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BIO-RAD Geenius Assay Providing a Breakthrough in Diagnosis of HIV-1 & HIV-2 Infection

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ABSTRACT

Where the HIV diagnostic tests are usually carried out in India by enzyme-linked immunosorbent assay(ELISA) and Western Blot(WB) as a confirmatory test, the Geenius HIV-1/2 complemental assay provides a complete system for HIV diagnosis manufactured by Bio-Rad Laboratories, USA. Geenius has already been approved by the Center for Disease Control and Prevention(USA). The main differences that question the perpetuity of the current clinical methods are Gennius's simpler mechanism, faster results. On top of that, it is cheaper to use which makes it a quite attractive choice in low-resource settings. An accurate and early diagnosis is needed for HIV infection not only to provide adequate treatment to the patient which can prolong the patient's life significantly but also to prevent its metastasis in the community. A very significant problem of the current methods like WB or line immunoassay(INNO-LIA) is, they can't differentiate between HIV-1 and HIV-2 infection. This error in diagnosis can build resistance to the virus as both types of infection needs different therapeutic agents who target specific areas of the virus. In this review, we provided some results from researches done by different groups of researchers from different countries and diverse populations. These studies compared the results from WB, INNO-LIA, and Bio-Rad Geenius. By using statistical results, the studies found Geenius results as more sensitive, efficient, time-friendly, and can efficiently differentiate between HIV-1/2 infection that is a major drawback in the conventional methods. From the available results of the studies, we suggest the adaptation of Bio-Rad Geenius assay for simpler, cheaper, and rapid diagnosis of HIV infection.

Key Words : Geenius HIV-1/2 assay, Western Blot, sensitive, Time friendly, Differentiate HIV-1/2 Infection.

INTRODUCTION

There are nearly 2.5 million new cases of the human immunodeficiency virus (HIV) registered worldwide each year. Whereas, there is no drug or vaccine discovered that can eradicate this infection till now. Long-term treatment can provide survival but patients are often exposed to opportunistic infections and pre-existing infections that reactivates.[1] Therefore, early detection of the disease is crucial not only for the patient's survival but also for the prevention of spreading. The diagnosis of this infection consists of an enzyme-linked immunoassay (ELISA) which is for screening purposes, Western Blot (WB), and line immunoassay(INNO-LIA) for conformation. [2] But the problems with these conventional methods are their expensiveness, time-consuming nature, and requirement of sophisticated equipment which is difficult to obtain in a low-resource setting. The delay resulted in a significant detrimental effect on the treatment. This problem worked as an impetus for creating rapid diagnostic tests that can provide a semblance in our fast life. Bio-Rad genius HIV-1/2 confirmatory assay known as Geenius assay has claimed to provide a more sophisticated and sensitive result as compared to the already available ones. Also, they have the convenience of being inexpensive, less complex, and much faster. US-FDA and CE in Europe already approved Geenius assay as a confirmatory test for HIV. The diagnosis of the HIV type is an important step for further treatment as misdiagnosis can have serious repercussions. HIV-2 is resistant to non-nucleoside reverse transcriptase inhibitors(NNRTI) whereas drugs with NNRTI are the drug of choice in the treatment of HIV-1. Therefore, an HIV-2 infected patient who's misdiagnosed as HIV-1 infected and treated with NNRTIs can show drug resistance and long-term therapy failure. Bio-Rad multisport assay can be used as a confirmatory test as well as to differentiate the HIV type. Sample such as blood, serum, or plasma is processed separately in a closed cassette where synthetic or recombinant peptides specific for HIV-1/2 antigens are applied as discrete bands. [3] The results are available within 30 minutes following a three-step protocol. The adaptation of Geenius study in the HIV testing algorithm is proving to be more advantageous. In the following pages, some research works are comparing Geenius assay to other previously available methods which will demonstrate whether we should embrace this new technology or not.

STUDY-1

This study evaluated the applicability of Bio-Rad Geenius HIV-1/2 confirmatory assay as a substitute of INNO-LIA assay in samples that were reactive on primary screening Immunoassays submitted to the Israeli National HIV Reference Laboratory (INHRL). 191 samples(n=129;positive/n=69; negative) were taken in this study where the INNO-LIA and Geenius assays were carried out according to the manufacturer's instructions. [3,4] The Geenius study was repeated in cases where the results were discordant with the INNO-LIA. The percentage of correct assay samples was respectively high in the case of Geenius assay (85% [168/198]) when compared to INNO-LIA (75% [149/198]). On top of that Geenius study provided a lesser number of negative or indeterminate results from the HIV-positive individuals, thus is more sensitive and can detect new HIV infections. The efficiency of the Geenius study was superior to INNO-LIA in all parameters tested, however, the harmony between the two assays was good (kappa=0.87). Geenius assay also provides other advantages like minimizing the risk of contamination, usage of bar code for samples, the cassette reduces mistakes and using automated reader mitigate the chance

of individual mistake. In conclusion, the study recommended using Geenius assay as more sensitive and efficient than IMMUNO-LIA. [5]

Table 1: Performance of Geenius and INNO-LIA assay for HIV-Positive(n=129) and negative (n=69) individuals.

