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 2016Oct28

This panel focused on the impact of extended storage at different temperatures on quantitation. This is the first time we distributed the panel to users of the Hologic Panther platform

Storage Conditions	True Status (Pre-Maniulation) copies/mL [log_{10}]	Panel Sample	Labs Reporting Incorrect Final Status
Room Temperature (1 week)	1122[3.05]	D	**Incorrect Result/Interpretation** • V27
		H	
+37°C (26 hours)	1122 [3.05]	A	**Incorrect Result/Interpretation** • V27
		F	
-80°C	1122 [3.05]	C	
		G	
-80°C	TND	B	**Incorrect Result/Interpretation** • V10 • V45 • V27
		E	

Participants using the Abbott RealTime HIV-1 RNA PCR, Roche CAP/CTM HIV-1 Test v2.0 and the Hologic Panther Apitma HIV-1 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 B and E.
  Result: Target Not Detected with a Viral Load <LDL
  Interpretation: Target Not Detected

- **V27:** Incorrect result/interpretation for the negative samples B and E
  Result: Target Not Detected with a Viral Load <LDL
  Interpretation: Target Not Detected
  Undetected HIV-1 RNA in positive samples F and H.

- **V45:** Incorrect result/interpretation for the negative samples B and E.
  Result: Target Not Detected with a Viral Load <LDL
  Interpretation: Target Not Detected

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Final Report for Panel HIVVL 2016Oct28

Issued 18-Jan-2017

Introduction

The NLHRS distributed the 2016Oct28 panel and the 2017Apr19 panel on Oct 12th 2016. This final report is publicly available, however the identity of participants is not disclosed.

As an extension of the 2013-2015 panels, the 2016Oct28 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 that this is the first first panel to include laboratories that uses the Panther platform from Hologic.

Panel Samples, HIV Test Kits and Data Entry

1. *Panel Composition* – Panel 2016Oct28 (Table 1) contained the following:
   - One negative sample sent in duplicate (B and E); 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 (D/H) was stored at room temperature (RT) for 1 week and then returned to -80°C.
     - Set 2 (A/F) was stored +37°C for 26 hours and then returned to -80°C.
     - Set 3 (C/G) was stored at the recommended temperature of -80°C.

Table 1: Description of panel 2016Oct28 samples

Sample Identification	Sample Type	Sample Subtype	Storage Conditions	Viral Load copies/mL [log_{10}] Pre-Manipulation[1]
D H	HIV-1	B	Room Temperature (1 week)	1122 [3.05]
A F	HIV-1	B	+37°C (26 hours)	1122 [3.05]
C G	HIV-1	B	-80°C	1122 [3.05]
B E	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 25 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* – October 28th, 2016.

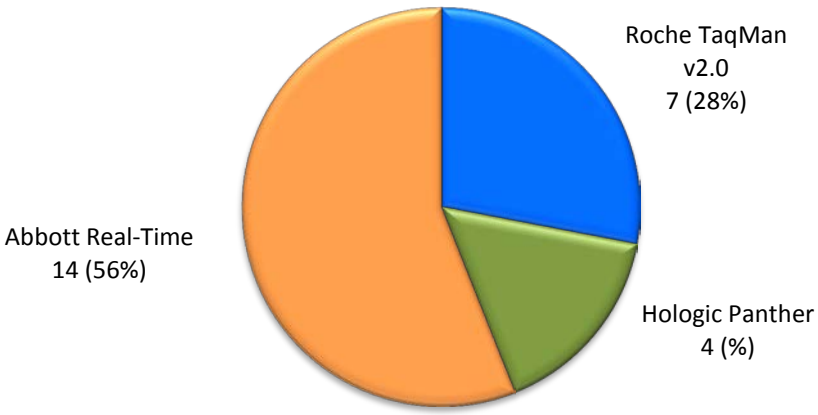

Figure 1: Breakdown of the assays used by the 25 participants in the NLHRS 2016Oct28 Viral Load Panel (excludes the NLHRS).

Return rate

Results were returned from 100% of participants (25/25).

