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 2018Oct26

2018Oct26 HIV Serology Panel			
Panel Sample	**True Status**	**Labs Reporting Incorrect Status**	
A	HIV-1/2 Ab/Ag Negative		
B	HIV-1 Ab Positive		
C	HIV-1/2 Ab/Ag Negative		
D	HIV-1 Ag Positive		
E	HIV-2 Ab Positive		

There were no incorrect interpretations observed for the 2018Oct26 panel.

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Final Report for Panel HIVSER 2018Oct26

Issued 2019-01-16

Introduction

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

Panel Samples, HIV Test Kits and Data Entry

- *Panel Composition*
  - 2018Oct26 HIV Serology Panel: Five samples; two HIV negative (A, C), one HIV-2 Ab positive (E), one HIV-1 Ab positive (B) and one HIV-1 Ag positive (D). Sample D 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 2018Oct26 panel was October 26th, 2018.

- *HIV Test Kits* –Nine different assays were used by the 42 participants (excluding the NLHRS) who returned results (Table 1, Figures 1 and 2). The majority of participants, 81% (34/42), used a 4th generation HIV immunoassay. One participant switched to the Ag/Ab version of the Alere Determine rapid test in their algorithm.

- *Data entry* - The NLHRS Quality Assessment Program used the web based Survey Monkey system to capture results. The options and the selection format for the Final Interpretation in Survey Monkey were changed to simplify the submission process for the end users. Participants were also asked to pilot a new NLHRS QAP website that will replace Survey Monkey in the future.

Table 1: Summary of the assays used in the 2017Oct27, 2018Apr19, and 2018Oct26 HIV panels (excludes the NLHRS).

Type	Assay	# of Users		
		2017Oct27	2018Apr19	2018Oct26
Screen – 4th Generation	Abbott ARCHITECT HIV Ag/Ab Combo CMIA	32	32	32
	Roche Elecsys HIV Combi ECLIA	2	2	2
	Siemens ADVIA Centaur HIV Ag/Ab Combo (CHIV) ChLIA Assay	2	2	2
	Bio-Rad Genscreen ULTRA HIV Ag/Ab	1	1	1
Screen – 3rd Generation	Bio-Rad GS HIV-1/HIV-2 PLUS O EIA	1	0	0
Screen – Rapid Test	bioLytical INSTI HIV-1/HIV-2 Antibody Test Kit	4	4	4
	Alere Determine HIV-1/2	0	1	0
	Alere Determine HIV-1/2 Ag/Ab	0	0	1
Confirmatory – p24	bioMerieux VIDAS HIV p24 II ELFA	2	2	2
	Bio-Rad Genscreen HIV-1 Ag EIA	1	1	1
Confirmatory – Ab	Bio-Rad Geenius HIV-1/2 Confirmatory Assay	11	11	11

