Aptima HIV-1 peer groups.

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 Roche, Abbott and Hologic users reported standard deviation (SD) of 0.21 or lower between duplicates.

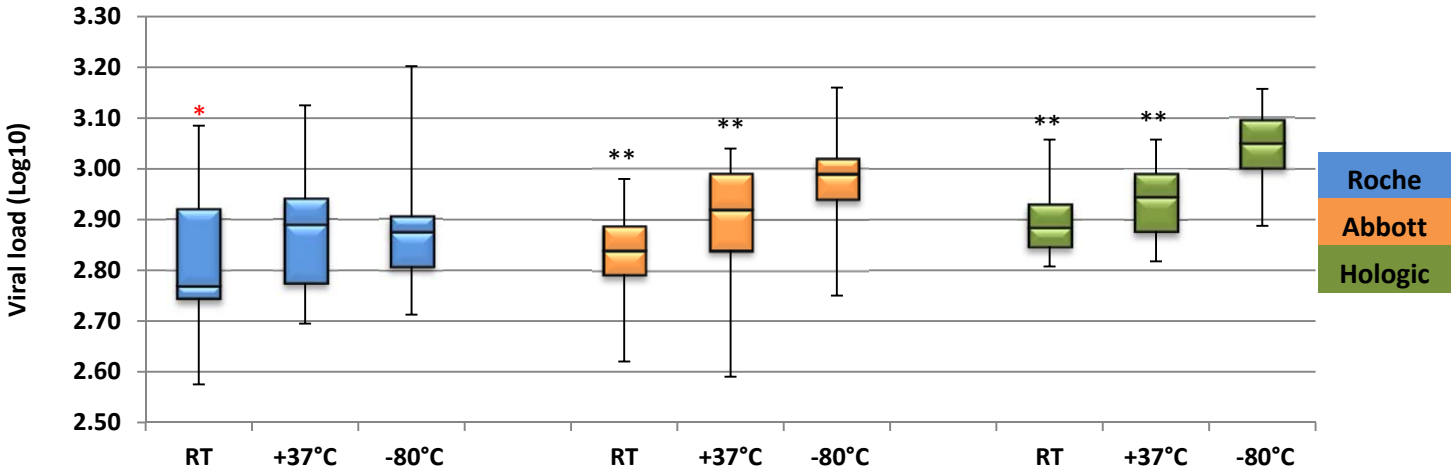

Figure 4: Effect of sample storage temperature on viral load values, 2017Apr19 HIV-1 VL panel
** Significant difference ($p < 0.05$) noted when compared to gold standard storage (-80˚C)
* Difference between the maximum and the min is > 0.5 $\log_{10}$

