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

HTLV Serology Quality Assessment Program

Summary for Panel HTLVSER 2016Apr21

Panel Sample	True Status	Labs Reporting Incorrect Status	
A	HTLV-II Positive		
B	HTLV Indeterminate	**Indeterminate with no recommendation**	**HV15**
C	HTLV-I/II Negative		
D	HTLV-I Positive	**Incorrect Final Status**	**HV15** **HV44**
E	HTLV-I/II Negative		

Incorrect interpretations based on their assay result(s):

- **HV15**
  Indeterminate final status for Samples B & D and made no recommendation.
- **HV16**
  Sample B: Indeterminate final status but made a recommendation.
- **HV18**
  Sample A, B, D: Did not provide a final status but made a recommendation.
- **HV21**
  Sample D: Unable to complete testing but made a recommendation.
- **HV22**
  Sample E: Unable to complete testing but made a recommendation.
- **HV44**
  Incorrect diagnosed Sample D as HTLV Negative.
- **HV55**
  Selected confirmatory assay INNO-LIA but did not submit results; possible data entry error.

Legend:

- Flagged: Incorrect Result.
- Flagged: Unresolved sample with no recommendation.
- No Flag (of interest): Unresolved sample but made a recommendation for further testing.

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Final Report for Panel HTVLSER 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, HTLV Test Kits and Data Entry

- *Panel Composition* – Panel 2016Apr21 consisted of five samples; two HTLV negative samples (C, E), one HTLV-I positive sample (D), one HTLV-II positive sample (A) and one indeterminate sample (B). Sample B was included to see how participants would report a challenging sample. Testing and characterization by the NLHRS are presented in Appendix 1. Panels were prepared and sent to 15 participants including the NLHRS April 4th, 2016. The deadline for data entry was April 21st, 2016.

- *HTLV Test Kits* – Three different assays were used by the 14 participants excluding the NLHRS (Table 1, Figure 1). The majority of participants, 86% (12/14) performed screen testing only. One laboratory performed confirmatory testing in the absence of a screen test. One lab (HV55) said they performed the INNO-LIA but no results were returned, possible transcription error. No participants used expired kits.

Table 1: Summary of the assays used in the NLHRS 2015Oct22 and 2016Apr21 HTLV Panels. (Excludes the NLHRS)

Type	Assay	# of Users		Use of Additional QC Material (2016Apr21)	
		2015Apr23	2015Oct22	Yes	No
Screen	Abbott ARCHITECT rHTLV-I/II CMIA	13	13	5	7
Confirmatory	Fujirebio INNO-LIA HTLV I/II Score	1*	1	1	-
	MP Diagnostics HTLV BLOT 2.4 WB	1	1	1	-

* HV55 said they perform the INNO-LIA but returned no results, they were not included in the count.

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

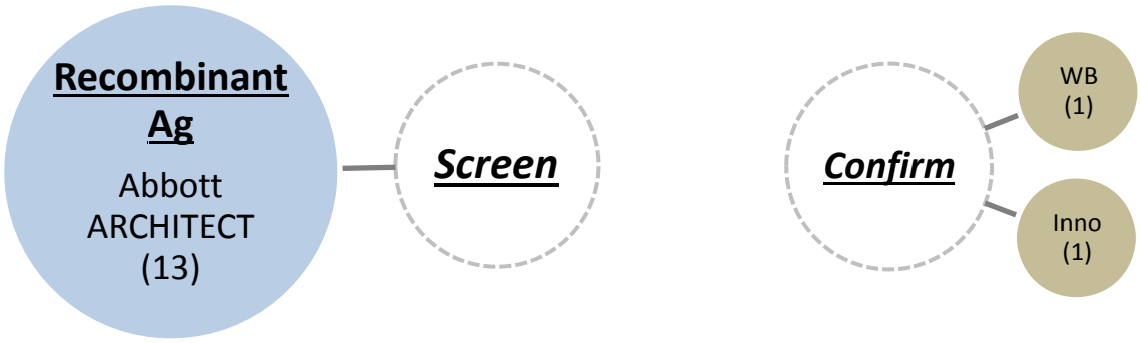

Figure 1: Breakdown of the assays used by the 14 participants in the NLHRS 2016Apr21 HTLV Panel (Excludes the NLHRS).

Results

- *Return rate* - Results were returned from 100% of participants (14/14).

- *Qualitative Group Analysis* (Table 2)
  - *Sample A (HTLV-II positive)* – All participants correctly identified the sample.
    14/14 participants provided either a correct serology status and/or recommendation.
    - **HV18**: Did not provide a final status but made a recommendation.
  - *Sample B (HTLV indeterminate)* – All participants correctly identified the sample.
    13/14 participants provided either a correct serology status and/or recommendation.
    - **HV15:** Had a final result of indeterminate and made no recommendation.
    - **HV16:** Had a final result of indeterminate but made no recommendation.
    - **HV18:** Did not provide a final status but made a recommendation.
  - *Sample C (HTLV negative)* – All participants correctly identified the sample.
    14/14 participants provided either a correct serology status and/or recommendation.
  - *Sample D (HTLV negative)* – 12/14 correctly identified the sample.
    12/14 participants provided either a correct serology status and/or recommendation.
    - **HV15:** Had a final result of indeterminate and made no recommendation.
    - **HV18:** Did not provide a final status but made a recommendation.
    - **HV21:** Had insufficient volume to complete their testing but made a recommendation.
    - **HV44:** Had a negative result for this positive sample.
  - *Sample E (HTLV-II positive)* – All participants correctly identified the sample.
    14/14 participants provided either a correct serology status and/or recommendation.
    - **HV22:** Had insufficient volume to complete their testing but made a recommendation.