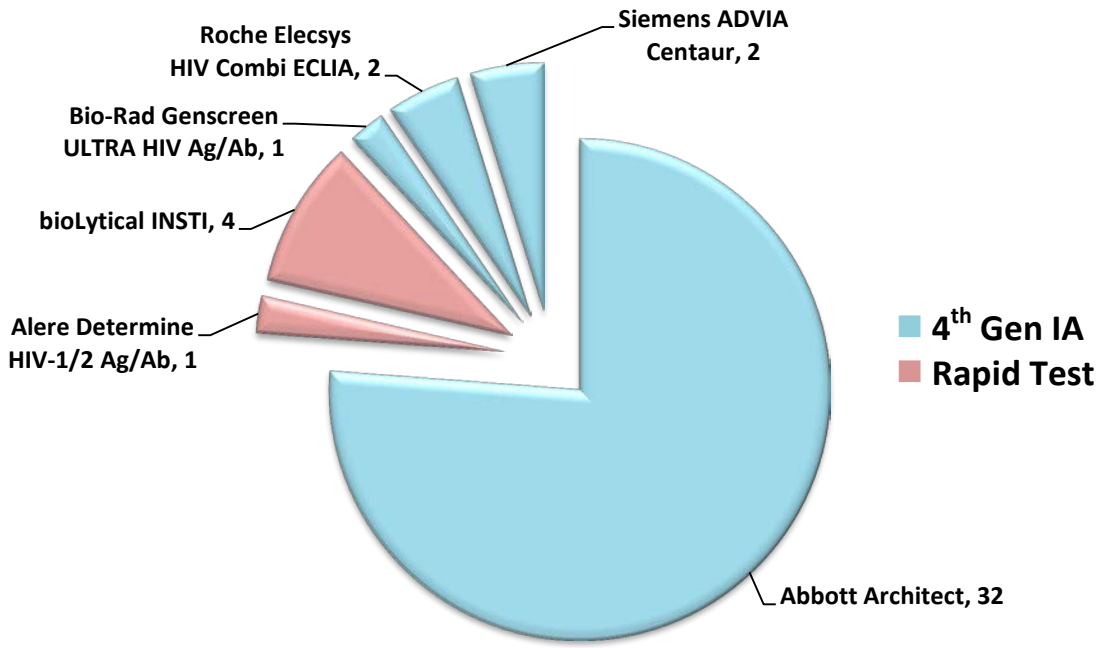

Figure 1: Breakdown of the screening assays used by 42 participants in the 2018Oct26 HIV serology panel (excludes the NLHRS).

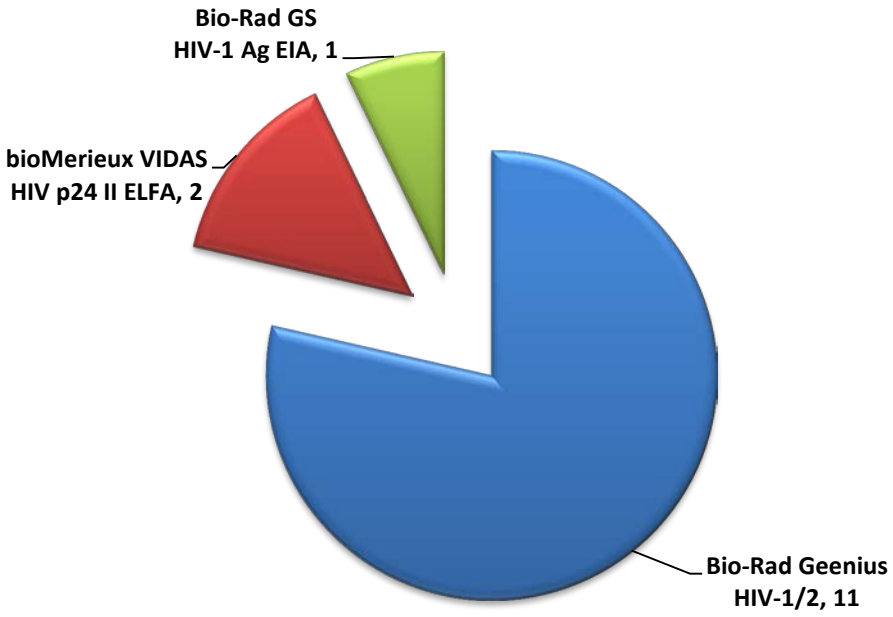

Figure 2: Breakdown of the confirmatory assays used by participants in the 2018Oct26 HIV serology panel (excludes the NLHRS).

Table 2: 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

Homogeneity and stability

- The homogeneity and stability of the 2018Oct26 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 3 and 4, 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.