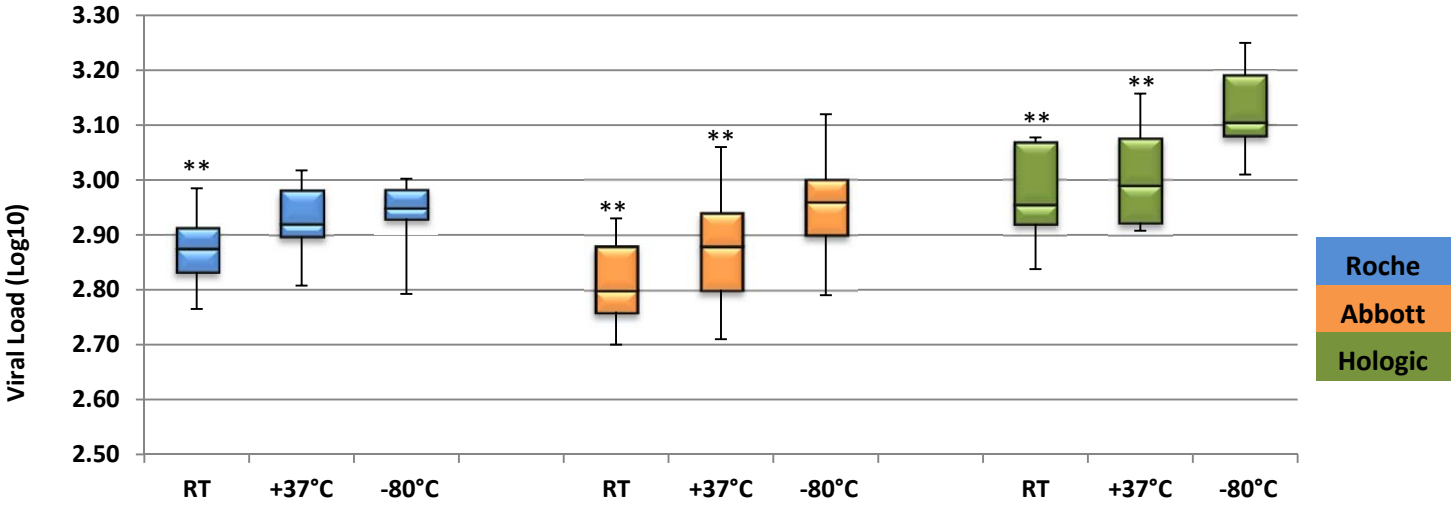

Figure 5: Effect of sample storage temperature on viral load values, 2016Oct28 HIV-1 VL panel.
** Significant difference ($p < 0.05$) noted when compared to gold standard storage (-80˚C)

3. **Effect of Suboptimal Storage** (Figure 4)

Storage at RT for 1 week (Samples B, E)

- *Abbott RealTime 0.6mL (n=10)* - Participant results (including the NLHRS) showed statistical difference between storage at RT for 1 week compared to -80°C ($p < 0.0001$). This is consistent to what was observed in the two previous HIV-1 viral load panels, 2016Apr21 and 2016Oct28
- *Roche CAP/CTM v2.0 (n=8)* - Participant results (including the NLHRS) showed no statistical difference between storage at RT for 1 week compared to -80°C ($p = 0.8063$). This is inconsistent with what was observed in the two previous HIV-1 viral load panels, 2016Apr21 and 2016Oct28
- Hologic Panther Apitma HIV-1 Quant (n=6)-Participants results showed statistical difference between storage at RT for week compared to -80°C ($p<0.0001$). This is consistent with what was observed in the previous HIV-1 viral load panel, 2016Oct28

Storage at +37°C for 26 hours (Samples C, H)

- *Abbott RealTime 0.6mL (n=10)* - Participant results (including the NLHRS) showed statistical difference between storage at +37°C for 26 hours compared to -80°C ($p = 0.0027$). This is consistent with what was observed in the two previous HIV-1 viral load panel, 2016Apr21 and 2016Oct28
- *Roche CAP/CTM v2.0 (n=8)* - Participant results (including the NLHRS) showed no statistical difference between storage at +37°C for 26 hours compared to -80°C ($p =0.6817$). This is consistent with what was observed in the two previous HIV-1 viral load panel, 2016Apr21 and 2016Oct28
- Hologic Panther Aptima HIV-1 Quant (n=6)-Participants results show statistical difference between storage at +37°C for 26 hours compared to -80°C ($p=0.0014$). This is consistent with what was observed in the previous HIV-1 viral load anel, 2016Oct28

4. **Individual Analysis (Participant Statistics)** (Figures 6, 7, 8 and Tables 6A, 6B, 6C,6D,6E,6F,6G)

- 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.

