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INFECTIOUS DISEASES RESEARCH CENTRE

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 2019Oct31

2019Oct31 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/C/E/F	3.01[2], 2.95[3]	3.03[2], 2.98[3]	
B	D/H[4]	1.89[2], 2.00[3]	2.08[2], 1.99[3]	
N/A	B/G	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 Abbott RealTime HIV-1 0.6 mL assay.
4. Challenging samples; participants were not flagged based on their results.

There were no incorrect results observed for the 2019Oct31 panel.

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Final Report for Panel HIVVL 2019Oct31

Issue 2019-December-23

Introduction

The NLHRS distributed the 2019Oct31 and 2020Apr17 panels on October 16th, 2019. This final report is specific to the 2019Oct31 only and is publicly available; however, the identity of participants is not disclosed. The deadline for results submission is October 31st,2019. The preliminary report was issued on November 18, 2019.

Panel Samples, HIV Test Kits, and Data Entry

- *Panel Composition* – The 2019Oct31 panel contained the following:
  - One negative sample sent in duplicate (B and G); defibrinated human plasma.
  - One positive HIV-1 RNA sample (VQA150000 RNA copy control, subtype B) diluted to approximately 1000 cp/mL in defibrinated human plasma (Basemetrix 53, Seracare Life Sciences Inc.) aliquoted in four replicates (A, C, E, F) and stored at -80°C.
  - One positive HIV-1 RNA sample (VQA150000 RNA copy control, subtype B) diluted to approximately 100 cp/mL in defibrinated human plasma (Basemetrix 53, Seracare Life Sciences Inc.) aliquoted in two replicates (D, H) and stored at -80°C. This sample was designated as challenging; participants were not flagged for the results submitted.
  - The NLHRS characterized the positive panel members on both the Roche and Abbott platforms to assess the Log10 cp/mL value prior to panel send out (Summary page).
  - Panel were sent to 15 participants and to the NLHRS on October 16th, 2019

- *HIV Viral Load Test Kits* – Seven different assays were used by the participants (excluding the NLHRS) who returned results. One participant switched to the Roche COBAS 4800 platform.

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

Homogeneity and Stability

- The homogeneity of the 2019Oct31 HIV-1 viral load panel was assessed by using the Roche assay peer group (n=6) and the Abbott assay peer group (n=5) results in the positive duplicate sample set (A/C/E/F). 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 2019Oct31 HIV-1 viral load panel was assessed by comparing the group mean generated by the participants in the positive duplicate sample sets with the results from the characterization of the samples. The difference between both means did not exceed 0.5 Log10 cp/mL.

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)**
   - One outlier was detected and removed from analysis (Grubb's test).
   - All group comparisons were performed using the unpaired t test.
   - Since no significant differences ($p > 0.05$) were identified in the duplicate sets (A/C/E/F) between the Roche and Abbott users, their datasets were combined and analyzed together.
   - Analysis was not performed for small peer groups of n<=2 (Abbott 0.5 mL, Roche COBAS 6800, Roche COBAS 6800, Hologic Aptima, and Cepheid GeneXpert II).
   - 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/C/E/F).

   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).
     - 1.08 Log10 cp/mL for the Roche CAP/CTM HIV-1 v2, and 1.09 Log10 cp/mL for the Abbott RealTime (0.6 mL) peer groups.

