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 2016Apr21

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

Storage Conditions	True Status (Pre- Maniulation) copies/mL [log_{10}]	Panel Sample	Labs Reporting Incorrect Final Status
Room Temperature (1 week)	891 [2.95]	A	
		G	
+37°C (26 hours)	891 [2.95]	C	
		F	
-80°C	891 [2.95]	B	
		D	
-80°C	TND	E	**Incorrect Result/Interpretation** • V10 • V11 • V14 • V26 • V27
		H	

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

Incorrect test result:

- **V10:** Incorrect result/interpretation for the negative samples E and H.
  Result: Target Not Detected with a Viral Load <LDL
  Interpretation: Target Not Detected

- **V11** detected RNA (<LDL) in negative sample E.

- **V14:** Incorrect result/interpretation for the negative samples E and H.
  Result: Target Not Detected with a Viral Load <LDL
  Interpretation: Detected Below the Limit of Detection

- **V26** detected RNA in negative sample H.

- **V27:** Incorrect result/interpretation for the negative samples E and H.
  Result: Target Not Detected with a Viral Load <LDL
  Interpretation: Target Not Detected

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Final Report for Panel HIVVL 2016Apr21

Issued 2016-06-02

Introduction

The NLHRS distributed the 2016Apr21 panel on April 4th 2016. The 2016Oct20 panel will be shipped the first week of October 2016. This final report is publicly available, however the identity of participants is not disclosed.

As an extension of the 2013-2015 panels, the 2016Apr21 panel continued to look at the effect of suboptimal storage on the ability to quantitate viral loads on an HIV-1 subtype B sample.

Panel Samples, HIV Test Kits and Data Entry

1. *Panel Composition* – Panel 2016Apr21 (Table 1) contained the following:
   - One negative sample sent in duplicate (E and H); 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 (A, B, C, D, F and G) to reduce the effect of variation due to preparation. Each pair was stored under different storage conditions (listed in table 1).
     - Set 1 (A/G) was stored at room temperature (RT) for 1 week and then returned to -80°C.
     - Set 2 (C/F) was stored +37°C for 26 hours and then returned to -80°C.
     - Set 3 (B/D) was stored at the recommended temperature of -80°C.

Table 1: Description of panel 2015Apr23 samples

Sample Identification	Sample Type	Sample Subtype	Storage Conditions	Viral Load copies/mL [log_{10}] Pre-Manipulation[1]
A G	HIV-1	B	Room Temperature (1 week)	891 [2.95]
C F	HIV-1	B	+37°C (26 hours)	891 [2.95]
B D	HIV-1	B	-80°C	891 [2.95]
E H	TND	-	-80°C	TND

1. based on the Roche CAP/CTM v2.0 assay.

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

2. *HIV Viral Load Test Kits* – Three different assays were used by the 22 participants (excluding the NLHRS) who returned results (Figure 1).
3. *Data entry* - The NLHRS Quality Assessment Program used the web based Survey Monkey system to capture results.
4. *Submissions deadline* – April 21st, 2016.

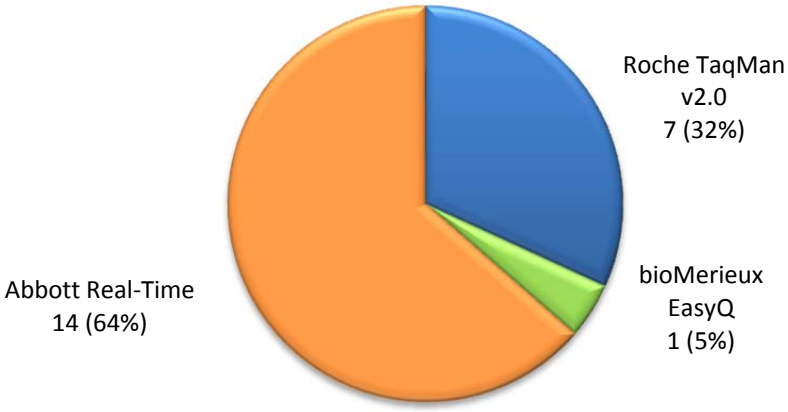

Figure 1: Breakdown of the assays used by the 22 participants in the NLHRS 2016Apr21 Viral Load Panel (Excludes the NLHRS).

