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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 2022Dec16

2022Dec16 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/D/F/G/H	3.02^3, 3.12^4, 3.01^5, 3.04^6	3.10^2, 2.94^3	
N/A	B/C/E	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

There were no incorrect results observed for the 2022Dec16 panel.

Note: The uncertainty of each participant's results for the 2022Dec16 panel is presented in Appendix 1, Table 2

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Final Report for Panel HIVVL 2022Dec16
Issue 2023-March-13

Introduction

The NLHRS distributed the 2022Dec16 and 2023Apr19 panels on November 29th, 2022. This final report is specific to the 2022Dec16 panel only and is publicly available; however, the identity of participants is not disclosed. The deadline for results submission was December 16th, 2022. The preliminary report was issued on January 20, 2023.

Panel Samples, HIV Test Kits, and Data Entry
- *Panel Composition* – The 2022Dec16 panel contained the following:
  - One negative sample sent in triplicate (B, C, E); 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, D, F, G, H) 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).
  - Panels were sent to 19 participants including NLHRS on November 29th, 2022.
  - Metrological tracebility is not applicable to this panel.
  - Uncertainty is applicable to this panel. Each participant's uncertainty for the 2022Dec16 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

- o The homogeneity of the 2022Dec16 HIV-1 viral load panel was assessed by using the Roche cobas 6800 peer group (n=5) in the positive sampled set (A/D/F/G/H). 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.
- o The stability of the 2022Dec16 HIV-1 viral load panel was assessed by comparing the group mean generated by the participants in the 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.

Results

- • *Evaluation Criteria:*
    - o Negative samples: Expected result to be "Target not detected".
    - o 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)**
    - o One outlier was detected and removed from analysis (Grubb's test).
    - o Analysis was not performed for small peer groups of n<=2 (Roche cobas 4800, Roche cobas 8800, Roche CAP/CTM).
    - o Negative samples were analyzed qualitatively.

2. **Group Analysis (Summary Statistics)** (Figure 1, Tables 1 and 2)
    - o The panel sample replicates were combined for the summary statistics (A/D/F/G/H).

    Inter-Lab Variation (Tables 1 and 2)
    - o Difference between the minimum and maximum results for each sample within a peer group (the maximum value divided by the minimum).
        - ▪ 1.11 Log10 cp/mL for the Roche cobas 6800, 1.08 Log10 cp/mL for the Cepheid GeneXpert II, 1.10 Log10 cp/mL for the Abbott RealTime (0.6 mL) and 1.08 Log10 cp/mL for the Hologic Panther peer group
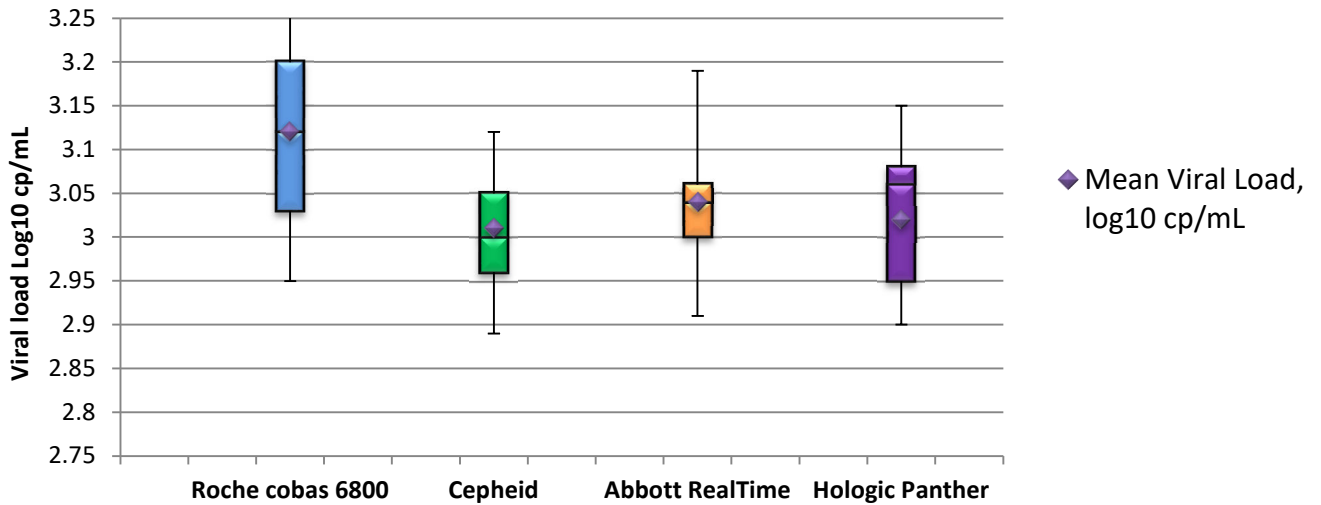
Figure 1: Viral load results for the Roche cobas 6800, Cepheid GeneXpert II, Abbott RealTime and the Hologic Panther peer group.

