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INFECTIOUS DISEASES RESEARCH CENTRE

National Laboratory for HIV Reference Services
Sexually Transmitted and Bloodborne Infections
National Microbiology Laboratory
Public Health Agency of Canada

HIV Viral Load Quality Assessment Program
Summary for Panel HIVVL 2023Apr19

2023Apr19 HIV-1 VL Panel				
Subtype	**Panel Sample Pair**	**Viral Load Consensus Mean[1]**	**Viral Load Mean Characterization by the NLHRS**	**Labs Reporting Incorrect Status**
B	A/B/D/E/G	3.06[3], 3.09[4], 3.01[5], 3.02[6]	3.15[2], 2.98[3]	V21, V27
N/A	C/F/H	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 the Hologic Aptima HIV-1 assay.
4. Based on the Roche cobas 6800 HIV-1 assay
5. Based on the Cepheid GeneXpert II
6. Based on the Abbott RealTime HIV-1 assay

Summary of findings results observed for the 2023Apr19 panel:

1) Participant V21 submitted a result of “Target detected but not quantifiable” for Sample C. Sample C is the negative replicates in the panel (C, F, H)

2) Participant V27 submitted a result of “Target Not Detected” for Sample G (positive replicates in the panel) and a result of “Target Detected for Sample H (negative replicates in the panel).

Note: The uncertainty of each participant’s results for the 2023Apr19 panel is presented in Appendix 1, Table 2

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Final Report for Panel HIVVL 2023Apr19

Issue 2023-June-22

Introduction

This final report is specific to the 2023Apr19 panel only and is publicly available. The NLHRS distributed the 2022Dec16 and 2023Apr19 panels on November 29th. The identity of the participants is not disclosed. The deadline for results submission was April 19, 2023. The preliminary report was issued on May 03, 2023.

Panel Samples, HIV Test Kits, and Data Entry

- *Panel Composition* – The 2023Apr19 panel contained the following:
    - One negative sample sent in triplicate (C, F, H); defibrinated human plasma.
    - One positive HIV-1 RNA sample (Accurun 315 series 500, subtype B) diluted to approximately 1000 cp/mL in defibrinated human plasma (Basemetrix 53, Seracare Life Sciences Inc.) aliquoted in five replicates (A, B, D, E, G) and stored at -80°C.
    - The NLHRS characterized the positive panel members on both the Roche CAP-CTM and Hologic Panther platforms to assess the Log10 cp/mL value prior to panel send out (Summary page).
    - The samples in the 2023Apr19 panel are the same samples used for the 2022Dec16 panel.
    - Panels were sent to 19 participants including NLHRS on November 29th, 2022.
    - Metrological traceability is not applicable to this panel.
    - Uncertainty is applicable to this panel. Each participant's uncertainty for the 2023Apr19 panel is presented in Appendix 1, table 2.

- *HIV Viral Load Test Kits* – Seven different assays were used by the participants (including the NLHRS) who returned results.

- *Data entry* - Results entry for this panel utilized an in-house developed website.

Homogeneity and Stability

- The homogeneity of the 2023Apr19 HIV-1 viral load panel was assessed by using the Roche cobas 6800 peer group (n=5) for the positive sample set (A/B/D/E/G). All participants were able to detect HIV-1 RNA and the results were within ± 0.5 Log10 cp/mL of the group mean (Appendix 1). There is no indication of heterogeneity in the panel samples.
- The stability of the 2023Apr19 HIV-1 viral load panel was assessed by comparing the group mean generated by the participants forthe positive sample replicates with the results from the characterization of the samples. The difference between both means did not exceed ± 0.5 Log10 cp/mL, meeting the stability criteria.

Results

- *Evaluation Criteria:*
  - Negative samples: Expected result to be “Target not detected”.
  - Positive samples: Expected viral load results to be in Log10 cp/mL and within ± 0.5 Log10 cp/mL of their respective peer group.

1. Statistical Analysis (General)

- No outlier was detected in this event (Grubb’s test).
- Analysis was not performed for small peer groups of n<=2 (Roche cobas 4800, Roche cobas 8800, Roche CAP/CTM).
- Negative samples were analyzed qualitatively.

