JC WILT INFECTIOUS DISEASES RESEARCH CENTRE

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 2019Oct31

2019Oct31 HTLV Serology Panel		
Panel Sample	**True Status**	**Labs Reporting Incorrect Status**
A	HTLV-I Ab Positive	1 participant reported incorrect status
B	HTLV-I Ab Positive	1 participant reported incorrect status
C	Negative	
D	HTLV-II Ab Positive	1 participant reported incorrect status
E	Negative	

One participant reported incorrect results for samples A, B, and D.

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HTLV Serology Quality Assessment Program
Final Report for Panel HTVLSER 2019Oct31

Issued 2019-December-23

Introduction

The NLHRS distributed the 2019Oct31 and 2020Apr17 panels on October 16th, 2019. This final report is specific to the 2019Oct31 panel only and is publicly available; however, the identity of participants is not disclosed. The deadline for results submission is October 31st, 2019. The preliminary report was issued on November 15, 2019.

Panel Samples, HTLV Test Kits, and Data Entry

- *Panel Composition*
  - The 2019Oct31 panel consisted of five samples; two HTLV negative (C, E), two HTLV-I positive (A, B), and one HTLV-II positive sample (D). Samples A, B, and D were diluted 1 in 2 with defibrinated human plasma (Basematrix 53, Seracare Life Sciences). Testing and characterization by the NLHRS are presented in Appendix 1. Panels were sent to 16 participants and to the NLHRS on October 16th, 2019.

- *HTLV Test Kits*
  - Four different assays were used by the 16 participants excluding the NLHRS (Appendix 2).

- *Data entry*
  - Results entry for this panel utilized an in-house developed website.

Homogeneity and Stability

- The homogeneity and stability of the 2019Oct31 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).

Results

- *Evaluation Criteria:*
  - Negative samples: HTLV non-reactive/negative in the final HTLV serology interpretation with assay results supporting the interpretation.
  - Positive samples: HTLV reactive/positive in the final HTLV serology interpretation with assay results supporting the interpretation. Participants must provide a recommendation for further action for samples that they could not determine the true serology status based on the assay used in their testing.

- *Qualitative Group Analysis (Figure 1)*:
  - Sample A (HTLV-I Ab Positive) – 16/17 participants provided either a correct serology status and/or recommendation.
  - Sample B (HTLV-I Ab Positive) – 16/17 participants provided either a correct serology status and/or recommendation.
  - Sample C (Negative) – 17/17 participants provided either a correct serology status and/or recommendation.
  - Sample D (HTLV-II Ab Positive) – 16/17 participants provided either a correct serology status and/or recommendation.
  - Sample E (Negative) – 17/17 participants provided either a correct serology status and/or recommendation.

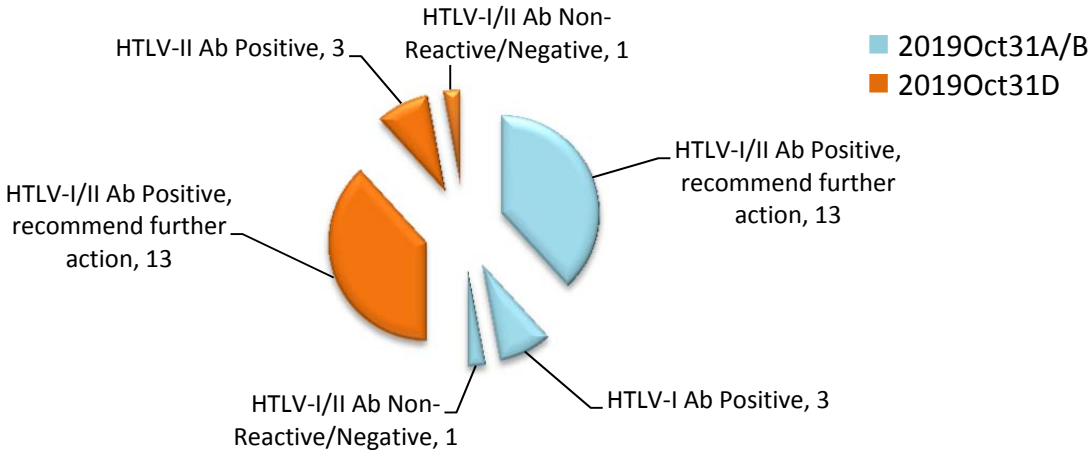

Figure 1: The final HTLV serology status of the positive samples in the 2019Oct31 HTLV serology panel submitted by participants using HTLV screening and confirmatory assays.