Return rate

Results were returned from 96% of participants (22/23).

- One participant (V28) was unable to participate due to shipping delays.
- Two participants (V25 and V42) were not shipped a panel because they were unable to complete the required shipping paperwork.
- Nine year average return rate of 89.7% (Figure 2).

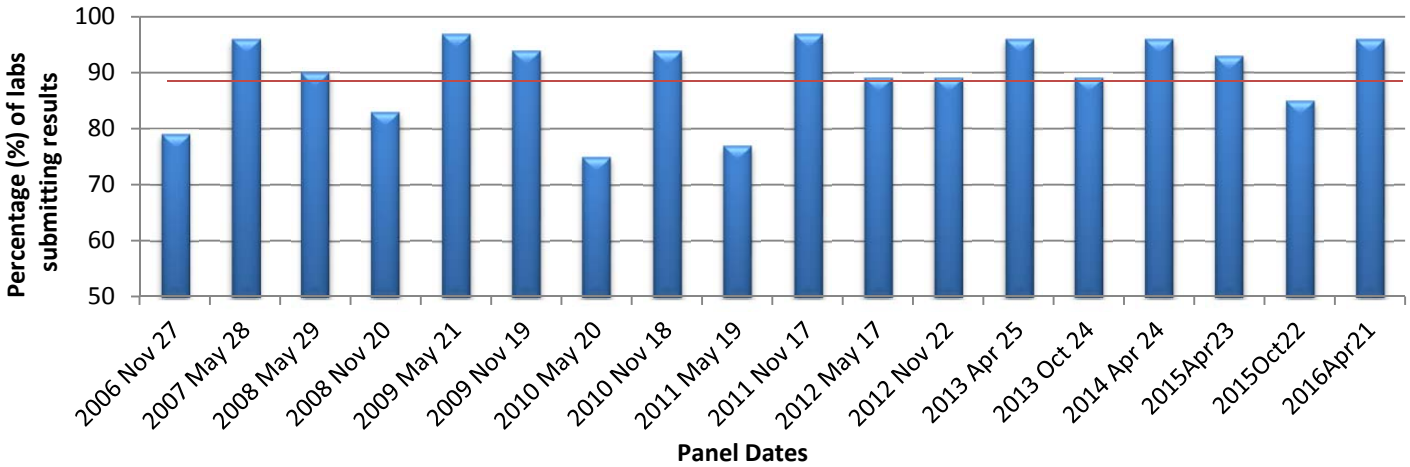

Figure 2: Percentage of HIV Viral Load Panel results submitted between 2006 and 2016

Flags

1. Incorrect test result.
   - **V11** detected RNA (<LDL) in negative sample E.
   - **V26** detected RNA in negative sample H.

2. Labs continue to implement interpretative criteria different from the kit insert for negative samples (E,H) on the Abbott RealTime HIV-1 RNA PCR and Roche CAP/CTM HIV-1 Test v2.0
   - **V10, V27** result of Target Not Detected, viral load <LDL with final interpretation of Not detected.
   - **V14** result of Target Not Detected, viral load <LDL with final interpretation Detected <LDL.

Table 2: Kit Insert Recommendations

Sample	Reported Result	Viral Load	Reported Interpretation
Negative *"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 < LDL
Positive	Detected	Value	Detected

Table 3: Incorrect Participant Interpretive Criteria for Negative Samples

Sample	Reported Result	Viral Load	Reported Interpretation
Negative	Target not detected	**< LDL**	Target not detected
Negative	Target not detected	**< LDL**	**Detected < LDL**

Red: Incorrect

Results

1. **Statistical Analysis (General)**
   - No outliers were detected (Grubb's test)
   - All group comparisons done using the Unpaired *t* test.
   - No significant difference ($p > 0.05$) between duplicate sets; A/G, B/D, C/F
     - Data for each set was combined and analyzed together.
   - No analysis for peer groups of n=1 (Abbott 0.2mL and bioMerieux EasyQ).
   - Negative samples are analyzed qualitatively.