True HIV status	INNO-LIA result	No. of Individuals with indicated Geenius assay result		
		Positive (n=100)	Indeterminate(n=11)	Negative (n=18)
Positive (n=129)	Positive (n=86)	84	2	0
	Indeterminate(n=21)	16	4	1
	Negative(n=22)	0	5	17
		Positive (n=0)	Indeterminate(n=1)	Negative(n=68)
Negative (n=69)	Positive(n=0)	0	0	0
	Indeterminate(n=6)	0	0	6
	Negative(n=63)	0	1	62

*HIV-1 positive, HIV-2 positive and Indeterminate HIV results found in both assays considered as positive.

STUDY-2

This study is a comparative evaluation of the Geenius-1/2 study with conventional western blots done in the Japanese population. The objective was a comparison for the confirmation and differentiation performance of NEW LAV BLOT 1& 2(NLB-1/2) also known as WB-1/2 and Geenius assay. They studied a total of 166 HIV-1 positive samples where 146 were from patients with chronic infection (73 were receiving antiretroviral therapy) and 20 from acutely infected patients. They had been diagnosed positive with Dainascreen HIV combo (HIV-1 p24 ag/HIV-1/2 Ab immunochromatographic test) or the Architect HIV Ag/Ab combo assay and used NLB-1&2 and the Cobas Ampliprep/Cobas TaqMan HIV-1 test as confirmatory tests. Table-2 compares Geenius, NLB-1/2 assays results for those 140 samples. Geenius provided 145 positive results with just one indeterminate result with 99.3% sensitivity. The NLB-1 showed 144 positive results and two indeterminate results providing a sensitivity of 98.6%. On the other hand, among those 20 acute infected HIV-1 patients Geenius confirmed 7 cases as positive infection among those who were shown indeterminate by NLB-1. Thus, Geenius had shown higher detection sensitivity than that of NLB-1.[6]

Table 2: comparison of NLB-1 &2 with Geenius results for chronic and acute HIV-1 samples.

	WB		Geenius						
			HIV-1 positive	HIV-1 Indeterminate	HIV-2 Positive	HIV-2 Indeterminate	HIV positive Untypeable	HIV Negative	Total
Established HIV-1 Infection[a] (n=146)	NLB 1	Positive	143	0	0	0	1	0	144
		Indeterminate	1[c]	1[c]	0	0	0	0	2
		Negative	0	0	0	0	0	0	0
		Total	144	1	0	0	1	0	146
	NLB 2	Positive	18	0	0	0	0	0	18
		Indeterminate	122	1	0	0	1	0	124
		Negative	4	0	0	0	0	0	4
		total	144	1	0	0	1	0	146
Acute HIV-1 Infection[b] (n=20)	NLB 1	Positive	0	0	0	0	0	0	0
		Indeterminate	7	6	0	0	0	3	16
		Negative	0	0	0	0	0	4	4
		Total	7	6	0	0	0	7	20
	NLB 2	Positive	0	0	0	0	0	0	0
		Indeterminate	5	3	0	0	0	0	8
		Negative	2	3	0	0	0	7	12
		Total	7	6	0	0	0	7	20

[a]NLB-1 and Nucleic Acid Test(NAT) positive.

[b]NLB-1 negative but NAT positive while sample was collected.

[c]on anti-retroviral therapy while sample was collected.

Again researchers included 30 samples of two commercial HIV-2 panels which were used to compare NLB1, NLB 2, and Geenius. [Table3] Geenius gave 28 HIV-2 positive and 2 positive but untypable results with sensitivity of 100% [95% cl, 88.4-100.0], NLB 2 also provides a sensitivity of 100 % [95% Cl, 88.4-100.0] whereas NLB1 gave only 2 positive results with 28 indeterminable ones and the false positivity rate is around 6.7%.

Table 3: Comparison of Geenius, NLB1 and NLB2 for HIV-2 positive samples.

		Geenius					
		HIV-1 positive	HIV-2 positive	HIV-2 positive with HIV-1 cross reactivity	HIV positive untypable	HIV negative	Total
NLB1	Positive	0	0	2	0	0	2
	Indeterminate	0	10	16	2	0	28
	Negative	0	0	0	0	0	0
	Total	0	10	18	2	0	30
NLB2	Positive	0	10	18	2	0	30
	Indeterminate	0	0	0	0	0	0
	Negative	0	0	0	0	0	0
	Total	0	10	18	2	0	30

From the results, the researchers concluded that Geenius is undoubtedly an emerging alternative than that of western blot for not an only confirmation but also differentiation of HIV-1 and HIV-2 patients. Further, they also added that it is also an approach with rapid and accurate results at a low cost.