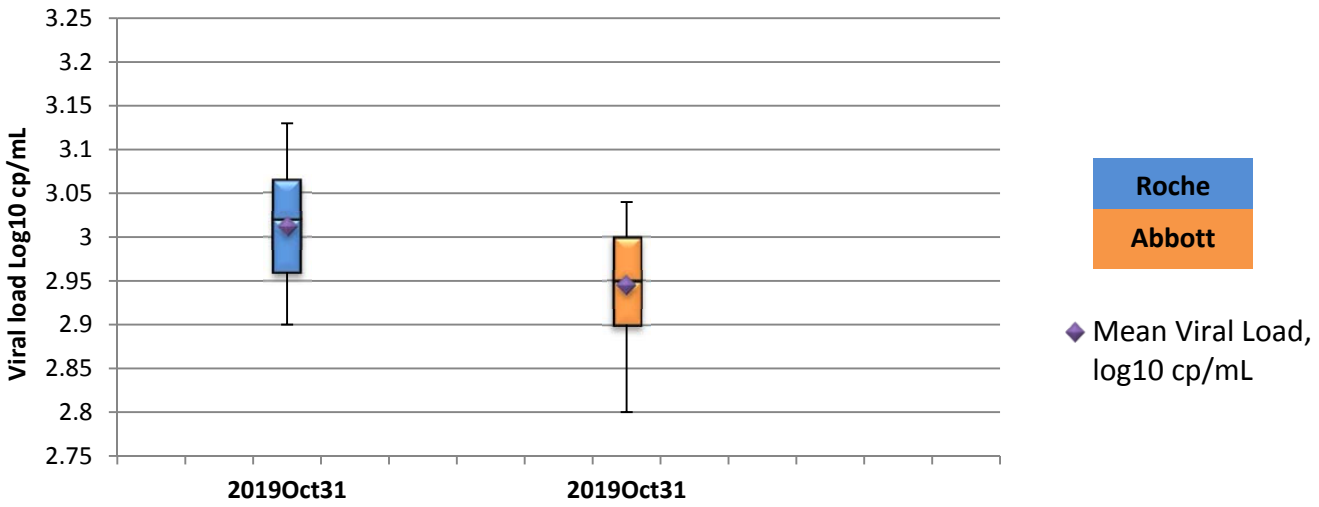

Figure 1: Viral load results for the Roche CAP-CTM HIV-1 v2.0 and the Abbott RealTime HIV-1 0.6 mL groups for the 2019Oct31 panel.

Reproducibility

- This is an important aspect of viral load testing; required to quantify changes in viral load.
- To assess intra-reproducibility, 4 replicates of the positive samples were included in the panel. The standard deviation of the 4 replicates is illustrated in Figure 2.

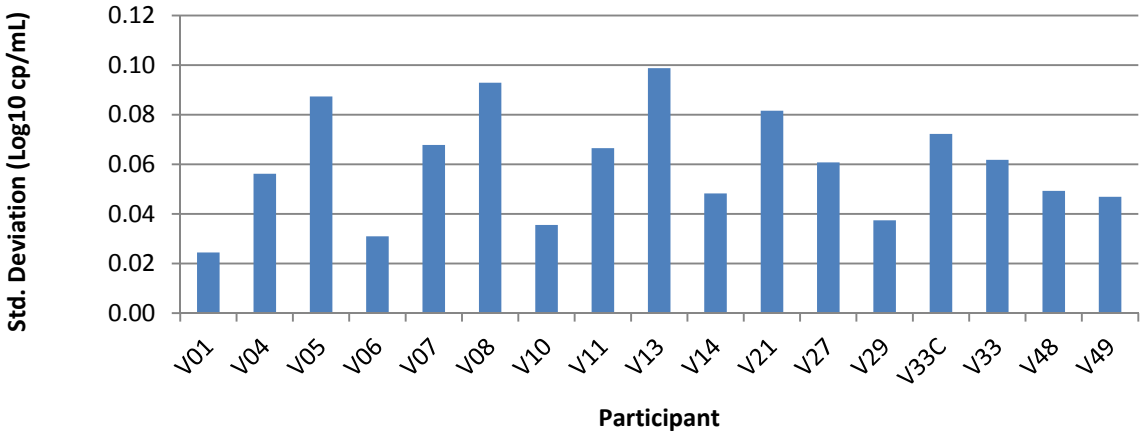

Figure 2: Participants' standard deviation for the sample group A/C/E/F.

3. **Comparison Between the Major and Minor Peer Groups** (Figure 3)
   - The results between the major peer group (Roche and Abbott 0.6 mL users) and the minor peer group (n<=2; i.e. Cepheid GeneXpertII, Hologic Aptima HIV-1, Roche COBAS 6800, Roche COBAS 4800, and Abbott 0.5 mL) for the sample group A/C/E/F 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.