Reproducibility

o This is an important aspect of viral load testing; required to quantify changes in viral load.
o 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.
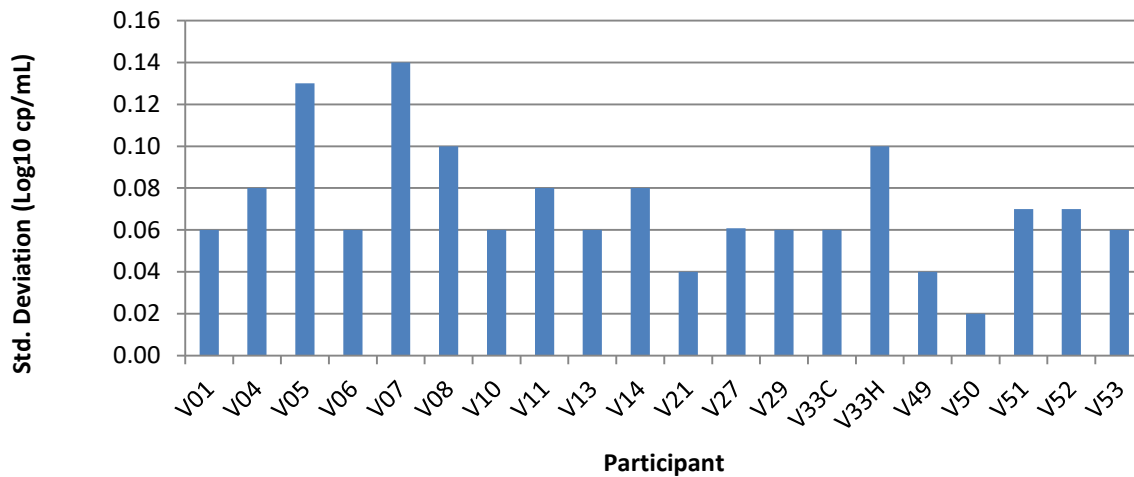

Figure 2: Participants' standard deviation for the sample group A/D/F/G/H.

3. **Comparison Between the Major and Minor Peer Groups** (Figure 3)
   o 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/D/F/G/H were comparable (within ± 0.5 Log10 cp/mL).
   o A proper and fair comparison between the different peer groups would require more users of the platforms within the minor peer group.
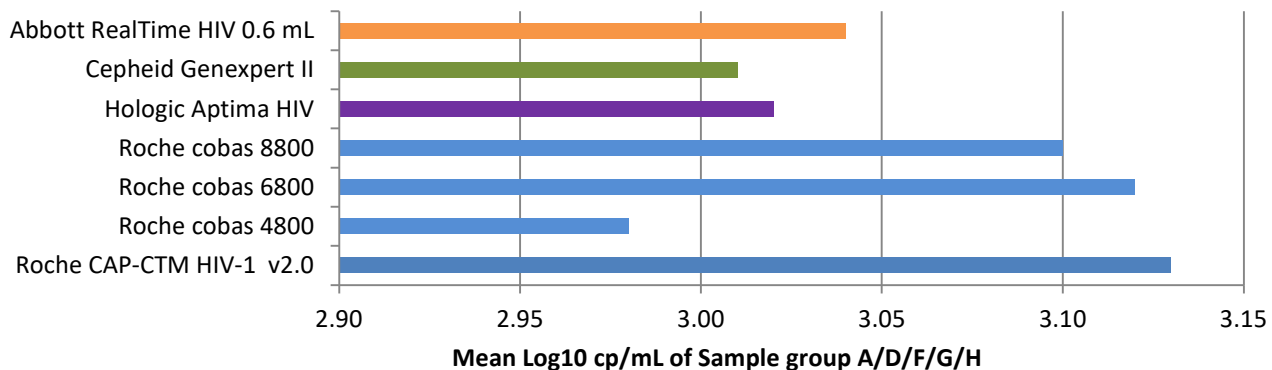

