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 2017Apr19

2017Apr19 HTLV Serology Panel		
Panel Sample	**True Status**	**Labs Reporting Incorrect Status**
A	Negative	
B	HTLV-II Ab Positive	
C	Negative	
D	HTLV-I Ab Positive	
E	HTLV-I Ab Positive	

All participants were able to provide either the correct serology status and/or recommendation

Errors observed based on results submitted(s):

No errors were observed based on results submitted by all participants.

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Final Report for Panel HTLVSER 2017Apr19

Issued 2017-06-27

Introduction

The NLHRS distributed the 2016Oct28 panel and the 2017Apr19 panel on October 12th 2016. This report is specific to the 2017Apr19 panel only and 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 2017Apr19 consisted of five samples; two HTLV negative samples (A, C), two HTLV-I positive samples (D, E) and one HTLV-II positive sample (B). Testing and characterization by the NLHRS are presented in Appendix 1. Panels were prepared and sent to 15 participants including the NLHRS on October 12th, 2016. The deadline for results submission for panel 2017Apr19 was April 19th, 2017.

- *HTLV Test Kits* – Three different assays were used by the 14 participants excluding the NLHRS (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.

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

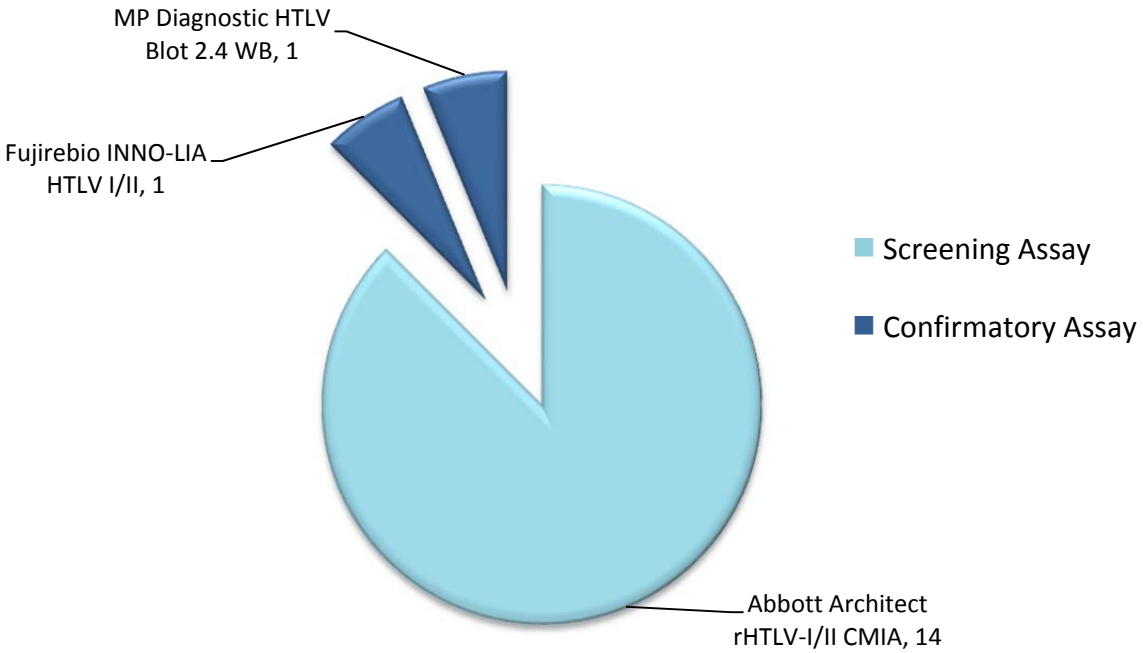

Figure 1: Breakdown of the assay used by the participants in the NLHRS 2017Apr19 HTLV serology panel

(Excludes the NLHRS)

Results

- *Return rate* - Results were returned from 100% of participants (15/15).