- Two participant (V26 and V37) was unable to participate due to shipping delays.
- One participants (V44) was unable to participate due to logistic delays.
- Ten year average return rate of 90.3% (Figure 2).

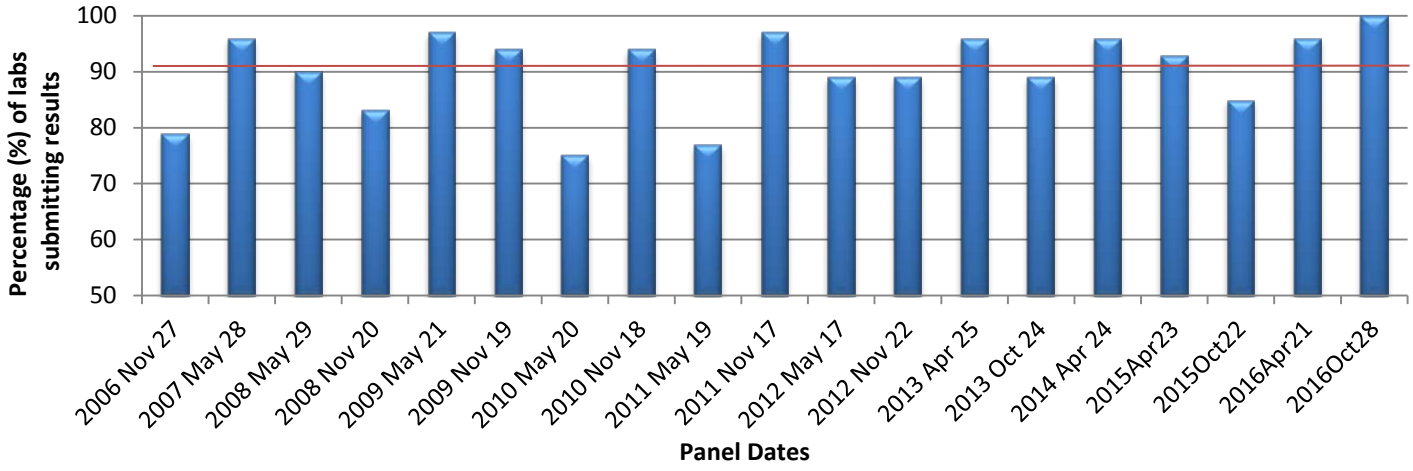

Figure 2: Percentage of HIV Viral Load Panel results submitted between 2006 and 2016

Flags

1. Incorrect test result.
   - **V27** Did not detect HIV-1 RNA in positive sample H and F.

2. Labs continue to implement interpretative criteria different from the kit insert for negative samples (B,E) on the Abbott RealTime HIV-1 RNA PCR, Roche CAP/CTM HIV-1 Test v2.0, Hologic Panther Aptima HIV-1
   - **V10, V27, and V45** result of Target Not Detected with viral load <LDL with final interpretation of Not detected.

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

Red: Incorrect

Results

1. **Statistical Analysis (General)**
   - One outlier was detected and removed from further analysis (Grubb's test)
   - All group comparisons done using the Unpaired *t* test.
   - No significant difference ($p > 0.05$) between duplicate sets; A/F, C/G, D/H
     - Data for each set was combined and analyzed together.
   - No analysis for peer groups of n=1 (Abbott 0.2mL).
   - Users of the Hologic Panther Apitma HIV-1 Quant are included in the anaylsis even though they are a small group (n=4)
   - Negative samples are analyzed qualitatively.

2. **Individual Analysis (Participant Statistics)** (Figures 5, 6, 7 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, 5C)
   - The duplicate panel samples were combined for the summary statistics (A/F, C/G, D/H).

   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 1.08 for the Roche CAP/CTM v2., 1.11 for the Abbott RealTime (0.6mL) and 1.08 for Hologic Panther Aptima HIV-1 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 ,Abbott and Hologic users reported standard deviation (SD) of 0.19 or lower between duplicates.