2. **Individual Analysis (Participant Statistics)** (Figures 4, 5, 6 and Tables 5A, 5B, 5C)
   - This is 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.

Results ***(continued)***

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

   Inter-Lab Variation
   - Difference between the minimum and maximum results for each sample within a peer group (the maximum value divided by minimum).
   - Average of 1.12 for the Roche CAP/CTM v2.0 and 1.20 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 Roche and Abbott users reported standard deviation (SD) of 0.22 or lower between duplicates.

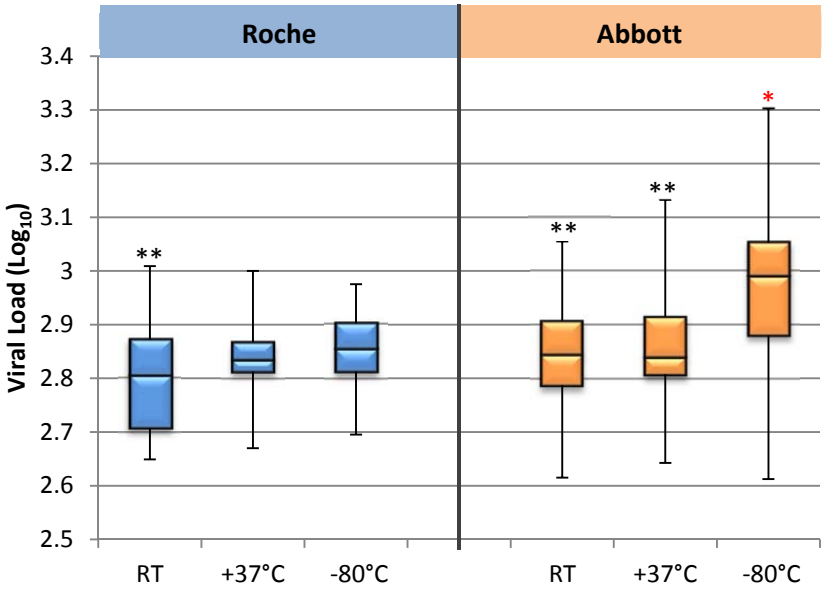

Figure 3: Effect of sample storage temperature on viral load values.

* Difference between the maximum and the min is > 0.5 $\log_{10}$

** Significant difference ($p < 0.05$) noted when compared to gold standard storage (-80˚C)

4. **Effect of Suboptimal Storage**

   Storage at RT for 1 week (Samples A, G)
   - *Abbott RealTime 0.6mL (n=14)* - Participant results (including the NLHRS) showed statistical difference between storage at RT for 1 week compared to -80°C ($p < 0.007$).
   - *Roche CAP/CTM v2.0 (n=8)* - Participant results (including the NLHRS) showed statistical difference between storage at RT for 1 week compared to -80°C ($p < 0.038$).

   Storage at +37°C for 26 hours (Samples C, F)
   - *Abbott RealTime 0.6mL (n=14)* - Participant results (including the NLHRS) showed statistical difference between storage at +37°C for 26 hours compared to -80°C ($p = 0.003$).
   - *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 ($p > 0.42$).