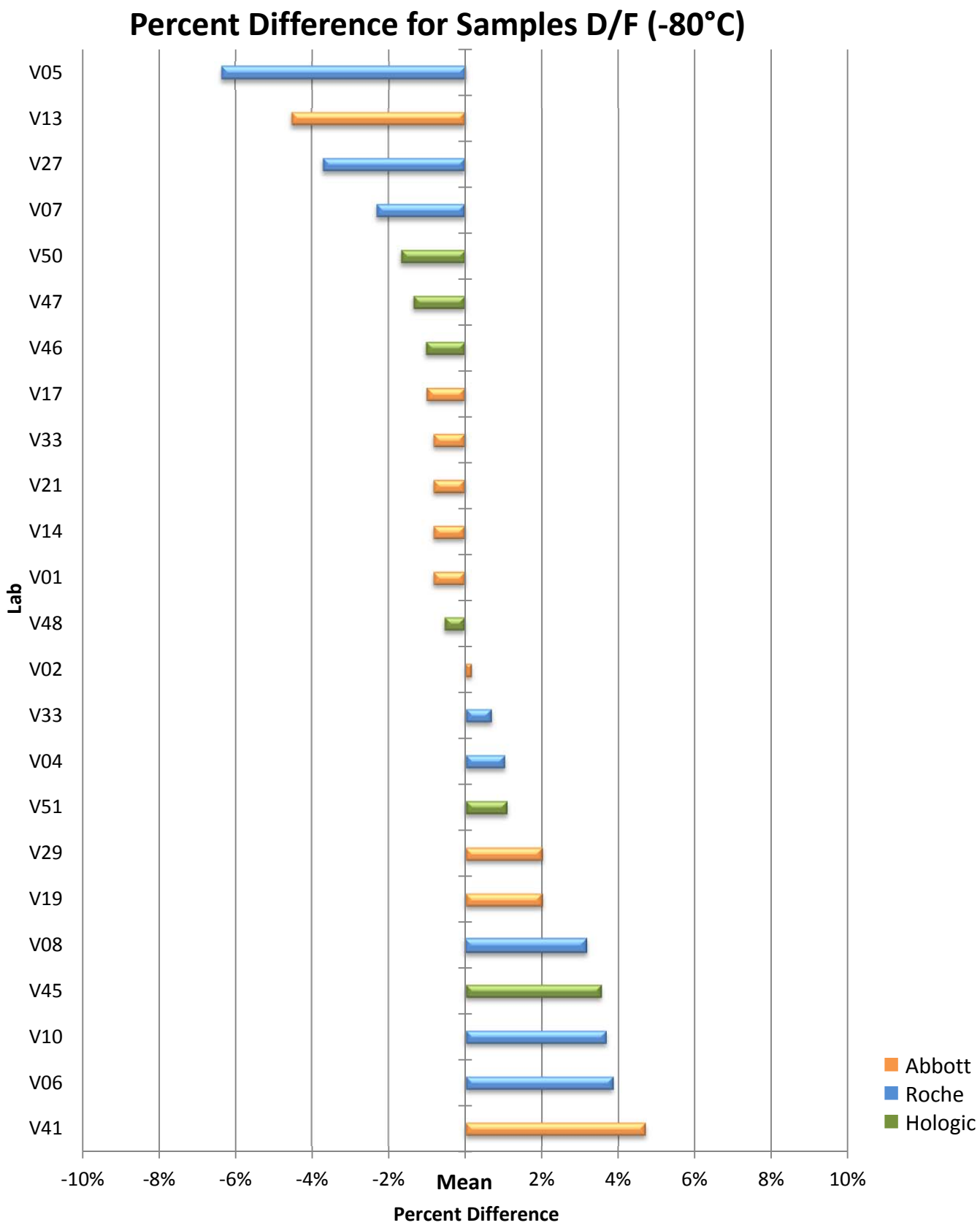

Figure 6: Percent Difference from the Peer Group Mean of D/F.

