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 HIVV428 2015Oct22

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

Storage Conditions	True Status copies/mL [log_{10}]	Panel Sample	Labs Reporting Incorrect Final Status
-20°C (13 months)	1080 [3.03]	C E	**Incorrect Result** • V28
-20°C (8 months)	1080 [3.03]	A D	
-80°C	1080 [3.03]	F H	
-80°C	TND	B G	**Incorrect Result/Interpretation** • V05 • V08 • V26 • V27

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:

- **V05:** Detected RNA in the negative samples B and G.
  Result: Below the Limit of Detection
  Interpretation: Detected <LDL
- **V08** detected RNA (<LDL) in negative sample B.
- **V26** detected RNA in negative sample B.
- **V27:** Incorrect result/interpretation for the negative samples B and G.
  Result: Below the Limit of Detection
  Interpretation: Target Not Detected
- **V28** had a result of <LDL on positive sample E.

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Final Report for Panel HIVV428 2015Apr23

Introduction

The NLHRS distributed 2 HIV serology panels in April 2015 with the second panel to be tested after the test event open date of October 6th, 2015.

As with the previous 2013-2015 panels, the NLHRS continued to examine the effect of storage temperature on quantitation.

Panel Samples, HIV Test Kits and Data Entry

1. *Panel Composition* – Panel 2015Oct22 (Table 1) contained the following:
   - One negative sample sent in duplicate (B and G); defibrinated human plasma.
   - One positive sample was diluted and aliquoted for 6 identical samples (A, C, D, E, F and H).
     - Each pair was stored under different storage conditions (listed in table 1).
     - HIV-1 RNA subtype B diluted to approximately 1000 copies/mL in defibrinated human plasma (Basemetrix 53, Seracare Life Sciences Inc.).
     - Identical to the 2014Apr24, 2014Nov23 and 2015Apr23 panel sample (aliquoted at the same time)

Table 1: Description of panel 2015Apr23 samples

Sample Identification	Sample Type	Sample Subtype	Storage Conditions	Viral Load copies/mL [log_{10}] Pre-Manipulation[1]
C* E*	HIV-1	B	-20°C (13 months)	1080 [3.03]
A* D*	HIV-1	B	-20°C (8 months)	1080 [3.03]
F* H*	HIV-1	B	-80°C	1080 [3.03]
B G	TND	-	-80°C	TND

** These samples were prepared at the same time as the 2014Apr24, 2014Nov23 and 2015Apr23 panels.*
1. based on the Roche CAP/CTM v2.0 assay.

2. *HIV Viral Load Test Kits* – Three different assays were used by the 24 participants (excluding the NLHRS) who returned results (Figure 1).

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

3. *Data entry* - The NLHRS HIV Viral Load Quality Assessment Program used the computerized web based system Oneworld Accuracy to capture and analyze results.
4. *Submissions deadline* – October 22nd, 2015.
5. *Laboratory Specific Report (LSR) issued by Oneworld Accuracy* – November 28th, 2015.

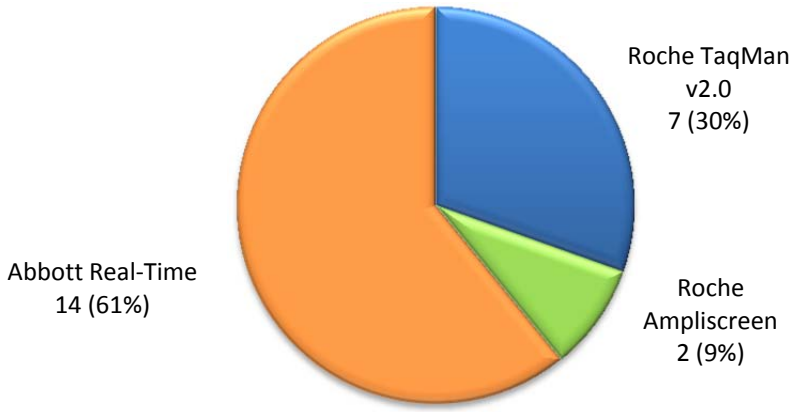

Figure 1: Breakdown of the assays used by the 23 participants in the NLHRS 2015Oct22 Viral Load Panel (Excludes the NLHRS).

