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 2018Apr19

2018Apr19 HTLV Serology Panel		
Panel Sample	**True Status**	**Labs Reporting Incorrect Status**
A	Negative	
B	HTLV-II Ab Positive	
C	HTLV-I Ab Positive	
D	Negative	
E	HTLV-I Ab Positive	

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

All participants reported the correct final status for all samples in the 2018Apr19 HTLV serology panel.

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Final Report for Panel HTVLSER 2018Apr19

Issued August-15-2018

Introduction

The NLHRS distributed the 2017Oct27 and 2018Apr19 panels on October 11th 2017. This final report is specific to the 2018Apr19 panel only and it is publicly available; however the identity of participants is not disclosed.

Panel Samples, HTLV Test Kits and Data Entry

- *Panel Composition* – Panel 2018Apr19 is the relabelled 2017Oct27 panel, consisting of five samples; two HTLV negative samples (A, D), two HTLV-I positive samples (C, 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 16 participants including the NLHRS on October 11th, 2017. The data entry deadline for the 2018Apr19 panel was April 19th, 2018.

- *HTLV Test Kits* – 4 different assays were used by the 16 participants excluding the NLHRS (Figure 1). The majority of participants, 86% (13/16) 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.

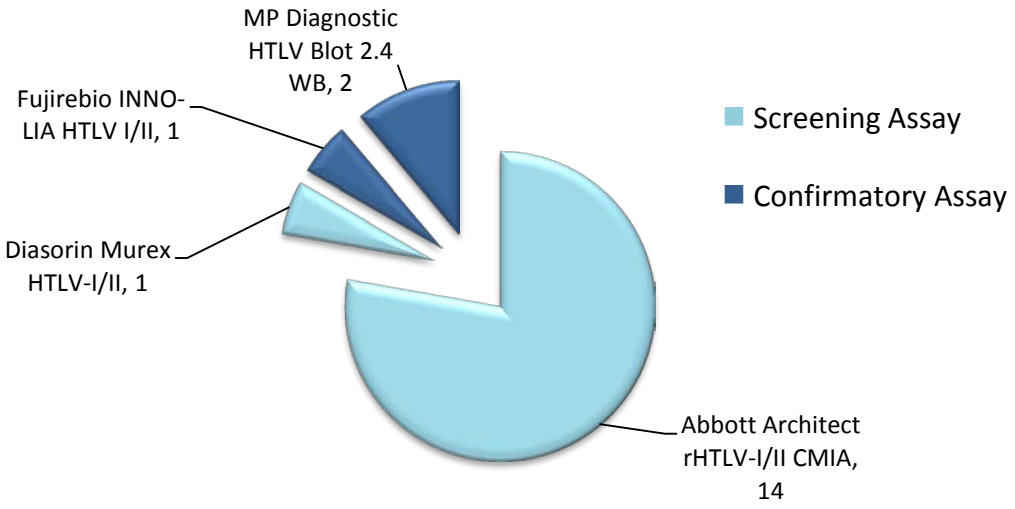

Figure 1: Assays used by the participants in the NLHRS 2018Apr19 HTLV serology panel (excludes the NLHRS)

Homogeneity and stability

- Panel members of the 2018Apr19 HTLV serology panel were randomly selected for testing by the NLHRS during the 2018Apr19 test event. The homogeneity and stability of the panel samples is assessed by comparing the 2018 results with the participant's 2017 results.
- There is no indication of heterogeneity or instability of the panel samples as the results submitted by the participants are consistent with the expected results from the NLHRS' characterization of each panel member (Table 1 and Appendix 1).

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.
   - 8 participants (50%, 8/16) reported using external QC material.

Figure 2: Sources of external quality control used in the 2018Apr19 HTLV serology panel

External QC and QA activities

2. *Quality Assurance (QA) programs*- Allows participants to evaluate their overall use of the assay and reporting of the results.
   - 13 participants (81.3%, 13/16) reported participation in other quality assurance programs (Figure 3).

