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 2023Oct31

2023Oct31 HIV Serology Panel		
Panel Sample	**True Status**	**Labs Reporting Incorrect Status**
A	HIV-1/2 Ag/Ab Negative	
B	HIV-1 Ab Positive	
C	HIV-1/2 Ag/Ab Negative	**HV57**
D	HIV-2 Ab Positive	
E	HIV-1 Ab Positive	

Summary of findings observed for the 2023Oct31 panel:

1) Participant HV57 submitted an erroneous result for Sample C as “HIV-1/2 Reactive” (Sample C is HIV-1/2 Ag/Ab negative).

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Final Report for Panel HIVSER 2023Oct31

Issued 2024-01-29

Introduction

The NLHRS distributed the 2023Oct31 panel and the 2024Apr16 panel on October 17, 2023. This final report is specific to the 2023Oct31 panel only and is publicly available; however, the identity of participants are not disclosed. The deadline for results submission was October 31, 2023. The preliminary report was issued on November 20, 2023.

Panel Samples, HIV Test Kits, and Data Entry

- *Panel Composition:*
  - The 2023Oct31 panel consisted of five samples: two HIV negative (A, C), two HIV-1 Ab positive (B, E) and one HIV-2 Ab positive (D). Sample E was 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 is presented in Appendix 2. Panels were sent to 39 participants including the NLHRS on October 17 2023.
  - The metrological traceability and uncertainty is not applicable for this panel.

- *HIV Test Kits*
  - Ten different assays were used by 39 participants (including 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 2023Oct31 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-1/2 Ag/Ab Negative)* – 39/39 participants (including NLHRS) provided either a correct serology status and/or recommendation.
  - *Sample B (HIV-1 Ab Positive)* – 39/39 participants (including NLHRS) provided either a correct serology status and/or recommendation.
  - *Sample C (HIV-1/2 Ag/Ab Negative)* – 38/39 participants (including NLHRS) provided either a correct serology status and/or recommendation.
  - *Sample D (HIV-2 Ab Positive)* – 39/39 (including NLHRS) participants provided either a correct serology status and/or recommendation.
  - *Sample E (HIV-1 Ab Positive)* – 39/39 (including NLHRS) participants provided either a correct serology status and/or recommendation.

