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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 2021Apr19

2021Apr19 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/E/F	3.04[2], 2.90[3]	3.03[2], 2.98[3]	
B	B/G[4]	1.96[2], 2.03[3]	2.06[2], 1.88[3]	
N/A	C/H	TND	TND	**V07**

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.

Summary of findings observed for the 2021Apr19 test event:

1) Participant V07 reported a result of “Detected but Non-Quantifiable” for sample H (expected result: Target Not Detected).

2) Participant V33 did not meet the submission deadline.

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Final Report for Panel HIVVL 2021Apr19

Issued 2021-July-19

Introduction

The NLHRS distributed the 2020Oct30 and 2021Apr19 panels on October 14, 2020. This final report is specific to the 2021Apr19 panel only and is publicly available; however, the identity of participants has not been disclosed. The deadline for results submission was April 19, 2021. The preliminary report was issued on May 5, 2021.

Panel Samples, HIV Test Kits, and Data Entry

- *Panel Composition* – The 2021Apr19 panel contained the following:
  - One negative sample sent in duplicate (C and H); 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, D, 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 (B, G), 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).
  - The samples in the 2021Apr19 panel are the same samples used for previous panels (2019Oct31, 2020Apr17, 2020Oct30).
  - The panel was sent to 17 participants including the NLHRS on October 14, 2020.

- *HIV Viral Load Test Kits* – Seven different assays were used by the participants (excluding 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 2021Apr19 HIV-1 viral load panel was assessed by using the Roche assay peer group (n=5) and the Abbott assay peer group (n=4) results in the positive duplicate sample set (A/D/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 2021Apr19 HIV-1 viral load panel was assessed by comparing to the participant generated group mean for the positive duplicate samples from 2020Oct30. 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)**
   - No outliers were detected in the 2021Apr19 results (Grubb's test).
   - Oultiers were detected and removed (Grubb's test) from the combined results for all test events from 2019-2021 before further analysis in the Roche CAP-CTM and Abbott 0.6 mL peer groups.
   - Since no significant differences ($p > 0.05$) were identified in the duplicate sets (A/D/E/F), the results were combined and analyzed together.
   - Analysis was not performed for small peer groups of $n \leq 2$ (Abbott 0.5 mL, Roche COBAS 6800, Roche COBAS 4800, 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/D/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).
     - 1.23 Log10 cp/mL for the Roche CAP/CTM HIV-1 v2 and 1.15 Log10 cp/mL for the Abbott RealTime (0.6 mL) peer groups.

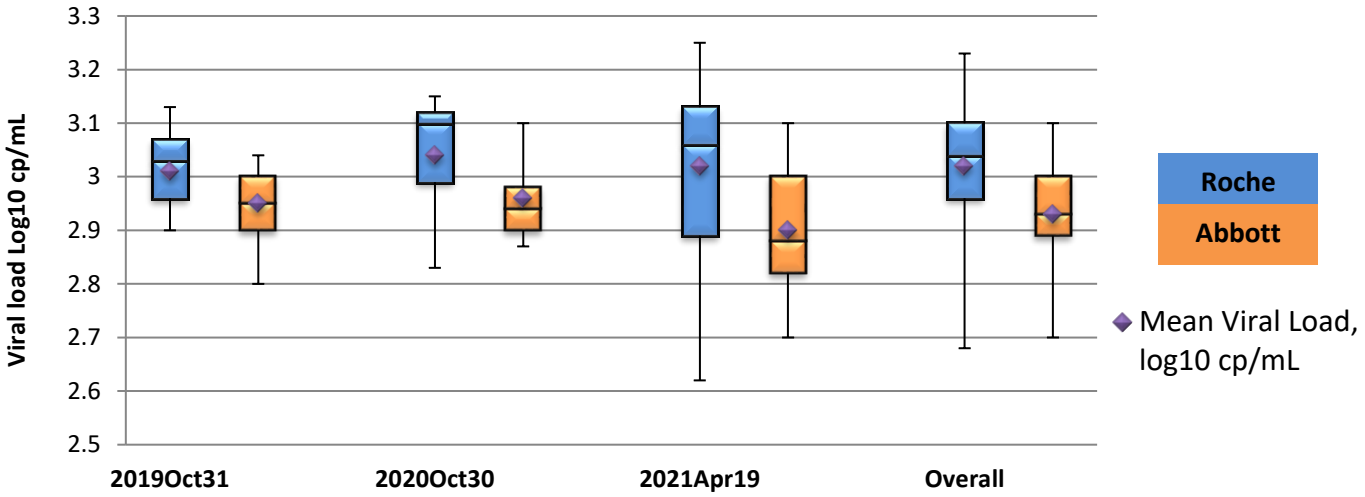

Figure 1: Viral load results from each of the test events from 2019-2021 for the Roche CAP-CTM and the Abbott 0.6 mL peer groups.

