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 HTLS425 2015Oct22

Panel Sample	True Status	Labs Reporting Incorrect Status	
A	HTLV-I Positive		
B	HTLV-I/II Negative		
C	HTLV-II Positive		
D	HTLV-I/II Negative		
E	HTLV-II Positive		

All participants returned correct results for the 2015Oct22 HTLV Serology panel.

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HTLV Serology Quality Assessment Program
Final Report for Panel HTLS425 2015Oct22

Introduction

The NLHRS distributed 2 HTLV serology panels April 7th 2015, with the second panel to be tested after the test event open date of October 6th, 2015.

Panel Samples, HTLV Test Kits and Data Entry

- *Panel Composition* – Panel 2015Oct22 consisted of five samples; two HTLV negative samples (B, D), one HTLV-I positive sample (A) and two HTLV-II positive samples (C, E). Testing and characterization by the NLHRS prior to shipment are presented in Appendix 1. Panels were then prepared and sent to 15 participants including the NLHRS on April 7th. The deadline for data entry was October 22nd, 2015.

- *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. No participants used expired kits.

Table 1: Summary of the assays used in the NLHRS 2015Apr23 and 2015Oct22 HTLV Panels. (Excludes the NLHRS)

Type	Assay	# of Users		Use of Additional QC Material (2015Oct22)	
		2015Apr23	2015Oct22	Yes	No
Screen	Abbott ARCHITECT rHTLV-I/II CMIA	13	13	8	5
Confirmatory	Innogenetics INNO-LIA HTLV I/II Score	1	1	1	-
	MP Diagnostics HTLV BLOT 2.4 WB	1	1	1	-

* The participant who uses this assay was unable to obtain kits for testing and did not participate in the panel.

- *Data entry* - The NLHRS HIV Serology Quality Assessment Program used the computerized web based system Oneworld Accuracy to capture and analyze results. After the October22nd, 2015 deadline for submission of results, a preliminary Laboratory Specific Report (LSR) reflecting the statistical analysis of the data was then issued by Oneworld Accuracy to all participants November 28th, 2015.

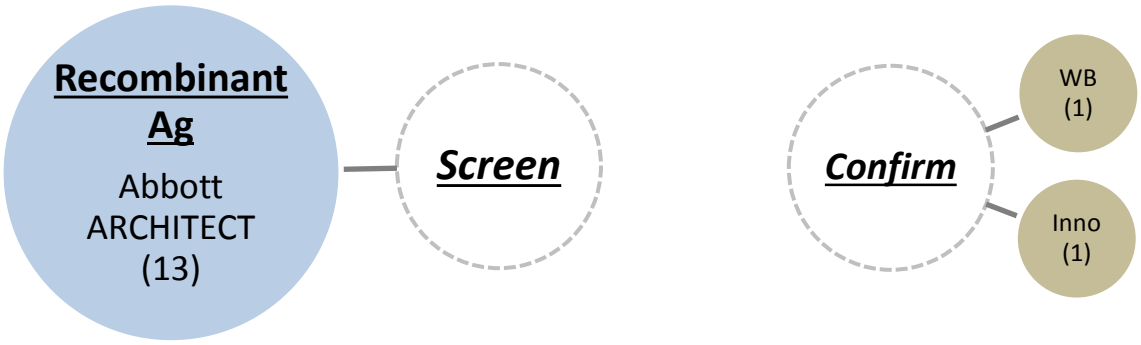

Figure 1: Breakdown of the assays used by the 14 participants in the NLHRS 2015Oct22 HTLV Panel (Excludes the NLHRS).

Results

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

- *Group Analysis* (Table 2)
  - *Sample A (HTLV-I positive)* – All participants correctly identified the sample.
    14/14 participants provided either a correct serology status and/or recommendation.
  - *Sample B (HTLV negative)* – All participants correctly identified the sample.
    14/14 participants provided either a correct serology status and/or recommendation.
  - *Sample C (HTLV-II positive)* – All participants correctly identified the sample.
    14/14 participants provided either a correct serology status and/or recommendation.
  - *Sample D (HTLV negative)* – All participants correctly identified the sample.
    14/14 participants provided either a correct serology status and/or recommendation.
  - *Sample E (HTLV-II positive)* – All participants correctly identified the sample.
    14/14 participants provided either a correct serology status and/or recommendation.

Table 2: 2015Oct22 HTLV Panel final status reported from participants.

