



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 2020Oct30

2020Oct30 HIV Serology Panel		
Panel Sample	True Status	Labs Reporting Incorrect Status
A	HIV-2 Ab Positive	HV16
B	HIV-1/2 Ag/Ab Negative	HV16
C	HIV-1 Ag Positive	
D	HIV-1 Ab Positive	HV04 HV05 HV16 HV79
E	HIV-1/2 Ag/Ab Negative	HV03 HV04 HV05 HV16 HV79

There were several erroneous results observed for the 2020Oct30 panel:

- 1) One Participant submitted “Not tested” as the final result for samples A, B, D, E despite completing all the testing
- 2) Three Participants have Sample D and Sample E mixed up in the pre-testing and/or testing stage which resulted in submitting the wrong final results for Sample D and E
- 3) One Participant submitted “HIV-1 Positive” as the result for Sample E despite a non-reactive result from their HIV screening assay.



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## **HIV Serology Quality Assessment Program**

### **Final Report for Panel HIVSER 2020Oct30**

*Issued 2021-March-05*

#### **Introduction**

The NLHRS distributed the 2020Oct30 panel and the 2021Apr19 panel on Oct 14<sup>th</sup> 2020. This final report is specific to the 2020Oct30 panel only and is publicly available; however the identity of participants is not disclosed. The deadline for results submission was October 30<sup>st</sup>, 2020. The preliminary report was issued on November 24, 2020.

#### **Panel Samples, HIV Test Kits and Data Entry**

- *Panel Composition:*
  - 2020Oct30 panel consists of five samples; two HIV negative (B, E), one HIV-2 Ab positive (A), one HIV-1 Ag positive (C) and one HIV-1 Ab positive (D). Sample A and D 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 (Appendix 2). Panels were sent to 41 participants and to the NLHRS on Oct 14<sup>th</sup>, 2020.
- *HIV Test Kits* –Nine different assays were used by the 41 participants (excluding the NLHRS) who returned results (Appendix 3).
- *Data entry* –Results entry for this panel utilized an in-house developed website.

#### **Homogeneity and stability**

- The homogeneity and stability of the 2020Oct30 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 is 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. Expect the participants to provide a recommendation for further action on samples that they could not determine the true serology status based on the assay used in their testing.
  
- *Qualitative Group Analysis (Figure 1 and 2)*
  - *Sample A (HIV-2 Ab Positive)* – 40/41 participants provided either a correct serology status and/or recommendation.
  - *Sample B (HIV-1/2 Ag/Ab Negative)* – 40/41 participants provided either a correct serology status and/or recommendation.
  - *Sample C (HIV-1 Ag Positive)* – 41/41 participants provided either a correct serology status and/or recommendation.
  - *Sample D (HIV-1 Ab Positive)* – 37/41 participants provided either a correct serology status and/or recommendation
  - *Sample E (HIV-1/2 Ag/Ab Negative)* – 36/41 participants provided either a correct serology status and or recommendation