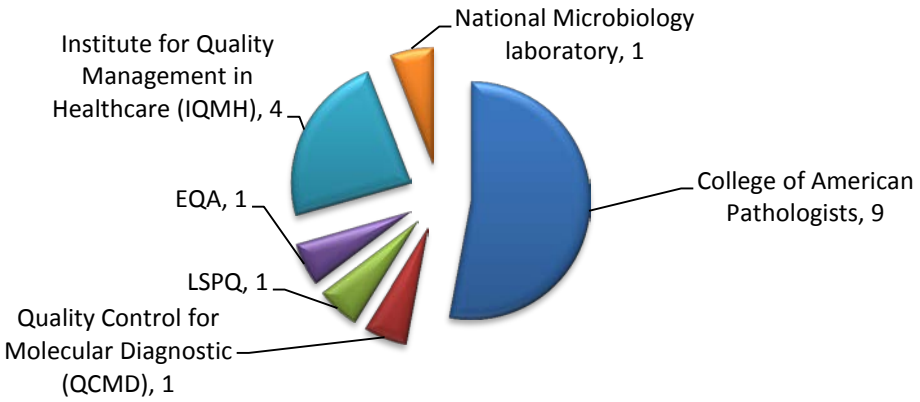

Figure 3: Distribution of external quality assurance programs which participants are enrolled in other than the NLHRS QAP

Participant's feedback

- 15 of 16 participants provided feedback for the 2018Apr19 HTLV serology panel. 14 participants liked the changes made to the survey compared to the previous iteration (Figure 4).
- Several areas of improvement for the next survey was identified by the participants (Figure 5).
- 4 participants were satisfied with the current format while 2 participants had no comments on areas the NLHRS could improve upon (Figure 5).

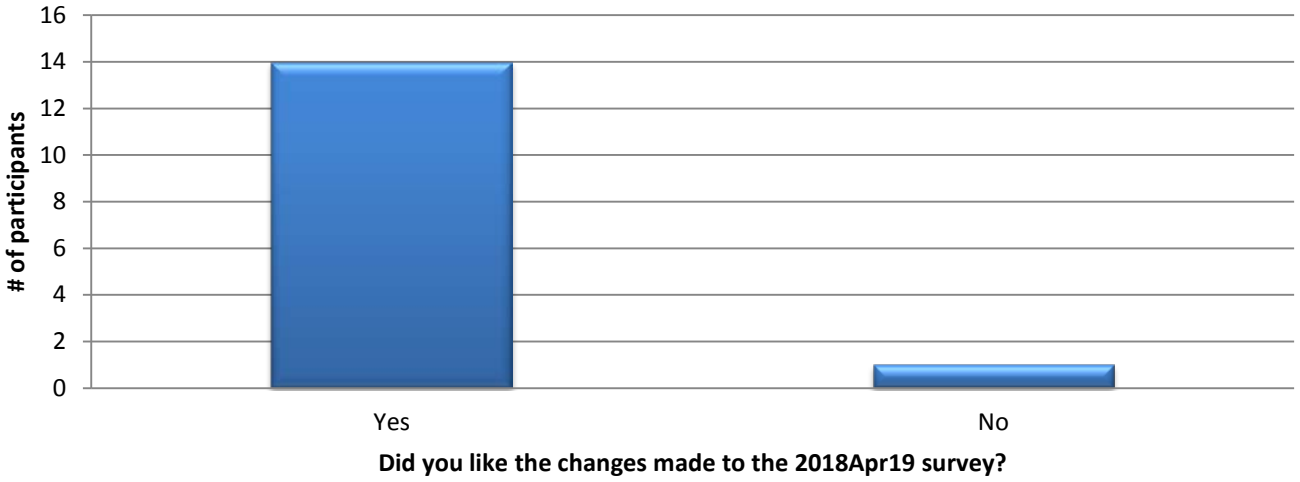

Figure 4: Participants' responses when ask if they liked the changes made to the 2018Apr19 survey

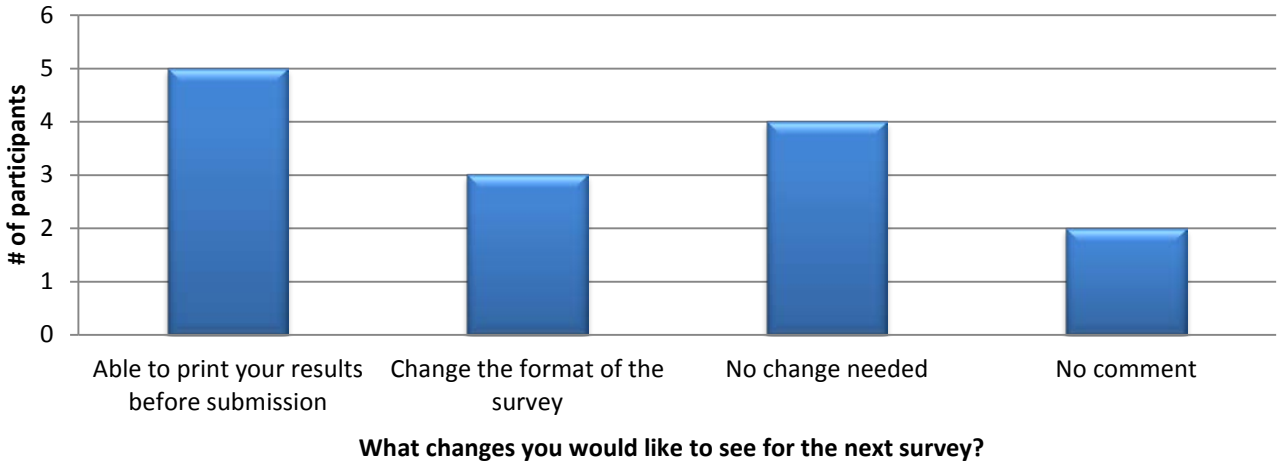

Figure 5: Participants' responses to which area requires improvement in the NLHRS HTLV serology survey