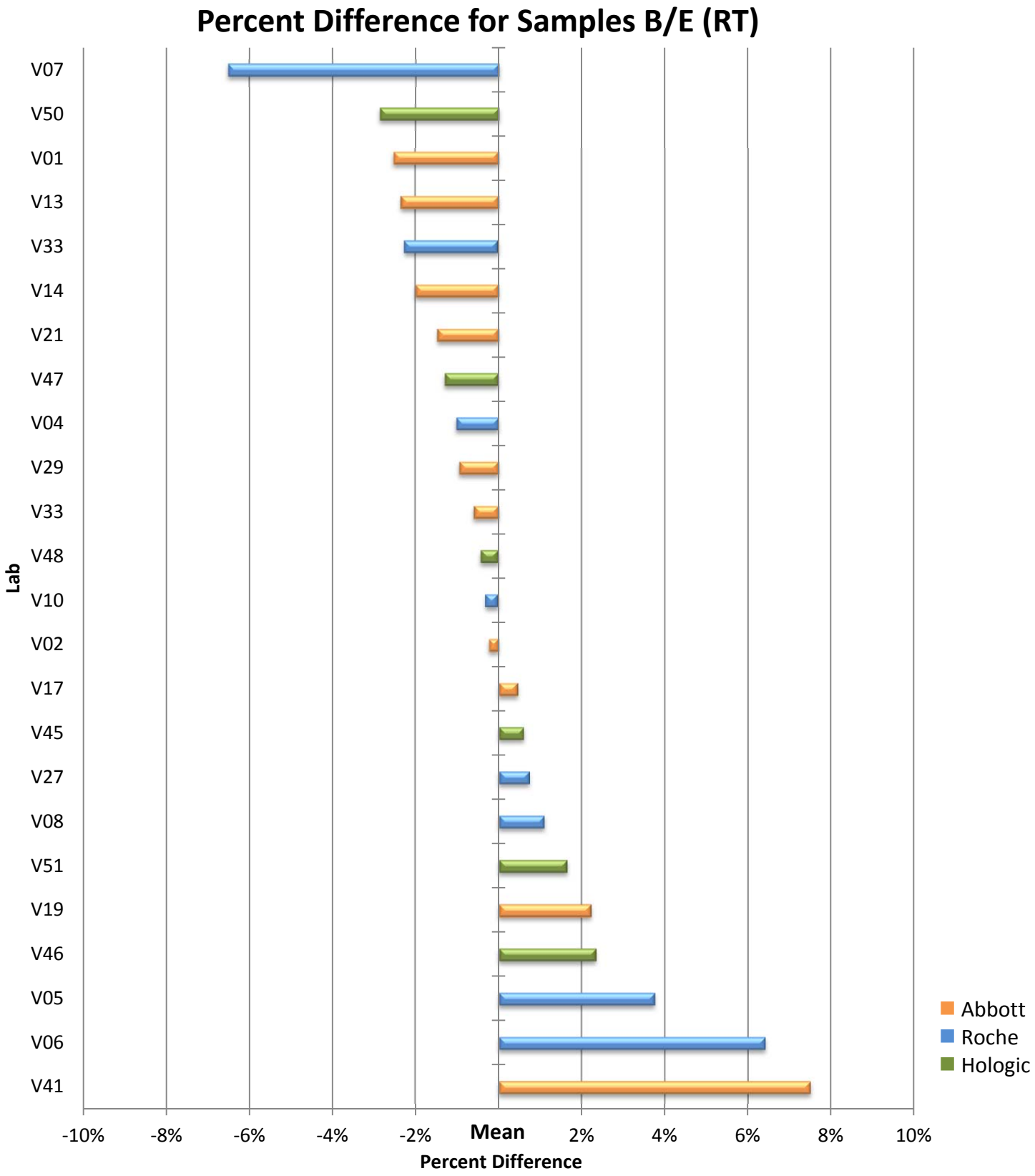

Figure 7: Percent Difference from the Peer Group Mean of B/E.