Reproducibility

- This is an important aspect of viral load testing; required to quantify changes in viral load.
- To assess intra-assay variability, four replicates of a positive sample was included in the panel. Inter-assay variability was also assessed by combining data points of the four replicates together from the past three test events. The standard deviations are illustrated in Figure 2.

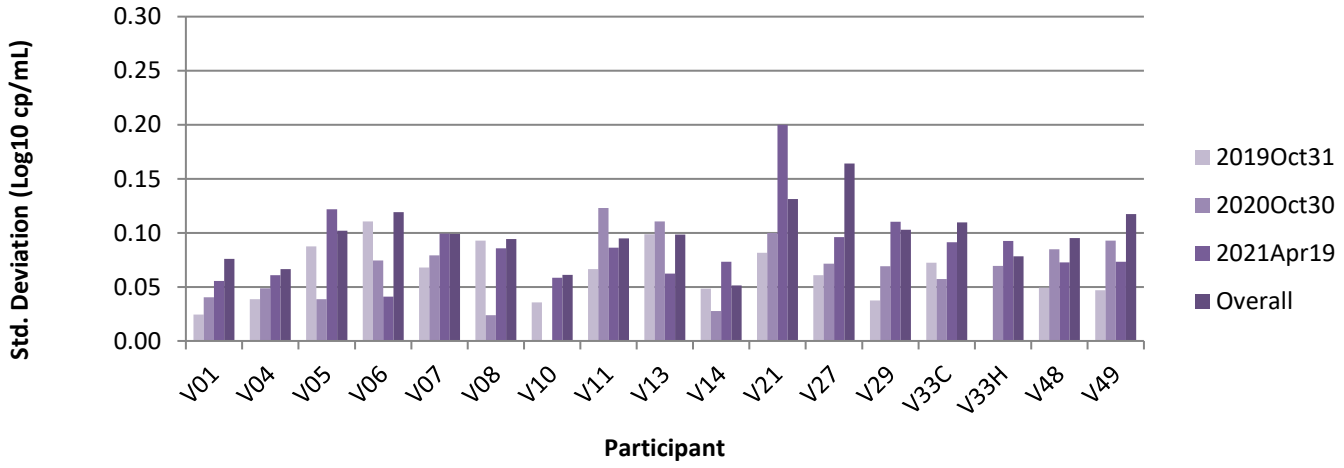

Figure 2: Participants' standard deviations for the four positive replicates from 2019-2021.

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 GeneXpert II, Hologic Aptima HIV-1, Roche COBAS 6800, Roche COBAS 4800, and Abbott 0.5 mL) for the sample group A/D /E/F were comparable (within ± 0.5 Log10 cp/mL).
   - The low number of participants within the minor group may not allow for generalizability.

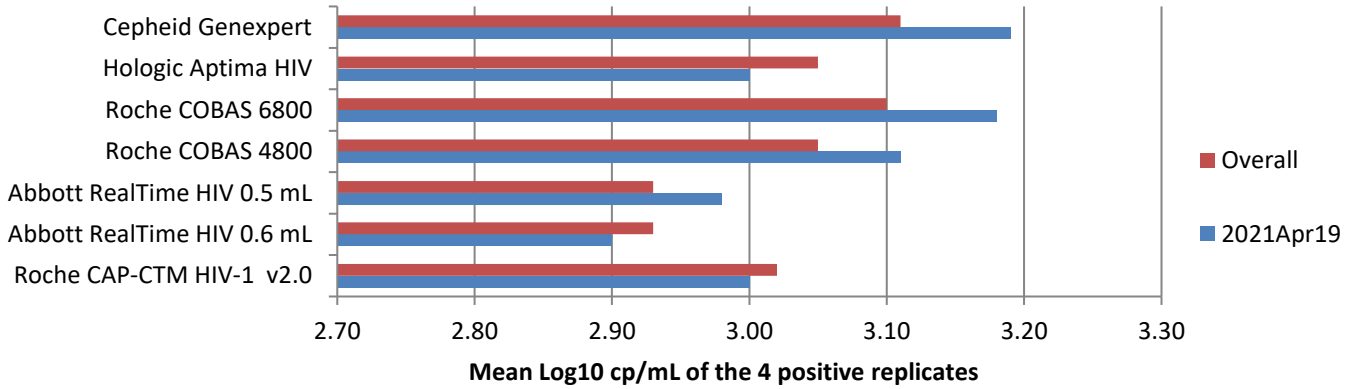

Figure 3: Viral load comparison between the different viral load platforms for the 4 positive replicates.