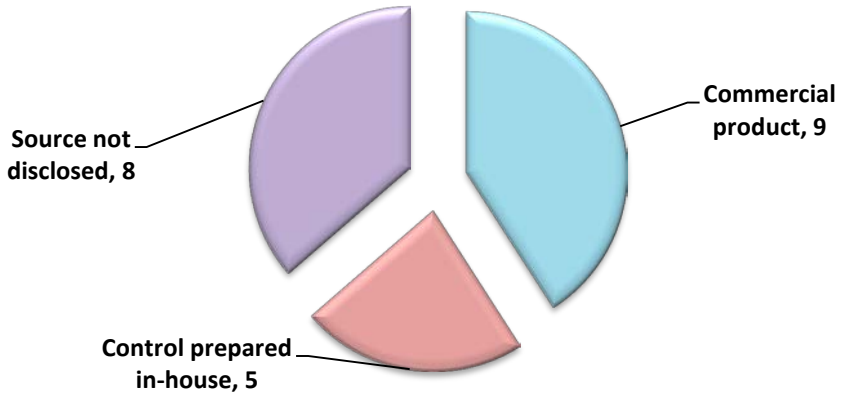

Figure 3: Source of external quality control used for the 2018Oct26 HIV serology panel.

2. *Quality Assurance (QA) programs* – Participation in QA programs allows participants to evaluate their overall use of the assay and reporting of the results.
   - 37 of 42 participants reported participation in other quality assurance program (Figure 4).

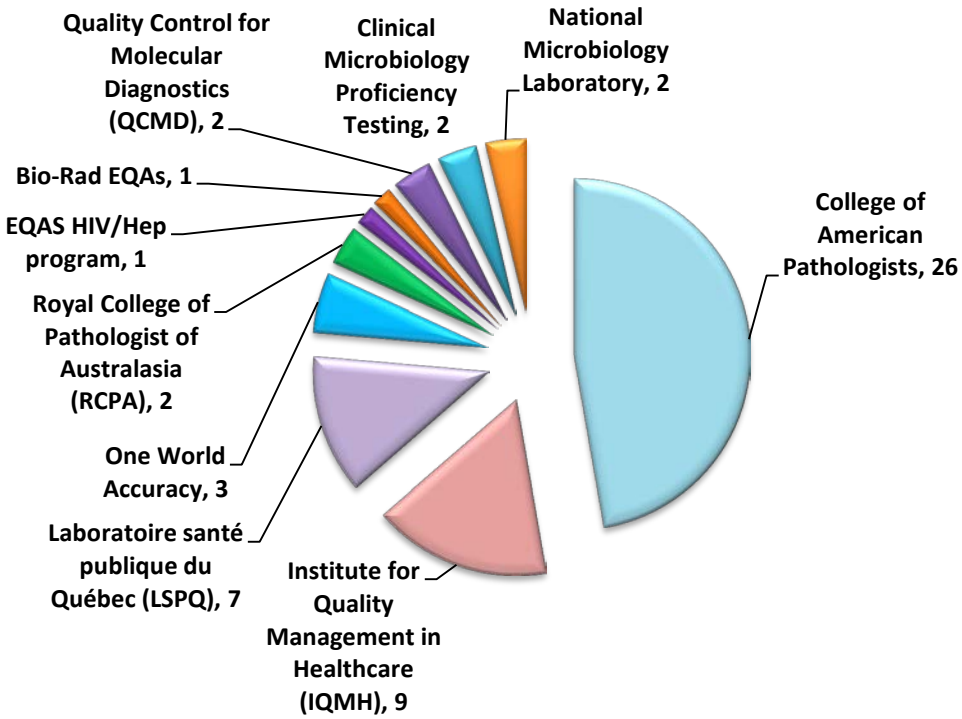

Figure 4: Distribution of external quality assurance programs which participants are enrolled in other than the NLHRS QAP.

Participants' feedback collected from Survey Monkey and the beta testing of the new QAP website

- Of the 42 participants, 41 provided feedback in Survey Monkey. Thirty-eight participants were satisfied with the changes made in Survey Monkey (Figure 5 and 6), 3 participants found the survey easier to complete with the changes made, and 3 participants did not find the changes to be useful.
- Thirteen participants did not comment when asked to provide suggestions for improvement; 15 participants were satisfied with the current format.
- Of the 42 participants, 40 participated in the beta testing of the new NLHRS QAP website. Feedback on the new NLHRS QAP website is still being collected.
- Suggestions for improvement collected in Survey Monkey have been incorporated into the new NLHRS QAP website which will streamline the results entry process and improve overall functionality.

