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 2019Apr16

2019Apr16 HTLV Serology Panel		
Panel Sample	**True Status**	**Labs Reporting Incorrect Status**
A	HTLV-I Ab Positive	
B	Negative	
C	HTLV-I Ab Positive	
D	Negative	
E	HTLV-II Ab Positive	

There were no incorrect results observed for the 2019Apr16 panel.

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Final Report for Panel HTVLSER 2019Apr16

Issued 2019- August-02

Introduction

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

Panel Samples, HTLV Test Kits, and Data Entry

- *Panel Composition*
  - 2019Apr16 is the relabelled 2018Oct26 HTLV Serology Panel, consisting of five samples; two HTLV negative (B, D), two HTLV-I positive (A, C), and one HTLV-II positive sample (E). Testing and characterization by the NLHRS are presented in Appendix 1. Panels were sent to 18 participants including the NLHRS on October 10th, 2018. The data entry deadline for the 2019Apr16 panel was April 16th, 2019.

- *HTLV Test Kits* – Six different assays were used by the 17 participants excluding the NLHRS (Figure 1). One participant has switched from using the Abbott Architect rHTLV-I/II CMIA to the Diapro HTLV-I/II Ab ELISA from Advanced Laboratory Diagnostic for HTLV screening test for this panel.

- *Data entry* - The NLHRS Quality Assessment Program (QAP) switched from the web based Survey Monkey system to an in-house developed website for results entry in this panel

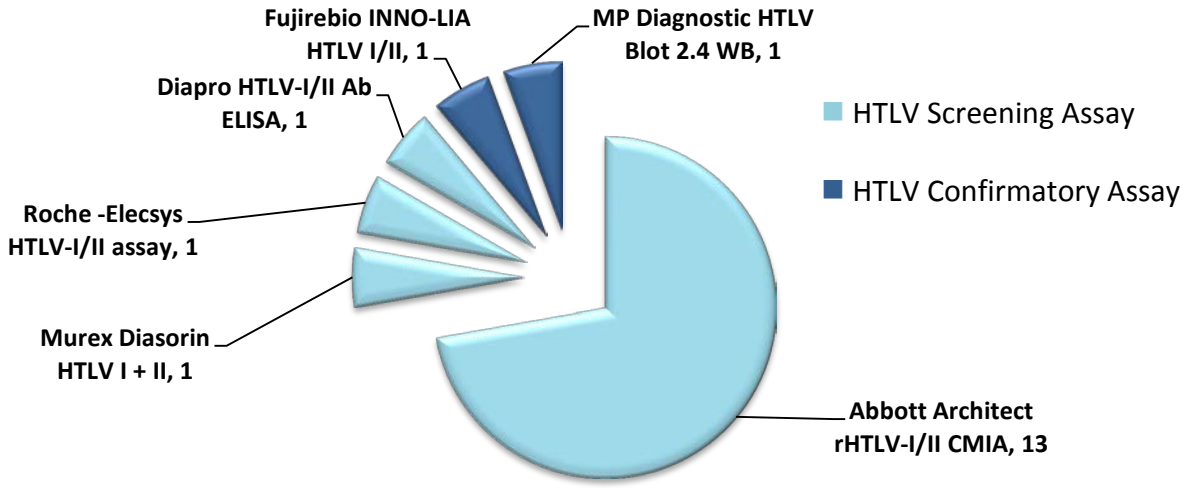

Figure 1: Assays used by the participants in the NLHRS 2019Apr16 HTLV serology panel (excludes the NLHRS).

Homogeneity and stability

- The homogeneity and stability of the 2019Apr16 HTLV serology panel was assessed by comparing the participants' results (including the NLHRS) with the results of the panel's characterization performed by the NLHRS prior to the test event.
- 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).
- The source material (Access Biological) for the positive panel members is the same source material used for the 2017-2018 HTLV serology panels.

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.
   - Eight participants (58.8%, 8/17) reported using external QC material.