4. **Challenging Samples** (Figure 4)
   - Sample pair B/G was diluted to a lower Log10 cp/mL than previous panel samples in order to challenge participants with low viral load samples.
   - In total, six challenging samples have been tested by participants from 2019-2021. Figure 4 summarizes the results of the challenging samples submitted by each participant.

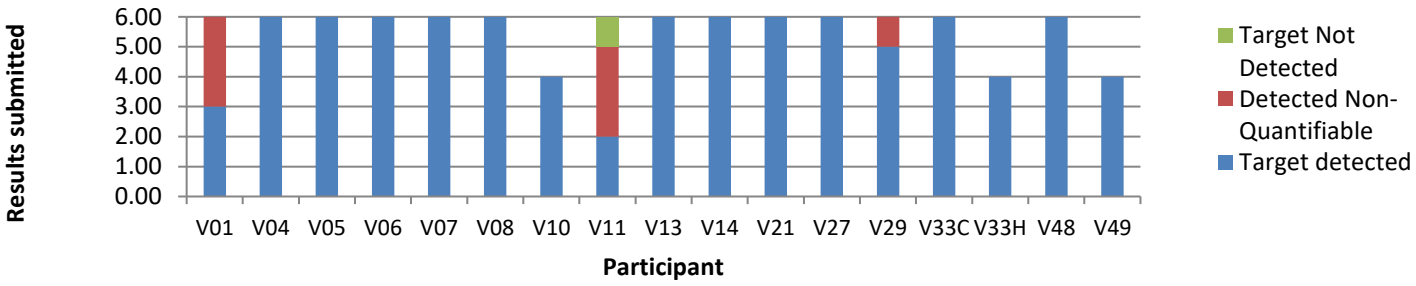

Figure 4: Results submitted by the participants for the challenging samples from 2019-2021.

5. **Individual Analysis (Participant Statistics)** (Figure 5)
   - 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.

