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 2019Apr16

2019Apr16 HIV Serology Panel		
Panel Sample	**True Status**	**Labs Reporting Incorrect Status**
A	HIV-1 Ab Positive	
B	HIV-1/2 Ag/Ab Negative	
C	HIV-1 Ag Positive	
D	HIV-1/2 Ag/Ab Negative	
E	HIV-2 Ab Positive	

There were no incorrect results observed for the 2019Apr16 panel.

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Final Report for Panel HIVSER 2019Apr16

Issued 2019-August-02

Introduction

The NLHRS distributed the 2018Oct26 panel and the 2019Apr16 panel on Oct 10th 2018. This final report is specific to the 2019Apr16 panel only and is publicly available; however the identity of participants is not disclosed.

Panel Samples, HIV Test Kits and Data Entry

- *Panel Composition:*
  - 2019Apr16 is the relabelled 2018Oct 26HIV Serology Panel, consisting of five samples; two HIV negative (B, D), one HIV-1 Ab positive (A), one HIV-1 Ag positive (C) and one HIV-2 Ab positive (E). Sample C was diluted 1 in 10 with defibrinated human plasma (Basematrix 53, Seracare Life Sciences). The source material for Samples B, D, and E is the same source material used for the creation of the 2017-2018 HIV serology panels. Testing and characterization by the NLHRS prior to shipment are presented in Appendix 2. Panels were sent to 42 participants including the NLHRS on Oct 10th, 2018. The deadline for data entry for the 2019Apr16 panel was April 16th, 2019.

- *HIV Test Kits* –Nine different assays were used by the 42 participants (excluding the NLHRS) who returned results (Figure 1).

- *Data entry* - The NLHRS Quality Assessment Program switched from the web based Survey Monkey system to an in-house developed website for results entry in this panel.

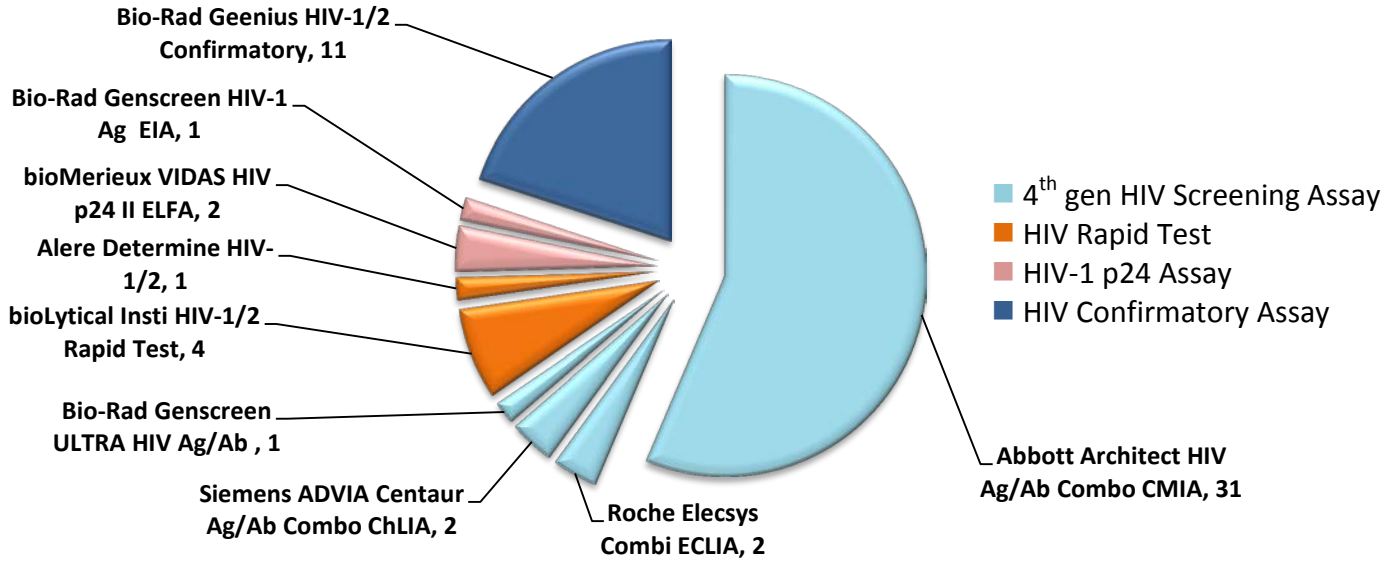


Figure 1: Assays used by the participants in the NLHRS 2019Apr16 HIV serology panel (excludes the NLHRS).

