JC WILT INFECTIOUS DISEASES RESEARCH CENTRE

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

HIV Serology Quality Assessment Program

Summary for Panel HIVSER 2022Apr19

2022Apr19 HIV Serology Panel		
Panel Sample	**True Status**	**Labs Reporting Incorrect Status**
A	HIV-2 Ab Positive	
B	HIV-1 Ag Positive	**HV21 HV54 HV74**
C	HIV-1/2 Ag/Ab Negative	
D	HIV-1/2 Ag/Ab Negative	
E	HIV-1 Ab Positive	

Summary of findings observed for the 2022Apr19 panel:

1) Participant HV21 did not report a recommendation for Sample B when submitting a final result of “HIV-1/2 Non-Reactive”.

2) Participant HV54 and HV74 failed to provide the correct results for Sample B.

3) Participants HV22, HV28 and HV80 did not provide results for this event.

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Final Report for Panel HIVSER 2022Apr19

Issued 2022-July-14

Introduction

The NLHRS distributed the 2021Oct29 panel and the 2022Apr19 panel on October 20, 2021. This final report is specific to the 2022Apr19 panel only and is publicly available; however, the identity of participants are not disclosed. The deadline for results submission was April 19, 2022. The preliminary report was issued on May 20, 2022.

Panel Samples, HIV Test Kits, and Data Entry

- *Panel Composition:*
  - The 2022Apr19 panel consisted of five samples: two HIV negative (C, D), one HIV-2 Ab positive (A), one HIV-1 Ag positive (B), and one HIV-1 Ab positive (E). Samples A and E were diluted 1 in 2 with defibrinated human plasma (Basematrix 53, Seracare Life Sciences). Testing and characterization of the panel by the NLHRS prior to shipment are presented in Appendix 2. Panels were sent to 43 participants including the NLHRS on October 20, 2021.
  - The metrological traceability and uncertainty is not applicable for this panel.

- *HIV Test Kits*
  - Nine different assays were used by 39 participants (excluding the NLHRS) who returned results (Appendix 3).

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

Homogeneity and stability

- The homogeneity and stability of the 2022Apr19 HIV serology panel was assessed by comparing the participants' results with the panel characterization results obtained by the NLHRS prior to the panel send-out.
- There was no indication of heterogeneity or instability of the panel samples as the data submitted by the participants was consistent with the expected results from the NLHRS characterization of each panel member (Figures 1, 2, and Appendix 2).

Results

- *Evaluation Criteria:*
  - Negative samples: HIV non-reactive/negative in the final HIV serology interpretation with assay results supporting the final serology interpretation.
  - Positive samples: HIV reactive/positive in the final HIV serology interpretation with assay results supporting the final serology interpretation. Participants must provide a recommendation for further action for samples that they could not determine the true serology status for based on the assay used in their testing.

- *Qualitative Group Analysis (Figures 1 and 2)*
  - *Sample A (HIV-2 Ab Positive)* – 39/39 participants provided either a correct serology status and/or recommendation.
  - *Sample B (HIV-1 Ag Positive)* – 36/39 participants provided either a correct serology status and/or recommendation.
  - *Sample C (HIV-1/2 Ag/Ab Negative)* – 39/39 participants provided either a correct serology status and/or recommendation.
  - *Sample D (HIV-1/2 Ag/Ab Negative)* – 39/39 participants provided either a correct serology status and/or recommendation.
  - *Sample E (HIV-1 Ab Positive)* – 39/39 participants provided either a correct serology status and/or recommendation.