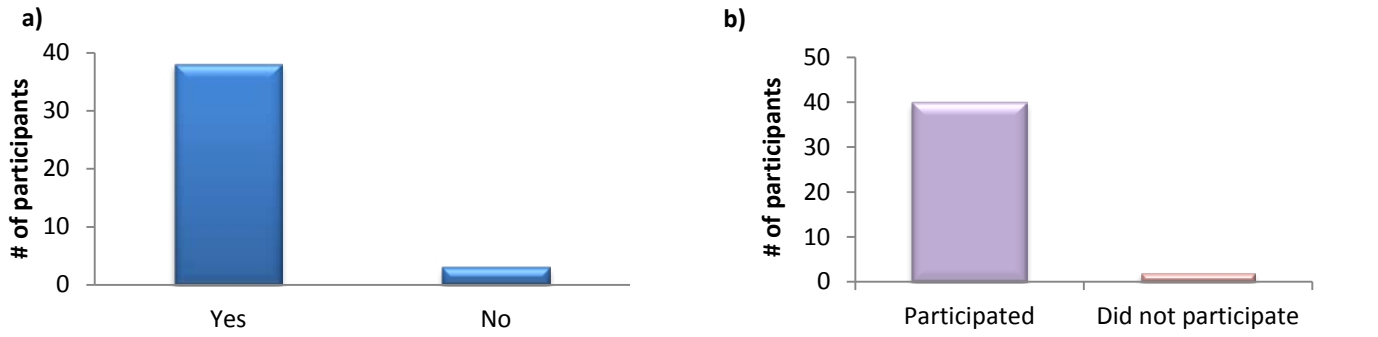

Figure 5: Number of participants' who a) liked the changes to Survey Monkey and b) used the new QAP website

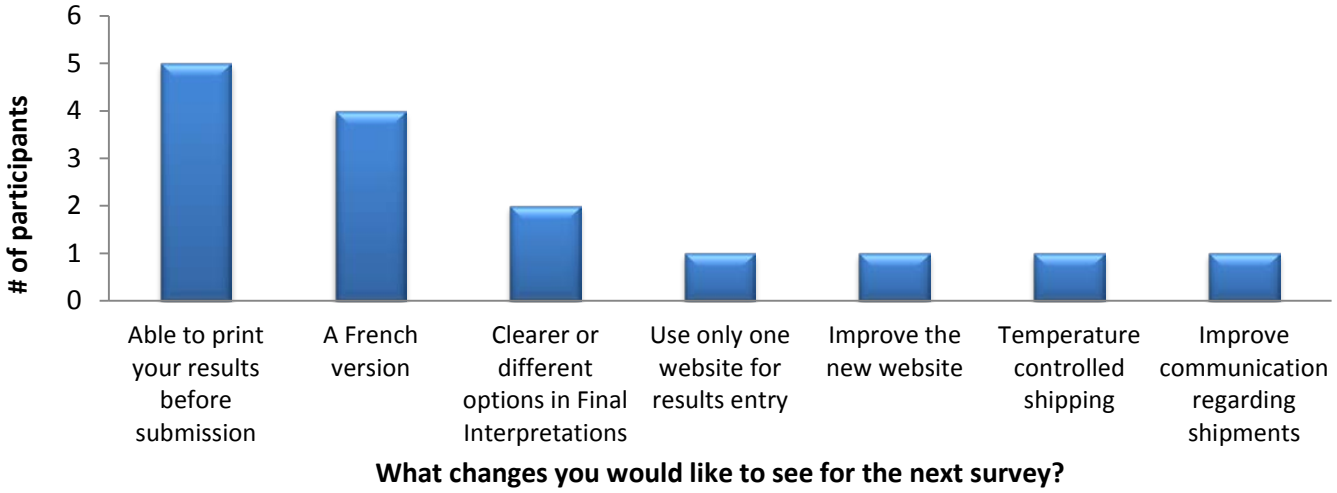

Figure 6: Participants' responses when asked which area requires improvement in the NLHRS HIV serology survey.