Homogeneity and stability

- The homogeneity and stability of the 2019Apr16 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 (Tables 1 and 2, Appendix 2).
- The source material (Access Biological) for the positive panel members is the same source material used for the 2017-2018 HIV Serology panel.

External QC and QA activities

1. *External quality control (QC) material* - Used in addition to controls provided in kits. External QC material allows users to detect technical problems and assay sensitivity from lot to lot.
   - 22 participants (52.3%, 22/42) reported using external QC material.

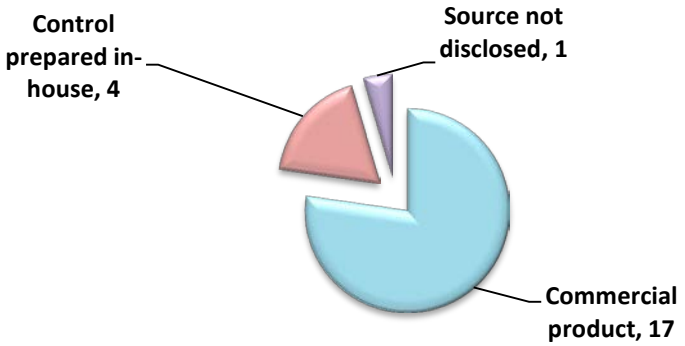


Figure 2: Source of external quality control used for the 2019Apr16 HIV serology panel.

2. *Quality Assurance (QA) programs* – Participation in QA programs allows participants to evaluate their overall use of the assay and reporting of the results.
   - 38 of 42 participants reported participation in other quality assurance program (Figure 3).

