Analytical Performance of the VITROS ® Immunodiagnostic Products Anti-HTLV I/II Assay*

C. Noeson, S. Clark, L. Colt, P. Contestable, R. Fazio, B. Novick, A. Patru, R. Polimeni, L. Sprague

Ortho Clinical Diagnostics, Rochester, NY

Objective

This study was designed to assess the analytical performance of the VITROS Immunodiagnostic Products Anti-HTLV I/II assay (VITROS Anti-HTLV) on the VITROS ECi/ECiQ Immunodiagnostic Systems, the VITROS 3600 Immunodiagnostic System and the VITROS 5600/ XT 7600 Integrated Systems. The assay detects antibodies to HTLV Types I and II.

Methods

Antibody detection in VITROS Anti-HTLV is achieved using recombinant HTLV antigens coated onto the well. Sample is added to the coated wells in the first stage of the reaction and HTLV antibody from the sample is captured. After washing, HRP conjugated recombinant HTLV antigens are added. Following a final wash, bound HRP conjugates are detected using the VITROS signal reagent. The assay cut-off for VITROS Anti-HTLV is 1.00; values above the cut-off are Reactive for HTLV antibodies and values below 1.00 are Non-reactive.

Analytical sensitivity was evaluated using two commercially available performance panels and 25 fresh spiked patient samples. Analytical specificity was assessed consistent with CLSI EP7 and also by testing patient samples known to include potentially interfering substances. Assay reproducibility was assessed consistent with CLSI EP05 using two reagent lots with a 7-member panel. VITROS Anti-HTLV was tested for equivalence in serum and EDTA, Heparin and Sodium Citrate plasmas – including serum and lithium heparin separator tubes. College of American Pathologists (CAP) Viral Marker series 3 (VM3) was tested in triplicate to confirm compatibility with VITROS Anti-HTLV I/II.

Supported Sample Types and Sample Stability

Anti-HTLV negative and spiked anti-HTLV positive samples prepared in Serum, Lithium Heparin plasma, Sodium Citrate plasma, Serum Separator Tubes and Lithium Heparin Plasma Separator Tubes have been tested and shown to generate stable, clinically equivalent results even when stored for up to 24 hours at room temperature (up to 30 °C (86 °F), 7 days at 2-8 °C (36-46 °F) or frozen (≤ -20 °C (-4 °F) with up to five freeze-thaw cycles.

Analytical Sensitivity

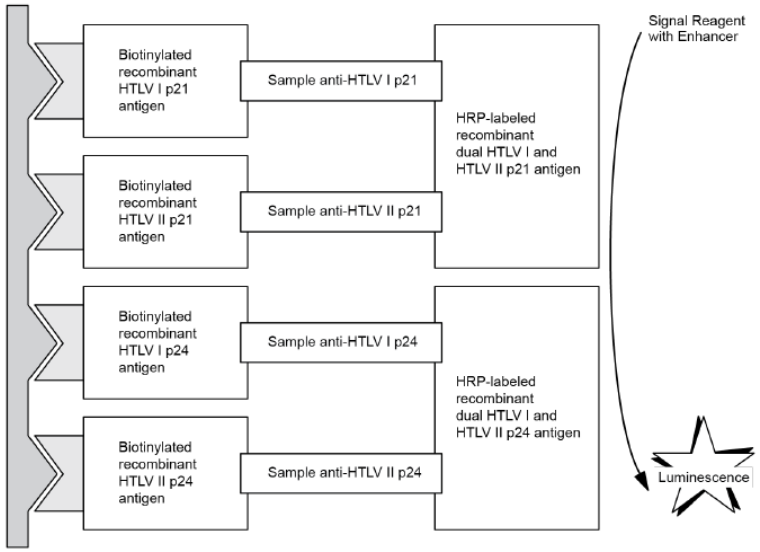
Analytical sensitivity was evaluated using 2 commercially available performance panels and College of American Pathologists Viral Marker Series 3 (CAP VM3). Results for both panels and the CAP samples were concordant with the package insert. Results from one of the commercial panels are shown below.

Qualification Panel (QRP713)

Panel Member	Panel Classification**	VITROS 5600 Master Lot 1 (S/C)	VITROS 3600 Master Lot 2 (S/C)	VITROS Eci/ECiQ Master Lot 1 (S/C)	VITROS XT7600 Master Lot 2 (S/C)
QRP713-01	HTLV I Reactive	45.9	36.3	45.7	38.5
QRP713-02	Non-reactive	0.01	0.01	0.01	0.01
QRP713-03	HTLV I Reactive	62.4	49.9	63.2	53.5
QRP713-04	HTLV II Reactive	27.1	22.2	25.7	24.7
QRP713-05	HTLV I Reactive	28.2	22.2	27.4	24.5
QRP713-06	HTLV II Reactive	20.2	16.4	19.1	17.6

** Data from SeraCare Anti-HTLV I/II AccuTrak™ Qualification Panel Data Sheet.

Assay Architecture



Potentially Interfering Substances

Potentially interfering compounds were evaluated consistent with CLSI document EP7. Anti-HTLV negative and spiked anti-HTLV positive samples were prepared and spiked with the following compounds at the concentrations listed. None of the compounds were found to interfere with the clinical interpretation of test results.