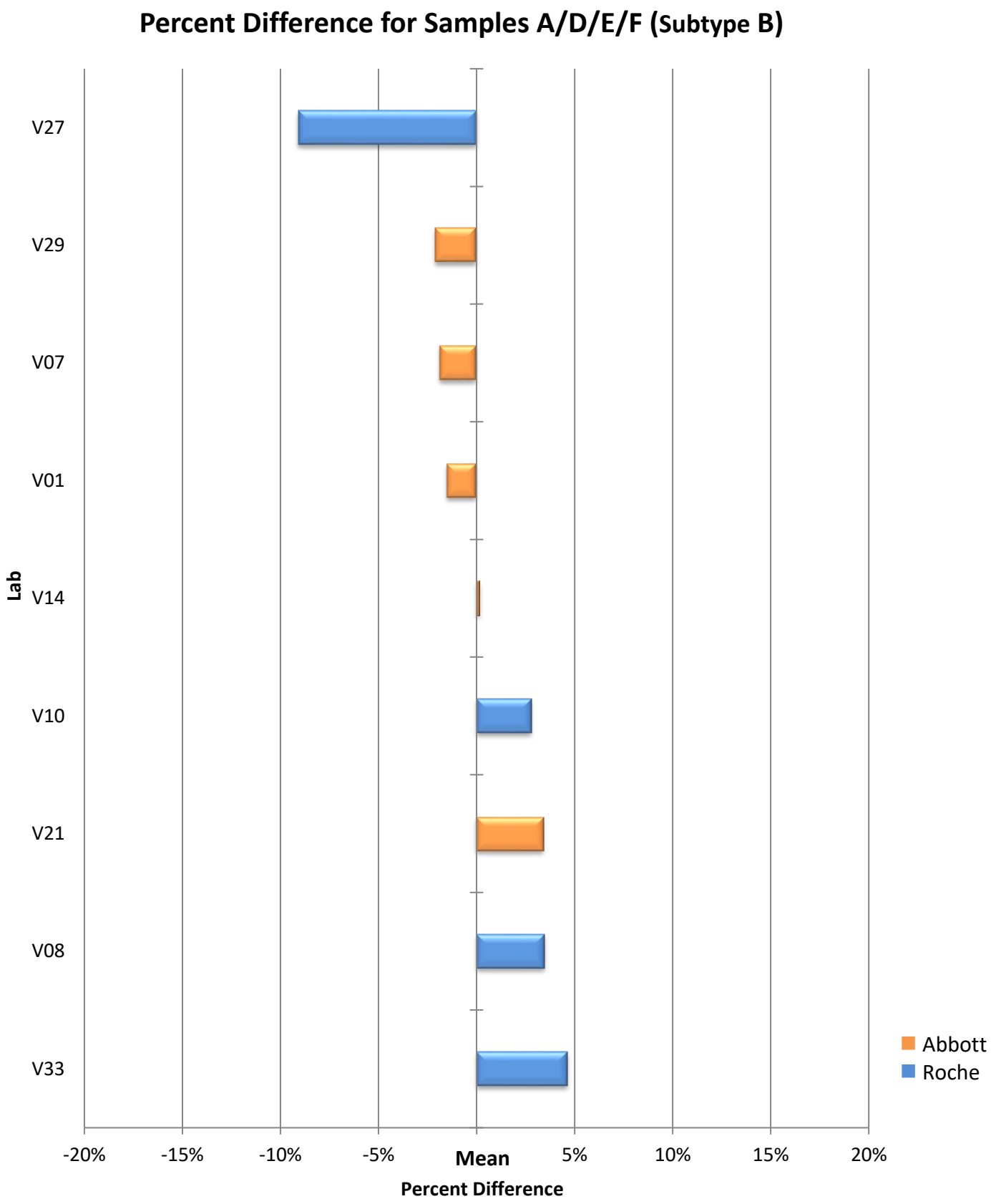

Figure 5: Percent difference from the peer group mean for A/D/E/F.

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 except for one participant. This participant provided a "Detected but non-quantifiable" result on sample H, one of the two negative samples in the panel. Sample contamination is the likely cause of this erroneous result as there was a weak positive sample (sample G) before the negative sample.

The four positive replicates found in each panel from 2019 to 2021 were included in order to assess the precision of viral load results over time. All participants demonstrated good precision as the overall standard deviation for the four replicates ranged from 0.05 to 0.16 log10 cp/mL.

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 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:

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Thank you for your participation in the NLHRS Quality Assurance Program

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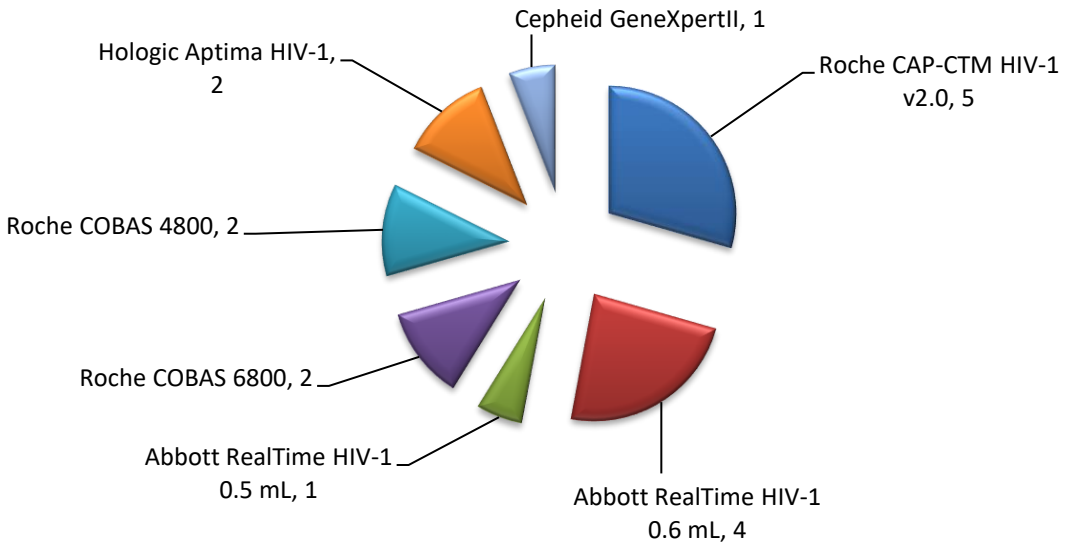
Appendix 1: Summary of the 2021Apr19 viral load results

Table 1: Abbott RealTime HIV-1 Result (0.6 mL)(Log10 HIV RNA cp/mL)		
Sample Code	A/D/E/F	B/G
Mean	2.90	2.02
Minimum	2.70	1.74
Median	2.88	1.86
Maximum	3.10	2.81
% CV	4.39	19.9
SD	0.13	0.40
Inter-lab Variation	1.15	1.61
Measurement of Uncertainty 2019-2021 (n=48)	0.14	N/A

Table 2: Roche CAP/CTM HIV-1 v2.0 Result (Log10 HIV RNA cp/mL)		
Sample Code	A/D/E/F	B/G
Mean	3.00	1.96
Minimum	2.62	1.76
Median	3.05	1.99
Maximum	3.23	2.19
% CV	5.79	7.22
SD	0.17	0.14
Inter-lab Variation	1.23	1.24
Measurement of Uncertainty 2019-2021 (n=56)	0.23	N/A

Appendix 2: Summary of assays used by the participants (including the NLHRS) in the 2021Apr19 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.