2. Group Analysis (Summary Statistics) (Figure 1, Tables 1 and 2)

- The duplicate panel samples were combined for the summary statistics (A/B/D/E/G).

Inter-Lab Variation (Tables 1 and 2)

- Difference between the minimum and maximum results for each sample within a peer group (the maximum value divided by the minimum).
  - 1.17 Log10 cp/mL for the Roche cobas 6800, 1.07 Log10 cp/mL for the Cepheid GeneXpert II, 1.07 Log10 cp/mL for the Abbott RealTime (0.6 mL) and 1.12 Log10 cp/mL for the Hologic Panther peer group.

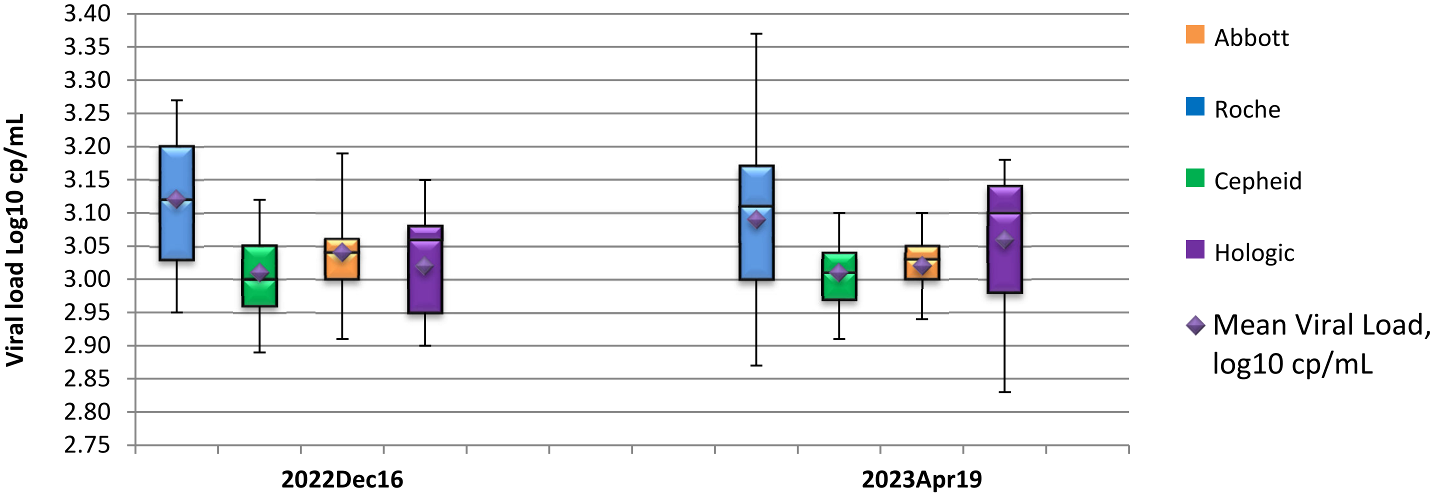

Figure 1: Viral load results for the Roche cobas 6800, Cepheid GeneXpertII, Abbott RealTime and the Hologic Panther peer group.

Reproducibility

- This is an important aspect of viral load testing; required to quantify changes in viral load.
- To assess intra-reproducibility, five replicates of the positive samples were included in the panel. The standard deviation of the five replicates are illustrated in Figure 2.