- *Qualitative Group Analysis* (Table 1)

  Starting with the 2017Apr19 panel and onward, the NLHRS will no longer flag participant that reported without a serology status but made a recommendation for further action.

  - *Sample A (HTLV-I/II Ab negative)* – All participants correctly identified the sample. 15/15 participants provided either a correct serology status and/or recommendation
  - *Sample B (HTLV-II Ab positive)* – All participants provided either a correct serology status and/or recommendation
  - *Sample C (HTLV-I/II Ab negative)* – All participants correctly identified the sample. 15/15 participants provided either a correct serology status and/or recommendation.
  - *Sample D (HTLV-I Ab positive)* – All participants provided either a correct serology status and/or recommendation
  - *Sample E (HTLV-I Ab positive)* – All participants provided either a correct serology status and/or recommendation.

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

Table 1: 2017Apr19 HTLV Panel final status reported from participants (excludes the NLHRS).					
LAB	**SAMPLE A** **Negative**	**SAMPLE B** **HTLV-II Ab Positive**	**SAMPLE C** **Negative**	**SAMPLE D** **HTLV-I Ab Positive**	**SAMPLE E** **HTLV-I Ab Positive**
HV01	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab positive[1]
HV02	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab positive[1]
HV03	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab positive[1]
HV12	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab positive[1]
HV15	HTLV-I/II Ab negative	HTLV-II Ab positive	HTLV-I/II Ab negative	HTLV-I Ab positive	HTLV-I Ab positive
HV16	HTLV-I/II Ab negative	HTLV-II Ab positive	HTLV-I/II Ab negative	HTLV-I Ab positive	HTLV-I Ab positive
HV17	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab positive[1]
HV18	HTLV-I/II Ab negative	No status provided[1]	HTLV-I/II Ab negative	No status provided[1]	No status provided[1]
HV20	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab positive[1]
HV21	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab positive[1]
HV22	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]	HTLV-I/II AB positive[1]
HV44	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab positive[1]
HV50	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab positive[1]
HV55	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab positive[1]
HV76	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab positive[1]

[1] Further action recommended by participant; "Refer for further HTLV testing or request follow-up samples".

Table 2: Level of the different flags and the causes of 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 resulted in misinterpretation of the true status of the sample, unresolved status but made a recommendation

External QC and QA activities

1. *External quality control (QC) material* - Used in addition to controls provided in kits allows users to detect technical problems and assay sensitivity from lot to lot.
   - Six participants (40%, 6/15) reported using external QC material, a decrease from last year (53.3%, 8/15)

2. *Quality Assurance (QA) programs* - Allow participants to evaluate their overall use of the assay and reporting of the results. One participant provided no response.
   - Eleven participants (73.3%, 11/15) reported participation in QA programs other than the NLHRS panels, a noticeable increase from last year (53.3%, 8/15).

Discussion

All participants returned the correct result for all samples in the 2017Apr19 panel. Participants were able to detect the HTLV-II in sample B. The two participants performing the confirmatory testing were able to differentiated sample B as an HTLV-II positive. The NLHRS noticed there is a decrease in the number of participants reporting the use of external control in their assay. The NLHRS would like to encourage participants to use external controls when performing their assay as it help to detect technical problems and the potential variability between different reagent lots. It is to be noted that the NLHRS will no longer flag participant who reported without a serology status by recommend further action by the participant.

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 2017Apr19 HTLV Panel Samples

The NLHRS 2017Apr19 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-I/II Ab negative	Negative	-	-	-	-	-	-	-
B	HTLV-II Ab positive	HTLV-II Ab Positive	+/-	+/-	++	++	-	-	++
C	HTLV-I/II Ab negative	Negative	-	-	-	-	-	-	-
D	HTLV-I Ab positive	HTLV-I Positive	++	++	++	++	+	++	-
E	HTLV-I Ab positive	HTLV-I Positive	++	+/-	++	++	+	++	-

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.