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 2016Apr21

Blue Panel			
Panel Sample	**True Status**	**Labs Reporting Incorrect Status**	
A	HIV-1 Ab Positive	**Incorrect Final Status**	• HV02
B	HIV-1/2 Ab Negative		
C	HIV-1 Ab Positive	**Incorrect Final Status**	• HV02
D	HIV-2 Ab Positive	**Incorrect Final Status**	• HV01 • HV02
E	HIV-1/2 Ab Negative		
Labs: HV01, HV02, HV13, HV15, HV16, HV18, HV19, HV20, HV21, HV24, HV75.			

Regular Panel			
Panel Sample	**True Status**	**Labs Reporting Incorrect Status**	
A	HIV-1 Ab Positive	**Incorrect Final Status**	• HV07 • HV17 • HV53 • HV79
B	HIV-1/2 Ab Negative	**Incorrect Final Status**	• HV17 • HV44 • HV53 • HV79
C	HIV-1 Ab Positive	**Incorrect Final Status**	• HV07 • HV17 • HV53 • HV79
D	HIV-1 Ab Positive	**Incorrect Final Status**	• HV07 • HV17 • HV53 • HV79
E	HIV-1/2 Ab Negative	**Incorrect Final Status**	• HV17 • HV53 • HV79
Labs: HV03, HV04, HV05, HV07, HV12, HV14, HV17, HV22, HV23, HV26, HV27, HV28, HV30, HV31, HV43, HV44, HV45, HV48, HV49, HV50, HV53, HV54, HV55, HV56, HV57, HV59, HV63, HV64, HV68, HV74, HV75, HV76, HV79.			

Incorrect interpretations based on their assay result(s):

HV44
Provided 2 conflicting final status results; HIV positive and HIV negative.

HV53
Final status: Not Tested (B,E had no recommendation while A,C,D had a recommendation).

HV79
Incorrect Final Status: Using 3rd generation Ab only test, final status included Ag which was not run.

HV01, HV02
Provided screen results although a confirmatory assay was performed.

HV07, HV17
Incorrect Final Status: Using 4th generation Ab/Ag test, final status missing Ag.

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Final Report for Panel HIVSER 2016Apr21

Issued 2016-06-02

Introduction

The NLHRS distributed the 2016Apr21 panel on April 4th 2016. The 2016Oct20 panel will be shipped the first week of October 2016. This final report is publicly available, however the identity of participants is not disclosed.

Panel Samples, HIV Test Kits and Data Entry

- *Panel Composition*
  - Panel 2016Apr21 Blue: Five samples; two HIV negative (B, E), two HIV-1 positive (A, C) and one HIV-2 positive (D) sample. Sent to labs HV01, HV02, HV13, HV15, HV16, HV18, HV19, HV20, HV21, HV24, HV75.
  - Panel 2016Apr21 Regular: Five samples; two HIV negative (B, E) and three HIV-1 positive (A, C, D). Sent to labs HV03, HV04, HV05, HV07, HV12, HV14, HV17, HV22, HV23, HV26, HV27, HV28, HV30, HV31, HV43, HV44, HV45, HV48, HV49, HV50, HV53, HV54, HV55, HV56, HV57, HV59, HV63, HV64, HV68, HV74, HV75, HV76, HV79.
  - The blue panel was unique because sample D was HIV-2 Ab positive.
  - Testing and characterization by the NLHRS prior to shipment are presented in Appendix 2. Panels were sent to 43 participants including the NLHRS April 4th, 2016. The deadline for data entry was April 21st, 2016.

- *HIV Test Kits* – Eleven different assays were used by the 42 participants excluding the NLHRS who returned results (Table 1, Figure 1). The majority of participants, 83% (35/42) reported using one screen test only. Of these 35 participants, 88% (31/35) used 4th generation assays. Six participants continue to use 3rd generation assays which raises potential issues with the ability of labs to detect acute infections. Eight labs are running an HIV-1 only confirmatory assay which raises potential issues with the ability of labs to detect HIV-2 infections.