4. **Effect of Suboptimal Storage**

   Storage at RT for 1 week (Samples D, H)
   - *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 =0.0001). This effect was also observed in the previous HIV-1 viral load panel, 2016Apr21
   - *Roche CAP/CTM v2.0 (n=7)* - Participant results (including the NLHRS) showed statistical difference between storage at RT for 1 week compared to -80°C (p= 0.0002). This effect was also observed in the previous HIV-1 viral load panel, 2016Apr21
   - Hologic Panther Apitmal HIV-1 Quant (n=4)-Participants results showed statistical difference between storage at RT for week compared to -80°C (p = 0.009).

   Storage at +37°C for 26 hours (Samples A, 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 = 0.0009). This effect was also observed in the previous HIV-1 viral load panel, 2016Apr21
   - *Roche CAP/CTM v2.0 (n=7)* - Participant results (including the NLHRS) showed no statistical difference between storage at +37°C for 26 hours compared to -80°C (p =0.1361).
   - Hologic Panther Aptima HIV-1 Quant (n=4)-Participants results show statistical difference between storage at +37°C for 26 hours compared to -80°C (p=0.0073).

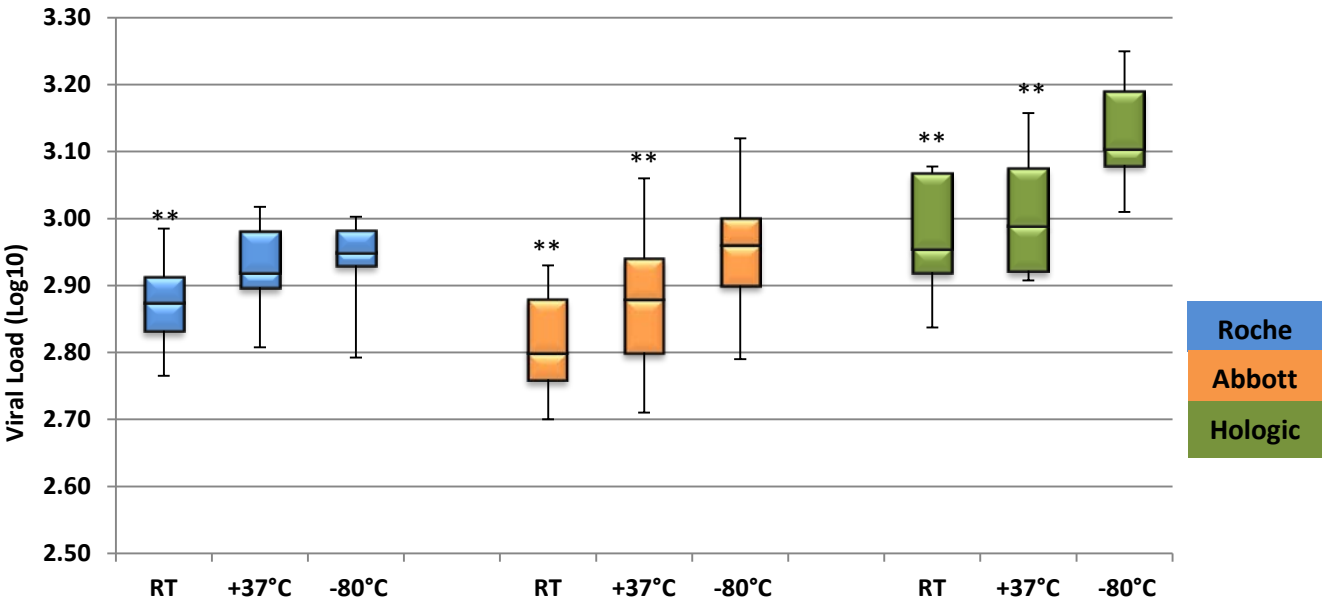

Figure 3: Effect of sample storage temperature on viral load values, 2016Oct28 HIV-1 VL panel