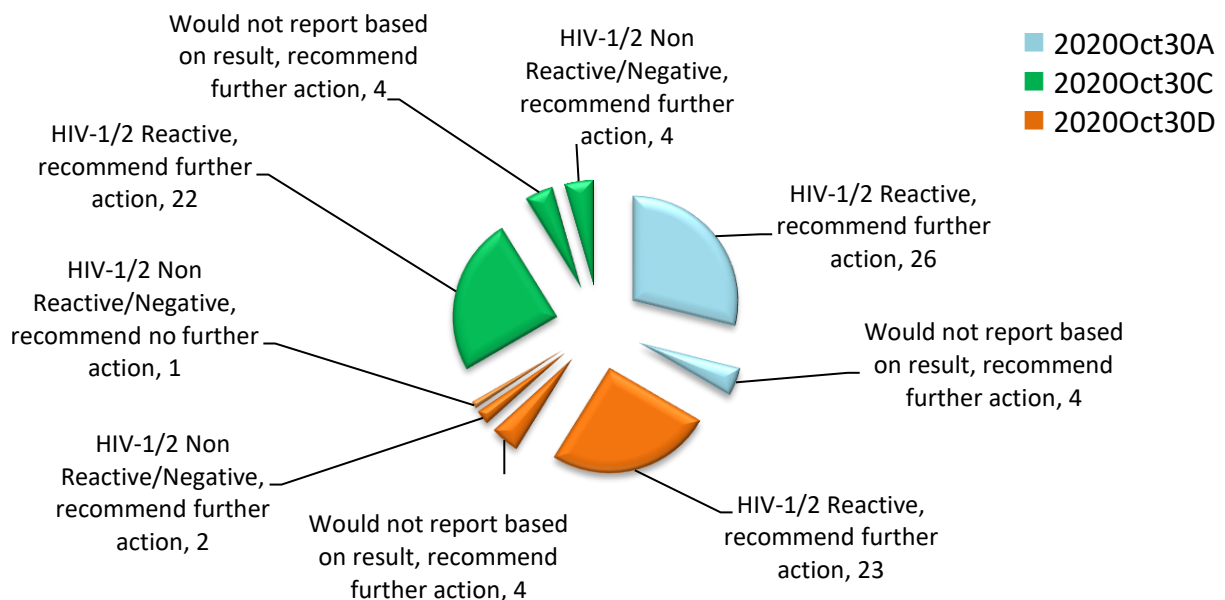
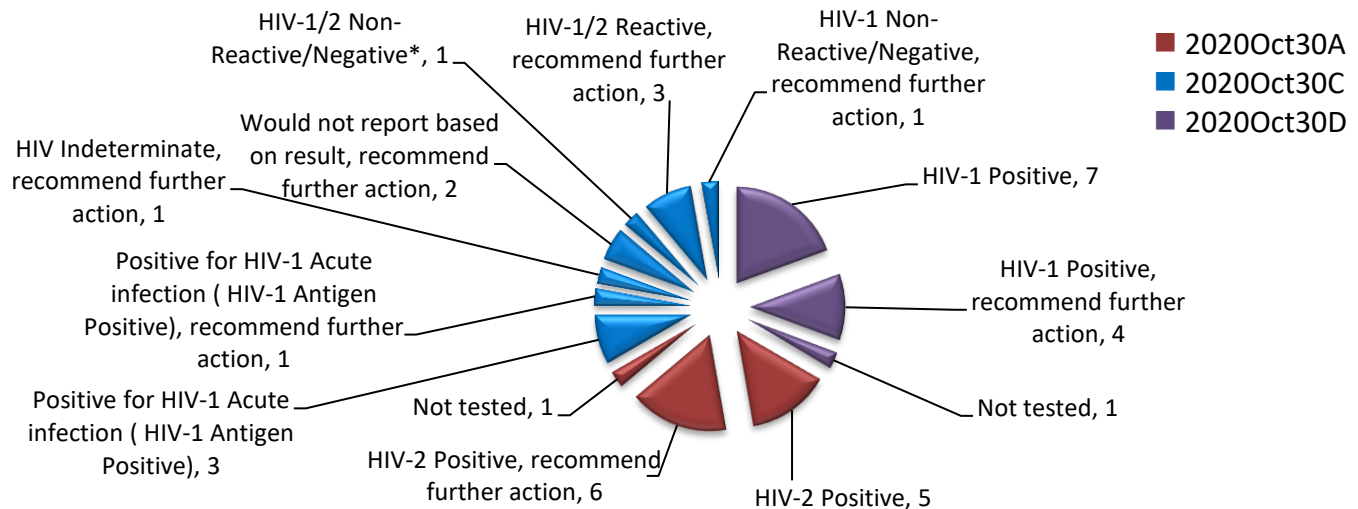


Figure 1: The final HIV serology status of the positive samples in the 2020Oct30 HIV serology panel submitted by participants using HIV screening assay.



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

Figure 2: The final HIV serology status of the positive samples in the 2020Oct30 HIV serology panel submitted by participants (include NLHRS) using HIV screening and confirmatory assay.

## Findings

Several errors were identified in this test event. Three participants mixed up samples D and E during the pre-testing and/or testing stage, and two participants accidentally chose the wrong interpretation at the result submission step.