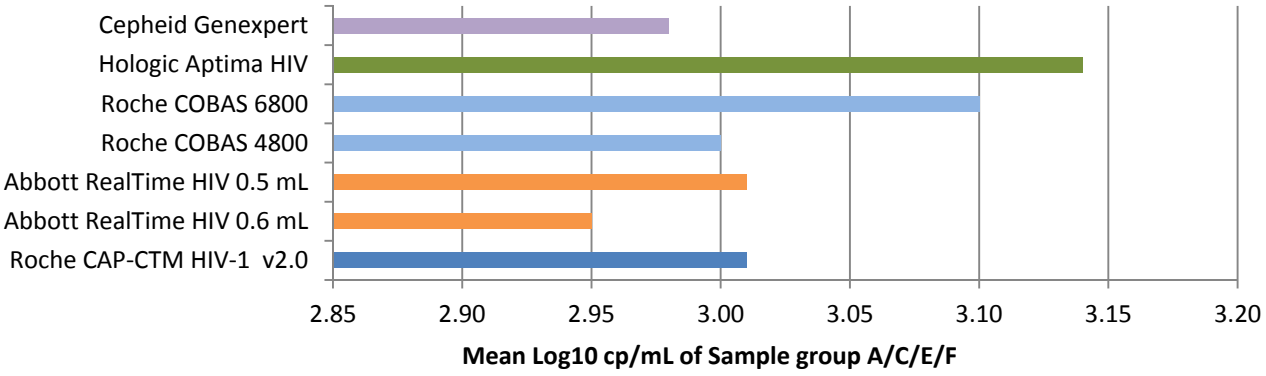

Figure 3: Viral load comparison between the different viral load platforms for sample group A/C/E/F.

4. **Challenging Samples**
   - Sample pair D/H was diluted to a lower Log10 cp/mL than previous panel samples in order to challenge participants with low viral load samples.
   - No participants reported “Target Not Detected”.
   - Three participants reported “Detected but non-quantifiable” for Sample H; all three participants use the Abbott platform. This is concordant with what was observed during the characterization of the samples with the Abbott platform.

5. **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.