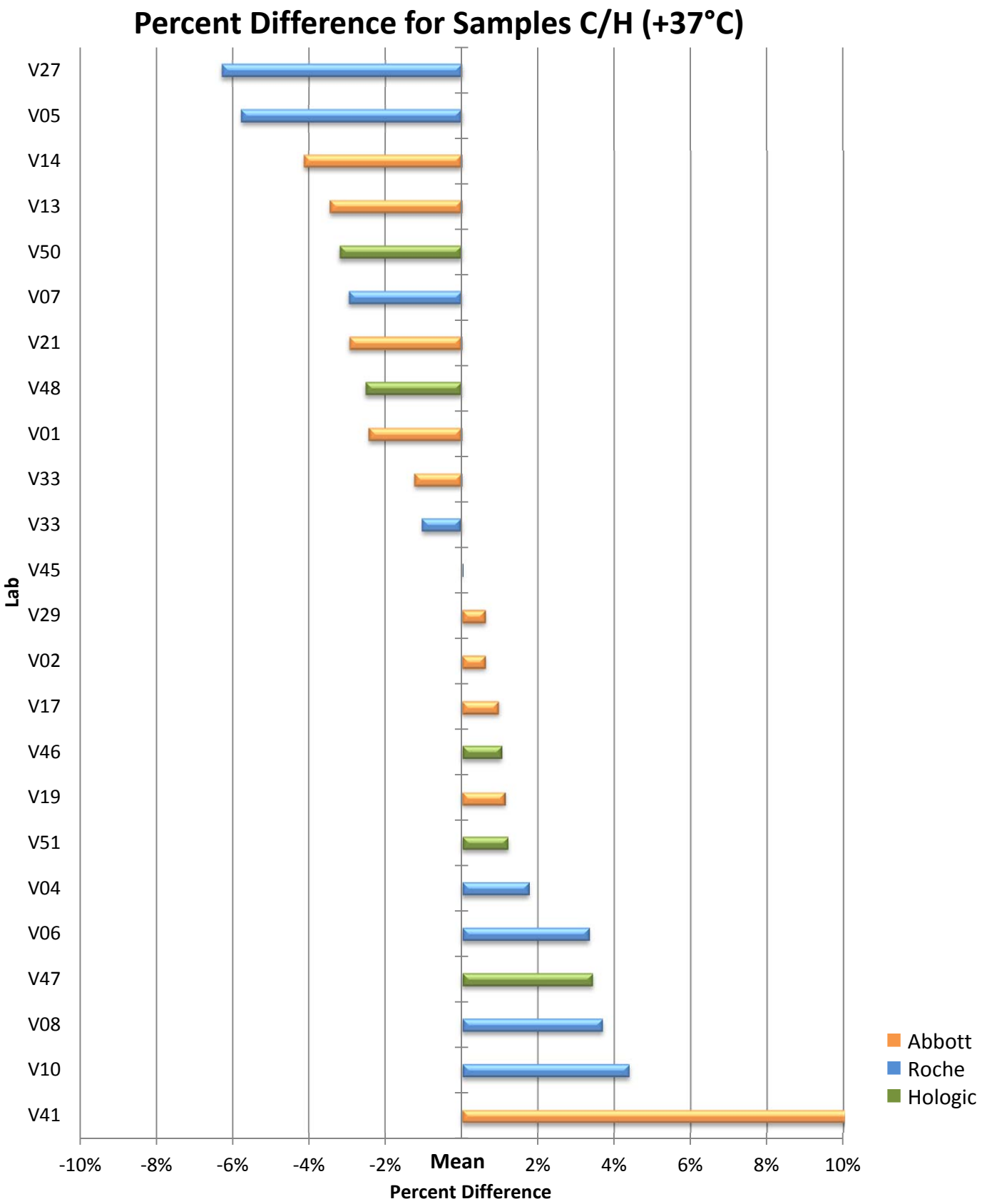

Figure 8: Percent Difference from the Peer Group Mean of C/H.

Table 4: Statistical comparison of results for Roche CAP/CTm v2.0, Abbott RealTime 0.6ml for (2013-2017 NLHRS panels) and Hologic Panther Aptima HIV-1(2016Oct28-2017Apr19) for samples stored at various temperature.