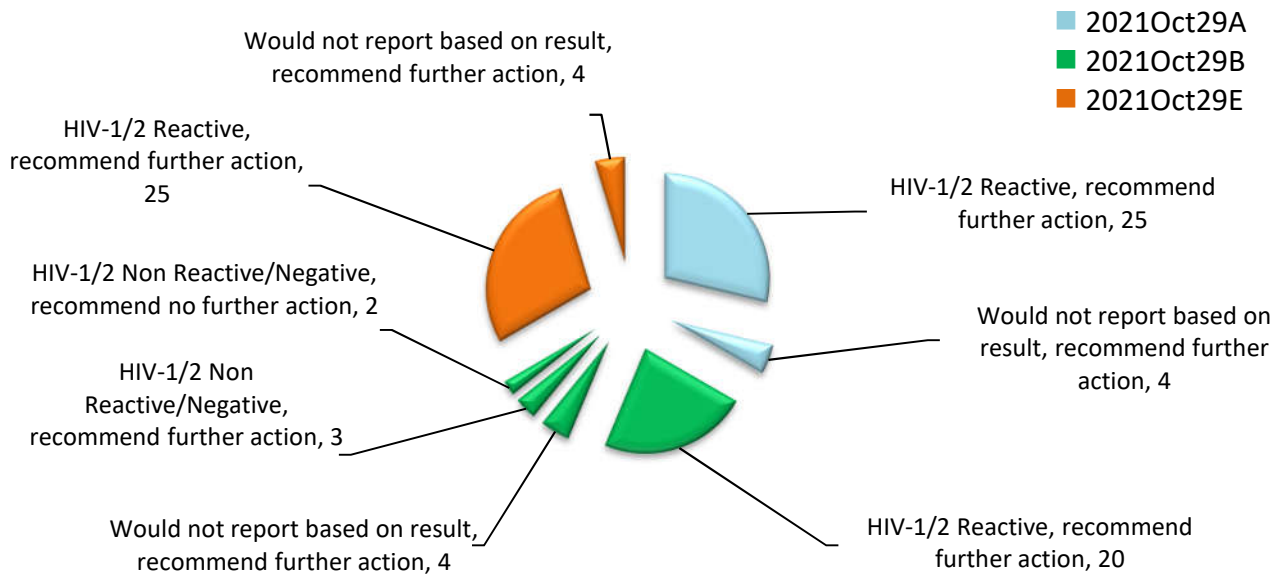
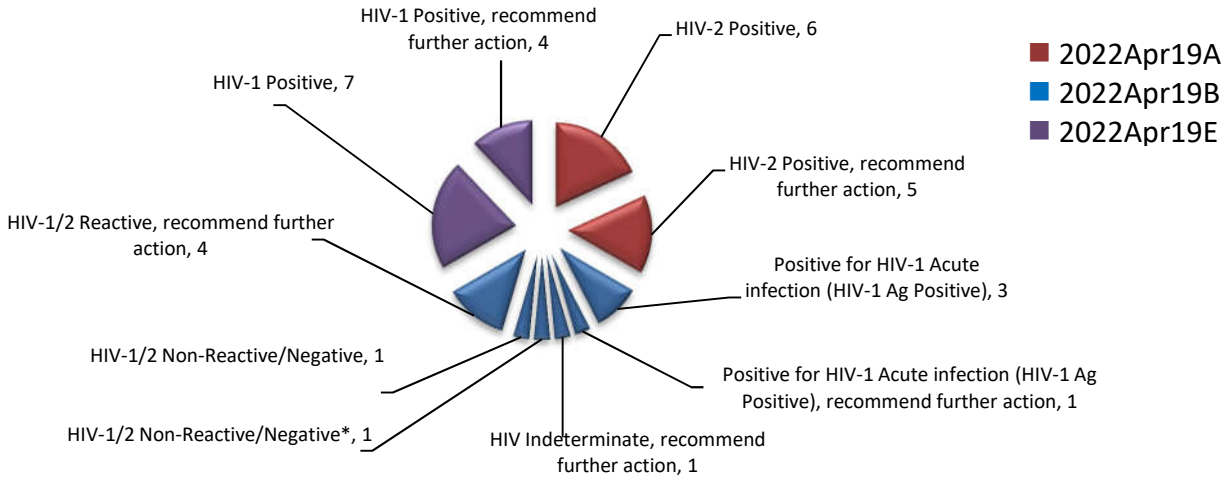


Figure 1: The final HIV serology status of the positive samples in the 2022Apr19 HIV serology panel submitted by participants using an HIV screening assay.



*Waived flag as the participant was only assessed on the confirmatory assay portion of their testing algorithm that used both molecular and serological assays.

Figure 2: The final HIV serology status of the positive samples in the 2022Apr19 HIV serology panel submitted by participants (including NLHRS) using HIV screening and confirmatory assays.

Findings

The majority of participants correctly identified the serology status and/or provided an appropriate recommendation for the panel samples included in the 2022Apr19 test event.

Two participants failed to detect the HIV-1 p24 Ag positive sample in this panel, 2022Apr19B. The material present in 2022Apr19B is the exact material used for the HIV-1 p24 Ag positive sample in previous panels (2019Oct31, 2020Oct30, 2021Apr) both participants tested reactive with the Vitros HIV Combo assay from Ortho Clinical Diagnostics with various kit lots used. In this event both participants used the same kit lot (lot#670). To determine if this was a lot specific issue, 5 HIV-1 p24 Ag positive panel samples were sent to the two participants for re-testing. All 5 panel samples were reactive on the Vitros HIV Combo assay when using a different kit lot but remains non-reactive when using lot#670. This suggests the two failed results for 2022Apr19 B was due to a lot specific issue with lot#670. The NLHRS will be sending the HIV-1 p24 Ag positive material to Ortho Clinical Diagnostics to assist in their investigation for this particular kit lot.

An error was made by one participant during results submission for Sample B. They selected HIV-1 Non-Reactive with no recommendation for a follow-up as it was negative on the confirmatory assay, but reactive in their screening assay. Three participants did return results in this event.