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

Table 1: 2018Apr19 HTLV Panel final status reported from participants (includes the NLHRS).					
LAB	**SAMPLE A** **Negative**	**SAMPLE B** **HTLV-II Ab Positive**	**SAMPLE C** **HTLV-I Ab Positive**	**SAMPLE D** **Negative**	**SAMPLE E** **HTLV-I Ab Positive**
HV01	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab positive[1]	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]
HV02	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab positive[1]	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]
HV03	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab positive[1]	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]
HV12	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab positive[1]	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]
HV15	HTLV-I/II Ab negative	HTLV-II Ab positive	HTLV-I Ab positive	HTLV-I/II Ab negative	HTLV-I Ab positive
HV16	HTLV-I/II Ab negative	HTLV-II Ab positive	HTLV-I Ab positive	HTLV-I/II Ab negative	HTLV-I Ab positive
HV17	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab positive[1]	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]
HV18	HTLV-I/II Ab negative	No status provided[1]	No status provided[1]	HTLV-I/II Ab negative	No status provided[1]
HV20	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab positive[1]	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]
HV21	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab positive[1]	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]
HV22	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab positive[1]	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]
HV44	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab positive[1]	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]
HV50	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab positive[1]	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]
HV55	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab positive[1]	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]
HV75	HTLV-I/II Ab negative	HTLV-II Ab positive	HTLV-I Ab positive	HTLV-I/II Ab negative	HTLV-I Ab positive
HV76	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab positive[1]	HTLV-I/II Ab negative	HTLV-I/II Ab positive[1]
HV80	HTLV-I/II Ab negative	HTLV-II Ab positive	HTLV-I Ab positive	HTLV-I/II Ab negative	HTLV-I Ab positive

[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

Results

- *Return rate* - 100% of the participants returned results by the deadline (16/16).

- *Qualitative Group Analysis* (Table 1)
  - *Sample A (HTLV-I/II Ab negative)* – All 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 Ab positive)* – All participants correctly either a correct serology status and/or recommendation
  - *Sample D (HTLV-I/II Ab negative)* – 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.

Discussion

The 2018Apr19 HTLV serology panel is the relabelled 2017Oct27 HTLV serology panel where the samples were rearranged into a different order. All participants were able to correctly identify the HTLV-I Ab positive and the HTLV-II Ab positive sample either through HTLV screening assay or HTLV confirmatory assay. Sample A and D were correctly identified as HTLV Ab negative as well. Hence, all participants are exhibiting consistent performance and good technical expertise in HTLV serology testing.

The NLHRS made several changes to the 2018Apr19 HTLV serology survey such as streamlining the results entry process and allowing the user to review the results entry before results submission as it was suggested by our participants. Several areas were identified for improvements, such as allowing the participants to print their results before submission. The NLHRS will take the feedback and suggestions into consideration to improve the overall HTLV serology quality assurance program.

Conclusion

The NLHRS would like to highlight the importance of running external quality control material in serological assays. External quality control material allows users to detect technical problems and lot to lot variations in the assay's sensitivity. The NLHRS would like to suggest to the participants that are currently not using external quality control material in their assay to consider implementing external quality control material into their workflow.

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.

We value each laboratory's participation in these QA panels and your suggestions for improvement. The NLHRS is committed to improve all aspect of the HTLV serology proficiency testing program in order to provide quality proficiency testing services to our participants.

If you would like to make an appeal, please submit your concerns to:

phac.nlhrs-lnsrv.aspc@canada.ca

Thank you for your participation in the NLHRS Quality Assurance Program

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Appendix 1: Characterization

Summary of NLHRS Characterization of the 2018Apr19 HTLV Panel Samples

The NLHRS 2018Apr19 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 Positive	++	+++	+++	++	-	-	+++
C	HTLV-I Ab positive	HTLV-I Positive	+++	+++	+++	+++	++	+++	-
D	HTLV-I/II Ab negative	Negative	-	-	-	-	-	-	-
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.