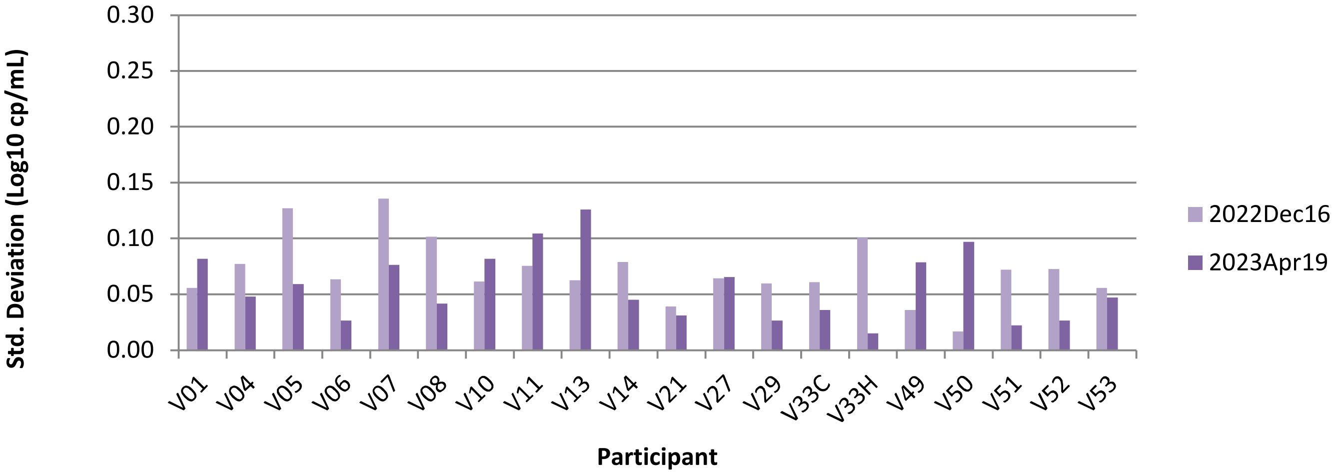

Figure 2: Participants' standard deviation for the positive replicates from 2022-2023

3. **Comparison Between the Major and Minor Peer Groups** (Figure 3)
    - The results between the major peer group (Roche cobas 6800, Cepheid GeneXpert II, Abbott RealTime and Hologic Panther) and the minor peer group (n<=2; i.e.Roche cobas 4800, Roche cobas 8800, and Roche CAP-CTM) for the sample group A/B/D/E/G were comparable (within ± 0.5 Log10 cp/mL).
    - A proper and fair comparison between the different peer groups would require more users of the platforms within the minor peer group.

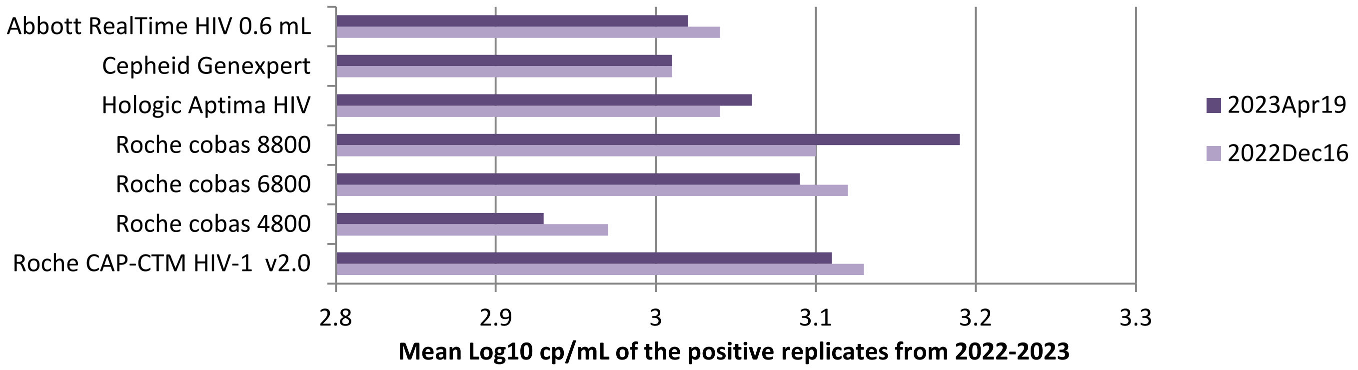

Figure 3: Viral load comparison between the different viral load platforms from 2022-2023

4. **Individual Analysis (Participant Statistics)** (Figure 4)
    - The percent difference (% D), the difference from the mean for participants in the major peer group, was calculated for each participant per sample pair.
    - No major differences were identified between the peer group mean and the participants’ results in this test event.