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

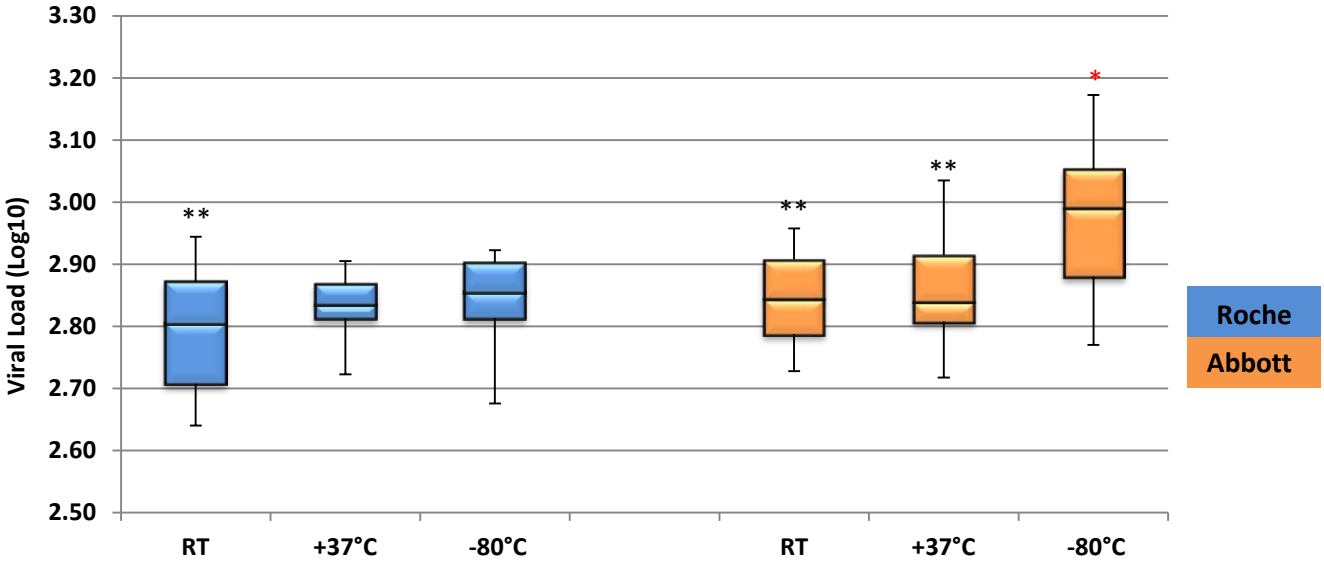

Figure 4: Effect of sample storage temperature on viral load values, 2016Apr21 HIV-1 VL panel.

* 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)