Sample	Storage Temperature vs -80C	Assay	Panel	p-value
Subtype B	RT for 1 week	Abbott RealTime 0.6ml	2017Apr19	<0.0001
		Roche CAP/CTM v2.0	2017Apr19	0.8063
		Hologic Panther HIV-1	2017Apr19	<0.0001
	+37°C for 26 hours	Abbott RealTime 0.6ml	2017Apr19	0.0027
		Roche CAP/CTM v2.0	2017Apr19	0.6817
		Hologic Panther HIV-1	2017Apr19	0.0014
Subtype B	RT for 1 week	Abbott RealTime 0.6ml	2016Oct28	0.0001
		Roche CAP/CTM v2.0	2016Oct28	0.0002
		Hologic Panther HIV-1	2016Oct28	0.0090
	+37°C for 26 hours	Abbott RealTime 0.6ml	2016Oct28	0.0009
		Roche CAP/CTM v2.0	2016Oct28	0.1361
		Hologic Panther HIV-1	2016Oct28	0.0073
Subtype B	RT for 1 week	Abbott RealTime 0.6ml	2016Apr21	0.0068
		Roche CAP/CTM v2.0	2016Apr21	0.0376
	+37°C for 26 hours	Abbott RealTime 0.6ml	2016Apr21	0.0030
		Roche CAP/CTm v2.0	2016Apr21	0.4281
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
	5 freeze thaws	Abbott RealTime 0.6ml	2014Oct23	0.0283
			2014Apr24	0.0133
		Roche CAP/CTM v2.0	2014Oct23	0.1184
			2014Apr24	0.4141
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
	+4°C for 6 days	Abbott RealTime 0.6ml	2013*	0.7960
		Roche CAP/CTM v2.0	2013Oct24	0.9125
			2013Apr25	0.6531

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

Conclusion

1. *Effect of Temperature*
   - Over the course of 5 years, we challenged 3 commercial viral load platforms with sub-optimal storage temperatures.
   - Outlined below in Table 5 are the conclusions of the storage temperatures for each platform.

Table 5: Impact of sub-optimal temperature 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	37°C	RT	4°C	-20°C(at various storage time)	5 freeze-thaw cycle
Abbott RealTime HIV-1 0.6 ml	Significant	Significant	Not Significant	Inconsistent results[2]	Significant
Roche CAP/CTM v2.0	Not Significant	Significant[1]	Not Significant	Not Significant	Not Significant
Hologic Panther Aptima HIV-1	Significant	Significant	No data	No data	No data

1. *The results from the surveys is suggestive that the effect of sub-optimal temperature is significant on HIV-1 quantitation when compare to optimal storage temperature.*
2. *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 different kit lot used, duration of the sub-optimal temperature storage and different technologist performing the assay must be taken into account.

2. Starting with the 2017Apr19 panel and onward, the NLHRS will no longer flag participants who report "below limit of detection" for a negative sample.

3. Proficiency testing is designed not only to test the examination stage but the overall process in patient testing. Errors in testing can also occur during the pre-examination stage which includes specimen collection and the post-examination stages (Appendix 2).

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** | **Negative sample detected <LDL** | **Outliers Removed**

Table 6A Roche CAP/CTM v2.0 Test Results (Log_{10} HIV RNA Copies/mL)

Lab ID #	Sample Code								Kit lot	Exp. date
	B	E	C	H	D	F	A	G		
V04	2.88	2.71	2.89	2.92	2.85	2.88			X05514	2018-05-31
V05	Error	2.93	Error	2.69	2.80	2.51	Error		X05405	2018-03-31
V06	2.92	3.09	3.01	2.89	3.00	2.89	<LDL	<LDL	W17057	2018-01-31
V07	2.58	2.70	2.81	2.73	2.87	2.67			X05405	2018-03-31
V08	2.96	2.75	2.94	2.98	2.97	2.88			X05405	2018-03-31
V10	2.89	2.74	3.02	2.94	2.92	2.96			X05432	2018-04-30
V27	2.77	2.92	2.76	2.59	2.83	2.63			X05432	2018-04-30
V33	2.76	2.76	2.86	2.79	2.81	2.90			X05405	2018-03-31
Mean	2.82		2.85		2.84					
Minimum	2.58		2.59		2.51					
Median	2.77		2.89		2.88					
Maximum	3.09		3.02		3.00					
% CV	4.62		4.35		4.62					
SD	0.11		0.13		0.13					
Inter-lab variation	1.20		1.17		1.20					

Table 6B Abbott RealTime Results (0.6mL) (Log_{10} HIV RNA Copies/mL)