- *Data entry* - The NLHRS Quality Assessment Program used the web based Survey Monkey system to capture results.

Table 1: Summary of the assays used in the NLHRS 2015Oct22 and 2016Apr21 HIV Panels. (Excludes the NLHRS)			
Type	**Assay**	**# of Users**	
		2015Oct22	**2016Apr21**
Screen – 4th Generation	Abbott ARCHITECT HIV Ag/Ab Combo CMIA	29	31
	Abbott AxSYM HIV Ag/Ab Combo MEIA	2	--
	Roche Elecsys HIV Combi ECLIA	2	2
	Siemens ADVIA Centaur HIV Ag/Ab Combo (CHIV) ChLIA Assay	2	2
Screen – 3rd Generation	Bio-Rad GS HIV-1/HIV-2 PLUS O EIA	2	2
	Abbott AxSYM HIV HIV 1/2 gO MEIA	1	--
Screen – Rapid	bioLytical INSTI HIV-1/HIV-2 Antibody Test Kit	4	4
Screen – HIV-2	Bio-Rad Genetic Systems HIV-2 EIA	1	2
Confirmatory – p24	bioMerieux VIDAS HIV p24 II ELFA	2	2
	Bio-Rad Genscreen HIV-1 Ag EIA	1	1
Confirmatory	Bio-Rad Multispot HIV-1/2 Rapid Test	1	1
	Genetic Systems HIV-1 Western Blot	6	8
	Fujirebio INNO-LIA HIV I/II Score	1	1