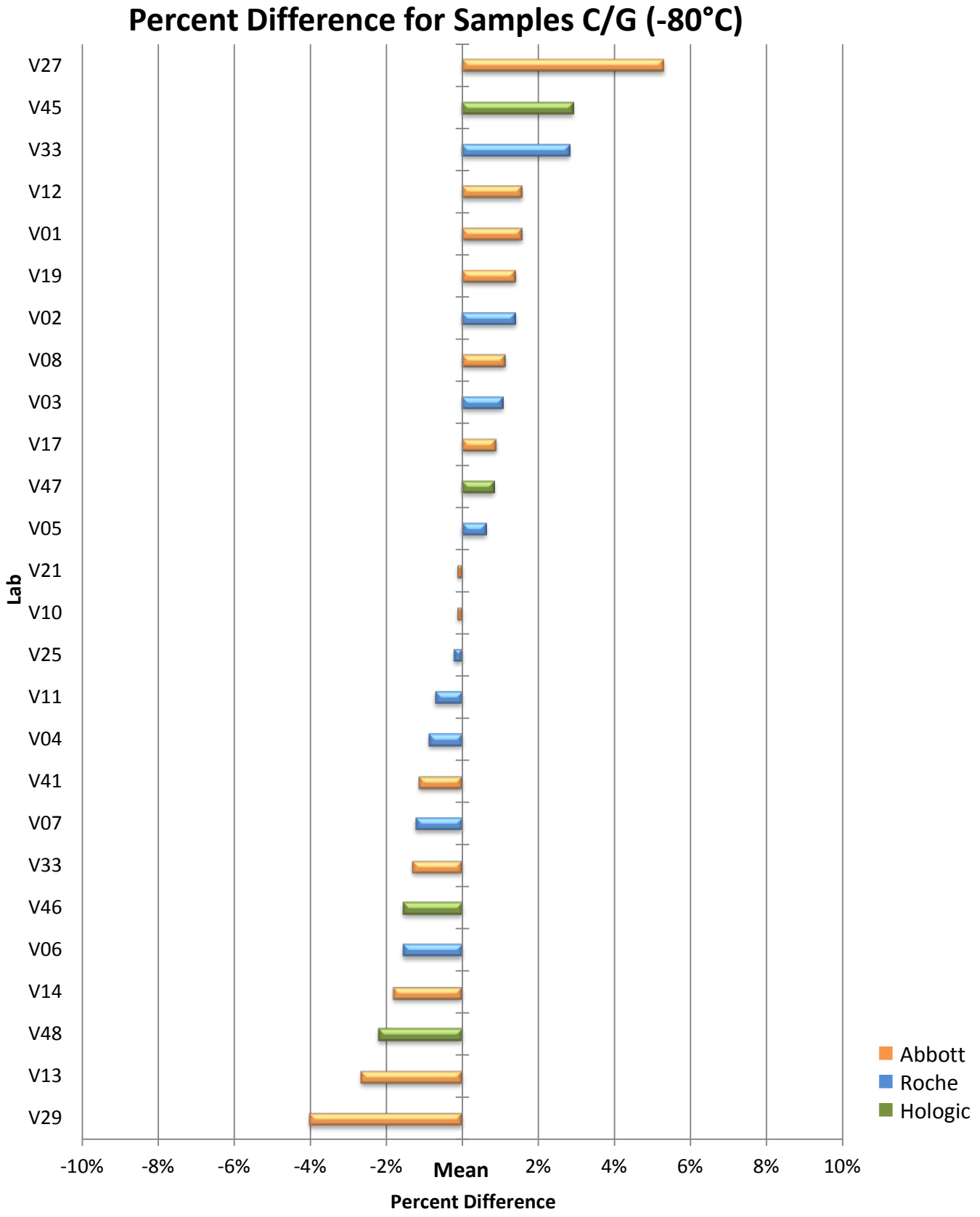

Figure 5: Percent Difference from the Peer Group Mean of C/G.