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

Table 3: 2018Oct26 HIV serology panel final status reported from participants using only a screening assay.

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

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

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

Table 4: 2018Oct26 HIV serology panel final interpretation reported by participants (includes the NLHRS) using both screening and confirmatory assays.

LAB	SAMPLE A HIV-1/2 Ab/Ag Negative	SAMPLE B HIV-1 Ab Positive	SAMPLE C HIV-1/2 Ab/Ag Negative	SAMPLE D HIV-1 Ag Positive	SAMPLE E HIV-2 Ab Positive
HV01	HIV-1/2 Non-Reactive/Negative[1]	HIV-1 Positive[1]	HIV-1/2 Non-Reactive/Negative[1]	HIV-1/2 Reactive (Screen)[1,2]	HIV-2 Positive[1]
HV02	HIV-1/2 Non-Reactive/Negative[1]	HIV-1 Positive[1]	HIV-1/2 Ab/Ag Negative[1]	HIV-1/2 Non-Reactive/ Negative (Screen)[1,2]	HIV-2 Ab Positive[1]
HV03	HIV-1/2 Non-Reactive/Negative[1]	HIV-1 Positive[1,2]	HIV-1/2 Non-Reactive/Negative[1]	HIV-1/2 Non-Reactive/Negative[1,2]	HIV-2 Positive[1,2]
HV13	HIV-1/2 Non-Reactive/Negative	HIV-1 Positive[2]	HIV-1/2 Non-Reactive/Negative	Positive for HIV-1 Acute infection[2]	HIV-2 Positive[2]
HV15[3]	HIV-1/2 Non-Reactive/Negative[1]	HIV-1 Positive[1,2]	HIV-1/2 Non-Reactive/Negative[1]	HIV-1/2 Non-Reactive/Negative[1]	HIV-2 Positive[1]
HV16	HIV-1/2 Non-Reactive/Negative	HIV-1 Positive[2]	HIV-1/2 Non-Reactive/Negative	Positive for HIV-1 Acute infection[2]	HIV-2 Positive
HV18	HIV-1/2 Non-Reactive/Negative[1]	HIV-1 Positive[1,2]	HIV-1/2 Non-Reactive/Negative[1]	HIV Indeterminate[1,2]	HIV-2 Positive[1,2]
HV19[4]	HIV-1/2 Non-Reactive/Negative	HIV-1 Positive[2]	HIV-1/2 Non-Reactive/Negative	Positive for HIV-1 Acute infection[2]	HIV-2 Positive[2]
HV20	HIV-1/2 Non-Reactive/Negative[1]	HIV-1 Positive[1,2]	HIV-1/2 Non-Reactive/Negative[1]	HIV-1/2 Reactive (Screen)[1,2]	HIV-2 Positive[1,2]
HV21	HIV-1/2 Non-Reactive/Negative[1]	HIV-1 Positive[1,2]	HIV-1/2 Non-Reactive/Negative[1]	HIV-1/2 Non-Reactive/Negative[1,2]	HIV-2 Positive[1,2]
HV22	HIV-1/2 Non-Reactive/Negative[1]	HIV-1 Positive[1]	HIV-1/2 Non-Reactive/Negative[1]	Would not report based on result[1,2]	HIV-2 Positive[1]
HV75	HIV-1/2 Non-Reactive/Negative	HIV-1 Positive	HIV-1/2 Non-Reactive/Negative	Positive for HIV-1 Acute infection	HIV-2 Positive
HV80[4]	HIV-1/2 Non-Reactive/Negative[1]	HIV-1/2 Reactive (Screen)[2]	HIV-1/2 Non-Reactive/Negative	HIV-1/2 Reactive (Screen)[1,2]	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 action recommended by participants; "Refer to provincial/reference laboratory for further testing" or "Request a follow-up sample".
[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 5: Expected results for the 2018Oct26 HIV serology panel.