Lab ID #	Sample Code								Kit lot	Exp. date
	B	E	C	H	D	F	A	G		
V01	2.70	2.84	2.92	2.81	2.96	2.94			472630	2017-11-02
V02	2.89	2.78	2.99	2.92	3.00	2.96			468098	2017-07-04
V13	2.82	2.73	2.83	2.84	2.88	2.80	<LDL	<LDL	471561	2018-01-12
V14	2.76	2.81	2.79	2.84	2.96	2.94			472630	2017-11-02
V17	2.85	2.86	3.03	2.90	2.99	2.90			472630	2017-11-02
V19	2.93	2.88	3.00	2.94	3.05	3.02			472630	2017-11-02
V21	2.80	2.80	2.80	2.90	2.90	3.00			470215	2017-11-25
V29	2.79	2.84	2.94	2.97	2.99	3.08			472646	2017-11-02
V33	2.77	2.88	2.90	2.90	3.01	2.89			471561	2018-01-12
V41	3.05	3.06	3.24	3.27	3.21	3.02			461383	2017-10-10
Mean	2.84		2.92		2.98					
Minimum	2.70		2.79		2.80					
Median	2.83		2.90		2.98					
Maximum	3.06		3.24		3.21					
% CV	3.23		3.54		2.89					
SD	0.09		0.10		0.09					
Inter-lab variation	1.13		1.16		1.15					

Appendix 1: Test Results

Legend: **Incorrect result** | **Negative sample detected <LDL** | **Outliers Removed**

Table 6C Hologic Panther Aptima HIV-1 (Log_{10} HIV RNA Copies/mL)										
Lab ID #	**Sample Code**								**Kit lot**	**Exp. date**
	B	**E**	**C**	**H**	**D**	**F**	**A**	**G**		
V45	2.93	2.87	2.90	2.98	3.11	3.21			Not provided	
V46	2.97	2.93	2.98	2.96	3.01	3.03			181541	2018-01-15
V47	2.85	2.84	3.05	3.03	3.05	2.97			111363	2018-11-15
V48	2.91	2.83	2.86	2.87	2.98	3.09			181541	2018-01-15
V50	2.88	2.72	2.81	2.88	3.06	2.94			176748	2018-06-15
V51	2.97	2.89	3.02	2.93	3.05	3.12			Not provided	
Mean	2..88		2.94		3.05					
Minimum	2.72		2.81		2.94					
Median	2.89		2.95		3.05					
Maximum	2.97		3.05		3.21					
% CV	2.40		2.59		2.44					
SD	0.07		0.08		0.07					
Inter-lab variation	1.09		1.09		1.09					

Table 6D Abbott RealTime (0.2mL) Results (Log_{10} HIV RNA Copies/mL)										
Lab ID #	**Sample Code**								**Kit lot**	**Exp. date**
	B	**E**	**C**	**H**	**D**	**F**	**A**	**G**		
V36	2.89	2.93	3.09	3.01	3.09	3.03			*Not provided*	

Table 6E Abbott RealTime (0.5mL) Results (Log_{10} HIV RNA Copies/mL)										
Lab ID #	**Sample Code**								**Kit lot**	**Exp. date**
	B	**E**	**C**	**H**	**D**	**F**	**A**	**G**		
V11	2.73	2.79	2.80	2.76	2.87	2.97			*472630*	*2017-11-02*

Table 6F Cepheid GeneXpert Results (Log_{10} HIV RNA Copies/mL)										
Lab ID #	**Sample Code**								**Kit lot**	**Exp. date**
	B	**E**	**C**	**H**	**D**	**F**	**A**	**G**		
V49	2.66	2.76	2.84	2.90	2.97	2.98			*14902*	*2017-04-23*

Table 6G bioMeriux BV NucliSens EASYQ HIV-1 Results (Log_{10} HIV RNA Copies/mL)										
Lab ID #	**Sample Code**								**Kit lot**	**Exp. date**
	B	**E**	**C**	**H**	**D**	**F**	**A**	**G**		
V26	2.38	2.51	2.62	2.81	2.78	2.64	<1.30	<1.30	*16011301*	*2017-04-28*

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.