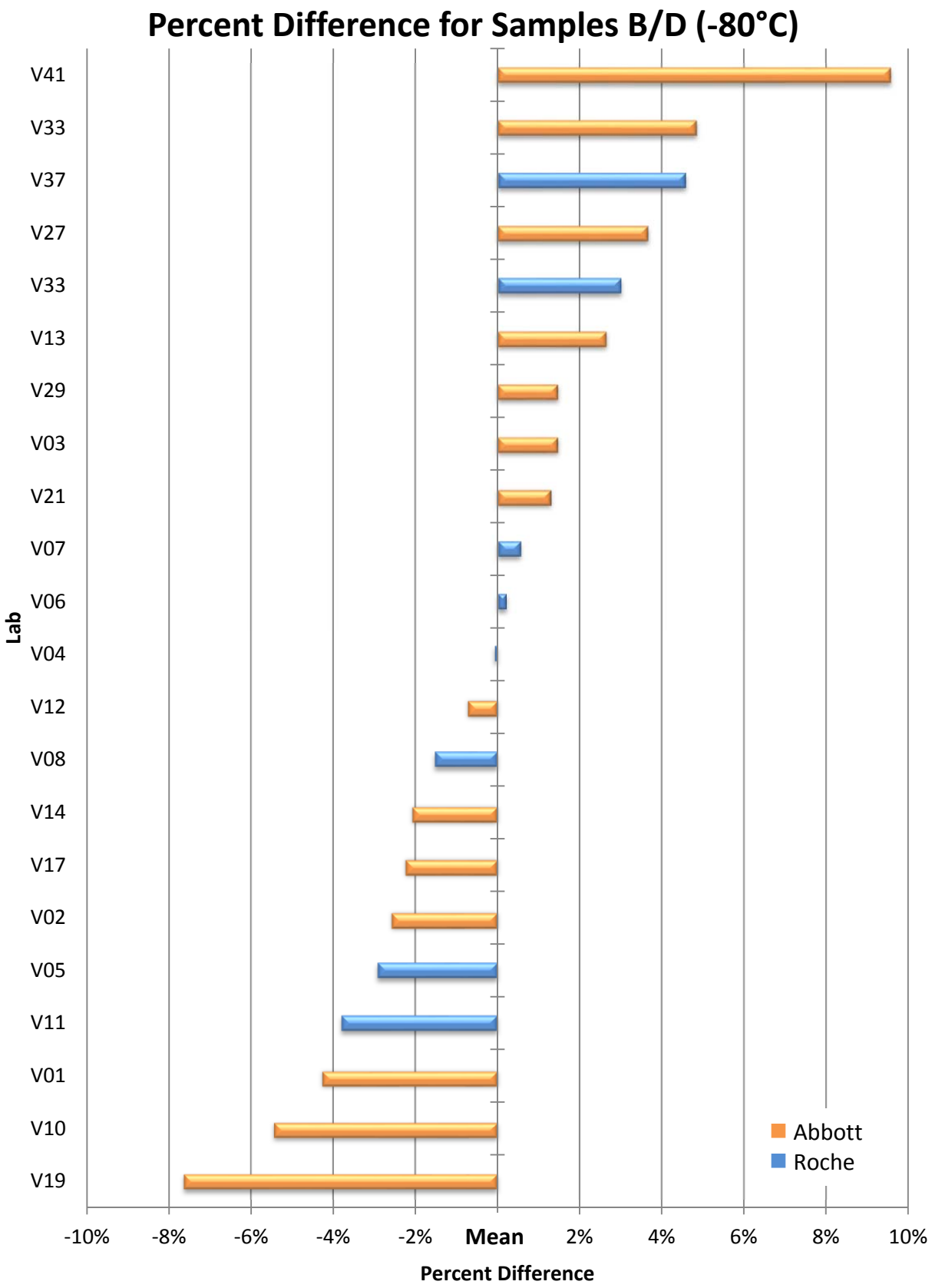

Figure 4: Percent Difference from the Peer Group Mean of B/D.