Results

1. *Return rate* - Results were returned from 85% of participants (23/27).
   - Four participants (V25, V37, V39, V43) did not return results.
   - Nine year average return rate of 89.4% (Figure 2).
2. *Results* - Viral loads are listed in Appendix 1 (Tables 5A-G)

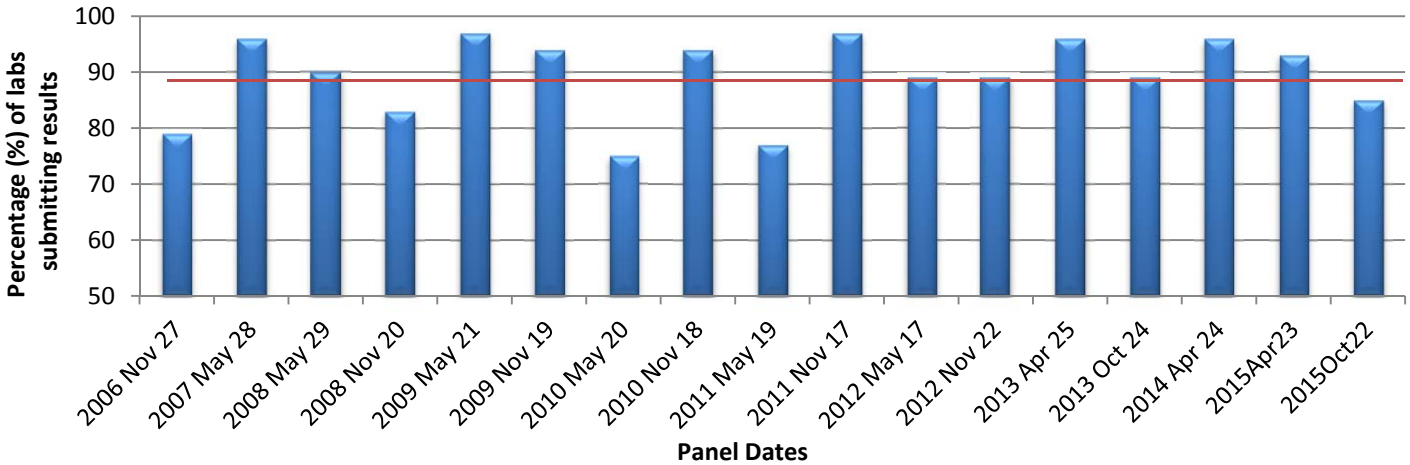

Figure 2: Percentage of HIV Viral Load Panel results submitted between 2006 and 2015

Flags

1. Incorrect test result.
   - **V08** detected RNA (<LDL) in negative sample B.
   - **V26** detected RNA in negative sample B.
   - **V28** had a result of detected <LDL on positive sample E.

2. Labs continue to implement interpretative criteria different from the kit insert for negative samples on the Abbott RealTime HIV-1 RNA PCR and Roche CAP/CTM HIV-1 Test v2.0
   - **V05** detected RNA in negative samples B,G. However it is unclear if this is due to interpretive criteria that does not follow the kit insert.
   - **V27** entered had a result of <LDL on negative samples B,G with final interpretation of Not detected

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

Table 3: Incorrect Participant Interpretive Criteria for Negative Samples		
Sample	**Reported Result**	**Reported Interpretation**
Negative	**LDL**	**Detected < LDL**
Negative	**< LDL**	**Detected < LDL**
Negative	**< LDL**	**Detected**
Negative	**< LDL**	Not detected