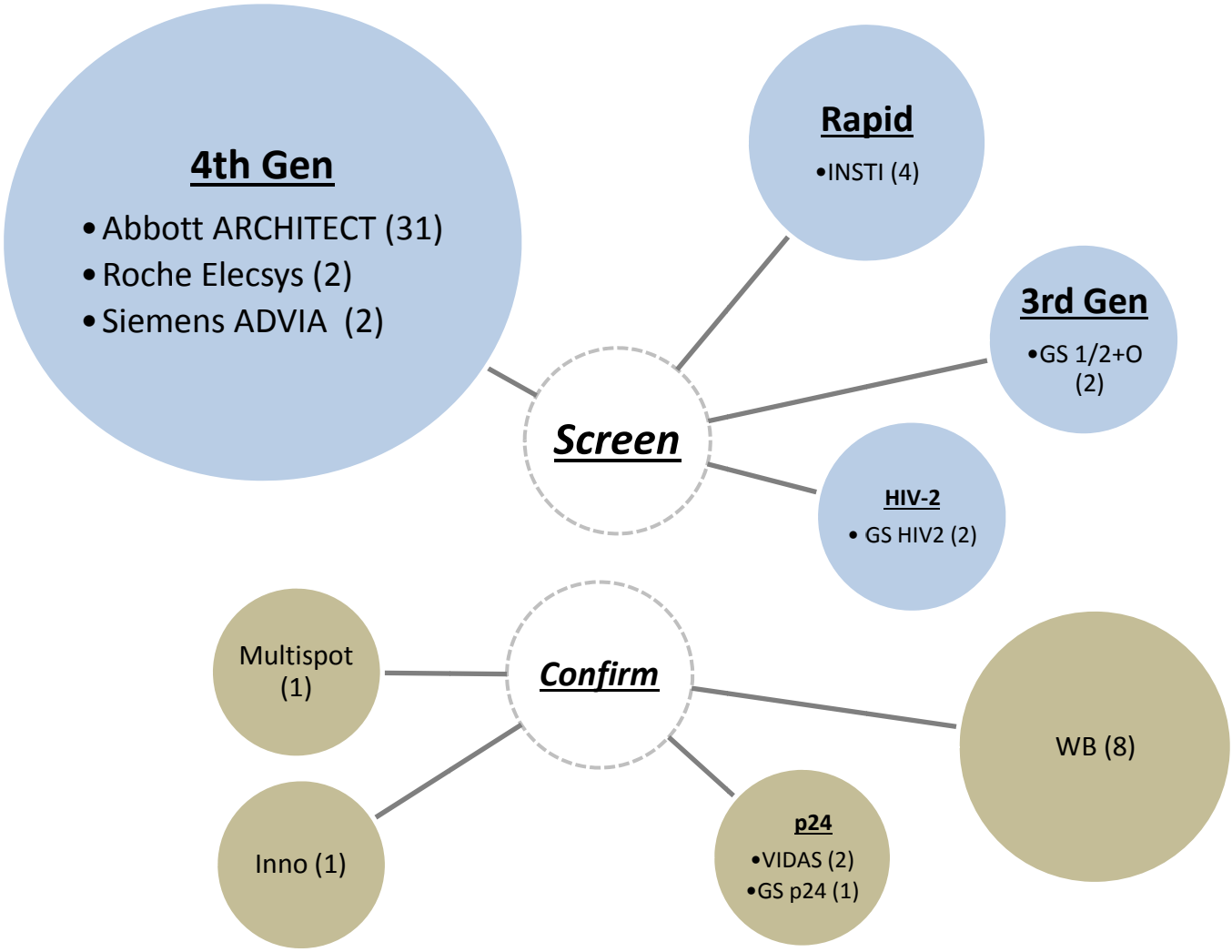

Figure 1: Breakdown of the assays used by the 42 participants in the NLHRS 2016Apr21 HIV Panel *(Excludes the NLHRS)*

Results

- *Return rate* - Results were returned from 100% of participants (42/42).

- *Group Analysis*

  Panel 2016Apr21 Blue (Table 2)

  - *Sample A (HIV-1 Ab positive)* – All participants correctly identified the sample.

    10/11 participants provided either a correct serology status and/or recommendation.

    HV02: Ran a confirmatory assay but gave final interpretation based on the screen test only.
  - *Sample B (HIV-1/2 Ab/Ag negative)* – All participants correctly identified the sample.
  - *Sample C (HIV-1 Ab positive)* – All participants correctly identified the sample.

    10/11 participants provided either a correct serology status and/or recommendation.

    HV02: Ran a confirmatory assay but gave final interpretation based on the screen test only.
  - *Sample D (HIV-2 Ab positive)* – **3/10** participants correctly identified the sample.

    10/11 participants provided either a correct serology status and/or recommendation.

    HV01, HV02: Ran a confirmatory assay but gave final interpretation based on the screen test only.
  - *Sample E (HIV-1/2 Ab/Ag negative)* – All participants correctly identified the sample.

Table 2: 2016Apr21 Blue Panel final status reported from participants.

LAB	SAMPLE A HIV-1 Positive	SAMPLE B Negative	SAMPLE C HIV-1 Positive	SAMPLE D HIV-2 Positive	SAMPLE E Negative
HV01	HIV-1 Ab positive	HIV-1/2 Ag/Ab negative	HIV-1 Ab positive	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative
HV02	HIV-1/2 Ag/Ab positive	Non-Reactive	HIV-1/2 Ag/Ab positive	HIV-1/2 Ag/Ab positive[1]	Non-Reactive
HV13	HIV-1/2 Ab positive[1]	HIV-1/2 Ab negative	HIV-1/2 Ab positive[1]	HIV-2 Ab positive[1] HIV-1 Ag negative	HIV-1/2 Ab negative
HV15	HIV-1 Ab positive	HIV-1/2 Ab negative	HIV-1 Ab positive	HIV-2 Ab positive[1]	HIV-1/2 Ab negative
HV16	HIV-1 Ab positive	HIV-1/2 Ag/Ab negative	HIV-1 Ab positive	HIV-1/2 Ab indeterminate[1] HIV-1 Ag negative	HIV-1/2 Ag/Ab negative
HV18	HIV-1 Ab positive	HIV-1/2 Ag/Ab negative HIV-1 Ag negative	HIV-1 Ab positive	HIV-1/2 Ag/Ab indeterminate[1]	HIV-1/2 Ag/Ab negative HIV-1 Ag negative
HV19	HIV-1 Ab positive[1]	HIV-1/2 Ag/Ab negative	HIV-1 Ab positive[1]	HIV-2 Ab positive[1] HIV-1 Ag negative	HIV-1/2 Ag/Ab negative
HV20	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative[1]	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ab positive[1]	HIV-1/2 Ag/Ab negative[1]
HV21	HIV-1 Ab positive[1]	HIV-1/2 Ag/Ab negative	HIV-1 Ab positive[1]	HIV-1 Ab indeterminate[1]	HIV-1/2 Ag/Ab negative
HV24	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative
HV75	HIV-1 Ab positive HIV-1 Ag negative	HIV-1/2 Ab negative HIV-1 Ag negative	HIV-1 Ab positive HIV-1 Ag negative	HIV-1/2 Ab positive-untypable[1] HIV-1 Ag negative	HIV-1/2 Ab negative HIV-1 Ag negative