Since the 2020Oct30 test event, we have noticed several of the Abbott Architect users adopting the newer Abbott Alinity platform. We anticipate this trend will continue in the future as more laboratories adopt the newer Abbott platform.

In closing, 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 serology proficiency-testing program in order to provide quality proficiency testing to our participants.

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

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

Thank you for your participation in the NLHRS HIV Serology QA Program

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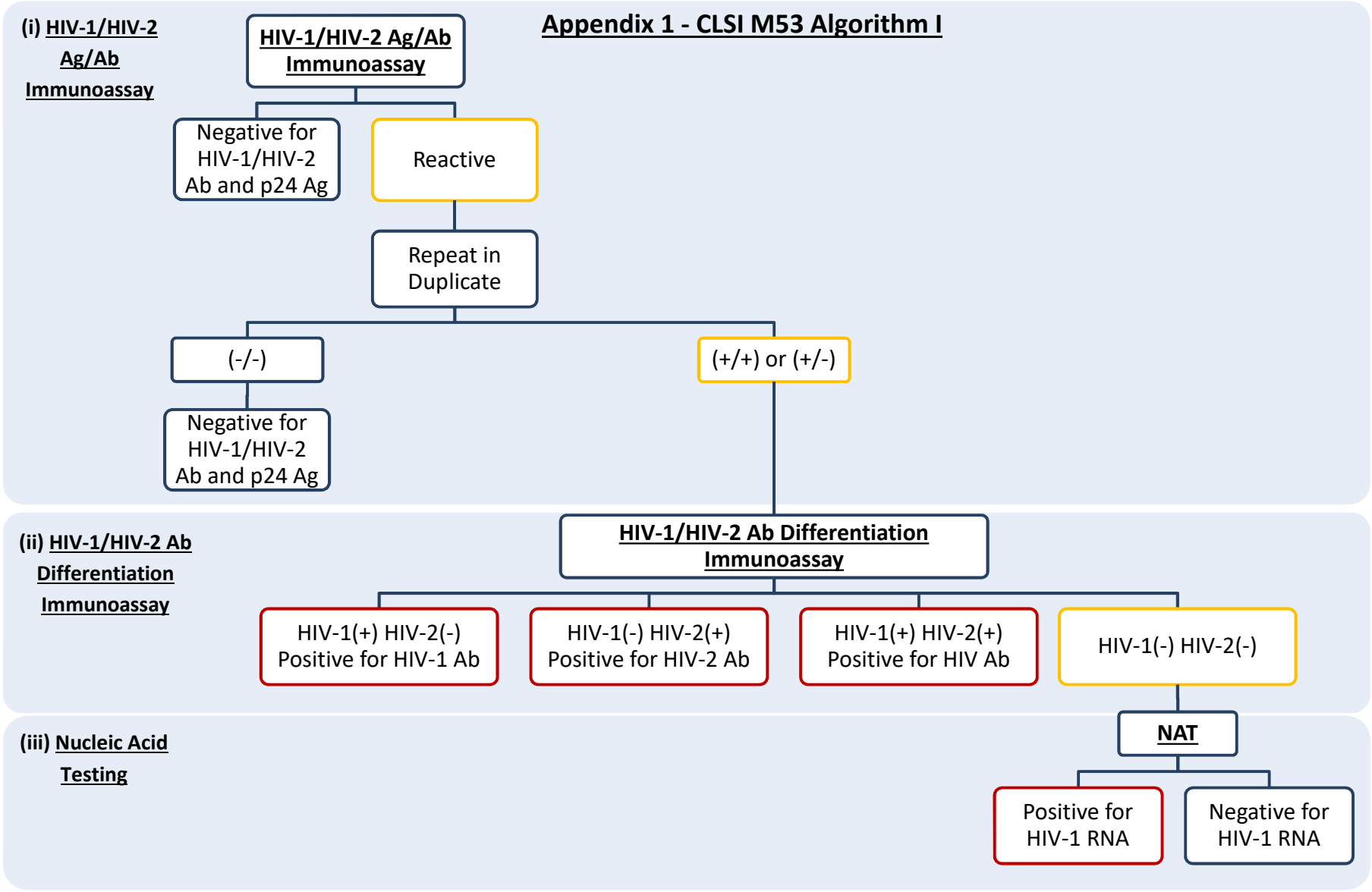
Appendix 1 - CLSI M53 Algorithm I