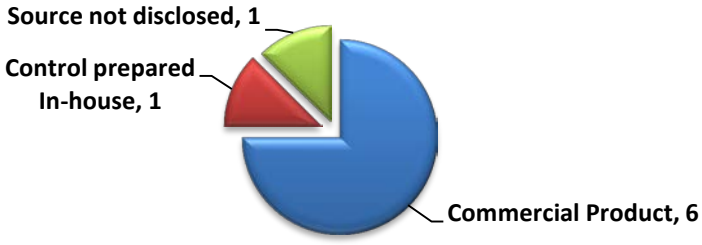

Figure 2: Source of external quality control used for the 2019Apr16 HTLV serology panel.

2. *Quality Assurance (QA) programs*- Allows participants to evaluate their overall use of the assay and reporting of the results.
   - Twelve participants (70.6%, 12/17) reported participation in other quality assurance programs (Figure 3).

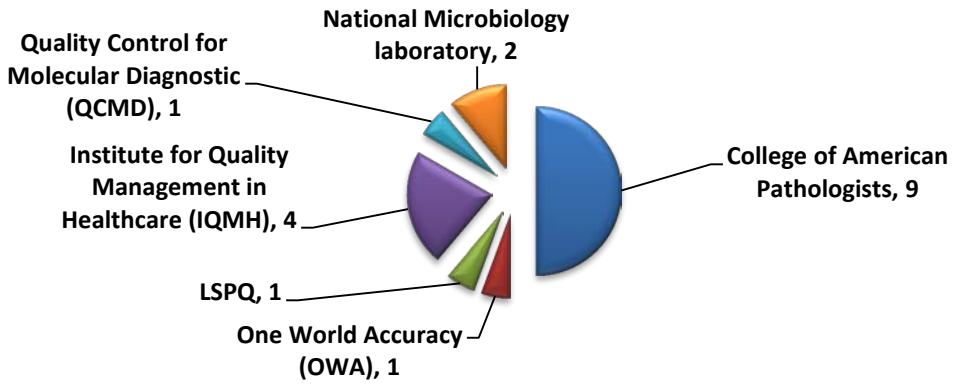

Figure 3: Distribution of external quality assurance programs which participants are enrolled in other than the NLHRS QAP.

Participants' feedback collected in the new QAP website

- Of the 17 participants, 2 provided feedback (Figure 4)

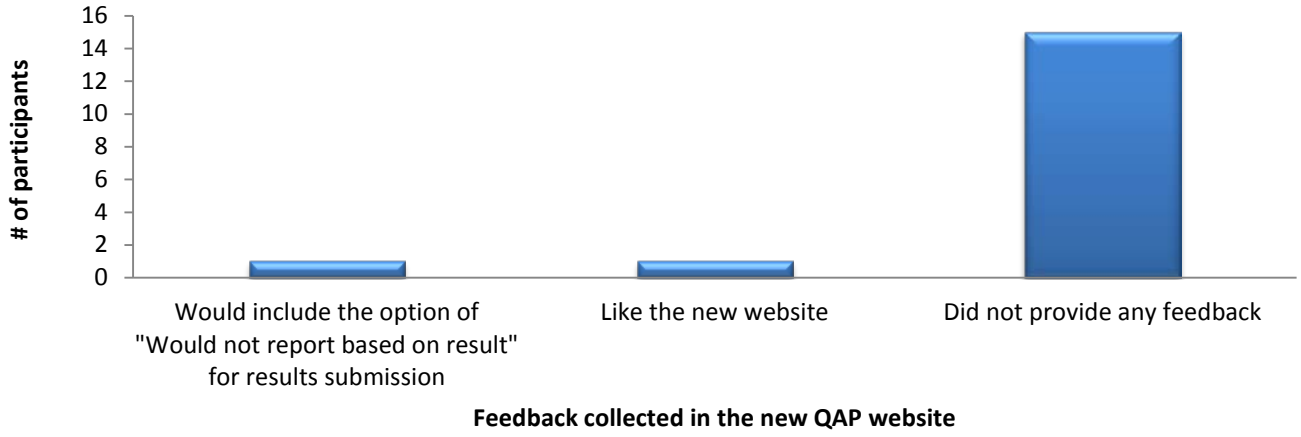

Figure 4: Feedback provided by the participant in the 2019Apr16 HTLV serology survey.

