JC WILT INFECTIOUS DISEASES RESEARCH CENTRE

National Laboratory for HIV Reference Services
National HIV and Retrovirology Laboratories
National Microbiology Laboratory
Public Health Agency of Canada

HIV Serology Quality Assessment Program

Summary for Panel HIVSER 2021Oct29

2021Oct29 HIV Serology Panel		
Panel Sample	**True Status**	**Labs Reporting Incorrect Status**
A	HIV-1/2 Ag/Ab Negative	**HV79**
B	HIV-1 Ab Positive	**HV30**
C	HIV-1 Ag Positive	**HV79**
D	HIV-1/2 Ag/Ab Negative	**HV57 HV79**
E	HIV-2 Ab Positive	**HV30**

Summary of findings observed for the 2021Oct29 panel:

1) Participant HV57 incorrectly selected “HIV-1/2 Reactive” for Sample D

2) Participant HV79 incorrectly selected “HIV-1/2 Reactive” for Sample A, D and C (non-reactive result using only the bioLytical HIV-1/2 INSTI Rapid test)

3) Participant HV30 incorrectly selected “HIV-1 Positive” for Sample B and Sample E (reactive result using only the bioLytical HIV-1/2 INSTI Rapid test)

4) Participant HV80 was not able to return results by the submission due date

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Final Report for Panel HIVSER 2021Oct29

Issued 2021-December-02

Introduction

The NLHRS distributed the 2021Oct29 panel and the 2022Apr19 panel on October 20, 2021. This final report is specific to the 2021Oct29 panel only and is publicly available; however, the identity of participant is not disclosed. The deadline for results submission was November 5, 2021. The preliminary report was issued on November 22, 2021.

Panel Samples, HIV Test Kits, and Data Entry

- *Panel Composition:*
  - The 2021Oct29 panel consisted of five samples: two HIV negative (A, D), one HIV-1 Ab positive (B), one HIV-1 Ag positive (C), and one HIV-2 Ab positive (E). Samples B 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.

- *HIV Test Kits*
  - Ten different assays were used by 41 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 2021Oct29 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)* – 40/41 participants provided either a correct serology status and/or recommendation.
  - *Sample B (HIV-1 Ab Positive)* – 40/41 participants provided either a correct serology status and/or recommendation.
  - *Sample C (HIV-1 Ag Positive)* – 40/41 participants provided either a correct serology status and/or recommendation.
  - *Sample D (HIV-1/2 Ag/Ab Negative)* – 39/41 participants provided either a correct serology status and/or recommendation.
  - *Sample E (HIV-2 Ab Positive)* – 40/41 participants provided either a correct serology status and/or recommendation.