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

Results *(continued)*

Panel 2016Apr21 Regular (Table 4)

- *Sample A (HIV-1 Ab positive)* – All participants correctly identified the sample.
  29/33 participants provided either a correct serology status and/or recommendation.
  - **HV07,HV17:** Final status for Antibody only instead of Antibody/Antigen (ran 4th gen).
  - **HV53:** Final status of Not Tested but made a recommendation.
  - **HV79:** Final status for Antibody/Antigen instead of Antibody only (ran 3rd gen).
- *Sample B (HIV-1/2 Ab negative)* – All participants correctly identified the sample.
  29/33 participants provided either a correct serology status and/or recommendation.
  - **HV17:** Final status for Antibody only instead of Antibody/Antigen (ran 4th gen).
  - **HV44:** Conflicting final status; HIV positive and HIV negative.
  - **HV53:** Final status of Not Tested with no recommendation.
  - **HV79:** Final status for Antibody/Antigen instead of Antibody only (ran 3rd gen).
- *Sample C (HIV-1 Ab positive)* – All participants correctly identified the sample.
  29/33 participants provided either a correct serology status and/or recommendation.
  - **HV07,HV17:** Final status for Antibody only instead of Antibody/Antigen (ran 4th gen).
  - **HV53:** Final status of Not Tested but made a recommendation.
  - **HV79:** Final status for Antibody/Antigen instead of Antibody only (ran 3rd gen).
- *Sample D (HIV-1 Ab positive)* – All participants correctly identified the sample.
  29/33 participants provided either a correct serology status and/or recommendation.
  - **HV07,HV17:** Final status for Antibody only instead of Antibody/Antigen (ran 4th gen).
  - **HV53:** Final status of Not Tested but made a recommendation.
  - **HV79:** Final status for Antibody/Antigen instead of Antibody only (ran 3rd gen).
- *Sample E (HIV-1/2 Ab negative)* – All participants correctly identified the sample.
  31/33 participants provided either a correct serology status and/or recommendation.
  - **HV17:** Final status for Antibody only instead of Antibody/Antigen (ran 4th gen).
  - **HV53:** Final status of Not Tested with no recommendation.
  - **HV79:** Final status for Antibody/Antigen instead of Antibody only (ran 3rd gen).

Discussion

- *HIV-2 Positive Sample (Blue panel sample D)*

  Labs running the western blot were unable to correctly identify the HIV-2 positive sample. This provides a great challenge in correctly diagnosing an HIV-2 positive sample. The Inno-LIA was able to correctly identify the sample while the Geenius identified it as positive but was unable to differentiate between HIV-1 and HIV-2.