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

Table 1: 2019Apr16 HTLV panel final status reported from participants (includes the NLHRS).

LAB	SAMPLE A HTLV-I Ab Positive	SAMPLE B HTLV Negative	SAMPLE C HTLV-I Ab Positive	SAMPLE D HTLV Negative	SAMPLE E HTLV-II Ab Positive
HV01	HTLV-I/II Ab Positive[1]	HTLV-I/II Ab Non-Reactive/Negative	HTLV-I/II Ab Positive[1]	HTLV-I/II Ab Non-Reactive/Negative	HTLV-I/II Ab Positive[1]
HV02	HTLV-I/II Ab Positive[1]	HTLV-I/II Ab Non-Reactive/Negative	HTLV-I/II Ab Positive[1]	HTLV-I/II Ab Non-Reactive/Negative	HTLV-I/II Ab Positive[1]
HV03	HTLV-I/II Ab Positive[1]	HTLV-I/II Ab Non-Reactive/Negative	HTLV-I/II Ab Positive[1]	HTLV-I/II Ab Non-Reactive/Negative	HTLV-I/II Ab Positive[1]
HV12	HTLV-I/II Ab Positive[1]	HTLV-I/II Ab Non-Reactive/Negative	HTLV-I/II Ab Positive[1]	HTLV-I/II Ab Non-Reactive/Negative	HTLV-I/II Ab Positive[1]
HV15	HTLV-I Ab Positive	HTLV-I/II Ab Non-Reactive/Negative	HTLV-I Ab Positive	HTLV-I/II Ab Non-Reactive/Negative	HTLV-II Ab Positive
HV16	HTLV-I Ab Positive	HTLV-I/II Ab Non-Reactive/Negative	HTLV-I Ab Positive	HTLV-I/II Ab Non-Reactive/Negative	HTLV-II Ab Positive
HV17	HTLV-I/II Ab Positive[1]	HTLV-I/II Ab Non-Reactive/Negative	HTLV-I/II Ab Positive[1]	HTLV-I/II Ab Non-Reactive/Negative	HTLV-I/II Ab Positive[1]
HV18	HTLV-I/II Ab Positive[1]	HTLV-I/II Ab Non-Reactive/Negative	HTLV-I/II Ab Positive[1]	HTLV-I/II Ab Non-Reactive/Negative	HTLV-I/II Ab Positive[1]
HV20	HTLV-I/II Ab Positive[1]	HTLV-I/II Ab Non-Reactive/Negative	HTLV-I/II Ab Positive[1]	HTLV-I/II Ab Non-Reactive/Negative	HTLV-I/II Ab Positive[1]
HV21	HTLV-I/II Ab Positive[1]	HTLV-I/II Ab Non-Reactive/Negative	HTLV-I/II Ab Positive[1]	HTLV-I/II Ab Non-Reactive/Negative	HTLV-I/II Ab Positive[1]
HV22	HTLV-I/II Ab Positive[1]	HTLV-I/II Ab Non-Reactive/Negative	HTLV-I/II Ab Positive[1]	HTLV-I/II Ab Non-Reactive/Negative	HTLV-I/II Ab Positive[1]
HV44	HTLV-I/II Ab Positive[1]	HTLV-I/II Ab Non-Reactive/Negative	HTLV-I/II Ab Positive[1]	HTLV-I/II Ab Non-Reactive/Negative	HTLV-I/II Ab Positive[1]
HV50	HTLV-I/II Ab Positive[1]	HTLV-I/II Ab Non-Reactive/Negative	HTLV-I/II Ab Positive[1]	HTLV-I/II Ab Non-Reactive/Negative	HTLV-I/II Ab Positive[1]
HV55	HTLV-I/II Ab Positive[1]	HTLV-I/II Ab Non-Reactive/Negative	HTLV-I/II Ab Positive[1]	HTLV-I/II Ab Non-Reactive/Negative	HTLV-I/II Ab Positive[1]
HV63	HTLV-I/II Ab Positive[1]	HTLV-I/II Ab Non-Reactive/Negative	HTLV-I/II Ab Positive[1]	HTLV-I/II Ab Non-Reactive/Negative	HTLV-I/II Ab Positive[1]
HV75	HTLV-I Ab Positive	HTLV-I/II Ab Non-Reactive/Negative	HTLV-I Ab Positive	HTLV-I/II Ab Non-Reactive/Negative	HTLV-II Ab Positive
HV76	HTLV-I/II Ab Positive[1]	HTLV-I/II Ab Non-Reactive/Negative	HTLV-I/II Ab Positive[1]	HTLV-I/II Ab Non-Reactive/Negative	HTLV-I/II Ab Positive[1]
HV80	HTLV-I/II Ab Positive[1]	HTLV-I/II Ab Non-Reactive/Negative	HTLV-I/II Ab Positive[1]	HTLV-I/II Ab Non-Reactive/Negative	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 result in misinterpretation of the true status of the sample, unresolved status but made a recommendation