Figure 3: Viral load comparison between the different viral load platforms for sample group A/D/F/G/H.

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

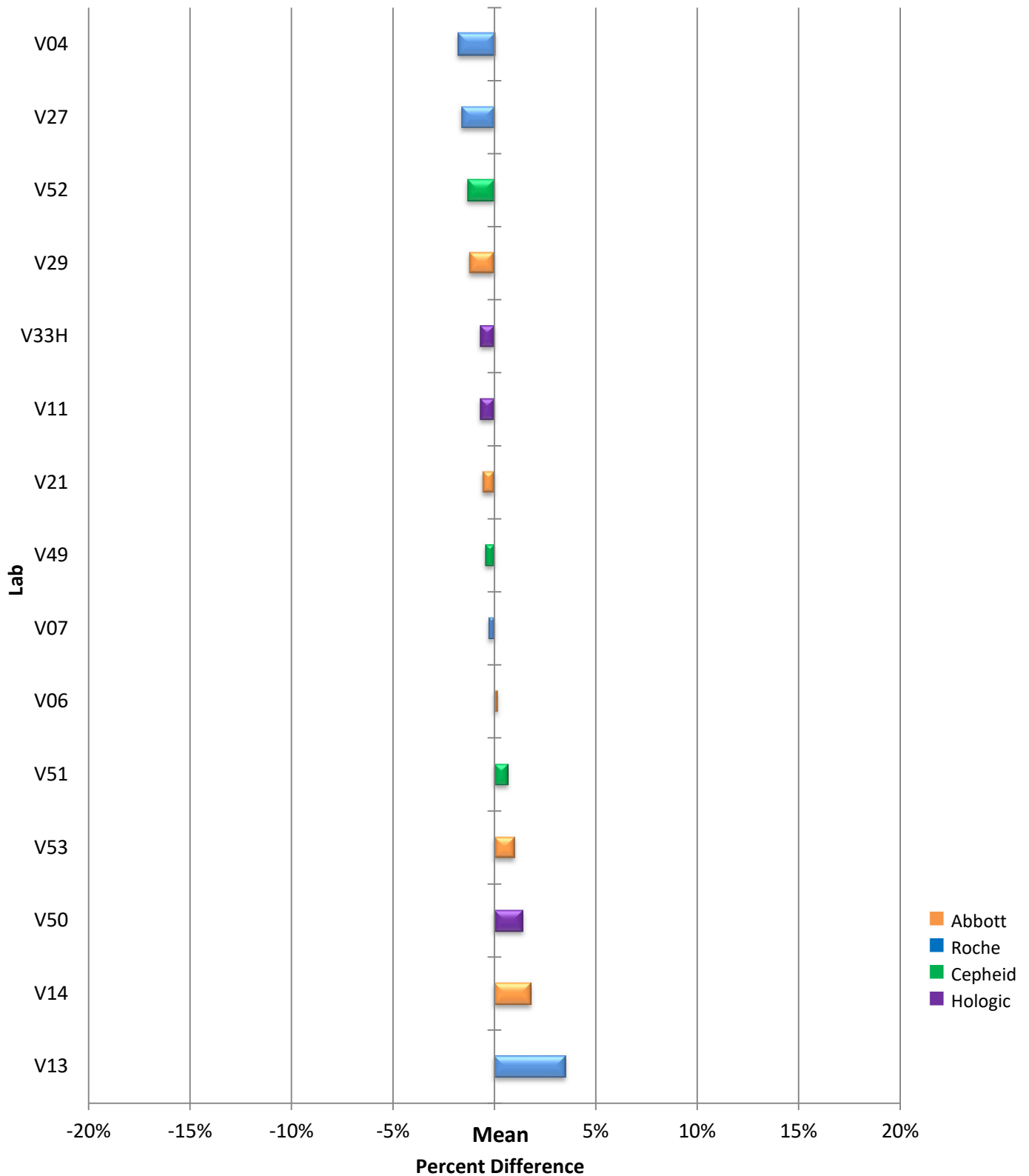## Percent Difference for Samples A/D/F/G/H (Subtype B)

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

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 instrument error for sample B. No other participants encountered this error for that specific sample. The participant may have encountered a random system error. The NLHRS will monitor whether this error re-occurs in the next test event.