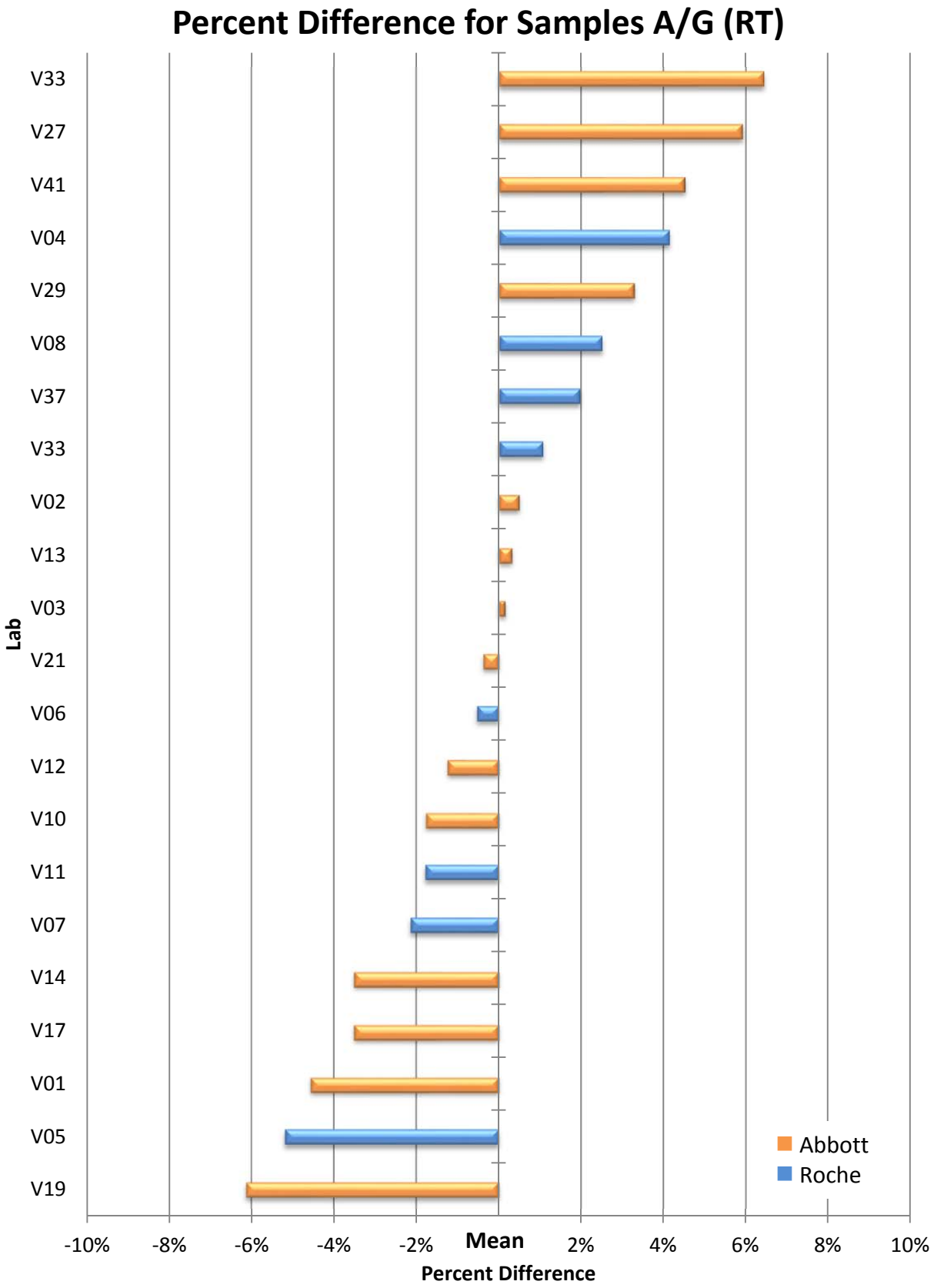

Figure 5: Percent Difference from the Peer Group Mean of A/G.