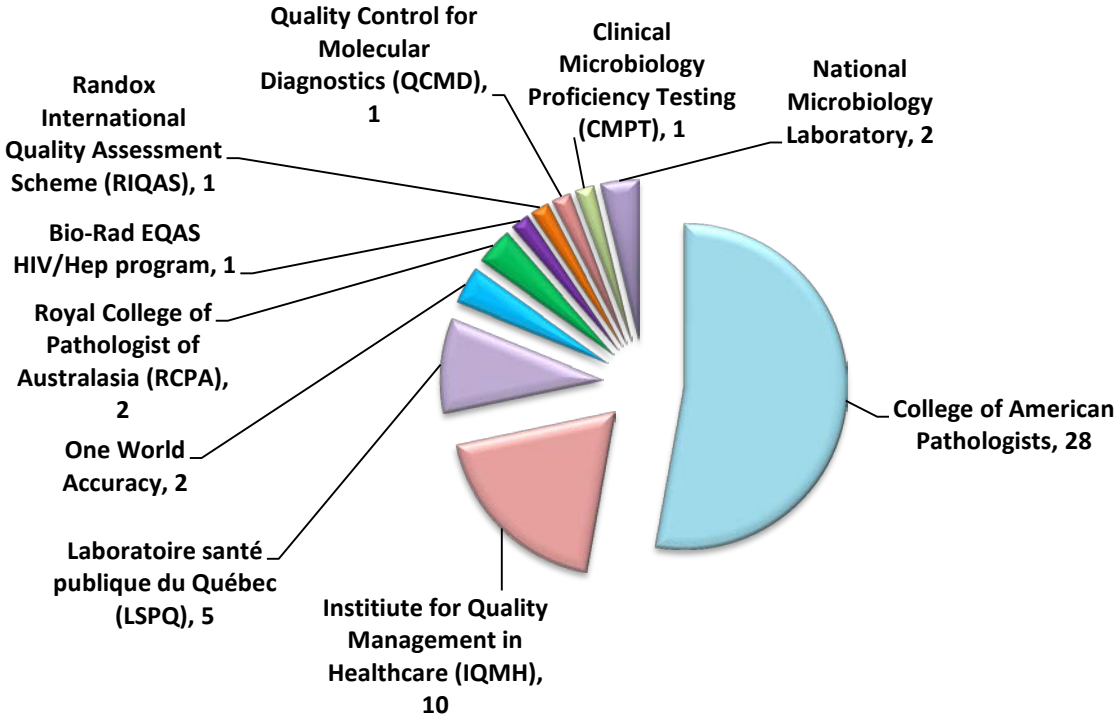


Figure 3: Distribution of external quality assurance programs which participants are enrolled in other than the NLHRS QAP.

Participants' feedback collected from the new QAP website

- Of the 42 participants, 6 provided feedback in the new QAP website. 2 participants found the survey easier to complete with the changes made (Figure 4)

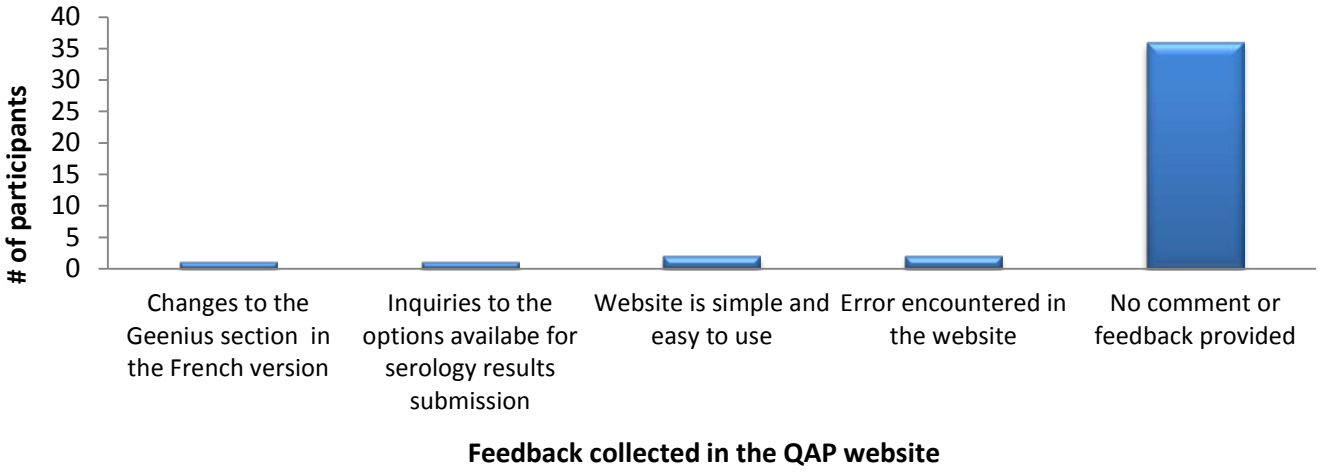


Figure 4: Feedback provided by the participant in the 2019Apr16 HIV serology survey.

Legend: **Major** **Intermediate** **Minor**

Table 1: 2019Apr16 HIV serology panel final status reported from participants using only a screening assay.