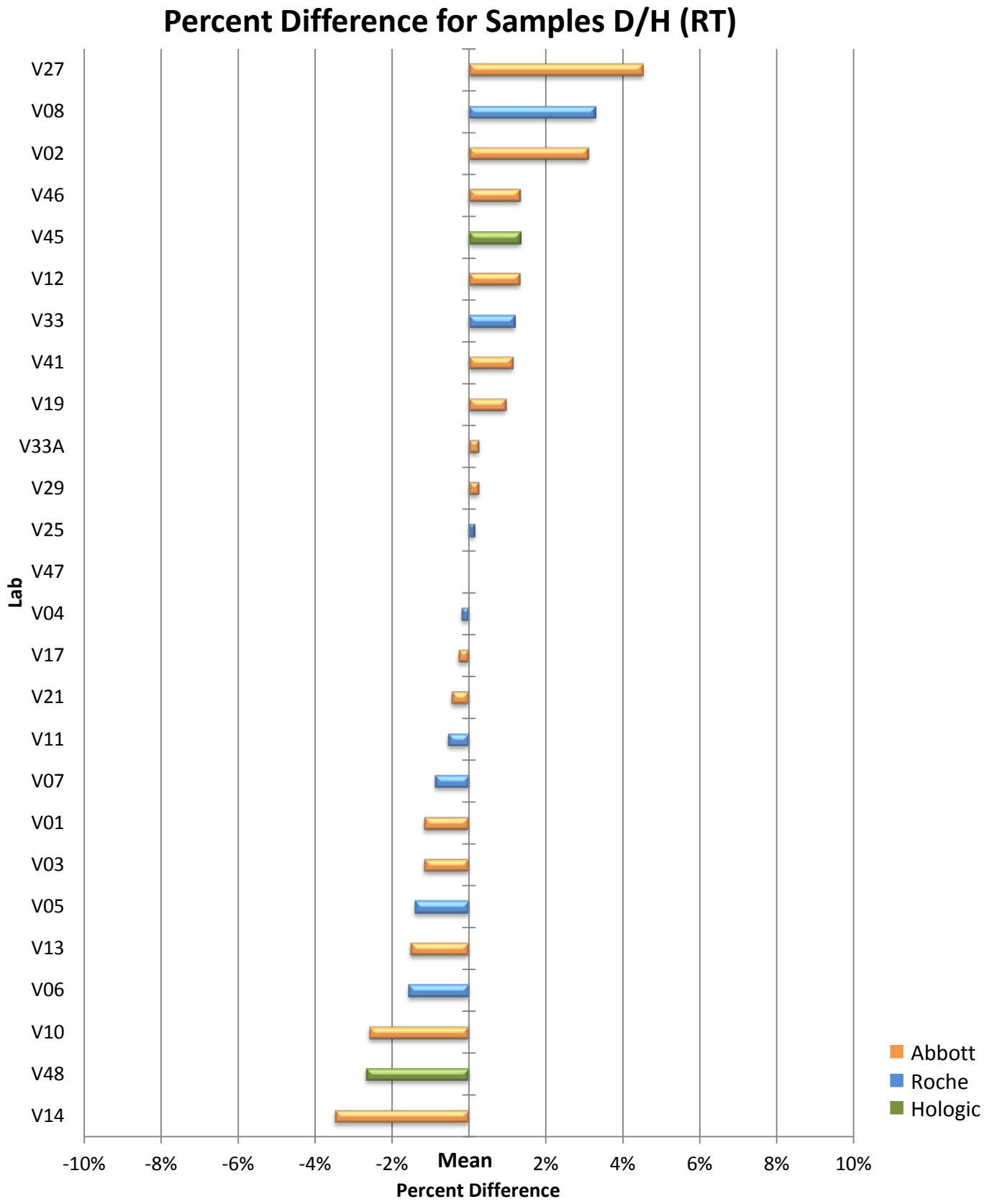

Figure 6: Percent Difference from the Peer Group Mean of D/H.