Assay	Sample A	Sample B	Sample C	Sample D	Sample E
bioLytical INSTI HIV-1/2-Rapid Test	HIV-1/2 Non-Reactive	HIV-1/2 Reactive	HIV-1/2 Non-Reactive	HIV-1/2 Non-Reactive	HIV-1/2 Reactive
Alere Determine HIV-1/2 Ag/Ab-Rapid Test	HIV-1/2 Non-Reactive	HIV-1/2 Reactive	HIV-1/2 Non-Reactive	HIV-1 Ag Reactive	HIV-1/2 Reactive
4th Gen HIV Screening Assay	HIV-1/2 Non-Reactive	HIV-1/2 Reactive	HIV-1/2 Non-Reactive	HIV-1/2 Reactive	HIV-1/2 Reactive
HIV Ab Confirmatory Assay	HIV Ab Negative	HIV-1 Ab Positive	HIV Ab Negative	HIV Ab Negative	HIV-2 Ab Positive
HIV-1 p24 Ag Test	HIV-1 p24 Ag Non-reactive	HIV-1 p24 Ag Non-Reactive	HIV-1 p24 Ag Non-Reactive	HIV-1 p24 Ag Positive	HIV-1 p24 Ag Non-Reactive

Results (Excluding the NLHRS)

- *Return rate*
  - Results were returned from 100% of participants.
  - HV74 was not able to participate this year as they were not able to finish their validation in time for the October test event.
- *Group Analysis (Tables 3 and 4)*
  - *Sample A (HIV-1/2 Ab/Ag Negative)* – 42/42 participants provided either a correct serology status and/or recommendation.
  - *Sample B (HIV-1 Ab Positive)* – 42/42 participants provided either a correct serology status and/or recommendation.
    - **HV01**: This participant did not correctly enter all the bands that were detected on their Geenius cartridge in Survey Monkey.
  - *Sample C (HIV-1/2 Ab/Ag Negative)* – 42/42 participants provided either a correct serology status and/or recommendation.
  - *Sample D (HIV-1 Ag Positive)* – 42/42 participants provided either a correct serology status and/or recommendation
    - **HV01**: This participant entered an incorrect Geenius interpretation of "HIV Ab Indeterminate" instead of "HIV Negative". In the case of this sample, the control band was the only detected band on their Geenius cartridge.
  - *Sample E (HIV-2 Ab Positive)* – 42/42 participants provided either a correct serology status and or recommendation.
    - **HV01:** This participant did not correctly enter all the bands that were detected on their Geenius cartridge in Survey Monkey.
- *Transcription Error*: ***HV01***
  HV01 incorrectly transcribed their Geenius results for samples B, D, and E in Survey Monkey. The NLHRS found that the Geenius band patterns submitted for samples B and E, and the Geenius interpretation for sample D, did not match the data entered in Survey Monkey.

Discussion

A discrepancy was detected in the Geenius results of the 2018Oct26 HIV serology panel. Three participants, including the NLHRS staff that participated in the survey, detected a gp41 band in sample E. The remaining 9 Geenius users did not. The additional gp41 band detected in sample E resulted in an interpretation of "HIV-2 Positive with HIV-1 cross reactivity". This result deviates from the initial characterization of the sample conducted by the NLHRS and from the majority of participants where the result for sample E was "HIV-2 Positive" with no cross reactivity with HIV-1.

Discussion (continued)

In addition, not all participants were able to detect the p31 and p24 bands with the Geenius assay. To investigate these discrepancies, the NLHRS tested 3 aliquots of samples B and E on the Bio-Rad HIV-1/2 Geenius Confirmatory Assay, the Fujirebio INNO-LIA HIV-1/2 Score, and the Bio-Rad HIV-1 Western Blot. The frequency of the bands detected on each of the assays for samples B and E, as well as the results from all Geenius users from the 2018Oct26 test event can be found in Appendix 3.