Legend:
- Flagged: Incorrect Result.
- Flagged: Unresolved sample with no recommendation.
  - Note: Unresolved sample but made a recommendation for further testing.

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

LAB	SAMPLE A HTLV-II Positive	SAMPLE B Indeterminate	SAMPLE C Negative	SAMPLE D HTLV-I Positive	SAMPLE E Negative
HV01	HTLV-I/II Ab positive[1]	HTLV-I/II Ab positive[1]	HTLV-I/II Ab Negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab Negative
HV02	HTLV-I/II Ab positive[1]	HTLV-I/II Ab positive[1]	HTLV-I/II Ab Negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab Negative
HV03	HTLV-I/II Ab positive[1]	HTLV-I/II Ab positive[1]	HTLV-I/II Ab Negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab Negative
HV15	HTLV-II Ab positive	HTLV-I/II Ab Ind	HTLV-I/II Ab Negative	HTLV-I/II Ab Ind	HTLV-I/II Ab Negative
HV16	HTLV-II Ab positive	HTLV-I/II Ab Ind[1]	HTLV-I/II Ab Negative	HTLV-I Ab positive	HTLV-I/II Ab Negative
HV17	HTLV-I/II Ab positive[1]	HTLV-I/II Ab positive[1]	HTLV-I/II Ab Negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab Negative
HV18	No Status Provided[1]	No Status Provided[1]	HTLV-I/II Ab Negative	No Status Provided[1]	HTLV-I/II Ab Negative
HV20	HTLV-I/II Ab positive[1]	HTLV-I/II Ab positive[1]	HTLV-I/II Ab Negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab Negative
HV21	HTLV-I/II Ab positive[1]	HTLV-I/II Ab positive[1]	HTLV-I/II Ab Negative	Unable to Participate[1]	HTLV-I/II Ab Negative
HV22	HTLV-I/II Ab positive[1]	HTLV-I/II Ab positive[1]	HTLV-I/II Ab Negative	HTLV-I/II Ab positive[1]	Not Tested[1]
HV44	HTLV-I/II Ab positive[1]	HTLV-II Ab positive	HTLV-I/II Ab Negative	HTLV-I/II Ab Negative	HTLV-I/II Ab Negative
HV50	HTLV-I/II Ab positive[1]	HTLV-II Ab positive	HTLV-I/II Ab Negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab Negative
HV55	HTLV-I/II Ab positive[1]	HTLV-I/II Ab positive[1]	HTLV-I/II Ab Negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab Negative
HV76	HTLV-I/II Ab positive[1]	HTLV-I/II Ab positive[1]	HTLV-I/II Ab Negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab Negative

[1] Further action required by participant; "Refer for further HTLV testing".

Discussion

- Twelve participants returned the correct result for all samples in the 2016Apr2016 panel.
- The screening labs were all able to detect the indeterminate sample (B). Both labs running confirmatory assays had a final result of indeterminate. Only HV16 made a recommendation for further testing while HV15 made no recommendation for both its indeterminate samples.
- HV22 had one false negative.
- Participants were surveyed on their use of Quality Control (QC) reagents in addition to those included in the commercial kits (Table 1). Half of participants (50%) reported using additional QC material on their assays.

Conclusion

Proficiency testing programs are designed not only to test the examination stage but the overall process in patient sample testing. As outlined in Appendix 2, errors in laboratory and medical testing can also occur during the pre-examination stage which includes all elements related to specimen collection.

The quality of HTLV antibody testing overall in Canada remains very high.

Thank you for your participation in the NLHRS Quality Assurance Program

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Appendix 1: Characterization

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

The NLHRS 2016Apr21 HTLV Panel Sample Testing Results									
Sample	**Final Status**	**NLHRS Testing**							
		Fujirebio INNO-LIA HTLV I/II Score							
		Interpretation	p19 I/II	p24 I/II	gp46 I/II	gp21 I/II	p19 I	gp46 I	gp46 II
A	HTLV-II Positive	HTLV-II	+	+	++	++	-	-	++
B	HTLV Indeterminate	HTLV Indeterminate	-	-	-	++	-	-	-
C	HTLV-I/II Negative	Neg	-	-	-	-	-	-	-
D	HTLV-I Positive	HTLV-I	-	-	+	+	-	+	-
E	HTLV-I/II Negative	Neg	-	-	-	-	-	-	-

N/T: Not tested

Appendix 2: 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.