LAB	SAMPLE A HIV-1 Ab Positive	SAMPLE B HIV-1/2 Ab/Ag Negative	SAMPLE C HIV-1 Ag Positive	SAMPLE D HIV-1/2 Ab/Ag Negative	SAMPLE E HIV-2 Ab Positive
HV04	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]
HV05	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]
HV07	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]
HV12	Would not report based on result[1]	HIV-1/2 Non-Reactive/Negative	Would not report based on result[1]	HIV-1/2 Non-Reactive/Negative	Would not report based on result[1]
HV14	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]
HV17	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]
HV23	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]
HV24	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]
HV26	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]
HV27	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]
HV28	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative [1]	HIV-1/2 Non-Reactive/Negative[1]	HIV-1/2 Non-Reactive/Negative[1]	HIV-1/2 Reactive (Screen)[1]
HV30	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative [1]	HIV-1/2 Non-Reactive/Negative[1]	HIV-1/2 Non-Reactive/Negative[1]	HIV-1/2 Reactive (Screen)[1]
HV31	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative [1]	HIV-1/2 Non-Reactive/Negative[1]	HIV-1/2 Non-Reactive/Negative[1]	HIV-1/2 Reactive (Screen)[1]
HV43	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]
HV44	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]
HV45	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]
HV48	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]
HV49	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]
HV50	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]
HV53	Would not report based on result[1]	HIV-1/2 Non-Reactive/Negative	Would not report based on result[1]	HIV-1/2 Non-Reactive/Negative	Would not report based on result[1]
HV54	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]
HV55	Would not report based on result[1]	HIV-1/2 Non-Reactive/Negative	Would not report based on result[1]	HIV-1/2 Non-Reactive/Negative	Would not report based on result[1]
HV56	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]
HV57	Would not report based on result[1]	HIV-1/2 Non-Reactive/Negative	Would not report based on result[1]	HIV-1/2 Non-Reactive/Negative	Would not report based on result[1]
HV59	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]
HV63	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]
HV64	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]
HV68	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]
HV76	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]
HV79	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative [1]	HIV-1/2 Non-Reactive/Negative[1]	HIV-1/2 Non-Reactive/Negative[1]	HIV-1/2 Reactive (Screen)[1]

[1] Further actions recommended by participant; “Refer to reference/provincial laboratory for further testing”, “Request a follow-up sample” or “Perform NAT”.

Legend: **Major** **Intermediate** **Minor**

Table 2: 2019Apr16 HIV serology panel final interpretation reported by participants (includes the NLHRS) using both screening and confirmatory assays.

LAB	SAMPLE A HIV-1 Ab Positive	SAMPLE B HIV-1/2 Ab/Ag Negative	SAMPLE C HIV-1 Ag Positive	SAMPLE D HIV-1/2 Ab/Ag Negative	SAMPLE E HIV-2 Ab Positive
HV01	HIV-1 Positive	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-2 Positive
HV02	HIV-1 Positive	HIV-1/2 Non-Reactive/Negative	Would not report based on result[1,2]	HIV-1/2 Non-Reactive/Negative	HIV-2 Positive
HV03	HIV-1 Positive[2]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1,2]	HIV-1/2 Non-Reactive/Negative	HIV-2 Positive[2]
HV13	HIV-1 Positive[2]	HIV-1/2 Non-Reactive/Negative	Positive for HIV-1 Acute infection[2]	HIV-1/2 Non-Reactive/Negative	HIV-2 Positive[2]
HV15[3]	HIV-1 Positive	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Non-Reactive/Negative[1]	HIV-1/2 Non-Reactive/Negative	HIV-2 Positive
HV16	HIV-1 Positive	HIV-1/2 Non-Reactive/Negative	Positive for HIV-1 Acute infection[2]	HIV-1/2 Non-Reactive/Negative	HIV-2 Positive
HV18	HIV-1 Positive	HIV-1/2 Non-Reactive/Negative	HIV Indeterminate[1,2]	HIV-1/2 Non-Reactive/Negative	HIV-2 Positive[2]
HV19	HIV-1 Positive[2]	HIV-1/2 Non-Reactive/Negative	Positive for HIV-1 Acute infection[2]	HIV-1/2 Non-Reactive/Negative	HIV-2 Positive[2]
HV20	HIV-1 Positive[2]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1,2]	HIV-1/2 Non-Reactive/Negative	HIV-2 Positive[2]
HV21	HIV-1 Positive[2]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Non-Reactive/Negative[1,2]	HIV-1/2 Non-Reactive/Negative	HIV-2 Positive[2]
HV22	HIV-1 Positive	HIV-1/2 Non-Reactive/Negative	Would not report based on result[1,2]	HIV-1/2 Non-Reactive/Negative	HIV-2 Positive
HV75	HIV-1 Positive	HIV-1/2 Non-Reactive/Negative	Positive for HIV-1 Acute infection	HIV-1/2 Non-Reactive/Negative	HIV-2 Positive
HV80[4]	HIV-1/2 Reactive (Screen)	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)

[1] Did not perform the stand alone HIV-1 p24 testing to confirm the presence of HIV-1 p24 antigen.
[2] Further actions recommended by participants; "Refer to provincial/reference laboratory for further testing", "Request a follow-up sample" or"Perform NAT".
[3] This participant used a modified confirmatory algorithm which does not account for p24 detection.
[4] Participant performed both an HIV-1/2 Ag/Ab rapid test and a 4th gen HIV immunoassay.