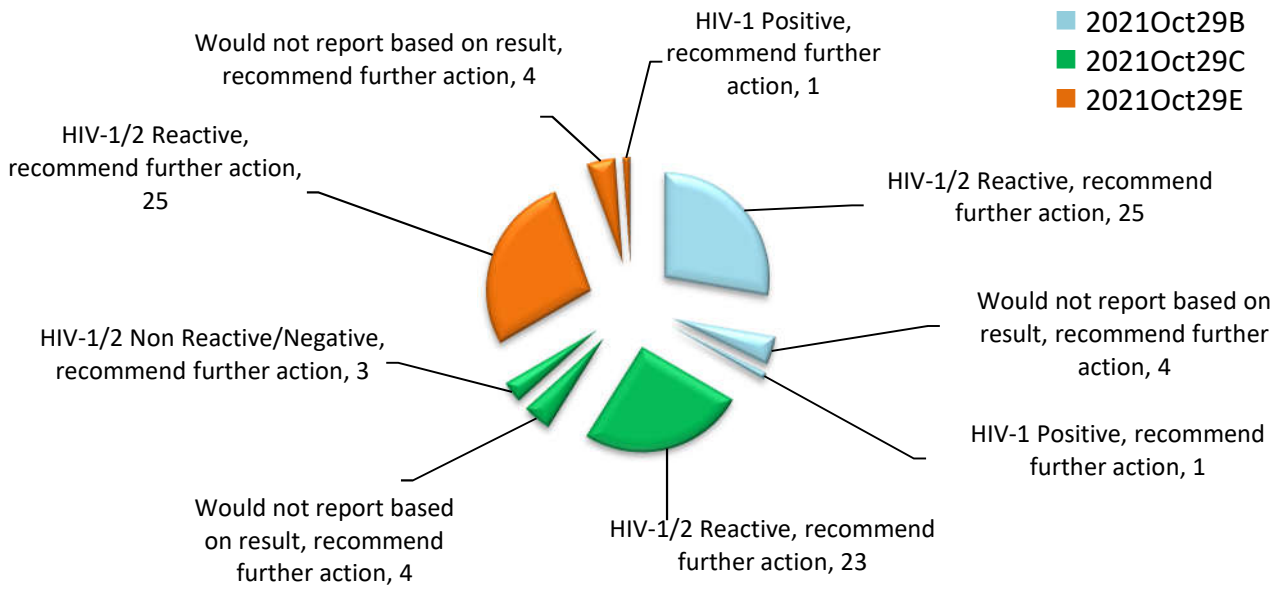
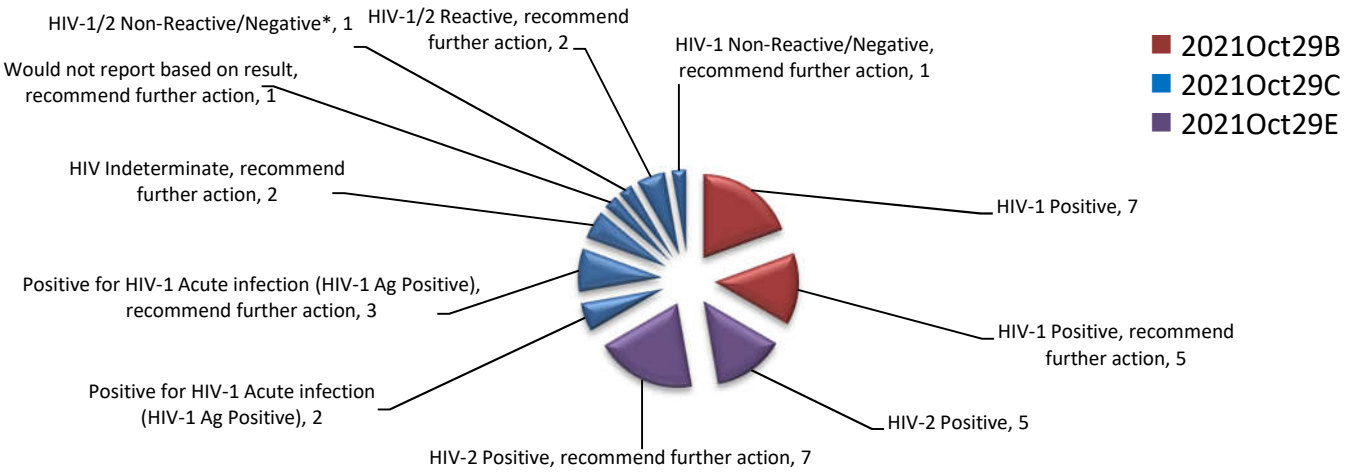


Figure 1: The final HIV serology status of the positive samples in the 2021Oct29 HIV serology panel submitted by participants using an HIV screening assay.



*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 2021Oct29 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 2021Oct29 test event.

Two users of the bioLytical HIV-1/2 INSTI® Rapid Test made an error when selecting the final interpretation. One participant selected "HIV-1/2 Reactive" for Sample A, C, and D even though they have provided the expected assay results with the bioLytical HIV-1/2 INSTI® Rapid Test (Appendix 2). Because the bioLytical HIV-1/2 INSTI® Rapid Test is not a HIV confirmatory assay, it is incorrect to select "HIV-1 Positive" for the reactive samples in the panel as the other participant have done in this test event

One participant made an error during results submission for Sample D when they accidentally selected "HIV-1/2 Reactive" even though the sample was non-reactive on their assay. One participant was not able to return results by the submission due date.