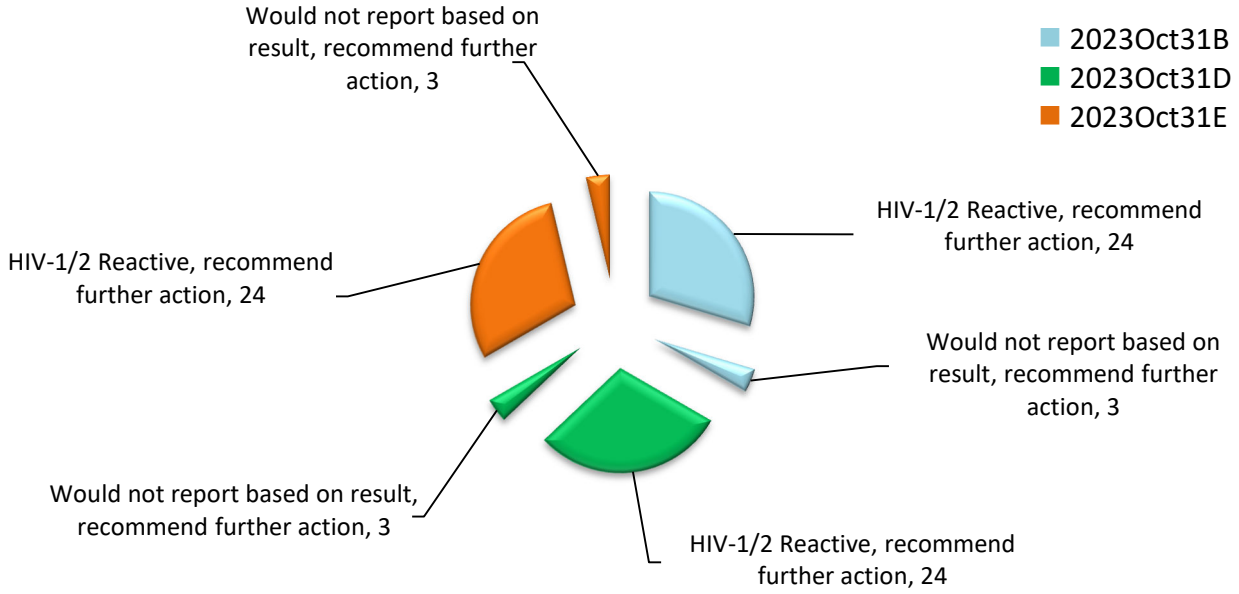

Figure 1: The final HIV serology status of the positive samples in the 2023Oct31 HIV serology panel submitted by participants using an HIV screening assay.

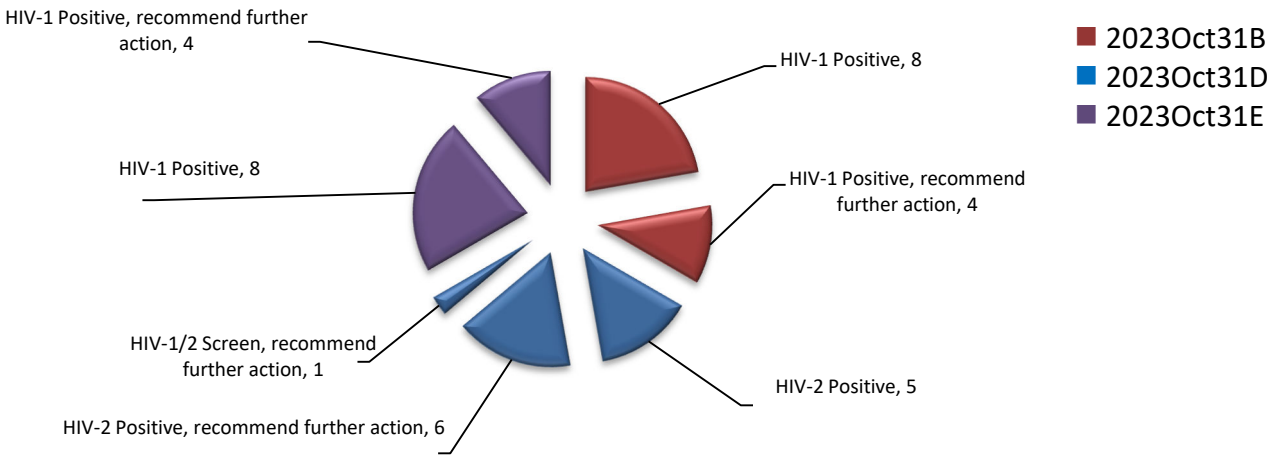

Figure 2: The final HIV serology status of the positive samples in the 2023Oct31 HIV serology panel submitted by participants (including NLHRS) using HIV screening and confirmatory assays.

Findings

One participant returned an incorrect result for Sample C as they have tested Sample C as "HIV-1/2 Reactive" on the Abbott Architect but Sample A was characterized as "HIV-1/2 Non-Reactive". Sample A and C are the negative duplicate samples present in the 2023Oct31 HIV serology panel (See Appendix 2). This prompted an investigation to determine the cause of this erroneous result.

Several retention aliquots of Sample A and C were tested in-house with the Abbott Alinity, and were non-reactive. Two sets of the 2023Oct31 panel were sent to this participant to be tested again to see if the false reactivity re-occurred on Sample C. The new set of the 2023Oct31 panel were tested several times by the participant in sequential order. Sample C was tested as "HIV-1/2 reactive" but Sample A remained "HIV-1/2 Non Reactive". Based on the results gathered so far, it is likely Sample C was cross-contaminated with either of the positive samples (Sample B, D, E) in the panel during the pre-analytical stage. This is supported by the consistent non-reactive results on Sample A. A new set of panel samples were sent to the participant for further troubleshooting.