Table 3: Level of the different flags and causes for the flag.

Level of flag	Causes for flagging
Major	Incorrect result/status provided
Intermediate	Deviation from kit insert, unresolved status without recommendation
Minor	Minor errors that do not result in misinterpretation of the true status of the sample, unresolved status but made a recommendation

Table 4: Expected results for the 2019Apr16 HIV serology panel.					
Assay	**Sample A**	**Sample B**	**Sample C**	**Sample D**	**Sample E**
bioLytical INSTI HIV-1/2-Rapid Test	HIV-1/2 Reactive	HIV-1/2 Non-Reactive	HIV-1/2 Non-Reactive	HIV-1/2 Non-Reactive	HIV-1/2 Reactive
Alere Determine HIV-1/2 Ab-Rapid Test	HIV-1/2 Reactive	HIV-1/2 Non-Reactive	HIV-1/2 Non-Reactive	HIV-1/2 Non-Reactive	HIV-1/2 Reactive
4th Gen HIV Screening Assay	HIV-1/2 Reactive	HIV-1/2 Non-Reactive	HIV-1/2 Reactive	HIV-1/2 Non-Reactive	HIV-1/2 Reactive
HIV Ab Confirmatory Assay	HIV-1 Ab Positive	HIV Ab Negative	HIV Ab Negative	HIV Ab Negative	HIV-2 Ab Positive
HIV-1 p24 Ag Test	HIV-1 p24 Ag Negative	HIV-1 p24 Ag Negative	HIV-1 p24 Ag Positive	HIV-1 p24 Ag Negative	HIV-1 p24 Ag Negative

Results (Excluding the NLHRS)

- *Return rate*
  - Results were returned from 100% of participants.
  - HV74 was not able to participate.

- *Group Analysis (Tables 1 and2)*
  - *Sample A (HIV-1 Ab Positive)* – 42/42 participants provided either a correct serology status and/or recommendation.
  - *Sample B (HIV-1/2 Ag/Ab Negative)* – 42/42 participants provided either a correct serology status and/or recommendation.
  - *Sample C (HIV-1 Ag Positive)* – 42/42 participants provided either a correct serology status and/or recommendation.
  - *Sample D (HIV-1/2 Ag/Ab Negative)* – 42/42 participants provided either a correct serology status and/or recommendation
  - *Sample E (HIV-2 Ab Positive)* – 42/42 participants provided either a correct serology status and or recommendation.

Discussion

The discrepancy that was detected in the 2018Oct26 HIV serology panel with the Geenius assay did not occur in the 2019Apr16 serology panel despite both panels shared the exact same samples. In the 2018Oct26 test event, 3 participants, including the NLHRS staff that participated in the survey, detected a gp41 band in the HIV-2 Ab positive sample, resulting in the Geenius interpretation of HIV-2 positive with a cross reactivity with HIV-1. This issue did not occur in the 2019Apr16 test event as all 12 Geenius users (including the NLHRS) did not detect the gp41 band in the HIV-2 Ab positive sample; aligning with the initial characterization result of the HIV-2 Ab positive sample.

In addition, a majority of the Geenius users were able to detect the p31 and p24 bands in the HIV-1 Ab positive samples unlike in the 2018Oct26 test event. The frequency of the bands detected and the Geenius kit lots used in the 2019Apr16 and the 2018Oct26 test events can be found in Appendix 3.