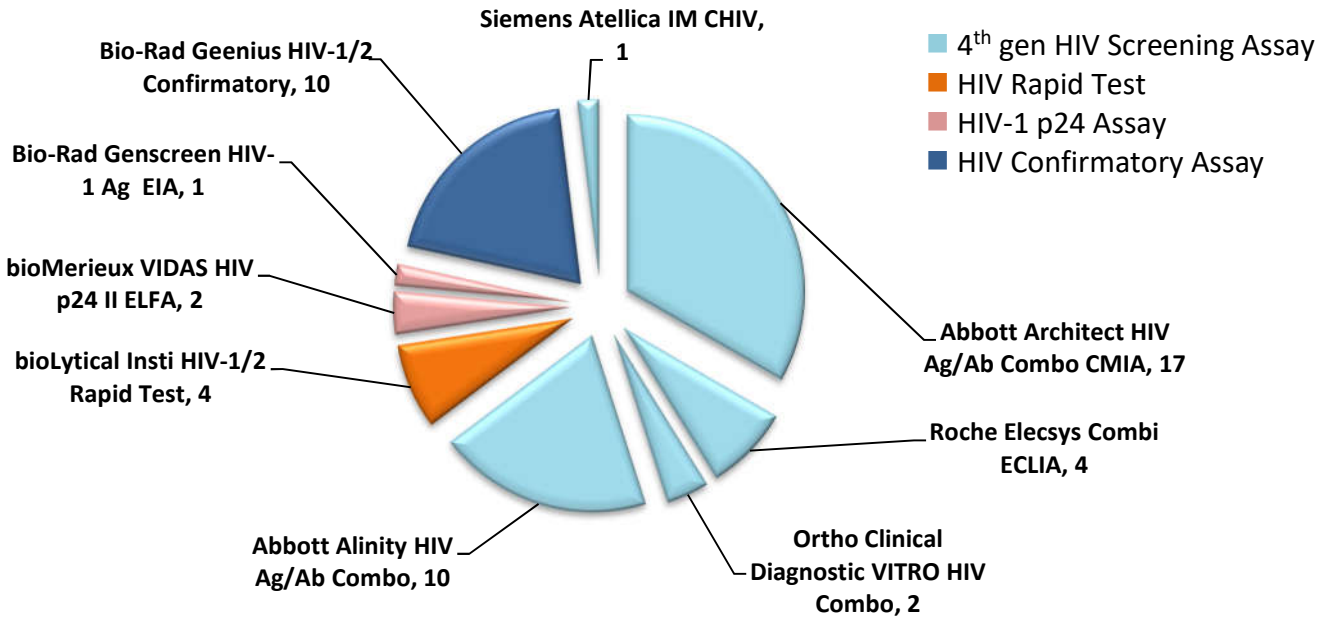


Appendix 1: Adaptation of the Clinical and Laboratory Standards Institute (CLSI) M53-*Criteria for Laboratory Testing and Diagnosis of Human Immunodeficiency Virus Infection: Approved Guideline* Algorithm I.

Appendix 2: Summary of NLHRS characterization of the 2022Apr19 HIV serology panel samples

Sample		C/D (Duplicate)	A	B	E
Final HIV Status		HIV-1/2 Ag/Ab Negative	HIV-2 Ab Positive	HIV-1 Ag Positive	HIV-1 Ab Positive
bioLytical INSTI® HIV-1/2 Rapid Test	**Result**	Non-Reactive	Reactive	Non-Reactive	Reactive
Bio-Rad GS HIV p24	**Result**	Non-Reactive	Non-Reactive	Reactive	Non-Reactive
Bio-Rad GS HIV p24 Confirmatory	**Result**	Not Tested	Not Tested	99.6% Neutralization	Not Tested
Fujirebio INNO-LIA HIV-I/II Score	**Result**	Negative	HIV-2	Negative	HIV-1
	sgp120	-	-	-	++
	gp41	-	-	-	++
	p31	-	++	-	++
	p24	-	+/-	-	+++
	p17	-	-	-	+++
	sgp105	-	+	-	-
	gp36	-	++	-	-
Bio-Rad Geenius HIV-1/HIV-2 Supplemental Assay	**Result**	Negative	HIV-2	Negative	HIV-1
	gp36	-	+	-	-
	gp140	-	+	-	+
	p31	-	+	-	+
	gp160	-	-	-	+
	p24	-	-	-	+
	gp41	-	-	-	+
	CTRL	+	+	+	+

Appendix 3: Summary of assays used by the participants in the 2022Apr19 HIV serology test event



Appendix 4: Summary of bands detected for samples A, B, and E by the Bio-Rad Geenius HIV-1/2 confirmatory assay in the 2022Apr19 HIV serology test event (including NLHRS)

Bio-Rad Geenius	Frequency of Bands Detected						
Sample	gp36	gp140	p31	gp160	p24	gp41	CTRL
2022Apr19A	11	11	11	-	-	-	11
2022Apr19B	-	-	-	-	-	-	11
2022Apr19E	-	2	11	11	11	11	11

Appendix 5: 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.