Since the 2020Oct30 test event, we have noticed some of the Abbott Architect users have switched over to the Abbott Alinity platform. We anticipate this trend will continue in the future as more laboratory make the switch to 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:

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

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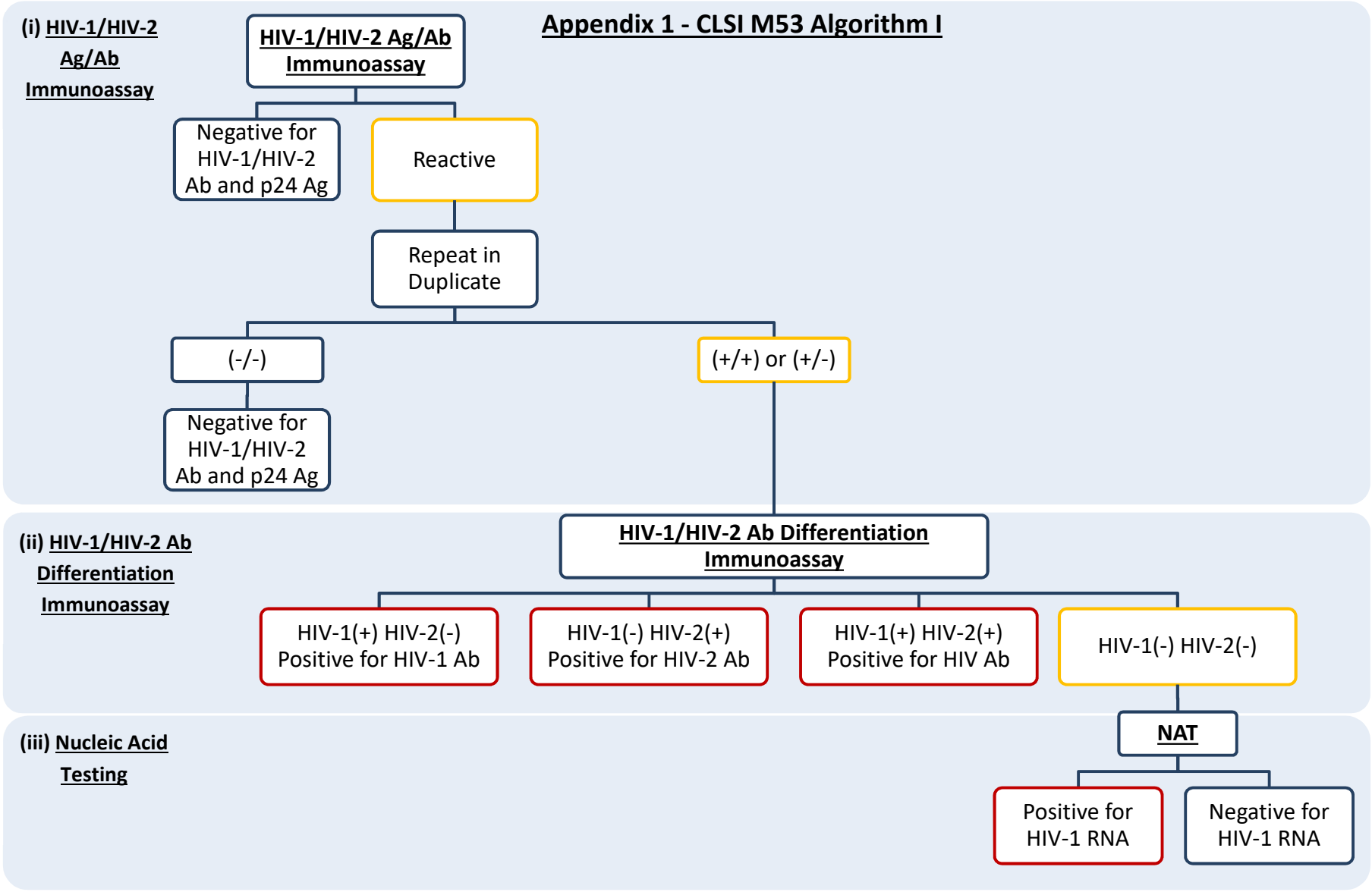
Appendix 1 - CLSI M53 Algorithm I