The potential cross reactivity and sensitivity issue of the Geenius assay that was identified in the 2018Oct26 test event may likely attributed to a random cartridge in a specific kit lot. The Geenius kit lot used in this current test event are different to the kit lots that were used in the 2018Oct26 test event but not all users that used lot 7B0028 and 7F0029 have detected the gp41 band in the HIV-2 positive sample. Further investigation is needed to determine the cause of the cross reactivity and sensitivity issue that was found in the 2018Oct26 test event.

Conclusion

There were no discordant findings in the participants' results for the 2019Apr16 test event. All participants returned the correct test results. In this test event, we have switched over to the new QAP website from Survey Monkey to address the lack of printing functionality for the participant and the option for the participant to be to enter their results in French. We appreciate your feedback provided in the survey and recognize that participants would like additional improvements to our reporting format and the timeliness of the dissemination of the report. To that end, the NLHRS is exploring to utilize the features of the new QAP website to reduce the turnaround time of the dissemination of the final report to the participants.

We value each laboratory's participation in these QA panels 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.

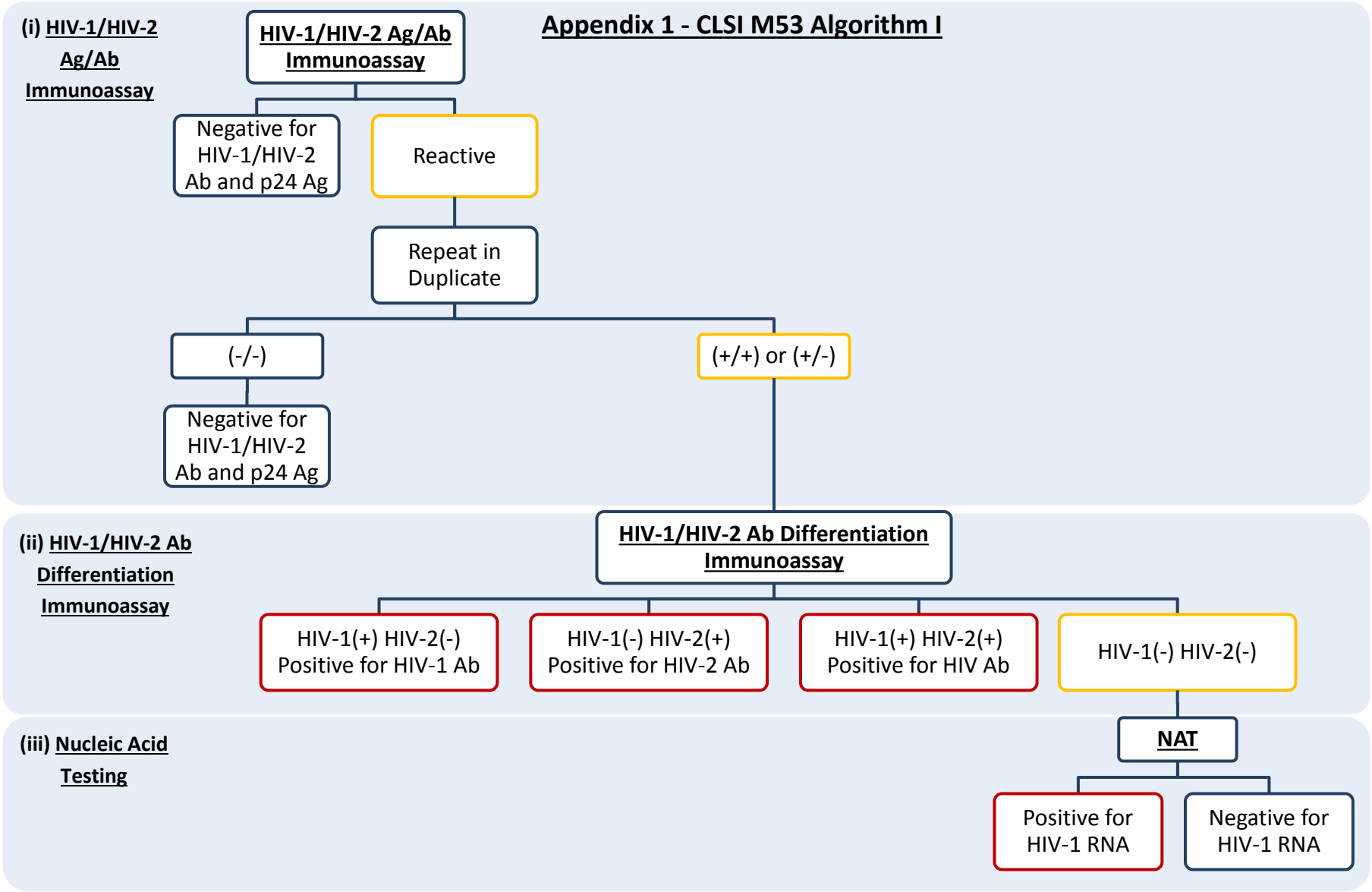
If you have any comments or concerns, please contact us at:

