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Section: Serology Manual	Subject Title: Human T-Lymphotropic Virus Type 1 (HTLV-1) EIA	
Issued by: LABORATORY MANAGER	Original Date: March 14, 2001	
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HUMAN T-LYMPHOTROPIC VIRUS TYPE 1 (HTLV-1) EIA

I. Introduction

The Adaltis DETECT-HTLV™ EIA Test Kit is a solid phase enzyme immunoassay utilizing a mixture of synthetic peptides for the detection of antibodies to HTLV-I and II in human serum or plasma.

II. Specimen Collection and Processing

Blood is collected (5 mL for adults and 1 mL for neonates) in a serum separator tube and separated by centrifugation. The serum is stored at 4⁰C.

III. Procedure

i) Reagents:

Positive Control
 Negative control
 Anti-Human IgG Peroxidase Conjugate
 Conjugate Diluent
 Tetramethylbenzidine (TMB) Reagent
 Peroxide Reagent
 Stop Solution
 2x 96 well Microplate coated with HTLV-I & HTLVII peptide

ii) Other Materials :

P Lab Analyser System
 5 ml graduated Pipettes
 10 ml graduated Pipettes
 1 ml graduated Pipettes
 10-100 ul pipettor
 Pipette tips
 55x12 (3.5 ml) tubes
 Automatic pipettor

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iii) Method:

A. Using P-Lab (Personal Lab)

Before beginning EIA assay:

1. Allow all components to reach room temperature.
Prepare wash solution:
Add 100 ml of 25x wash solution to 2400 ml of distilled water.
Fill up P-Lab wash bottle. Place bottle in 'Buffer #1' position in P-Lab.
Turn hard drive and monitor. Wait for software to be fully loaded before turning the P-Lab 'ON'.
2. Click once on third square from the left: '**open session**'. Click on '**New**',
Type in assay & date performed, e.g. HTLV 280499(HTLV tested April 28,99). Note session # e.g.'31'. Click on writer Name, enter initial.
Click 'OK'
3. The screen will show e.g. **HTLV280499#31**.ssn highlighted , click 'OK'.
4. Click on 5 th square from the left: '**Profile Include**', click on ' DetectHTLV#0.
prf',click 'OK'.
5. Click on 7th square from the left : '**Sample programming**'.
First click on '**Clear sample rack**', then click on sample code, and enter lab # ,and load specimens onto specimen rack at the same time. Also load second half of the rack with dilution tubes.
Once finished, double click on 'Small Rack' icon in top left corner, "X's should appear next to all lab #s,click 'done'.
6. The screen will show plate format (# of wells needed). Take out the required # of strips and wells. Load plate onto the **left** side in the P-Lab.
Click on 'done'.
7. Click on 8 th square from the left : '**Setup Entry**'. Click on pulldown arrow under 'Lot".. If it is a new lot, Click on '**Change Date**' and specify kit expiration date if needed. Click 'OK'.
8. Click on last square '**Start Session**', 'Save document :using the new file...'will appear, click 'OK'.
9. **Profile-vial locations for controls or standards and reagents**' will appear. Right click on each colored position, will show where to load each diluent, controls, conjugate, substrate and stop solution. Click 'continue', and wait for instrument initialization.

Position **Reagent & Controls**

R3	Sample Diluent
R4	Conjugate
R5	Substrate
R6	Stop Solution
1	Negative Control
2	Positive Control

Prepare **Fresh (same Day) Conjugate** as follows (always make 1 extra strip):

Number of Strips	40X conjugate(ul)	Conjugate Diluent(ml)
1	30	1.2
2	50	2.0
3	80	2.9
4	100	3.9
5	130	4.9
6	150	5.9
7	180	6.8

Prepare **Fresh (Same Day) Substrate** as follows (always make 1 extra strip):

Number of Strips	TMB Reagent(ml)	Peroxide Reagent(ml)
1	0.2	1.0
2	0.4	2.0
3	0.6	3.0
4	0.8	4.0
5	1.0	5.0
6	1.2	6.0
7	1.4	7.0

- Click on 2 nd square from the left' **Maintenance**'. Click on **'Start'** to start up **'Self Test'**. Machine will do all checks, then asks: **'Print Self Test report'**, click on **'Yes'**. Staple printout with day list.
- Click on **'Daily Maintenance'**: click on **' Fill syringes'**, check for bubbles in syringes, and if no bubbles present, click 'No'.

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13. Click on '**Fill lung**', screen will ask you to open lid. Open lid, and click on 'OK'. Water level should fill up to just under the lower black line. Click 1-2 times to Add 250ul of water each time to raise the level to just above the lower black line. Close lid and click on 'OK'.
14. Click on '**Buffer 1 prime**', listen to the noise to make sure that the vacuum is working.
15. Click on 3rd square 'Tips' if need to replace pipette box. Click on 6th square from the left to '**execute**' the run, will ask to put sample rack in, click 'OK'. The P-lab will start processing. Click on '**Time chart**' to see what stages the testing is at.
16. After assay is finished, "**Session terminated**" will appear on screen, click 'OK'.
17. Click on 'Maintenance, and click on "**Endwork**". Will ask to fill in buffer 2 with DH₂O, open lid, make sure enough DH₂O in the bottle, also change buffer 1 bottle to DH₂O bottle. Click 'OK'. The machine is rinsing the tubing with DH₂O. Next will ask to 'empty waste', press on "**Black Button**" on the side of the machine, and hold it until almost all fluid is drained off and bubbles appear. Empty waste. Click 'OK'. 'Empty tips', click 'OK'. 'Don't forget to shut off P-Lab', click 'OK'.
18. Click on left top corner 'File', and 'Exit', will ask 'exit from P lab Processor?' click 'Yes'.
19. Click on 4th box from the top : '**Open Results**'. Click on 'New Result'. Then click on you session and 'OK'.
20. Click on 3rd box from the left, '**report**', click on "DetectHTLV", and also the square with the '**printer**',
Report will be printed out.
21. Close all windows by clicking on '**X**' at top right corner three times.
22. Turn off hard drive, monitor, P-lab and printer.

iv) **Validation**

9. Absorbance of the blank must be \leq or = 0.100.
10. The Positive Control mean must be \geq or = 0.800.
If the value is less than 0.80, the run must be repeated
11. Cut off = NCx (negative control mean) + 0.150

If the positive control fails, the test is invalid. Should inform Senior/Charge technologists and repeat testing.

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v) Interpretation of Results:

3. Absorbance value is < cut off: reported as Negative.
4. Absorbance value is > or =cut off: send to PHL for confirmation.

vi) Quality Control:

Two positive and three negative controls must be included with each run.

Run Virotrol® I as external control (Do not dilute) with each new lot.

All positive specimens are sent to PHL for confirmation.

Two negative specimens are sent to PHL every 6 months for verification. The PHL reports are filed in Reagent Lot binder.

vii) Reference :

Package insert from DETECT-HTLV™, EIA Test Kit for the Detection of Antibodies to Human T-Lymphotropic Viruses (HTLV) Types I and II. Adaltis, rev.06/02.