

BACKGROUND

Western Blot was introduced into HIV testing in 1984 and was proposed for systematic confirmation of reactive ELISA results in 1985. It has remained a principal confirmatory tool used worldwide.

OBJECTIVES

In this study, the performance of new Geenius™ HIV 1/2 Confirmatory assays (Bio-Rad, Marnes La Coquette, France) was compared to that

The use of recombinant peptides or proteins for the production of the strips (dot blot) has been employed in order to have an easier reading and interpretation.

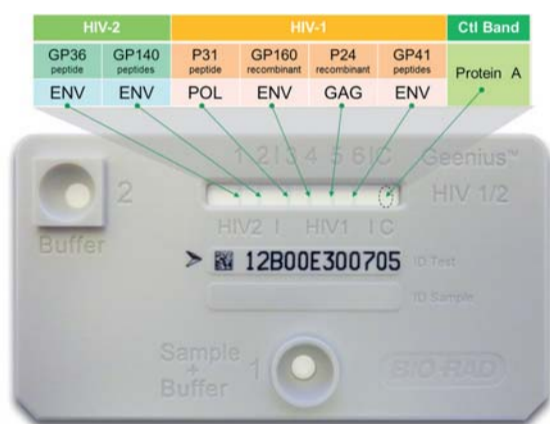
of NEW LAV BLOT I and NEW LAV BLOT II (Bio-Rad, Marnes La Coquette, France) routinely used in our laboratory.

MATERIALS AND METHODS

Assay design

The Geenius™ HIV 1/2 Confirmatory Assay employs HIV-1 and HIV-2 antigens bound to the membrane and colloidal gold Protein A (Fig. 1). The sample is applied to the sample + buffer well 1. After the sample and buffer have migrated onto the test strip (5 minutes), additional buffer is added to the buffer well 2 (20 minutes). In a reactive sample, the antibodies are captured by the antigens immobilized in the test area (test lines 1 to 6, Fig. 1). Geenius™ HIV 1/2 Confirmatory Assay cassette is then introduced in the Geenius™ HIV 1/2 Confirmatory reader, bands are automatically read and interpreted following Expert Software proprietary algorithm.

Fig. 1: Geenius™ HIV 1/2 Confirmatory Assay cassette



Test line 1 gp36 (HIV-2, envelop peptide)
 Test line 2 gp140 (HIV-2, envelop peptides)
 Test line 3 p31 (HIV-1, polymerase peptide)
 Test line 4 gp160 (HIV-1, full length envelop recombinant protein)
 Test line 5 p24 (HIV-1, full length core recombinant protein)
 Test line 6 gp41 (Group M & O) (HIV-1, envelop peptides)
 Test line 7 Control Band (Protein A)

The Table I shows criteria for interpretation of the band profiles of manufacturers.

Table I: Pack Insert validation Criteria

Positivity Criteria	Geenius HIV1/2 Confirmatory	NEW LAV BLOT HIV-1		NEW LAV BLOT HIV-2
		WHO*	CRSS**	
Positive HIV-1	At least : 1 ENV HIV-1 + (GAG or POL) OR 2 ENV HIV-1	2 ENV ± GAG ± POL	1 ENV + (1GAG or 1 POL)	
Positive HIV-2	2 ENV HIV-2			ENV*** + GAG + POL
Negative	No band	No band	No band	No band
Indeterminate	Bands that do not qualify as positive			

*WHO: World Health Organization

**CRSS: Consortium for Retrovirus Serology Standardization

Specimens for specificity and sensitivity evaluation

488 unselected serum samples, submitted to the laboratory for HIV confirmatory testing, were examined by Geenius™ HIV1/2 Confirmatory assay. Whenever one sample scored untypable by Geenius™, a differential assay with a synthetic peptide-based immunoassay Pepti LAV 1-2 (Bio-Rad, Marnes La Coquette, France) was used.

In addition, 92 frozen positive HIV-2 specimens (characterized by NEW LAV BLOT II and Pepti LAV 1-2, see (Table II) were included for the sensitivity of HIV-2 study.