Table 3: Expected results for the 2019Apr16 HTLV serology panel.					
Assay	**Sample A**	**Sample B**	**Sample C**	**Sample D**	**Sample E**
HTLV Screening Assay	HTLV-I/II Reactive	HIV-I/II Non-Reactive	HTLV-I/II Reactive	HTLV-I/II Non-Reactive	HTLV-I/II Reactive
HTLV Ab Confirmatory Assay	HTLV-I Ab Positive	HTLV Ab Negative	HTLV-I Ab Positive	HTLV Ab Negative	HIV-2 Ab Positive

Results (Excluding the NLHRS)

- *Return rate*
  - 100% of the participants returned results by the deadline (17/17).
- *Qualitative Group Analysis* (Table 1)
  - *Sample A (HTLV-I Ab Positive)* – All participants provided either a correct serology status and/or recommendation.
  - *Sample B (Negative)* – All participants provided either a correct serology status and/or recommendation.
  - *Sample C (HTLV-I Ab Positive)* – All participants provided either a correct serology status and/or recommendation.
  - *Sample D (Negative)* – All participants provided either a correct serology status and/or recommendation.
  - *Sample E (HTLV-II Ab Positive)* – All participants provided either a correct serology status and/or recommendation.

Discussion

All participants were able to correctly identify the HTLV-I Ab positive and the HTLV-II Ab positive samples either through an HTLV screening assay or HTLV confirmatory assay. Similarly, samples B and D were correctly identified as HTLV Ab negative by all participants. In addition, no post-analytical errors were made.

A new HTLV screening assay, the Diapro HTLV-I/II ELISA, was used in this test event. The NLHRS will follow closely with the performance of this assay in future test events to see if it is comparable to the Abbott Architect HTLV-I/II CMIA in which a majority of the participants uses for HTLV testing.

Conclusion

There are no discordant findings in the participants' results for the 2019Apr16 test event. All participants returned the correct results. In this test event, we have switched over to the new QAP website from Survey Monkey to address the lack of a printing function for the participants and the option for the participant to be

able to enter their results in French. We appreciate your feedback provided in the survey and recognize that participants would like additional improvements to our reporting format and the timeliness of the dissemination of the report. To that end, the NLHRS is exploring utilizing the features of the new QAP website to reduce the turnaround time of the dissemination of the final report to the participants.

We value each laboratory's participation in these QA panels and your suggestions for improvement. The NLHRS is committed to improving all aspects of the HTLV 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 HTLV Serology Quality Assurance Program

John Ho
Quality Assurance Program Coordinator
National Laboratory for HIV Reference Services
Public Health Agency of Canada
Tel: (204) 789-6522

Dr. John Kim
Laboratory Chief
National Laboratory for HIV Reference Services
Public Health Agency of Canada
Tel: (204) 789-6527

Appendix 1: Characterization

Summary of NLHRS Characterization of the 2019Apr16 HTLV Panel Samples

The NLHRS 2019Apr16 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 Ab Positive	HTLV-I Positive	+++	+++	+++	++	++	+++	-
B	Negative	Negative	-	-	-	-	-	-	-
C	HTLV-I Ab Positive	HTLV-I Positive	+++	+++	+++	+++	++	+++	-
D	Negative	Negative	-	-	-	-	-	-	-
E	HTLV-II Ab Positive	HTLV-II 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.