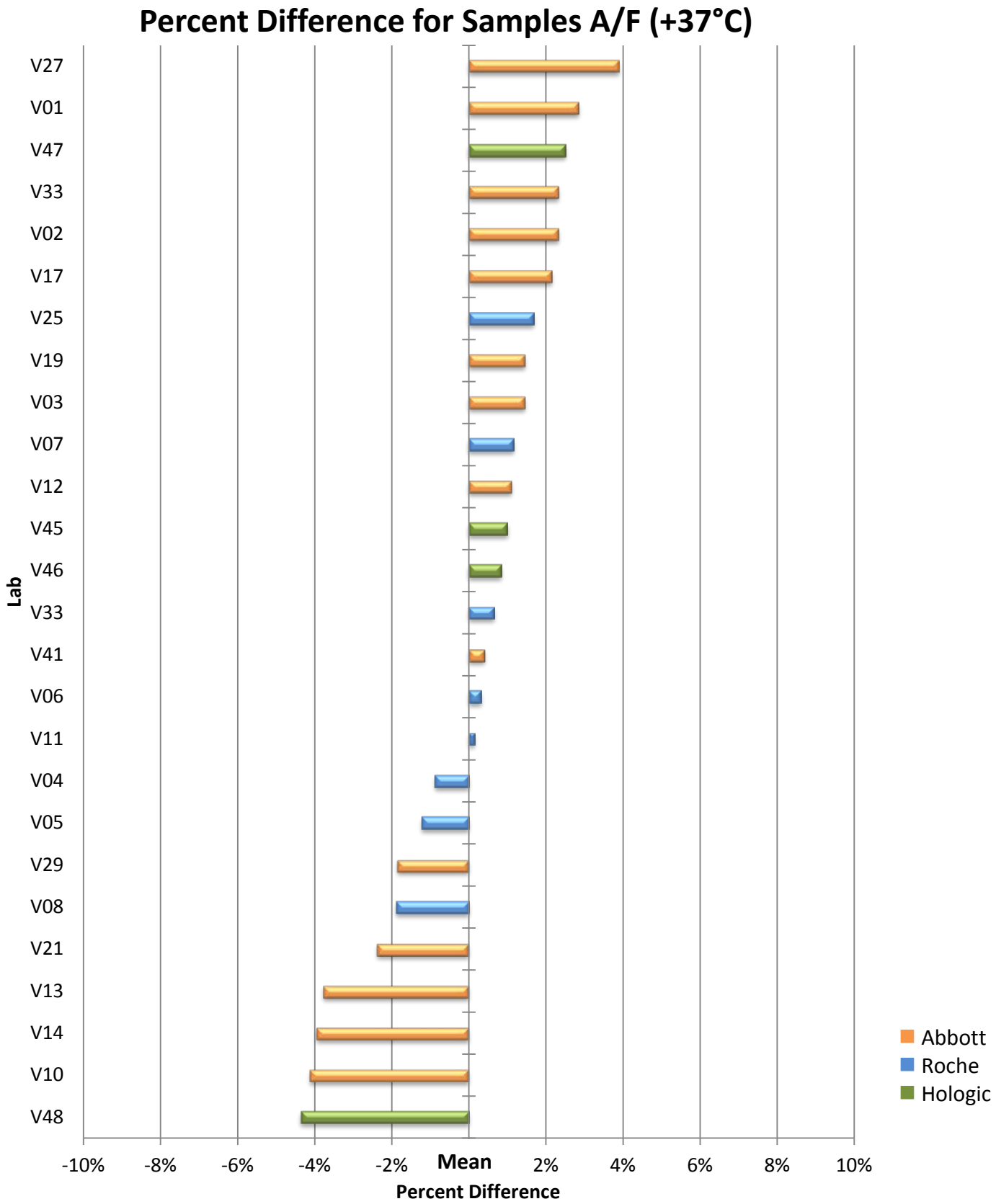

Figure 7: Percent Difference from the Peer Group Mean of A/F.

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

Sample	Storage Temperature vs -80˚C	Assay	Panel	p-value
Subtype B *1122 cp/mL Roche CAP/CTM v2.0*	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**
	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)*

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.
   - Eight participants (32%, 8/25) reported using external QC material, a slight increased from 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.
   - Eighteen participants (72%, 18/25) reported participation in QA programs other than the NLHRS panels, a slight increased from last survey.

Conclusion

1. *Effect of Temperature*
   - The Roche CAP/CTM v2.0 assay was significantly affected by storage at RT for 1 week (p=0.0002) but not for storage at +37°C for 26 hours (p = 0.1361) compared to storage at -80˚C.
   - The Abbott 0.6mL assay was significantly affected by both storage temperatures; RT for 1 week (p = 0.0001) and for +37°C for 26 hours (p = 0.0009) compared to storage at -80˚C.
   - The Hologic Panther Aptima HIV-1 assay was significantly affected by both storage temperatures; RT for 1 week (p=0.009) and for +37°C for 26 hours (p = 0.0073) compared to storage at -80˚C but the number of users is very small (n=4) when compared to Roche and Abbott users.
   - The effects of sub-optimal storage on both the Roche and Abbott assay observed in this panel is concordant in what was observed with the previous panel, 2016Apr21.
   - 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** **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	F	C	G	D	H	B	E		
V04	2.93	2.89	2.95	2.93	2.84	2.90			W011625	2017-08-31
V05	2.89	2.91	2.98	2.99	2.76	2.91			W077080	2017-06-30
V06	2.98	2.91	2.91	2.93	2.86	2.80			W091610	2017-02-28
V07	3.07	2.87	2.94	2.92	2.81	2.89			W091610	2017-02-28
V08	2.86	2.90	2.96	3.04	2.90	2.97			W091610	2017-02-28
V11	2.98	2.90	2.95	2.94	2.90	2.92			W091610	2017-02-28
V25	2.94	3.03	3.01	2.91	3.03	2.85			W017057	2017-02-28
V33	2.93	2.98	3.12	2.98	2.98	2.98			W03493	2017-01-31
Mean	2.94		2.97		2.88					
Minimum	2.86		2.91		2.76					
Median	2.92		2.95		2.88					
Maximum	3.07		3.12		2.98					
% CV	1.99		1.85		2.31					
SD	0.06		0.05		0.07					
Inter-lab variation	1.07		1.07		1.08					