Since the last test event in 2021Apr19, we have noticed several participants that originally used the Roche CAP-CTM and the Abbott RealTime System switch to other platforms. In summary, 5 participants switched to other platforms: the Roche cobas 6800 (2), the Roche cobas 8800 (2) and the Hologic Panther (1). We anticipate that more participants will switch to newer platforms 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 2022Dec16 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	3.12	3.01	3.04	3.02
Minimum	2.95	2.89	2.91	2.90
Median	3.12	3.00	3.04	3.05
Maximum	3.27	3.12	3.19	3.14
%CV	3.16	2.12	2.30	2.47
SD	0.10	0.06	0.07	0.07
Inter-lab Variation	1.11	1.08	1.10	1.08

Table 2: The viral load results with the expanded uncertainty at 95% confidence interval for the 2022Dec16 test event (Log10 HIV-1 RNA cp/mL)		
Lab code	**Average Log10 cp/mL**	**Expanded Uncertainty**
V01	3.12	0.13
V04	3.06	0.17
V05	2.98	0.14
V06	3.12	0.14
V07	3.11	0.30
V08	3.13	0.23
V10	3.09	0.14
V11	3.00	0.17
V13	3.23	0.17
V14	3.09	0.18
V21	3.02	0.09
V27	3.07	0.14
V29	3.00	0.13
V33C	3.12	0.14
V33H	3.00	0.22
V49	2.99	0.08
V50	3.06	0.04
V51	3.03	0.16
V52	2.97	0.16
V53	3.04	0.12

Appendix 2: Summary of platforms used by participants (including the NLHRS) in the 2022Dec16 HIV-1 viral load panel.
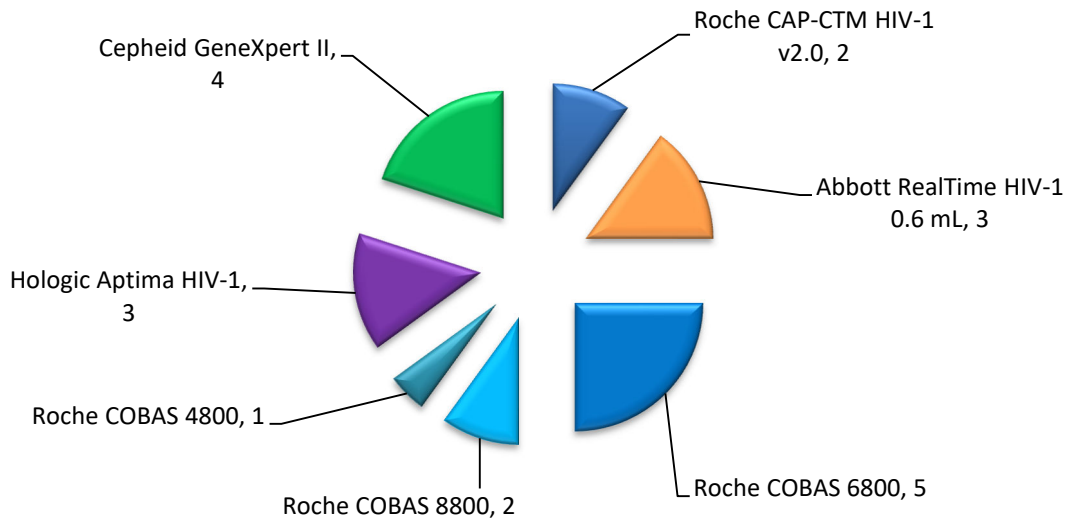
Appendix 3: Troubleshooting

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.