Red: Incorrect

Storage Temperature

As an extension of the 2013 and 2014 panels, the 2015 panels continued to look at the effect of storage at -20°C on the ability to quantitate viral loads on an HIV-1 subtype B sample (Figure 3 and Table 4).
The same HIV-1 subtype B sample from the 2014 panels was utilized for the 2015 panels to reduce the effect of variation due to preparation. Three sets of this identical positive sample were stored at the following temperatures for each of the 2015Apr23 and 2015Oct22 panels;

- Set 1 was stored at -20°C for 13 months and then returned to -80°C.
- Set 2 was stored -20°C for 8 months and then returned to -80°C.
- Set 3 was stored at the recommended temperature of -80°C.

Results

1. **Storage at -20°C for 13 months (Samples C, E)**
   - *Abbott RealTime 0.6mL (n=14)* - Participant results (including the NLHRS) showed statistical difference between storage at -20°C for 13 months compared to -80°C ($p<0.025$).
   - *Roche CAP/CTM v2.0 (n=7)* - Participant results (including the NLHRS) showed no statistical difference between storage at -20°C for 13 months compared to -80°C ($p>0.12$).

2. **Storage at -20°C for 8 months (Samples A, D)**
   - *Abbott RealTime 0.6mL (n=14)* - Participant results (including the NLHRS) showed statistical difference between storage at -20°C for 8 months compared to -80°C ($p<0.047$).
   - *Roche CAP/CTM v2.0 (n=7)* - Participant results (including the NLHRS) showed no statistical difference between storage at -20°C for 8 months compared to -80°C ($p>0.15$).

3. **Overall (Table 4, Figure 3)**
   - The Roche CAP/CTM v2.0 did not show a significant difference for storage at -20˚C for 8 or 13 months compared to storage at -80˚C ($p>0.12$) in both of the 2015 panels.
   - The Abbott 0.6mL assay showed a significant difference for storage at -20˚C for 8 months compared to storage at -80˚C ($p<0.47$) in the 2015 panels and for storage at -20˚C for 13 months ($p<0.25$) in the 2015Oct22 panel but not the 2015Apr23 panel ($p>0.19$).
   - The Abbott assay ran slightly lower than the Roche assay and generally had a tighter range.
   - Note; other assays were not evaluated due to the small number of participants.

Inter-Lab Variation (Tables 5A, 5B and 5D)

- Difference between the minimum and maximum results for each sample within a peer group.
- Not calculated for Table 5C as it only had one participant.
- Average of 1.15 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 0.24 or lower between the duplicates.

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.
   - Eleven participants (48%, 11/23) 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.
   - Eleven participants (48%, 11/23) reported participation in QA programs other than the NLHRS panels.

Table 4: Statistical comparison of results for Roche CAP/CTM v2.0 and Abbott RealTime 0.6mL for samples stored at various temperatures (2015, 2014 and 2013 NLHRS panels)

Sample	Storage Temperature vs -80˚C	Assay	Panel	p-value
Subtype B *1080cp/mL Roche CAP/CTM v2.0*	-20°C for 13 months	Abbott RealTime 0.6mL	2015Apr23	0.1927
			2015Oct22	**0.0243**
		Roche CAP/CTM v2.0	2015Apr23	0.9328
			2015Oct22	0.1262
	-20°C for 8 months	Abbott RealTime 0.6mL	2015Apr23	**0.0217**
			2015Oct22	**0.0469**
		Roche CAP/CTM v2.0	2015Apr23	0.24
			2015Oct22	0.1550
	-20°C for 35 days	Abbott RealTime 0.6mL	2014Oct23	0.06
			2014Apr24	0.9628
		Roche CAP/CTM v2.0	2014Oct23	0.897
			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*	**0.00761**
		Roche CAP/CTM v2.0	2013Oct24	0.40188
			2013Apr25	0.62019
	+4°C for 6 days	Abbott RealTime 0.6mL	2013*	0.79603
		Roche CAP/CTM v2.0	2013Oct24	0.91248
			2013Apr25	0.65307