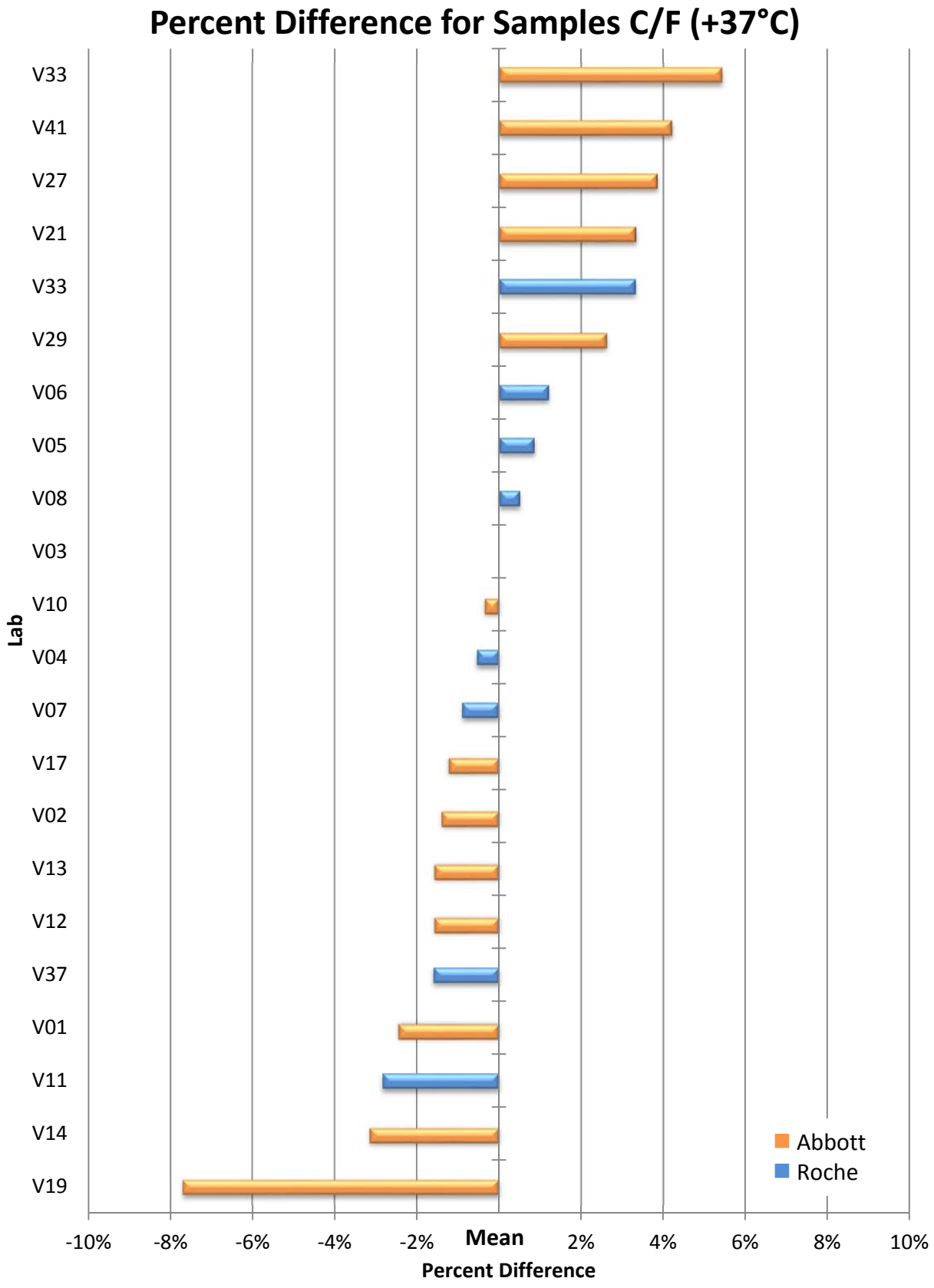

Figure 6: Percent Difference from the Peer Group Mean of C/F.

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.
   - Seven participants (32%, 7/22) reported using external QC material.

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.
   - Thirteen participants (62%, 13/21) reported participation in QA programs other than the NLHRS panels *(note, one lab excluded; data submitted manually and they were not asked this question).*

Table 4: Statistical comparison of results for Roche CAP/CTM v2.0 and Abbott RealTime 0.6mL for samples stored at various temperatures (2013-2016 NLHRS panels)

Sample	Storage Temperature vs -80˚C	Assay	Panel	p-value
Subtype B *891 cp/mL Roche CAP/CTM v2.0*	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 *1080cp/mL Roche CAP/CTM v2.0*	-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 *7800cp/mL Roche CAP/CTM v2.0*	-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*
   - The NLHRS examined 2 different short term storage conditions in the 2016 proficiency testing program; room temperature for 1 week and +37˚C for 26 hours. In each case the results were compared to the recommended storage of -80˚C.
   - The Roche CAP/CTM v2.0 assay was not significantly affected by storage at RT for 1 week ($p < 0.038$) but not for storage at +37°C for 26 hours ($p > 0.42$) compared to storage at -80˚C.
   - The Abbott 0.6mL assay was significantly affected by both storage methods; RT for 1 week ($p < 0.007$) and for +37°C for 26 hours ($p = 0.003$) compared to storage at -80˚C.
   - Contrary to previous panels (2014-2015), where the Roche assay generally had assay variability approaching 0.5 log, this panel, both assays saw this trend.
   - The NLHRS will continue to investigate sub-optimal storage methods.
2. The NLHRS will continue to monitor issues with the interpretation/reporting of “negative” as “below limit of detection” results as mentioned in the previous panels.
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