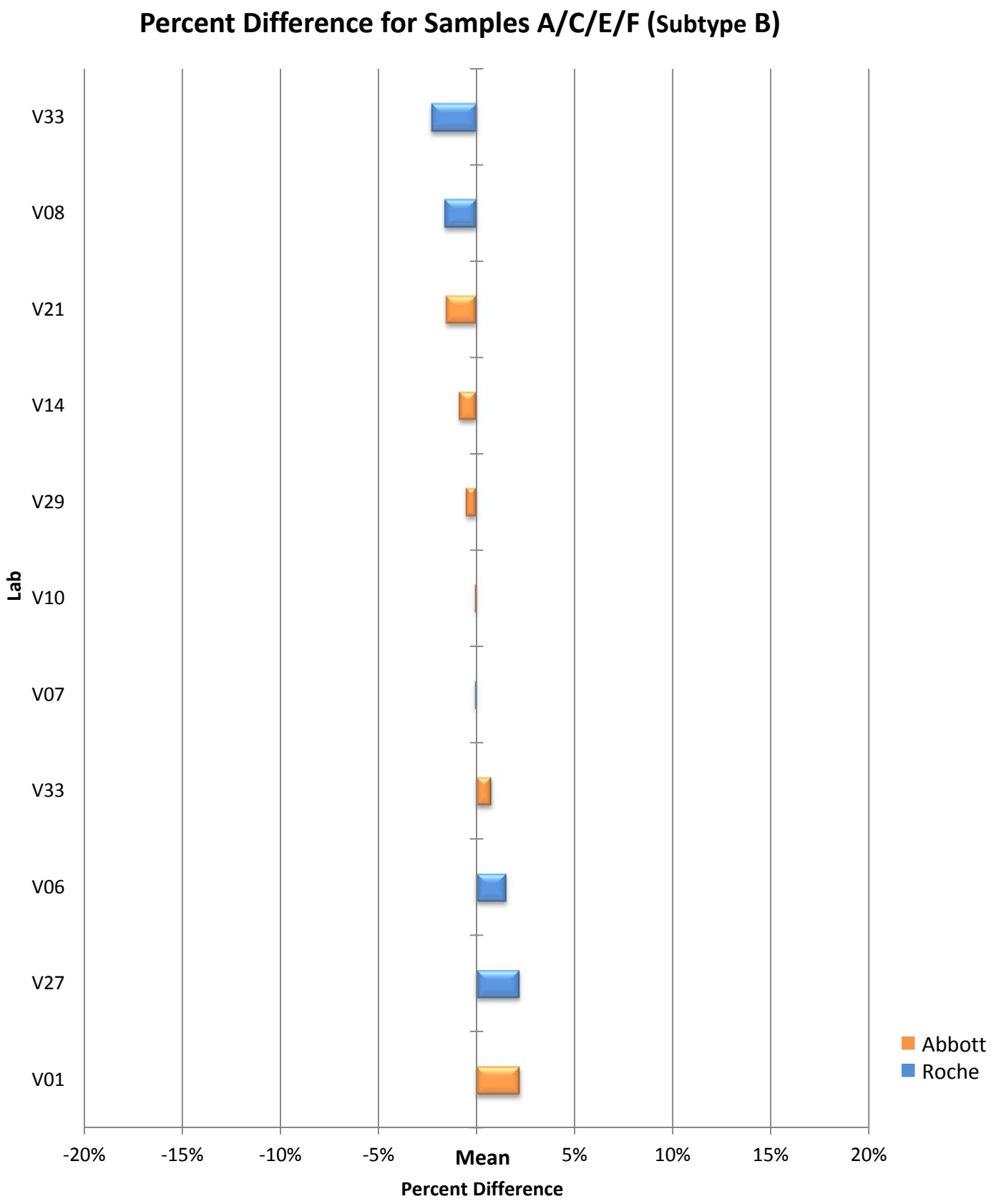

Figure 4: Percent difference from the peer group mean for A/C/E/F.

Findings

All participants were able to return results in Log10 cp/mL for the correct positive viral load samples and negative results for the correct negative samples. However, one participant encountered an invalid status for sample G and was not able to return a result. No other participants encountered this error for that specific sample. A retention aliquot for sample G and its replicate, sample B, were re-tested after the event; no errors were observed. The participant may have encountered a random system error. The NLHRS will monitor whether or not this error re-occurs in future test events.

The NLHRS continually monitors changes to the viral load platforms used by its participants. In regards to this test event, a single participant switched to the COBAS 4800, a new automated platform from Roche. We anticipate that more participants will switch to the newer platform resulting in changes to the viral load peer group for future test events.

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

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Thank you for your participation in the NLHRS Quality Assurance Program

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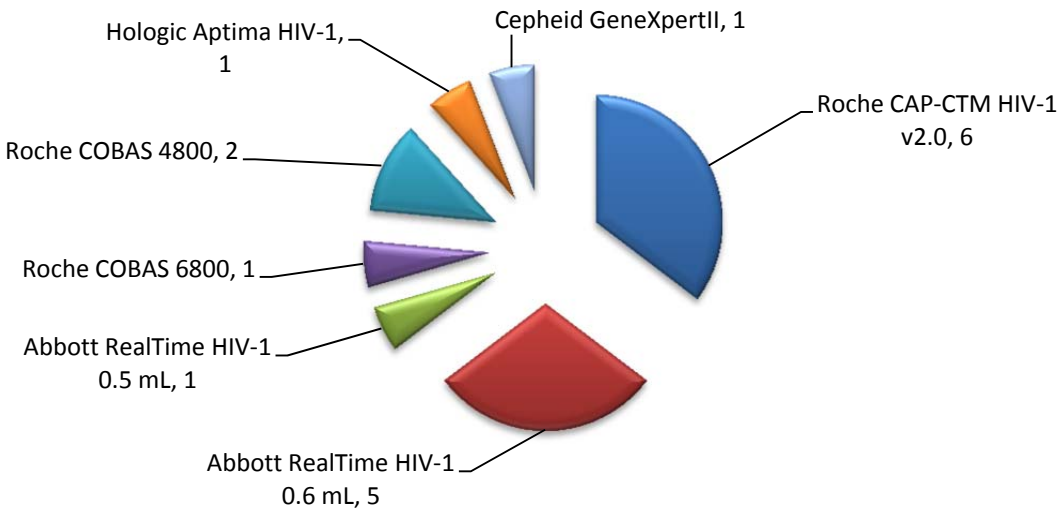
Appendix 1: Summary of the 2019Oct31 viral load results

Table 1: Abbott RealTime HIV-1 Result (0.6 mL)(Log10 HIV RNA cp/mL)		
Sample Code	A/C/E/F	D/H
Mean	2.95	2.00
Minimum	2.80	1.92
Median	2.95	2.00
Maximum	3.04	2.10
% CV	2.13	4.48
SD	0.06	0.09
Inter-lab Variation	1.09	1.09
Measurement of Uncertainty	0.14	N/A

Table 2: Roche CAP/CTM HIV-1 v2.0 Result (Log10 HIV RNA cp/mL)		
Sample Code	A/C/E/F	D/H
Mean	3.01	1.89
Minimum	2.90	1.60
Median	3.03	1.91
Maximum	3.13	2.16
% CV	2.41	8.21
SD	0.07	0.15
Inter-lab Variation	1.08	1.35
Measurement of Uncertainty	0.43	N/A

Appendix 2: Summary of assays used by the participants (includes the NLHRS) in the 2019Oct31 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 (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.