None of the assays were able to detect gp41 in sample E which is consistent with the result from the initial characterization performed by the NLHRS. Since the other confirmatory assays were unable to detect gp41 in this sample, the aberrant result is potentially due to cross reactivity that is specific to the Geenius assay.

The 3 confirmatory assays used in this investigation were able to detect p24 in all 3 sample B aliquots. Similarly, the Bio-Rad HIV-1 Western Blot and Fujirebio INNO-LIA HIV-1/2 Score assays also detected p31 in each aliquot, however, the Geenius assay only detected p31 in 2 out of 3 aliquots. The inconsistent result produced by the Geenius assay points towards a possible sensitivity issue and it will be further investigated.

Conclusion

Besides the minor transcription error made by one participant, HV01, no other errors were identified for the 2018Oct26 HIV serology panel. The absence of analytical errors is not surprising as all participants have consistently demonstrated good technical competency throughout each NLHRS HIV serology proficiency test event.

The total number of post-analytical errors has decreased when compared to the previous HIV serology test event (2018Apr19). The following changes made to the Final Interpretation section in Survey Monkey likely contributed to this reduction. First, the choices for the final interpretation were simplified to reduce confusion for the end user. Second, the selection format for the results was changed to a drop down menu format instead of the point and click format used in previous iterations of the survey. This change was made in order to minimize the likelihood of a participant making an incorrect selection based on the results submission format. It also reduced the complexity of the data entry interface making it less overwhelming for the participant. Lastly, the question regarding the presence of HIV-1 p24 Ag was removed as it was deemed a poorly worded question that participants may have found confusing. Overall, the majority of participants were satisfied with the changes made in Survey Monkey which they found to be more user friendly and easier to complete.

Conclusion (continued)

We appreciate participant feedback and recognize that participants would like additional improvements to our reporting format. To that end, the NLHRS is in the process of implementing a new results submission website that will address some of the feedback provided by our participants. We would like to express our gratitude to those that participated in the beta testing of this new data entry system.