** Combined the 2013Apr25 and 2013Oct24 panel results because there was no significant statistical difference between the panels. (p>0.2)*

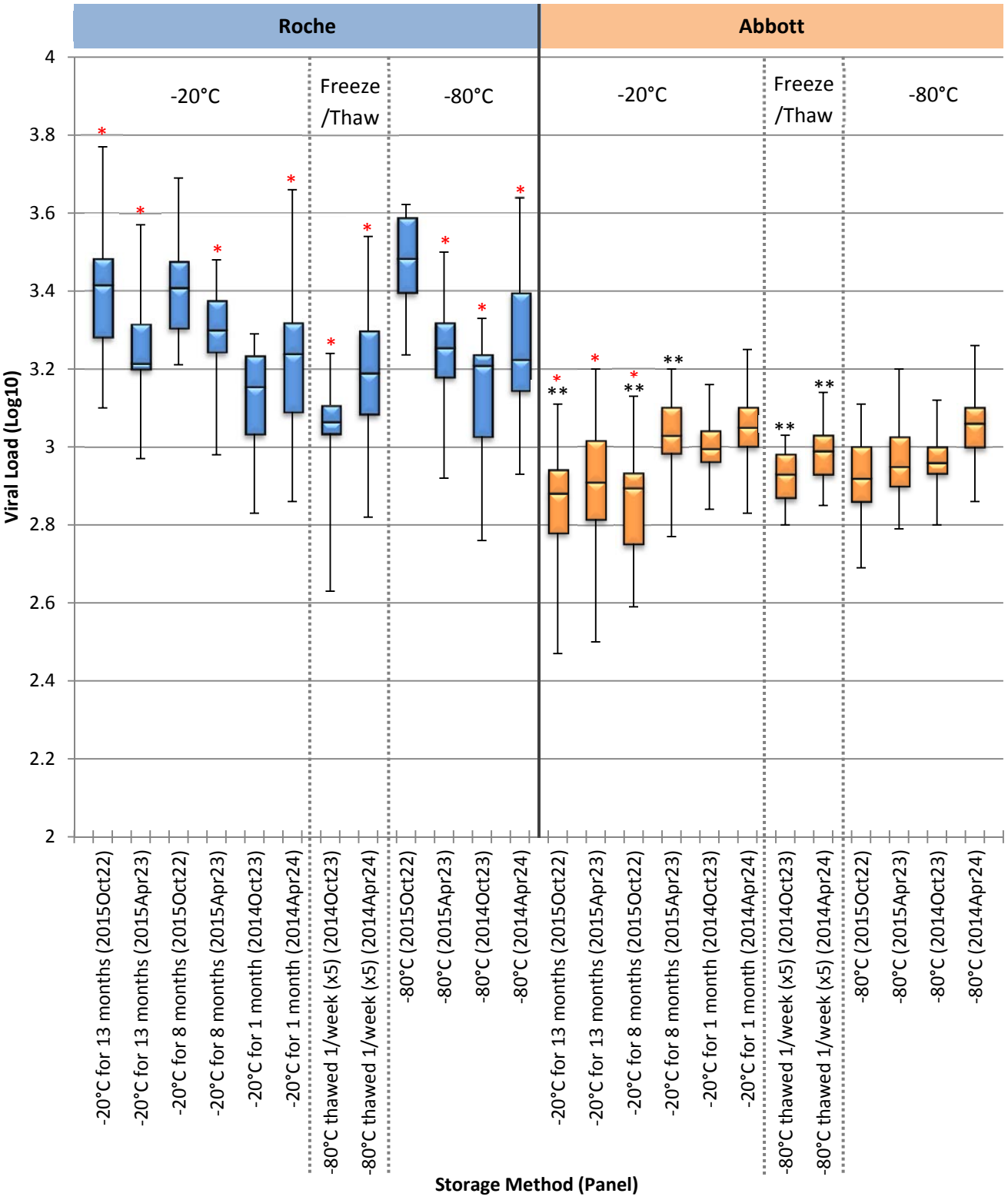

Figure 3: Effect of sample storage temperature on viral load values (Log_{10}) for the 2015Oct22, 2015Apr23, 2014Oct23 and 2014Apr24 HIV Viral Load Panels (Subtype B).