In this event, we did not observe any increase in the number of users of the Abbott Alinity platform. We will continue to monitor if the existing Abbott Architect users will switch over to the Alinity 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:

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

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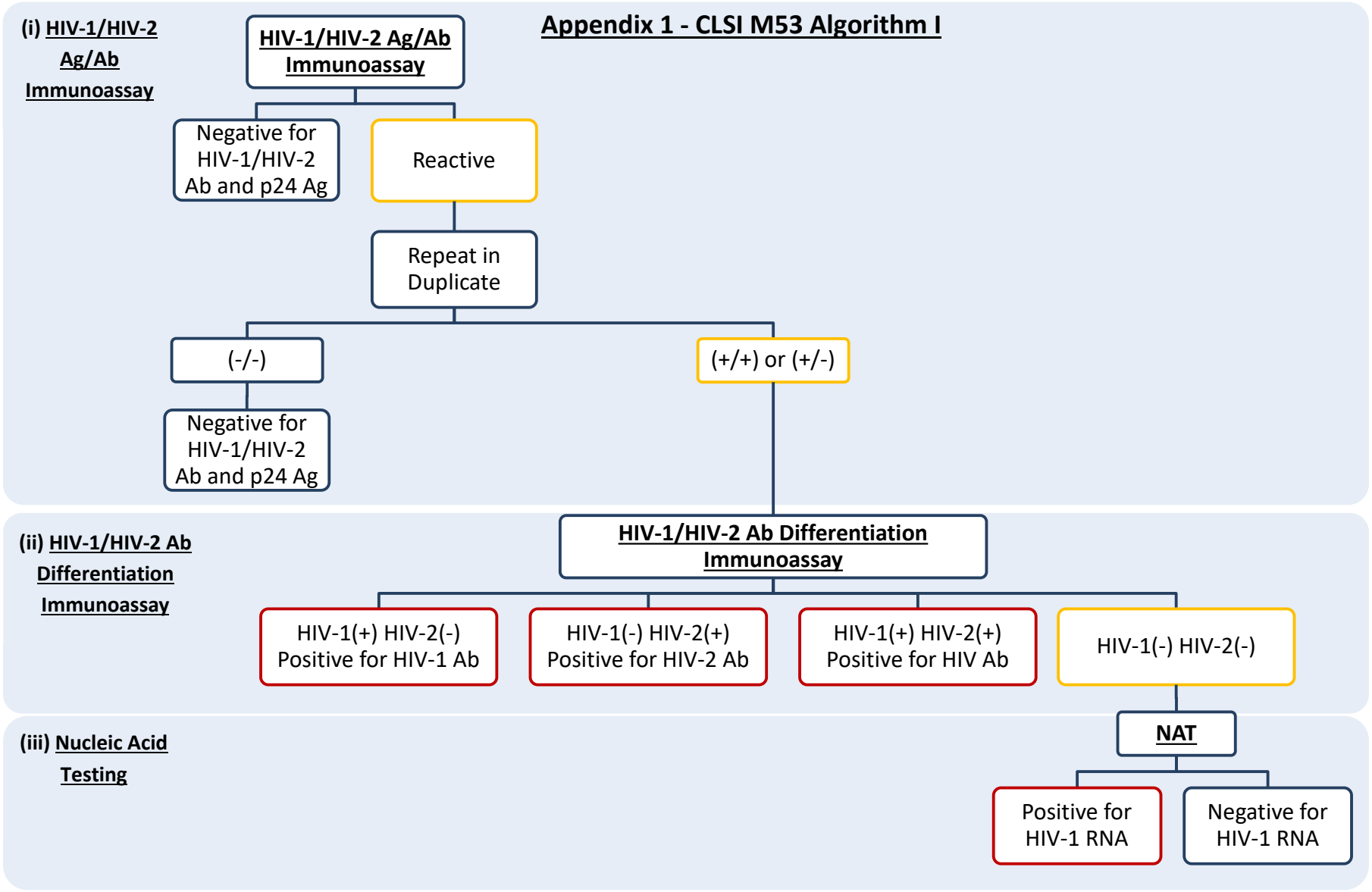
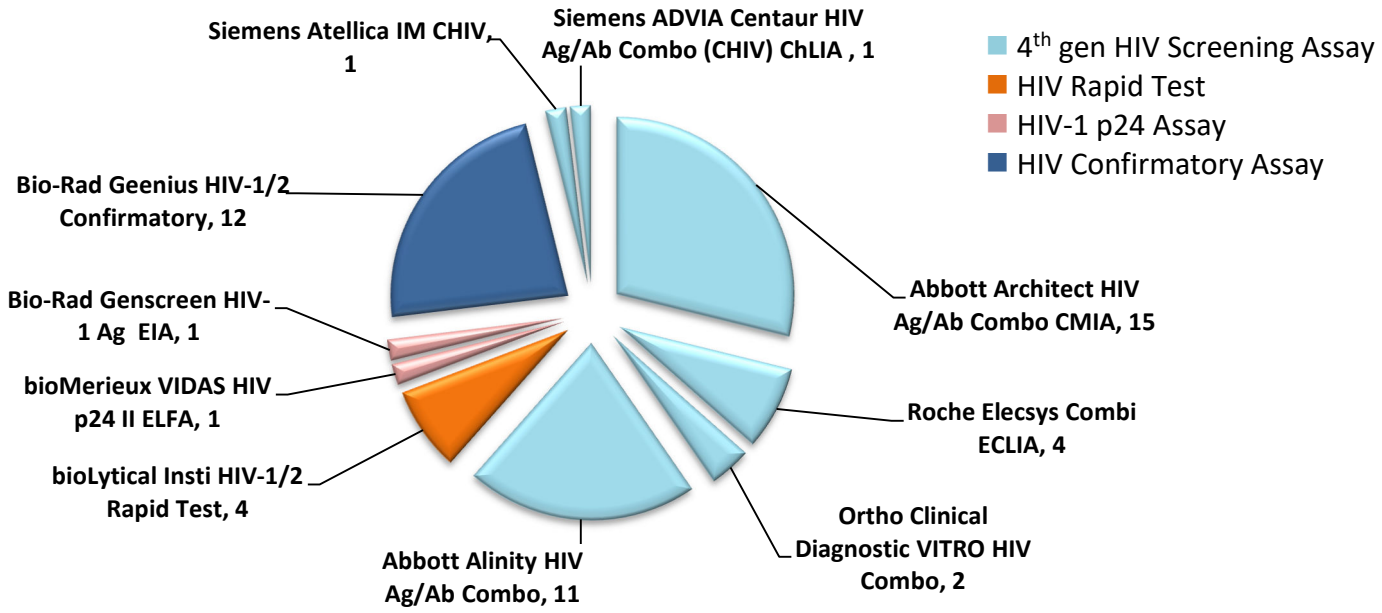


Appendix 1: Adaptation of the Clinical and Laboratory Standards Institute (CLSI) M53-*Criteria for Laboratory Testing and Diagnosis of Human Immunodeficiency Virus Infection: Approved Guideline* Algorithm I.

Appendix 2: Summary of NLHRS characterization of the 2023Oct31 HIV serology panel samples

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

Appendix 3: Summary of assays used by participants in the 2023Oct31 HIV serology test event



Appendix 4: Summary of bands detected for samples B, D, and E by the Bio-Rad Geenius HIV-1/2 confirmatory assay in the 2023Oct31 HIV serology test event (including NLHRS)

Bio-Rad Geenius	Frequency of Bands Detected						
Sample	gp36	gp140	p31	gp160	p24	gp41	CTRL
2023Oct31B	-	-	5	12	9	12	12
2023Oct31D	12	12	12	-	-	6	12
2023Oct31E	-	-	12	12	12	12	12

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.