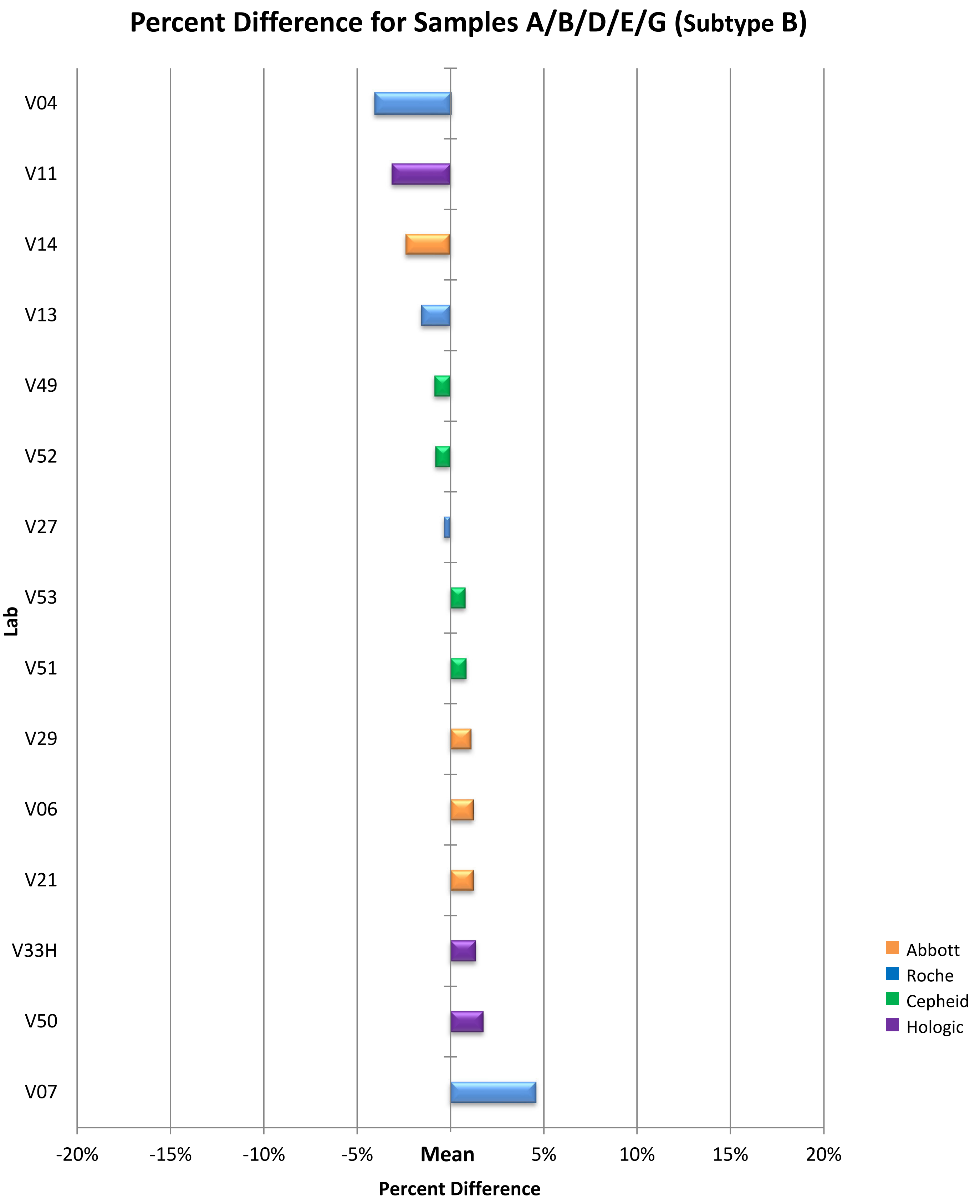

Figure 4: Percent difference from the peer group mean for A/B/D/E/G.

Findings

There were two findings for the 2023Apr19 test event. One participant (V21) reported Sample C (HIV-1 RNA negative) as "Detected". This erroneous result indicates a potential contamination issue. A remedial action was recommended to this participant and additional panels were sent to monitor if the issue has been resolved. One participant (V27) reported Sample G (HIV-1 RNA positive) as "Target Not Detected" and Sample H (HIV-1 RNA negative) as "Detected", this is possibly due to either a transcription error or sample mix up. This participant has been notified to investigate the cause of this error.

For this event, we have noticed one participant that originally used the Roche CAP-CTM has switched to the Roche cobas 4800. We anticipate that more participants will switch to newer platforms resulting in changes to the viral load peer group for future test events.