Findings

One participant reported HTLV-I/II non-reactive/negative for the 3 positive samples in the panel. Follow up with this participant revealed that the wrong samples were tested, indicative of a pre-analytical or analytical error (Appendix 4). We recommend verifying samples upon receipt and at the time of testing to ensure sample mix-ups do not occur.

If you have any comments, suggestions or concerns, please contact us at:

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Thank you for your participation in the NLHRS HTLV Serology Quality Assurance Program

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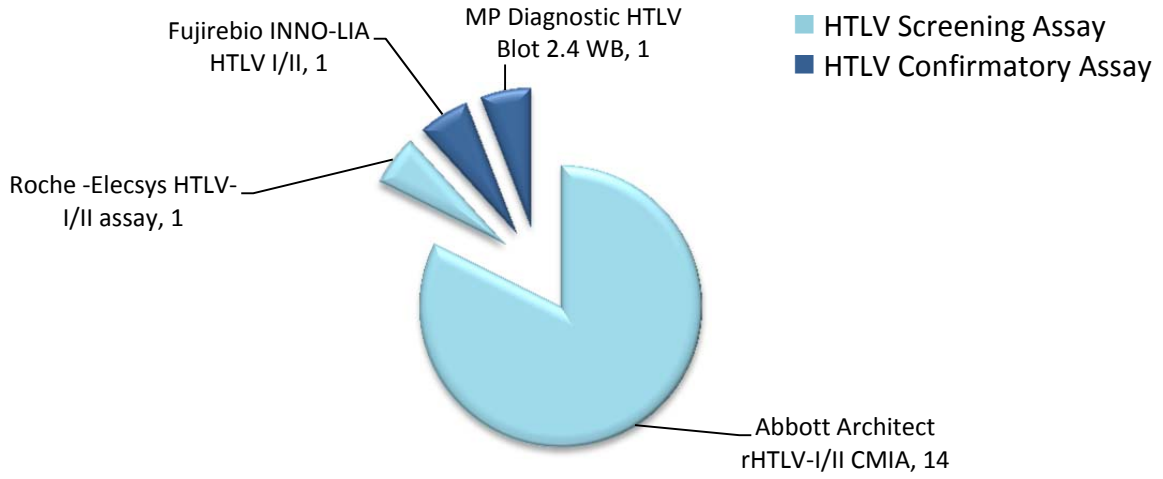
Appendix 1: NLHRS Characterization of the 2019Oct31 HTLV serology panel.

The NLHRS 2019Oct31 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	HTLV-I Ab Positive	HTLV-I Positive	+++	+++	+++	+++	++	+++	-
C	Negative	Negative	-	-	-	-	-	-	-
D	HTLV-II Ab Positive	HTLV-II Positive	++	+++	++	++	-	-	++
E	Negative	Negative	-	-	-	-	-	-	-

N/T: Not tested

Appendix 2: Summary of assays used by the participants in the 2019Oct31 HTLV serology panel.



Appendix 3: Summary of bands detected in samples A, B, and D by the Fujirebio INNO-LIA HTLV-I/II and MP Diagnostic HTLV Blot 2.4 WB assays in the 2019Oct31 HTLV serology panel.

Fujirebio INNO-LIA HTLV-I/II	Frequency of Bands Detected						
Sample	p19 I/II	p24 I/II	gp46 I/II	gp21 I/II	p19-I	gp46-I	gp46-II
2019Oct31A	2	2	2	2	2	2	-
2019Oct31B	2	2	2	2	2	2	-
2019Oct31D	2	2	2	2	-	-	2

MP Diagnostic HTLV Blot 2.4 WB	Frequency of Bands Detected										
Sample	rgp46-I	rgp46-II	p53	gp46	p36	p32	p28	P26	P24	P19	GD21
2019Oct31A	1	-	1	1	1	1	1	1	1	1	1
2019Oct31B	1	-	1	1	1	1	1	1	1	1	1
2019Oct31D	-	1	1	-	1	-	-	-	1	-	1

Appendix 4: 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.