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

[phac.nlhrs.qap-peq.lnsrv.aspc@canada.ca](mailto:phac.nlhrs.qap-peq.lnsrv.aspc@canada.ca)

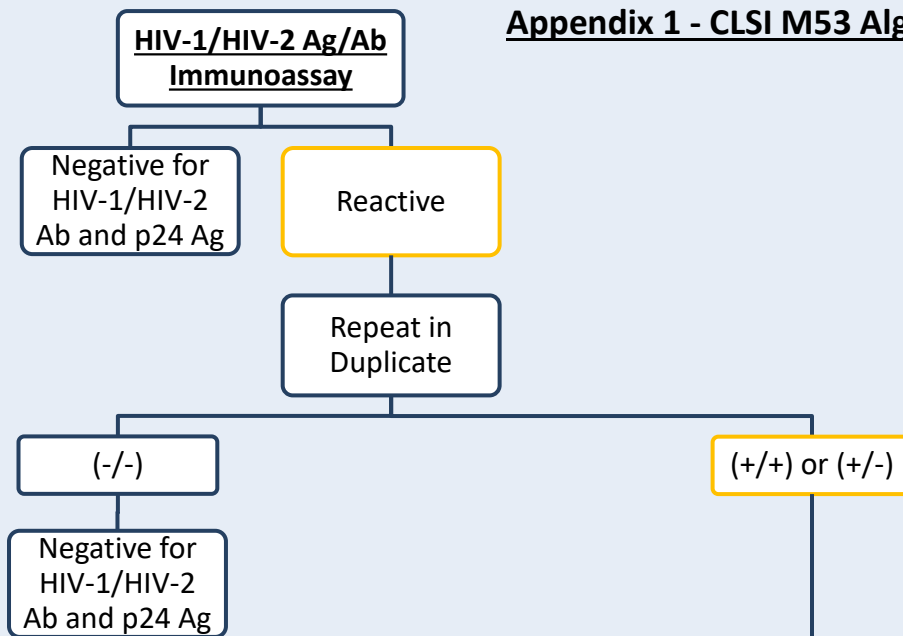
**Thank you for your participation in the NLHRS HIV Serology QA Program**

John Ho  
Quality Assurance Program Coordinator  
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Public Health Agency of Canada  
Tel: (204) 789-6518

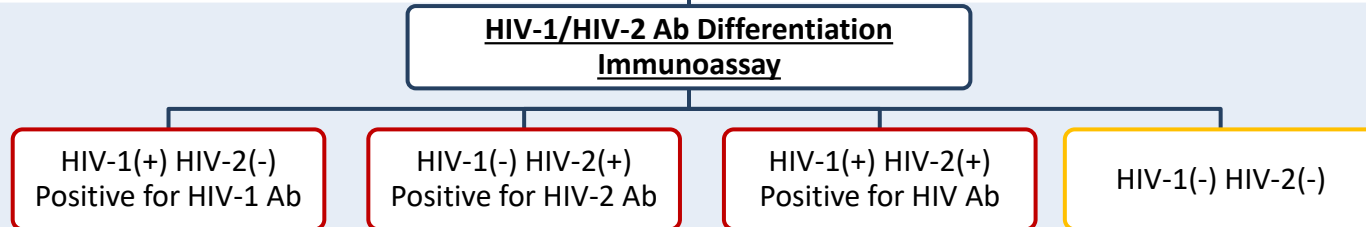
Dr. John Kim  
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**Appendix 1 - CLSI M53 Algorithm I**