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

Lab ID #	Sample Code								Kit lot	Exp. date
	A	F	C	G	D	H	B	E		
V01	2.87	3.03	3.05	2.95	2.74	2.82			11101701	2017-07-31
V02	2.90	2.97	3.05	2.94	2.91	2.89			11101701	2017-07-31
V03	2.89	2.93	2.96	3.01	2.80	2.76			10944341	2017-01-30
V10	2.75	2.75	3.04	2.86	2.74	Error	<1.6	<1.6	10944341	2017-01-30
V12	2.90	2.90	3.00	3.00	2.80	2.90			11101701	2017-07-31
V13	2.80	2.72	2.79	2.96	2.72	2.82			11068571	2017-04-30
V14	2.68	2.83	2.90	2.90	2.71	2.72			10944341	2017-01-31
V17	2.85	3.01	2.97	2.99	2.73	2.88			11101701	2017-07-31
V19	2.83	2.99	3.03	2.96	2.79	2.89			11101701	2017-07-31
V21	2.80	2.80	2.90	3.00	2.80	2.80			11101701	2017-07-31
V27	2.98	0	3.11	Error	2.94	0	<1.6	<1.6	10944341	2017-01-30
V29	2.68	2.95	2.78	2.89	2.78	2.86			11182901	2017-06-30
V33	2.90	2.97	2.89	2.94	2.88	2.76			10957671	2016-12-29
V41	2.88	2.88	2.92	2.25	2.92	2.77			109515081	2017-11-10
Mean	2.87		2.95		2.81					
Minimum	2.68		2.78		2.71					
Median	2.90		2.96		2.80					
Maximum	3.03		3.11		2.94					
% CV	3.40		2.64		2.49					
SD	0.10		0.08		0.07					
Inter-lab variation	1.13		1.12		1.08					

Appendix 1: Test Results

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

Table 5C Hologic Panther Aptima HIV-1 (Log_{10} HIV RNA Copies/mL)										
Lab ID #	**Sample Code**								**Kit lot**	**Exp. date**
	A	**F**	**C**	**G**	**D**	**H**	**B**	**E**		
V45	2.94	3.09	3.18	3.26	2.92	3.15	<1.4	<1.4	160165	2017-06-15
V46	3.07	2.95	3.11	3.05	2.98	3.09			160165	2017-06-15
V47	3.09	3.03	3.09	3.22	3.06	2.93			111363	2018-11-15
V48	2.84	2.87	3.10	3.02	2.91	2.92			160165	2017-06-15
Mean	2.99		3.13		3.00					
Minimum	2.84		3.02		2.91					
Median	2.99		3.11		2.96					
Maximum	3.09		3.26		3.15					
% CV	3.32		2.67		3.10					
SD	0.10		0.08		0.09					
Inter-lab variation	1.09		1.08		1.08					

Table 5D Abbott RealTime (0.2mL) Results (Log_{10} HIV RNA Copies/mL)										
Lab ID #	**Sample Code**								**Kit lot**	**Exp. date**
	A	**F**	**C**	**G**	**D**	**H**	**B**	**E**		
V36	2.91	2.94	3.02	3.02	2.60	2.84			*Not provided*	

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.