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
National Laboratory for HIV Reference Services
Public Health Agency of Canada
Tel: (204) 789-6522

Dr. John Kim
Laboratory Chief
National Laboratory for HIV Reference Services
Public Health Agency of Canada
Tel: (204) 789-6527



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 2019Apr16 HIV Panel Samples

Sample		B/D (Duplicate)	E	A	C
		Negative	**HIV-2 Ab Positive**	**HIV-1 Ab Positive**	**HIV-1 Ag Positive**
Final HIV Status		HIV-1/2 Ag/Ab Negative	HIV-2 Ab Positive	HIV-1 Ab Positive	HIV-1 Ag Positive
bioLytical INSTI HIV-1/2 Rapid Test	**Result**	NR	R	R	NR
Bio-Rad GS HIV Ag/Ab Combo	**Result**	Non-Reactive	Reactive	Reactive	Reactive
Bio-Rad GS HIV p24	**Result**	Non-Reactive	Non-Reactive	Non-Reactive	Reactive
Bio-Rad GS HIV p24 Confirmatory	**Result**	Not Tested	Not Tested	Not Tested	100% Neutralization
Bio-Rad GS HIV-1 Western Blot	**Result**	Neg	HIV IND	HIV-1	Neg
	gp160	-	-	++	-
	gp120	-	-	+	-
	p65	-	++	+/-	-
	p55	-	-	+	-
	p51	-	+	+/-	-
	gp41	-	-	++	-
	p40	-	+/-	++	-
	p31	-	++	++	-
	p24	-	+/-	++	-
	p18	-	-	+	-
Fujirebio INNO-LIA HIV-I/II Score	**Result**	Neg	HIV-2	HIV-1	Neg
	sgp120	-	-	++	-
	gp41	-	-	+++	-
	p31	-	+++	++	-
	p24	-	++	++	-
	p17	-	-	-	-
	sgp105	-	++	-	-
	gp36	-	+++	-	-
Bio-Rad Geenius HIV-1/HIV-2 Supplemental Assay	**Result**	Neg	HIV-2	HIV-1	Neg
	gp36	-	+	-	-
	gp140	-	+	-	-
	p31	-	+	+	-
	gp160	-	-	+	-
	p24	-	-	+	-
	gp41	-	-	+	-
	CTRL	+	+	+	+

Appendix 3: Summary of Bands Detected and the Geenius kit lot used in the 2019Apr16 and 2018Oct26 test events

Bio-Rad Geenius	Frequency of Bands Detected					
Sample	gp36	gp140	p31	gp160	p24	gp41
2019Apr16A[1]	0	0	10	12	12	12
2018Oct26B[1]	0	0	4	12	10	12
2019Apr16E[2]	12	12	12	0	0	0
2018Oct26E[2]	12	12	12	0	0	3

[1] HIV-1 Ab positive sample

[2] HIV-2 Ab positive sample

Kit lot of the Geenius HIV Confirmatory used in the 2019Apr16 and 2018Oct26 Test events					
Test Event	7B0028[1]	7F0029[1]	8A0030	8F0031	8H0032
2019Apr16	0	0	0	5	7
2018Oct26	2	5	4	1	0

[1] Geenius kit lot used that detected the gp41 band in the HIV-2 Ab Positive sample in 2018Oct26 test event

Appendix 4: 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.