We value each laboratory's participation in these QA test events and your suggestions for improvement. The NLHRS is committed to improve all aspects of the HIV-1 viral load proficiency testing program in order to provide quality proficiency testing to our participants.

If you have any comments, suggestions or concerns, please contact us at:

nlhrs.qap-peq.lnsrv@phac-aspc.gc.ca

Thank you for your participation in the NLHRS Quality Assurance Program

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Appendix 1: Summary of the 2023Apr19 viral load results

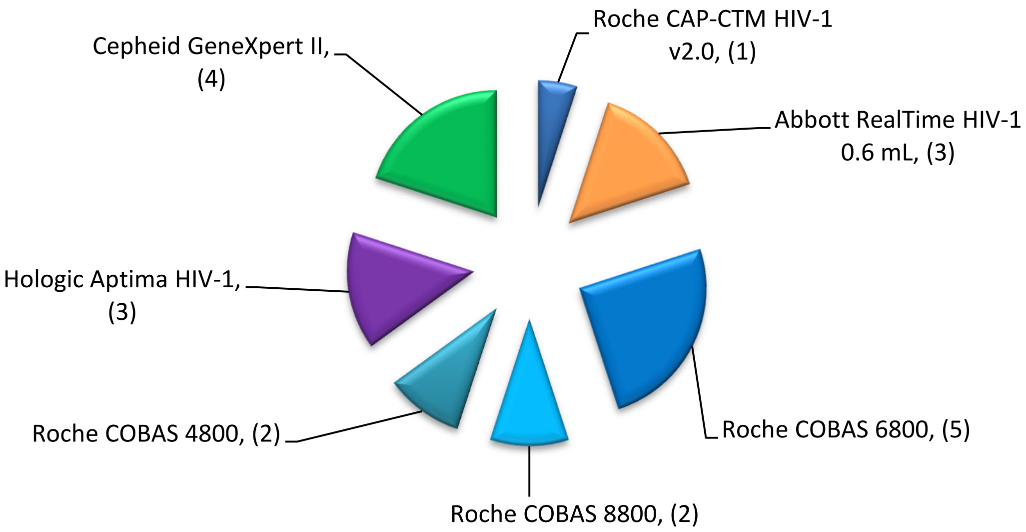
Table 1: Summary of HIV-1 viral load results of each respective peer group (Log10 HIV-1 RNA cp/mL)

	Roche cobas 6800	Cepheid GeneXpert II	Abbott RealTime HIV-1	Hologic Panther
Mean	3.09	3.01	3.02	3.06
Minimum	2.87	2.91	2.89	2.83
Median	3.11	3.01	3.03	3.10
Maximum	3.36	3.10	3.10	3.18
%CV	3.85	1.79	2.09	3.39
SD	0.12	0.05	0.06	0.10
Inter-lab Variation	1.17	1.07	1.07	1.12

Table 2: The viral load results with the expanded uncertainty at 95% confidence interval for the 2023Apr19 test event (Log10 HIV-1 RNA cp/mL)

Lab code	Average Log10 cp/mL	Expanded Uncertainty
V01	3.14	0.23
V04	2.96	0.22
V05	3.08	0.30
V06	3.13	0.06
V07	3.23	0.21
V08	2.77	0.12
V10	3.24	0.20
V11	2.97	0.23
V13	3.04	0.29
V14	2.95	0.12
V21	3.06	0.09
V27	3.08	0.15
V29	3.06	0.07
V33C	3.11	0.17
V33H	3.10	0.15
V49	2.98	0.15
V50	3.12	0.20
V51	3.03	0.09
V52	2.98	0.06
V53	3.03	0.13

Appendix 2: Summary of platforms used by participants (including the NLHRS) in the 2023Apr19 HIV-1 viral load panel.



Appendix 3: Troubleshooting

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 (<u>random error</u>)	<u>Sporadic test results identified as outlying and/or aberrant can be classified as random events. Possible causes of random error include:</u>			
	• 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 (<u>systematic error</u>)	<u>A series of test results identified as outlying and/or aberrant may be due to a systematic problem. Systematic problems may be due to:</u>			
	• 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.