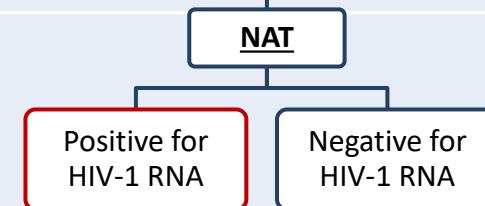
**(i) HIV-1/HIV-2 Ag/Ab Immunoassay**



**(ii) HIV-1/HIV-2 Ab Differentiation Immunoassay**



**(iii) Nucleic Acid Testing**



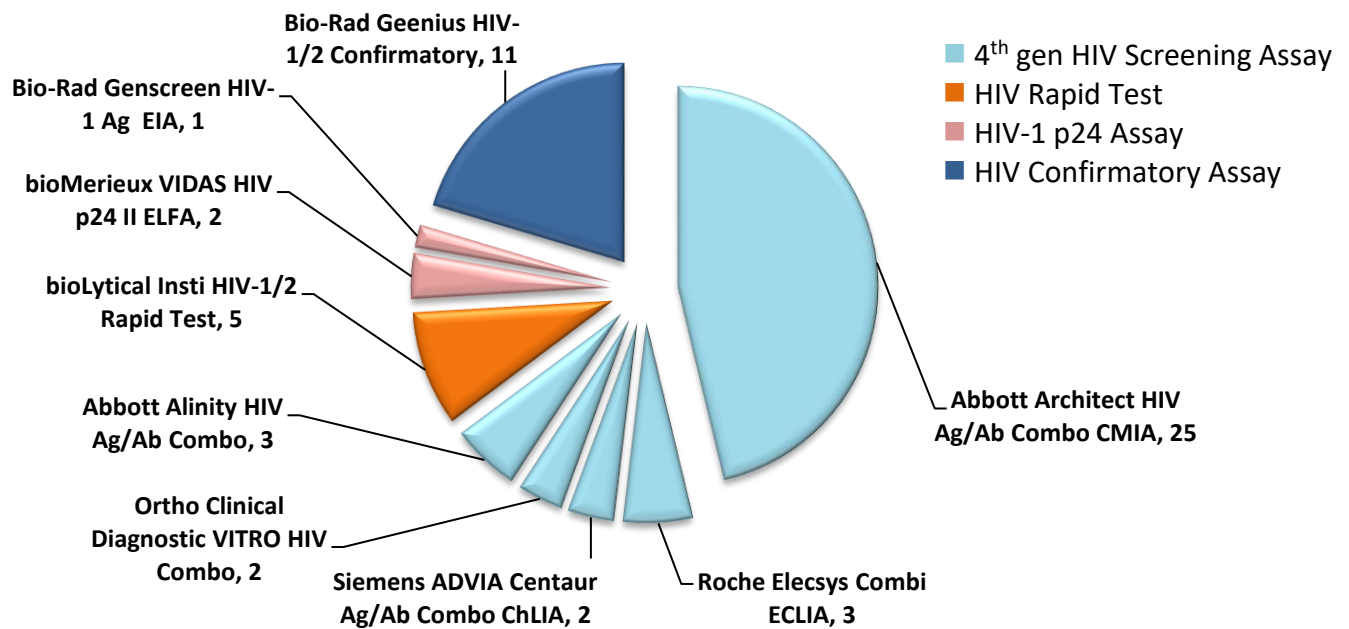
Appendix 1: Adaptation of the Clinical and Laboratory Standards Institute (CLSI) M53-*Criteria for Laboratory Testing and Diagnosis of Human Immunodeficiency Virus Infection: Approved Guideline* Algorithm I.

**Appendix 2: Characterization**

Summary of NLHRS Characterization of the 2020Oct30 HIV Panel Samples

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

**Appendix 3: Summary of assays used by the participants in the 2020Oct30 HIV serology panel**



**Appendix 4: Summary of bands detected in Sample A, B and D by the Bio-Rad Geenius HIV-1/2 confirmatory assay in the 2020Oct30 event.**

Bio-Rad Geenius Sample	Frequency of Bands Detected						
	gp36	gp140	p31	gp160	p24	gp41	CTRL
2020Oct30A	12	12	12	-	-	-	12
2020Oct30C	-	-	-	-	-	-	12
2020Oct30D	-	11	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 <sub>10</sub> 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 ( <u>random error</u> )	<u>Sporadic test results identified as outlying and/or aberrant can be classified as random events. Possible causes of random error include:</u>			
	• 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 ( <u>systematic error</u> )	<u>A series of test results identified as outlying and/or aberrant may be due to a systematic problem. Systematic problems may be due to:</u>			
	• 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.