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

Lab ID #	Sample Code A	G	B	D	F	C	E	H	Kit lot	Exp. date
V04	2.93	2.88	2.91	2.81	2.82	2.83			W07708	2017-06-30
V05	2.57	2.71	2.74	2.82	2.84	2.89			W01880	2017-02-28
V06	2.70	2.84	2.90	2.84	2.93	2.82			W01880	2017-02-28
V07	2.88	2.57	2.87	2.89	2.84	2.79			W01880	2017-02-28
V08	2.93	2.78	2.83	2.81	2.93	2.78			W01880	2017-02-28
V11	2.80	2.67	2.76	2.75	2.84	2.68	<20		W01880	2017-02-28
V33	2.76	2.87	3.02	2.88	2.86	3.01			W01880	2017-02-28
V37	2.81	2.87	2.99	3.00	2.83	2.76			W03493	2017-01-31
Mean	2.79		2.86		2.83					
Minimum	2.57		2.78		2.87					
Median	2.81		2.87		2.88					
Maximum	2.93		2.88		2.82					
% CV	4.10		2.82		2.86					
SD	0.11		2.76		2.76					
Inter-lab variation	1.14		2.95		2.94					

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

Lab ID #	Sample Code A	G	B	D	C	F	E	H	Kit lot	Exp. date
V01	2.79	2.67	2.92	2.75	2.83	2.74			464493	2017-03-21
V02	2.82	2.93	2.88	2.89	2.82	2.81			464493	2017-03-21
V03	2.85	2.88	2.98	3.03	2.85	2.86			462705	2016-11-11
V10	2.78	2.84	2.73	2.87	2.81	2.88	<1.6	<1.6	464493	2017-03-21
V12	2.75	2.90	3.00	2.88	2.80	2.82			462705	2016-11-11
V13	2.84	2.90	3.06	3.02	2.81	2.81			463792	2017-02-17
V14	2.73	2.79	3.01	2.79	2.80	2.73	<1.6	<1.6	463792	2017-02-17
V17	2.82	2.70	2.90	2.89	2.76	2.88			463792	2017-02-17
V19	2.73	2.64	2.84	2.63	2.68	2.59			464493	2017-03-21
V21	2.80	2.90	3.00	3.00	3.00	2.90			464493	2017-03-21
V27	3.06	3.00	3.09	3.05	2.97	2.96	<1.6	<1.6	464493	2017-03-21
V29	2.87	3.04	2.93	3.08	2.90	2.96			465076	2017-05-06
V33	3.06	3.03	3.11	3.10	3.03	2.99			465080	2016-10-29
V41	3.08	2.90	3.32	3.17	2.87	3.08			461383	2017-02-14
Mean	2.86		2.96		2.86					
Minimum	2.64		2.63		2.59					
Median	2.85		2.99		2.84					
Maximum	3.08		3.32		3.03					
% CV	4.26		4.89		3.80					
SD	0.12		0.14		0.11					
Inter-lab variation	1.17		1.26		1.17					

Table 5C Abbott RealTime (0.2mL) & bioMerieux NucliSens EASYQ v2.0 Results (Log_{10} HIV RNA Copies/mL)

Lab ID #	Sample Code A	G	B	D	C	F	E	H	Kit lot	Exp. date
V36 (Abbott)	2.87	3.06	3.08	3.07	2.9	3			*Not provided*	
V26 (bioMerieux)	3.17	2.51	2.88	2.77	2.39	2.68		1.6	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.