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

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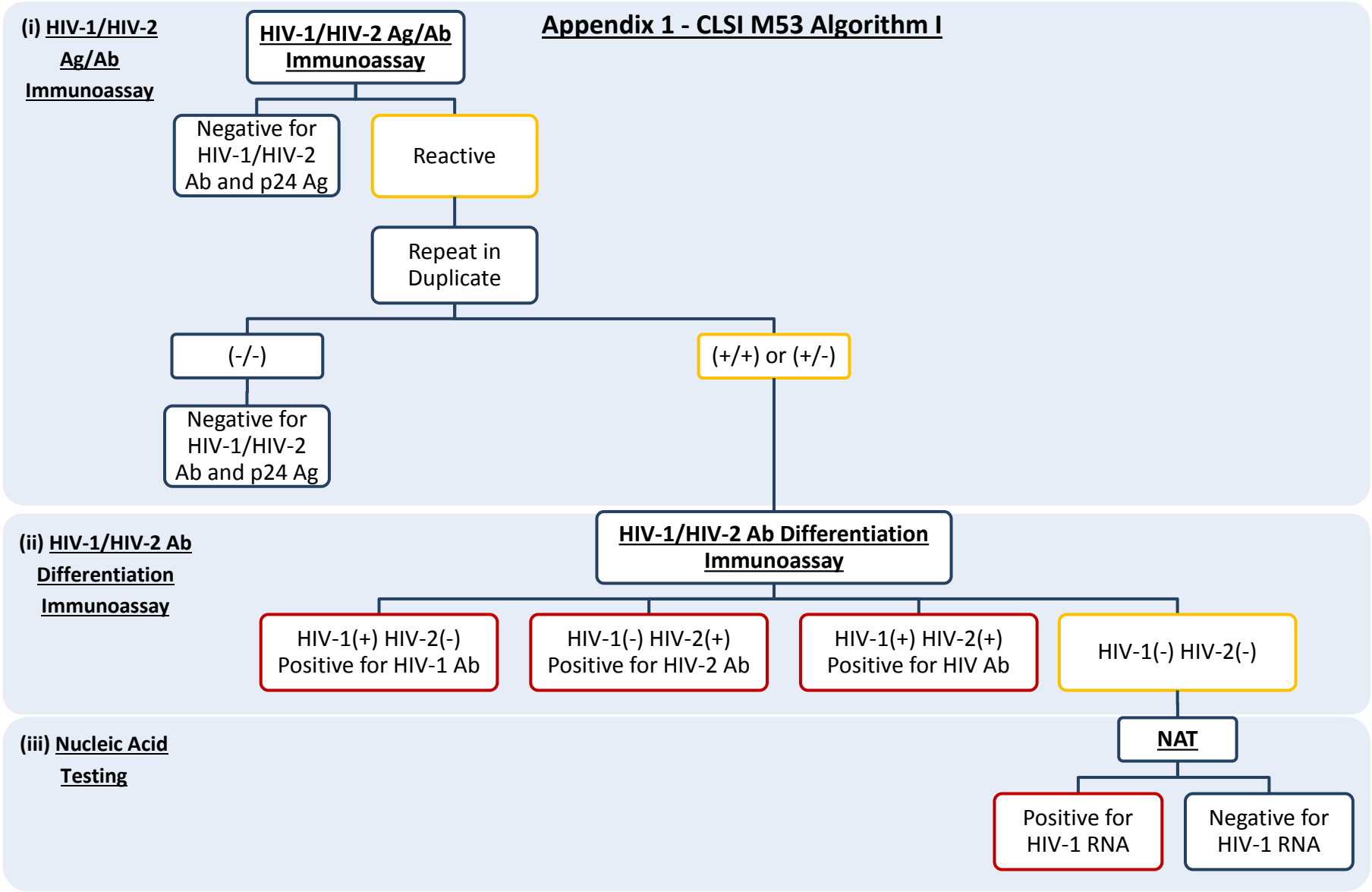

Appendix 1: Adaptation of the Clinical and Laboratory Standards Institute (CLSI) M53-*Criteria for Laboratory Testing and Diagnosis of Human Immunodeficiency Virus Infection: Approved Guideline* Algorithm I.

Appendix 2: Characterization

Summary of NLHRS Characterization of the 2018Oct26 HIV Panel Samples

Sample		A/C (Duplicate)	E	B	D
		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: Frequency of Bands Detected for 2018Oct26 Samples B and Sample E

Bio-Rad Geenius	Frequency of Bands Detected					
Sample	**gp36**	**gp140**	**p31**	**gp160**	**p24**	**gp41**
Sample B[1]	0	0	4	12	10	12
Sample B[2]	0	0	2	3	3	3
Sample E[1]	12	12	12	0	0	3
Sample E[2]	3	3	3	0	0	0

[1] Result from test event.

[2] Result from repeat testing at NLHRS.

Bio-Rad HIV-1 WB	Frequency of Bands Detected									
Sample	**gp160**	**gp120**	**p65**	**p55**	**p51**	**gp41**	**p40**	**p31**	**p24**	**p18**
Sample B[1]	3	3	3	3	0	3	3	3	3	3
Sample E[1]	0	0	3	0	3	0	3	3	3	0

[1] Result from repeat testing at NLHRS.

INNO-LIA HIV-1/2 Score	Frequency of Bands Detected						
Sample	**sgp120**	**gp41**	**p31**	**p24**	**p17**	**sgp105**	**gp36**
Sample B[1]	3	3	3	3	3	0	0
Sample E[1]	0	0	3	3	0	3	3

[1] Result from repeat testing at NLHRS.

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.