Compound	Concentration Tested	
Bilirubin (conjugated)	40 mg/dL	0.475 mmol/L
Bilirubin (unconjugated)	40 mg/dL	0.684 mmol/L
Biotin	3510 ng/ml	14391 nmol/L
Cholesterol	1242 mg/dL	32.16 mmol/L
Hemoglobin	1000 mg/dL	0.156 mmol/L
HAMA	2122 ng/mL	NA
IgG	2130 mg/dL	21.3 g/L
Intralipid	2000 mg/dL	NA
Rheumatoid Factor (RF)	2195 IU/dL	NA
Total Protein	15 g/dL	150 g/L
Triglycerides	1500 mg/dL	16.94 mmol/L

NA = Not Applicable (alternate units not provided).

Presented at AACC Virtual Meeting, Dec. 13 – 17, 2020

Potentially Interfering Disease States

Potentially cross-reactivity was assessed by testing samples from the following disease states. None was found to cross-react in the VITROS Anti-HTLV I/II test.

Sample Category	No. of Samples	Non-reactive	Reactive
Human Immunodeficiency Virus Type 1 (HIV-1)	24	24	0
Human Immunodeficiency Virus Type 2 (HIV-2)	25	25	0
Hepatitis A Virus (HAV), B Virus (HBV), C Virus (HCV)	5, 5, 5	5, 5, 5	0
Syphilis	18	18	0
Influenza Vaccine (Pre) and (Post)	5, 5	5, 5	0
Cytomegalovirus, Epstein-Barr Virus	5, 5	5, 5	0
Herpes	6	6	0
Rubella, Anti-nuclear antibodies, Toxoplasma gondii, Hemophilia	5, 5, 5, 5	5, 5, 5, 5	0
Multiple Leukocyte and Red Cell Alloantibodies	5, 5	5, 5	0
Monoclonal and Polyclonal Gammopathy	5, 5	5, 5	0
Pregnancy (1st Trimester), (2nd Trimester), (3rd Trimester)	8, 7, 9	8, 7, 9	0
Multiparous Women	5	5	0
Pediatric Samples (2-4 yrs), (5-9 yrs), (10-14 yrs), (15-17 yrs)	6, 5, 6, 6	6, 5, 6, 6	0

Precision

Precision was evaluated consistent with CLSI document EP05. Two replicates each of 1 negative patient sample pool, 4 diluted reactive patient sample pools, 1 negative control and 1 positive control sample were tested on 2 separate occasions per day on at least 20 different days. The experiment was performed using 2 reagent lots on one VITROS ECi/ECiQ Immunodiagnostic System, one VITROS 3600 Immunodiagnostic System, and one VITROS 5600 Integrated System. The data presented are a representation of the product performance on this system.

VITROS System	Mean (S/C)	Within-run[1]		Within-cal[2]		Within-lab[3]		No. Observations	No. Days
		SD	%CV	SD	%CV	SD	%CV		
VITROS ECi/CiQ	0.01	0.00	NA	0.01	NA	0.01	NA	80	20
	0.96	0.00	0.18	0.03	3.50	0.05	4.63	80	20
	1.35	0.02	1.81	0.05	4.05	0.07	4.76	80	20
	3.31	0.07	2.14	0.10	3.13	0.14	4.07	80	20
	4.63	0.06	1.31	0.11	2.42	0.16	3.41	80	20
	0.01	0.00	NA	0.00	NA	0.00	NA	80	20
	4.08	0.09	2.37	0.14	3.62	0.16	3.71	80	20

[1] Within-run (repeatability). Between duplicate precision averaged over all runs.
[2] Within-calibration. Total precision with weighted components of within-run, between-run, and between-day variation.
[3] Within-lab. A measure of the effect of recalibration on total precision, calculated within reagent lot, using data from at least 5 calibrations..

Conclusion

The VITROS Anti-HTLV I/II assay* demonstrates excellent analytical performance in the detection of HTLV I/II antibodies.

* Product availability subject to local regulatory requirements. Not approved or cleared for US Market.

Ortho Clinical Diagnostics