LAB	SAMPLE A HTLV-I Positive	SAMPLE B Negative	SAMPLE C HTLV-II Positive	SAMPLE D Negative	SAMPLE E HTLV-II Positive
HV01	HTLV-I/II Ab positive[1]	HTLV-I/II Ab Negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab Negative	HTLV-I/II Ab positive[1]
HV02	HTLV-I/II Ab positive[1]	HTLV-I/II Ab Negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab Negative	HTLV-I/II Ab positive[1]
HV03	HTLV-I/II Ab positive[1]	HTLV-I/II Ab Negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab Negative	Aspiration error
HV15	HTLV-I Ab positive	HTLV-I/II Ab Negative	HTLV-II Ab positive	HTLV-I/II Ab Negative	HTLV-II Ab positive
HV16	Would not Report[1]	HTLV-I/II Ab Negative	Would not Report[1]	HTLV-I/II Ab Negative	Would not Report[1]
HV17	HTLV-I/II Ab positive[1]	HTLV-I/II Ab Negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab Negative	HTLV-I/II Ab positive[1]
HV18	HTLV-I/II Ab positive[1]	HTLV-I/II Ab Negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab Negative	HTLV-I/II Ab positive[1]
HV20	HTLV-I/II Ab positive[1]	HTLV-I/II Ab Negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab Negative	HTLV-I/II Ab positive[1]
HV21	HTLV-I/II Ab positive[1]	HTLV-I/II Ab Negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab Negative	HTLV-I/II Ab positive[1]
HV22	HTLV-I/II Ab positive[1]	HTLV-I/II Ab Negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab Negative	HTLV-I/II Ab positive[1]
HV44	HTLV-I/II Ab positive[1]	HTLV-I/II Ab Negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab Negative	HTLV-I/II Ab positive[1]
HV50	HTLV-I/II Ab positive[1]	HTLV-I/II Ab Negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab Negative	HTLV-I/II Ab positive[1]
HV55	HTLV-I/II Ab positive[1]	HTLV-I/II Ab Negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab Negative	HTLV-I/II Ab positive[1]
HV76	HTLV-I/II Ab positive[1]	HTLV-I/II Ab Negative	HTLV-I/II Ab positive[1]	HTLV-I/II Ab Negative	HTLV-I/II Ab positive[1]

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

Discussion

- All participants returned the correct result for all samples in the 2015Oct22 panel.
- Participants were surveyed on their use of Quality Control (QC) reagents in addition to those included in the commercial kits (Table 1). Over half of the participants (67%) 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 HTLV Serology QA Program

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Appendix 1

Summary of NLHRS Characterization of the NLHRS 2015Oct22 HTLV Panel Samples

The NLHRS 2015Oct22 HTLV Panel Sample Testing Results											
Sample	**Final Status**	**NLHRS Testing**									
		Ortho HTLV-I/HTLV-II Ab Capture ELISA	**Avioq HTLV-I/II Microelisa System**	**Innogenetics 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	Reactive	*N/T*	HTLV-I	++	++	++	++	+/-	++	-
B	HTLV-I/II Ab Negative	*N/T*	Non-Reactive	Neg	-	-	-	-	-	-	-
C	HTLV-II Ab Positive	Reactive	*N/T*	HTLV-II	+	+	++	+++	-	-	++
D	HTLV-I/II Ab Negative	*N/T*	Non-Reactive	Neg	-	-	-	-	-	-	-
E	HTLV-II Ab Positive	Reactive	*N/T*	HTLV-II	+	+/-	++	++	-	-	++

N/T: Not tested

Appendix 2

Troubleshooting; common causes of outlying and/or aberrant results in Serology and Molecular Laboratories.

Type of Error	Possible Cause(s)	Pre-Examination Stage	Examination Stage	Post-Examination Stage
Sample mix-up	Two or more samples may have been interchanged, resulting in both outlying and aberrant results. Sample mix-up may occur during specimen reception or during testing.	√	√	
Transcription	Common causes of transcription errors include:			
	• Ordering of incorrect test by physician;	√		
	• Shipment of sample to incorrect laboratory;	√		
	• Selecting the wrong assay at laboratory;	√		
	• Interchanging the 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 log10 IU/mL);			√
	• Using a comma instead of a dot to denote a decimal point;			√
	• Selecting the incorrect assay interpretation.			√
	• Failure to recommend follow-up testing where necessary.			√
	It is recommended all results that are manually transcribed or entered (including Oneworld Accuracy) be checked by a second individual in order to avoid transcription errors.			
Outlying and/or aberrant test results due to random error.	Sporadic test results identified as outlying and/or aberrant can be classified as random events. Possible causes of random outlying and/or aberrant results include:			
	• Insufficient mixing of sample, especially following freezing;		√	
	• Poor pipetting;		√	
	• Ineffective or inconsistent washing;		√	
	• Transcription errors;	√		√
	• Sample mix-up;	√	√	
	• Cross-contamination or carryover;	√	√	
	• Presence of inhibitors to PCR		√	
Outlying and/or aberrant test results due to 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.