* 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)

Conclusion

1. *Effect of Temperature*
   - The NLHRS examined 6 different storage methods in the 2013-2015 proficiency testing programs, ranging from the effect of freeze thaws to short term storage at +4˚C/-20˚C and long term storage at -20˚C. 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 freeze thaws or any of the storage temperature methods ($p>0.1$).
   - The Abbott RealTime 0.6mL assay was significantly affected by freeze thaws ($p<0.03$), storage at -20˚C for 6 days ($p<0.01$) and storage at -20˚C for greater than 8 months ($p<0.025$). There was no significant difference between storage at -20˚C for 35 days or 13 months ($p>0.06$) and storage at +4˚C for 6 days ($p>0.79$).
   - ***Important Note:*** *While the group data analysis did not indicate a significant difference in storage methods for the Roche assay, this assay (Roche) did exhibit systematic high assay variability approaching or exceeding 0.5log.*

2. Our data suggests that samples stored at -20°C for up to 13 months tested on the Roche CAP/CTM v2.0 assay and samples stored at -20 for up to 1 month tested on the Abbott RealTime 0.6mL assay yield HIV-1 RNA results equivalent to samples stored at the -80˚C temperature stated in commonly used HIV viral load testing guidelines.
   - This may be beneficial to laboratories that do not have access to ultra-low freezers and/or inadvertently stored samples below -80°C.

3. 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.

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

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 HIV Serology QA Program

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Appendix 1: Test Results

Legend:
- Negative sample detected <LDL
- Incorrect result
- Statistical outlier, excluded from calculations

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

Lab ID #	Sample Code A	B	C	D	E	F	G	H	Kit lot	Exp. date
V04	3.41		3.37	3.37	3.44	3.48		3.62	T13803	2016-11-30
V05	3.43	<LDL	3.43	3.28	3.39	3.41	<LDL	3.40	T13803	2016-11-30
V06	3.22		3.77	3.47	3.48	3.38		3.58	T13803	2016-11-30
V07	3.54		3.52	3.69	3.55	3.51		3.61	T13803	2016-11-30
V08	3.48	<LDL	3.46	3.59	3.49	3.62		3.49	T13803	2016-02-29
V11	3.41		3.10	3.39	3.18	3.30		3.51	T13803	2016-11-30
V33	3.29		3.30	3.32	3.40	3.41		3.62	T13803	2016-11-30
V42	3.21		3.16	2.90	3.23	3.24		3.39	T07651	2016-07-31
Mean	3.37		3.39	3.44	3.39	3.42		3.53		
Minimum	3.21		3.10	3.28	3.18	3.24		3.39		
Median	3.41		3.40	3.39	3.42	3.41		3.55		
Maximum	3.54		3.77	3.69	3.55	3.62		3.62		
% CV	3.57		6.27	4.32	3.79	3.52		2.74		
SD	0.12		0.21	0.15	0.13	0.12		0.10		
Inter-lab variation	1.10		1.22	1.13	1.27	1.12		1.07		