Table 3: Confirmatory Assay results for HIV-2 Ab positive sample (Blue panel sample D)

Assay	# of Labs	Result
Western Blot	4	Negative
	3	Indeterminate
Inno-LIA	2	HIV-2 Positive
Multispot	1	HIV-2 Positive
Geenius	1	HIV positive, untypable

Discussion *(continued)*

- *Using Incorrect Final Status Terminology:* **HV07, HV17, HV79**

  Labs continue to provide interpretations that are not accurate based on the assays being used.
  - Labs using only a 4th generation screen assay should provide a final status of HIV-1/2 Ag/Ab;
  - Labs using only a 3rd generation screen assay should provide a final status of HIV-1/2 Ab.

Table 4: 2016Apr21 Regular HIV Panel final status reported from participants.

LAB	SAMPLE A HIV-1 Positive	SAMPLE B Negative	SAMPLE C HIV-1 Positive	SAMPLE D HIV-1 Positive	SAMPLE E Negative
HV03	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative
HV04	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative
HV05	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative
HV07	HIV-1/2 Ab positive[1]	HIV-1/2 Ag/Ab negative	HIV-1/2 Ab positive[1]	HIV-1/2 Ab positive[1]	HIV-1/2 Ag/Ab negative
HV12	Would not report[1]	HIV-1/2 Ag/Ab negative	Would not report[1]	Would not report[1]	HIV-1/2 Ag/Ab negative
HV14	Would not report[1]	HIV-1/2 Ag/Ab negative	Would not report[1]	Would not report[1]	HIV-1/2 Ag/Ab negative
HV17	HIV-1/2 Ab positive[1]	HIV-1/2 Ab negative	HIV-1/2 Ab positive[1]	HIV-1/2 Ab positive[1]	HIV-1/2 Ab negative[1]
HV22	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative
HV23	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative
HV26	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative
HV27	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative
HV28	HIV-1/2 Ab positive[1]	HIV-1/2 Ab negative[1]	HIV-1/2 Ab positive[1]	HIV-1/2 Ab positive[1]	HIV-1/2 Ab negative[1]
HV30	HIV-1/2 Ab positive[1]	HIV-1/2 Ab negative[1]	HIV-1/2 Ab positive[1]	HIV-1/2 Ab positive[1]	HIV-1/2 Ab negative[1]
HV31	HIV-1/2 Ab positive[1]	HIV-1/2 Ab negative[1]	HIV-1/2 Ab positive[1]	HIV-1/2 Ab positive[1]	HIV-1/2 Ab negative[1]
HV43	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative
HV44	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative *and* HIV-1/2 Ag/Ab positive	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative
HV45	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative
HV48	Would not report[1]	HIV-1/2 Ag/Ab negative	Would not report[1]	Would not report[1]	HIV-1/2 Ag/Ab negative
HV49	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative
HV50	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative
HV53	Not Tested[1]	Not Tested	Not Tested[1]	Not Tested[1]	Not Tested
HV54	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative
HV55	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative
HV56	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative
HV57	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative
HV59	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative
HV63	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative
HV64	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative
HV68	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative
HV74	HIV-1/2 Ab positive[1]	HIV-1/2 Ab negative	HIV-1/2 Ab positive[1]	HIV-1/2 Ab positive[1]	HIV-1/2 Ab negative[1]
HV75	HIV-1 Ab positive HIV-1 Ag negative	HIV-1/2 Ab negative HIV-1 Ag negative	HIV-1 Ab positive HIV-1 Ag negative	HIV-1 Ab positive HIV-1 Ag positive	HIV-1/2 Ab negative HIV-1 Ag negative
HV76	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative
HV79	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative[1]	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab positive[1]	HIV-1/2 Ag/Ab negative[1]

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

Conclusion

1. The publication of the CLSI M53 HIV testing guideline is anticipated to have a major impact in industrialized countries including Canada. The strength of these guidelines addresses several weaknesses from the original 1989 guidelines, which included the inability to diagnose acute infections, discriminate HIV-2 and the poor performance of the HIV-1 Western Blot.