STUDY-3

This study was done to evaluate the efficacy of Bio-Rad Geenius as a substitute of western blot for HIV-1/2. Here a total of 370 samples were collected from 356 patients and classified in the following Table 4. Where panel 1 had 57 HIV-1/2 negative samples. Panel 2 had 58 HIV-1 positive samples, panel 3 contains 36, HIV-2 positive samples where the infection was confirmed by radioimmunoprecipitation assay. Panel 4 had 9 HIV-infected patients but the results were untypable by Inno-Lia. Panel 5 had 110 samples from patients whose serum samples were indeterminate by Western Blot and Inno-Lia.

Table-4: performance of Geenius assay and western blot

HIV Serum parameter	Geenius	WB	P Value[b]
HIV negative panel (n=57)			
No. correctly identified/total no.	53/57	51/57	
No. indeterminate / total no.	4/57	6/57	0.6875
Specificity (%)	93	90	
Indeterminate rate (%)	7	10	
HIV11 positive panel (n=58)			
No. correctly identified/total no	58/58	50/58	
No. indeterminate / total no.	0/58	8/58	NA
Sensitivity(%)	100	86	
Indeterminate rate (%)	0	14	
HIV-2 positive panel (n=36)			
No. correctly identified / total no.	32/36		
No. untypeable / total no.	3/36		<0.0001
No. indeterminate / total no.	1/36[a]		
Sensitivity (%)	97		
Differentiation Capacity (%)	89		
Indeterminate rate (%)	3		
HIV Inno-Lia untypeable panel (n=9)			
No. correctly identified HIV-1 / total no.	7/9	NA	
No. correctly identified HIV-2 / total no.	2/9	NA	
No. indeterminate / total no.	0/9	9/9	
Sensitivity(%)	100	NA	
WB/Inno-Lia indeterminate status (n=110)			
HIV-1	11[a]/110	NA	NA
Indeterminate	13/110	110/110	
Negative	86/100	NA	

[a]patients presenting discordant results.

[b]McNemar's exact test may not be performed when one of the two assays presents only one result.

NA- Not applicable.

Table-4 panel 1 contains 57 samples that showed a specificity rate of 93% for Geenius and 90% for western blot. In panel 2, 58 HIV-1 positive samples showed a sensitivity of 100% for Geenius and

only 86% for western blot. In panel 3, among 36 HIV-2 positive patients, Gennius showed a sensitivity rate of 97% when compared to western blot which showed only 39% sensitivity. In panel 4, among 9 HIV untypeable patients Geenius confirmed seven as HIV-1 and two as HIV-2 infection. Lastly, the fifth panel with 110 samples with repeated indeterminate results for both Western blot and Immuno-Lia. Geenius showed only 3/110(22.7%) false-positive results.[7] This study was also carried out on cadaveric samples that makes this study atypical of the other studies. Here 80 blood samples were investigated as presented in the following Table 5.

Table-5: Geenius assay confirming HIV11 infection in cadaveric samples

HIV cadaveric sample parameter	**Geenius Result**
HIV negative(n=54)	
No. Correctly identified / total no.	52/54
NO. HIV-1 indeterminate / total no.	1/54
No. HIV-2 indeterminate / total no.	1/54
Specificity (%)	96
Indeterminate rate (%)	4
HIV positive (n=23)	
No. correctly identified / total no.	23/23
Sensitivity (%)	100
HIV-1 indeterminate (n=3) (no. / total no.)	
Negative	3/3

In these samples, Geenius showed a 100% sensitivity to the HIV-1 infection and showed a specificity of 95%. In a nutshell, the researchers suggested that Geenius can be used very effectively in place of western blot as it provides more accurate, rapid, detection of early infection and reduction in the number of indeterminate cases which is subversive to the previously available ones.

CONCLUSION

Based on the statistical results we already saw why Bio-Rad assay for HIV is being suggested by the researchers than the other methods like Western Blot or Immuno-Lia assay. The results are very promising and have the potential of wide use all over the world. It is also true that in low-resource settings where manpower is already inefficient, will they be able to operate this sophisticated method that needs proper training? Bio-Rad is very time friendly which can determine and confirm HIV-1 assay with sensitivity and specificity of 100% in most of the cases but it is also true that in many cases the already clinically active methods e.g. Western Blot is providing very close results to that of the Geenius. That can put administrations in a dilemma of whether the shift to a whole new testing method that also needs new investment and training to the technicians. They can think that is not worthy to implicate Geenius in place of already available ones that hinder the objective of this novel approach. So, further research is needed to make Geenius 100% sensitive to HIV-2 infections as well. It is also noted that even though a large number of samples can be tested in a short period, the cost of the test kit limits the usage, and in a low-budget setting that is a real issue. That's why Geenius is used as a confirmatory assay. We should find a solution where the test kits will have a reasonable price. In conclusion, we can claim that from the available results still

available, the Geenius assay is superior in terms of time, simplicity of handling, accurate results, sensitivity, specificity with low cost than many other previously available ones. So, it should be used widely for determination and confirmation of this inexorable disease in time that will save many lives and Geenius assay has made it easy.

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