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

Lab ID #	Sample Code A	B	C	D	E	F	G	H	Kit lot	Exp. date
V01	2.93		2.87	2.90	2.91	2.77		2.92	461383	2016-10-10
V02	2.76		2.84	2.89	2.79	2.90		2.91	461383	2016-10-10
V03	2.86		2.94	2.95	2.79	3.01		2.92	460251	2016-08-09
V10	2.92		2.94	2.79	2.81	2.82		2.94	457597	2016-04-06
V12	2.81		2.98	2.91	2.88	2.99		2.98	460251	2016-08-09
V13	2.94		2.81	2.90	2.75	2.81		2.91	460378	2016-08-16
V14	3.00		2.96	2.91	2.93	3.01		3.00	457108	2016-03-29
V17	2.70		2.47	2.59	2.55	2.76		2.51	461383	2016-10-10
V19	2.80		2.53	2.66	2.55	2.69		2.92	461383	2016-10-10
V21	3.00		2.90	2.90	2.70	2.80		3.00	*Not provided*	
V27	2.73	<LDL	2.90	2.73	2.89	2.76	<LDL	2.93	460251	2016-08-09
V29	2.62		2.73	2.70	2.88	3.01		3.02	461209	2016-10-02
V33	2.79		2.96	2.94	2.97	3.03		2.94	458404	2016-05-20
V41	3.10		3.11	3.13	3.03	2.92		3.11	461383	2016-10-10
Mean	2.85		2.85	2.85	2.82	2.88		2.93		
Minimum	2.62		2.47	2.59	2.55	2.69		2.51		
Median	2.84		2.90	2.90	2.85	2.86		2.94		
Maximum	3.10		3.11	3.13	3.03	3.03		3.11		
% CV	4.68		6.10	4.93	5.08	4.08		4.55		
SD	0.13		0.17	0.14	0.14	0.12		0.13		
Inter-lab variation	1.18		1.26	1.21	1.19	1.13		1.24		

Appendix 1: Test Results *(continued)*

Table 5C Abbott RealTime Results (0.2mL) (Log_{10} HIV RNA Copies/mL)

Lab ID #	Sample Code A	B	C	D	E	F	G	H	Kit lot	Exp. date
V36	2.94		2.81	2.96	2.93	2.95		2.99	*Not provided*	

Table 5D bioMerieux NucliSens EASYQ HIV-1 V2.0 (Log_{10} HIV RNA Copies/mL)

Lab ID #	Sample Code A	B	C	D	E	F	G	H	Kit lot	Exp. date
V26	2.76	3.08	2.86	2.8	2.92	2.94		3.11	14101601	2016-02-28
V28	2.50	<LDL	2.10	2.70	<LDL	2.10	<LDL	2.7	14112402	*Not provided*
Mean	2.63		2.48	2.75		2.52		2.91		
Minimum	2.50		2.10	2.70		2.10		2.70		
Median	2.63		2.48	2.75		2.52		2.91		
Maximum	2.76		2.86	2.80		2.94		3.11		
% CV	6.99		21.67	2.57		23.5		9.98		
SD	0.18		0.54	0.07		0.59		0.29		
Inter-lab variation	1.10		1.36	1.04		1.40		1.15		

Appendix 2: Troubleshooting

Common causes of outlying and/or aberrant results in Molecular Laboratories.

TYPE OF ERROR	POSSIBLE CAUSES
Pre-analytical	• Sample mix-up • Incorrect sample storage conditions/shipping conditions that affect sample suitability
Analytical - random	• Insufficient mixing of sample • Poor pipetting • Ineffective or inconsistent washing • Transcription errors • Sample mix-up • Cross-contamination or carryover of sample • Presence of inhibitors to PCR
Analytical - systematic	• Contaminated reagents, expired kits • Instrument error or malfunction • Insufficient washing • Cycling conditions suboptimal • Insufficient mixing/centrifuging before testing • Incorrect storage of test kit and/or reagent • Contamination of mastermix, extraction areas or equipment • Ineffective extraction process • Degradation of mastermix components
Post-analytical	• Transcription errors • Interchanging the results of two or more samples • Entering incorrect results • Selecting the wrong assay in Oneworld Accuracy • Entering values in incorrect units • Selecting incorrect assay interpretation

*This table was modified from a report produced by the National Reference Laboratory (NRL), Melbourne, Australia.