2. Most Canadian laboratories use the 4th generation EIA screen test, however, the six labs that continue to use 3rd generation assays as their primary/only screening method could miss a pre-seroconversion sample as previously demonstrated (Kadivar *et al.* J Clin Virol. 2013 and Panels 2015Apr23, 2014Oct23, 2013Oct24 and 2013Apr25).

3. At the recent CAHCLS meeting which include the provincial public health lab directors and the NLHRS, many of the labs performing Western blot testing will be switching to the recently approved Bio-Rad HIV Geenius assay.

4. The Geenius assay represents a significant improvement compared to the Western Blot for the confirmation of 4th generation HIV antibody reactive samples and the ability to identify HIV-2. As an example, the HIV-2 sample present in this panel was negative on Western blot but detected on the Geenius (albeit untypable).

4. The next panel for HIV serology will take into consideration that there will be a shift to the Bio-Rad Geenius test from the Western blot in laboratories performing antibody confirmatory testing.

Proficiency testing programs are designed not only to test the examination stage but the overall process in patient sample testing. As outlined in Appendix 3, errors in laboratory and medical testing can also occur during the pre and post examination stages.

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

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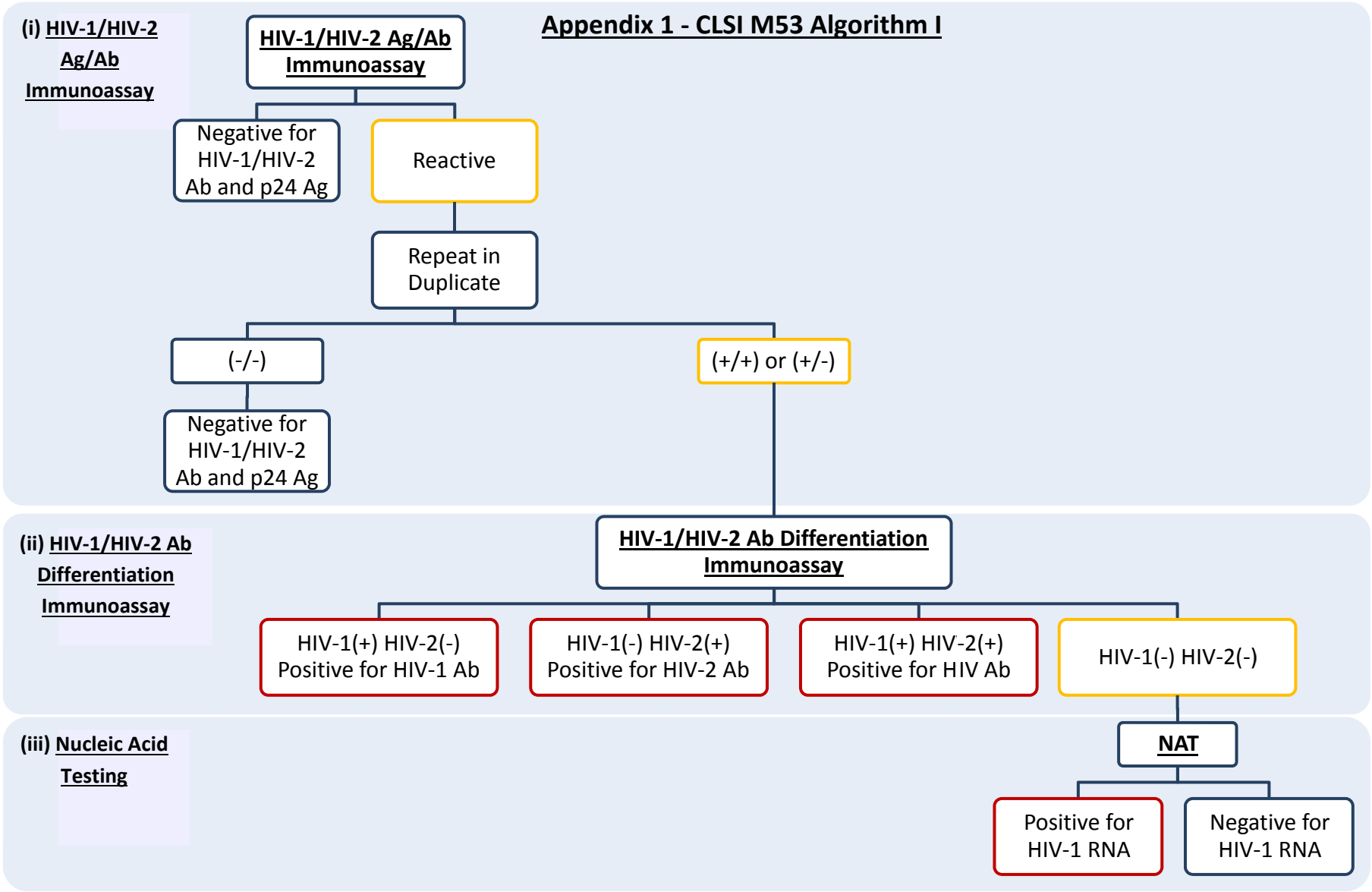