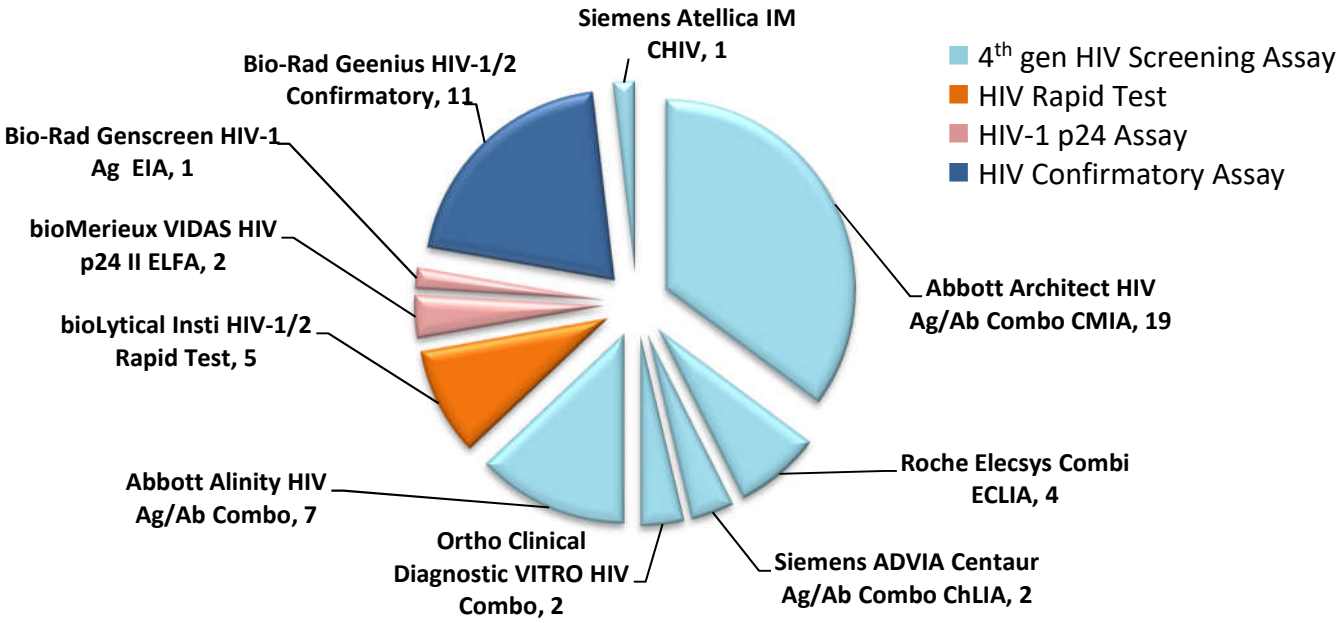


Appendix 1: Adaptation of the Clinical and Laboratory Standards Institute (CLSI) M53-*Criteria for Laboratory Testing and Diagnosis of Human Immunodeficiency Virus Infection: Approved Guideline* Algorithm I.

Appendix 2: Summary of NLHRS characterization of the 2021Oct29 HIV serology panel samples

Sample		A/D (Duplicate)	B	C	E
Final HIV Status		HIV-1/2 Ag/Ab Negative	HIV-1 Ab Positive	HIV-1 Ag Positive	HIV-2 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-1	Negative	HIV-2
	sgp120	-	++	-	-
	gp41	-	++	-	-
	p31	-	++	-	++
	p24	-	+++	-	+/-
	p17	-	+++	-	-
	sgp105	-	-	-	+
	gp36	-	-	-	++
Bio-Rad Geenius HIV-1/HIV-2 Supplemental Assay	**Result**	Negative	HIV-1	Negative	HIV-2
	gp36	-	-	-	+
	gp140	-	+	-	+
	p31	-	+	-	+
	gp160	-	+	-	-
	p24	-	+	-	-
	gp41	-	+	-	-
	CTRL	+	+	+	+

Appendix 3: Summary of assays used by the participants in the 2021Oct29 HIV serology test event



Appendix 4: Summary of bands detected for samples B, C, and E by the Bio-Rad Geenius HIV-1/2 confirmatory assay in the 2021Oct29 HIV serology test event

Bio-Rad Geenius	Frequency of Bands Detected						
Sample	gp36	gp140	p31	gp160	p24	gp41	CTRL
2021Oct29B	-	4	12	12	12	12	12
2021Oct29C	-	-	-	-	-	-	12
2021Oct29E	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.