CONCLUSION

In this study, Geenius™ HIV 1/2 Confirmatory Assay had an excellent sensitivity on HIV-1 samples better than NEW LAV BLOT I for HIV-1 positive samples. It reduced the number of indeterminate test results previously associated with the HIV-1 WB during the primary HIV-1 infection. However all indeterminate results should be interpreted with caution and the following samples (2 or 3 weeks after) are required to confirm the recent infection. The HIV-2 differentiation rate was 81.5% (75/92) with our HIV-2

Table II. Interpretation rules applied for the confirmation HIV-1 and HIV-2 in our lab

NEW LAV BLOT HIV-1 WHO	NEW LAV BLOT HIV-2 CRSS	NEW LAV BLOT HIV-2	Pepti Lav 1-2	HIV Interpretation in our lab
Negative	Negative	Negative	NT	Negative
Positive	Positive	Negative	NT	HIV-1 positive
Positive	Positive	Indeterminate	NT	HIV-1 positive
Indeterminate	Positive	Negative	NT	Indeterminate HIV-1
Indeterminate	Indeterminate	Negative	NT	Indeterminate HIV-1
Negative	Negative	Indeterminate	NT	Indeterminate HIV-2
Negative	Negative	Positive	NT	HIV-2 positive
Indeterminate	Indeterminate	Positive	NT	HIV-2 positive
Positive	Positive	Positive	HIV-2 Positive	HIV-2 positive

NT: no tested

RESULTS

Specificity and Sensitivity on 488 unselected samples for HIV confirmatory testing

The following Table III shows the results obtained with the Geenius™ HIV 1/2 Confirmatory assay in comparison with the NEW LAV BLOT I and NEW LAV BLOT II.

Table III: Results of 488 unselected samples.

Geenius HIV 1/2	New LAV BLOT I, II				
	Negative	HIV-1	HIV-2	Indeterminate	
Negative	257	0	0	0	257
HIV-1 Positive	0	205	0	10**	215
HIV-2 Positive	0	0	6	0	6
HIV-1 indeterminate	3*	0	0	0	3
HIV-2 indeterminate	2	0	0	0	2
HIV positive untypable	0	1	4	0	5
	262	206	10	10	488

*2 samples in HIV-1 primary infection (p24 Ag reactive)

**10 samples provided from 10 patients recently infected by HIV-1.

- Among 488 routine samples no false negative results were found.
- 3/260 (1.6%) negative samples (no reactive with Western Blot I/II and ARCHITECT HIV Combo) were found indeterminate results (1 HIV-1 indeterminate and 2 HIV-2 indeterminate).
- All 206 HIV-1 samples were found HIV-1 positive with Geenius™. One was classified as untypable.
- 7 (70%) of 10 HIV-2 positive samples were classified as dual HIV-1 and HIV-2 infection by NEW LAV BLOT I and II when only 4 (40%) were classified as HIV positive untypable with Geenius™ HIV 1/2 Confirmatory assay.
- 12/12 (100%) samples of 12 patients in HIV-1 primary infection were found positive (10 patients) and indeterminate (2 patients) by Geenius™ HIV1/2 Confirmatory assay in contrast with NEW LAV BLOT I which gave 10/12 (83%) and 2/10 (17%) negative.

Sensitivity of HIV-2 study

The following Table IV shows the results of 92 HIV-2 samples obtained with the Geenius™ HIV 1/2 Confirmatory assay:

Table IV: Results of 92 HIV-2 positive samples confirmed with NEW LAV BLOT II:

Number of samples	Geenius™ HIV 1/2 Confirmatory	NEW LAV BLOT HIV-1		NEW LAV BLOT HIV-2	Pepti Lav 1-2
		WHO	CRSS		
6	HIV-2 Positive	Indeterm.	Indeterm.	Positive	NT
23	HIV-2 Positive	Indeterm.	Positive	Positive	NT
46	HIV-2 Positive	Positive	Positive	Positive	Positive HIV-2
16	HIV positive untypable	Positive	Positive	Positive	Positive HIV-2
1	HIV positive untypable	Indeterm.	Positive	Positive	NT

NT: no tested

- No false negative and indeterminate results were found in the 92 HIV-2 well characterized samples (sensitivity 100%).
- 18.5% (17/92) were found HIV positive untypable by Geenius™ HIV1/2 Confirmatory assay. In contrast, 67.4% (62/92) were classified as dual HIV-1 and HIV-2 infections with NEW LAV BLOT I and II.

positive samples.

The Geenius has additional features including full traceability, sample and cassette barcoding that improve the quality management/assurance of HIV testing.

In addition, it is the first unitary assay for the confirmation and differentiation of HIV-1 and HIV-2 in serum, plasma or whole blood in less than 30 minutes.