Appendix 1: Adaptation of the Clinical and Laboratory Standards Institute (CLSI) M53-*Criteria for Laboratory Testing and Diagnosis of Human Immunodeficiency Virus Infection: Approved Guideline* Algorithm I.

Appendix 2: Characterization

Summary of NLHRS Characterization of the NLHRS 2016Apr21 HIV Panel Samples

The NLHRS 2016Apr21 HIV Panel Sample Serology Testing Results Note, Samples A, B, C and E were identical for both the regular and blue panels, only sample D was different.						
Sample		**A**	**B / E (Duplicate)**	**C**	**D (Regular Panel)**	**D (Blue Panel)**
		HIV-1 Positive	**Negative**	**HIV-1 Positive**	**HIV-1 Positive**	**HIV-2 Positive**
Final Status		HIV-1 Ab Positive	HIV-1/2 Ag/Ab Negative	HIV-1 Ab Positive	HIV-1 Ab Positive HIV-1 Ag Positive	HIV-2 Ab Positive
bioLytical INSTI HIV-1/2 Rapid Test	**Result**	R	NR	R	R	R
Bio-Rad GS HIV-1 p24	**Result**	Neg	Neg	Neg	Pos	Neg
Bio-Rad GS HIV-1 Western Blot	**Result**	Pos	Neg	Pos	Pos	Ind
	gp160	++	-	++	++	+/-
	gp120	++	-	++	++	-
	p65	++	-	++	+	-
	p55	++	-	++	++	-
	p51	++	-	++	+	-
	gp41	++	-	++	++	-
	p40	+	-	+	++	-
	p31	++	-	++	+/-	+/-
	p24	++	-	++	++	-
	p18	+/-	-	+/-	+	-
Fujirebio INNO-LIA HIV-I/II Score	**Result**	HIV-1	Negative	HIV-1	HIV-1	HIV-2
	sgp120	+++	-	+++	+++	-
	gp41	+++	-	+++	+++	+/-
	p31	+++	-	+++	-	-
	p24	+	-	+++	+++	-
	p17	++	-	++	+	+
	sgp105	-	-	-	-	+
	gp36	-	-	-	-	+++
Bio-Rad Geenius HIV-1/HIV-2 Supplemental Assay	**Result**	HIV-1	Negative	HIV-1	HIV-1	HIV untypable
	gp36	-	-	-	-	+
	gp140	-	-	-	-	+
	p31	+	-	+	-	-
	gp160	+	-	+	+	+
	p24	-	-	+	+	-
	gp41	+	-	+	+	+
Inhouse RIPA	**Result**	Ind	Neg	Pos	Pos	Ind

Appendix 3: 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.