

## Original Article

## Evaluation of HIV Indeterminate Confirmatory Test Results of Blood Donors in Northeast of Iran

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### ABSTRACT

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#### Keywords

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**Background and Aims:** Human Immunodeficiency virus (HIV) is spreading rapidly among the people worldwide. Infection with this virus leads to immune suppression and finally acquired immune deficiency syndrome. Early HIV detection is dependent on antibody screening against virus by enzyme linked immunosorbent assay (ELISA). Some confirmatory tests such as western blot and recombinant immunoblotting assay (RIBA) are used to verify viral infection. Many of the confirmatory test results are indeterminate. The aim of this study was to compare the frequency and patterns of indeterminate results of confirmatory tests in two groups; blood donors and patients with high risk behaviors in the northeast of Iran.

**Materials and methods:** This is a cross-sectional study from October 2009 to March 2014. A total number of 1055 serum samples with previous positive HIV ELISA test history were tested in our laboratory, some by RIBA and some by western blot method.

**Results:** Most of the indeterminate results belonged to blood donors and western blot analysis. The most reacting band was P24 in both methods and groups.

**Conclusion:** RIBA assay is more sensitive and reliable than western blot, but it is necessary to use other supplementary tests with less indistinctive results. It is necessary to pay attention to HIV glycoprotein reactivity in some methods, too.

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## Introduction

Human immunodeficiency virus (HIV) is spreading rapidly among the people worldwide [1]. Most of the infections are due to HIV1, which belongs to retrovirus family [2] and infects CCR5+, CD4+ T lymphocytes [3, 4]. It was first reported in United States of America in 1981 [5]. Infection with this virus leads to immune suppression and finally acquired immune deficiency syndrome (AIDS) [6]. Early HIV detection is dependent on antibody screening against virus by enzyme linked immunosorbent assay (ELISA) method [7]. Whenever the initial serodiagnostic test is positive, confirmatory tests such as nucleotide amplification test (NAT) [8,9], recombinant immunoblotting assay (RIBA) [10], western blot (WB) [1, 9,11-13] and recently polymerase chain reaction (PCR) should be used to verify viral infection [12,14].

The most practical confirmatory test and actually the gold standard method is WB [1, 14]. Results of this test are very important in the diagnosis of diseases; however, a large number of them are indeterminate. These results are very controversial, and their interpretation is not easy [11]. HIV infection induces inappropriate stress and complications for patients; those who are suspected to infection need more medical care and further tests for diagnosis [15]. Causes of indeterminate results are the following: infection with human T-lymphotropic virus type 1 (HTLV-I) or other retroviruses due to homologous region of virus surface glycoprotein [16], some medical conditions

such as leprosy, autoimmune disease, multiple blood transfusion, polyclonal gammopathy and hemodialysis [17, 18], and human contact with coprine arthritis encephalitis virus [19] due to cross reaction with P24 and other abnormal immune reactions [20, 21]. It has been reported that false positive results can occur in some certain conditions such as Lishmaniasis [22] and following influenza vaccination. In the case of the latter, it has been claimed that molecular protein resemblance of HIV1 envelope and influenza can be responsible for these false positive results [8].

In this study we compared the frequency of indeterminate results of two supplementary tests; RIBA and WB, in two groups; blood donors who were known as low risk group, and patients with high risk behaviors.

## Materials and Methods

From October 2009 to March 2014, a total number of 1055 serum samples with the history of previous positive HIV ELISA test were tested in our laboratory (the only laboratory which performs WB and RIBA test in North East of Iran). In this cross-sectional study, 395 samples belonging to blood donors and 610 samples belonging to the high risk group who had referred to health centers for treatment were enrolled. Testing algorithm is shown in Fig.1.

### Anti-HIV Assay

The vironostika HIV Ag/Ab kit (Biomerieux Sa, France) which is based on one step sandwich principle was used for anti-HIV

antibody detection according to the manufacturer's instructions.

### Western blot Analysis

Western blot was used as a supplemental test for samples found with repeatedly positive ELISA [23]. The HIV BLOT 2.2 WB assay (MP biomedical Asia pacific Company) was used according to the manufacturer's instructions. Interpretation of the results was performed according to the manufacturer's instructions, too. HIV infection was confirmed when positive results were obtained from WB assay.

### RIBA Analysis

The INNO-LIA HIVI/II SCORE (INNOGENETICS N.V, Belgium) was used

according to the manufacturer's instructions. Recombinant proteins and synthetic peptides from HIV-1 and HIV-2, and a synthetic peptide from HIV-1 group O were coated as discrete lines on a nylon strip with plastic backing. Five HIV-1 antigens were applied: sgp120 and gp41, which detect specific antibodies to HIV-1, and p31, p24, and p17, which may also cross-react with antibodies to HIV-2. HIV-1 group O peptides were present in the HIV-1 sgp120 band. The antigens gp36 and sgp105 were applied to detect antibodies to HIV-2. The results were interpreted based on the manufacturer's instructions, too [24].

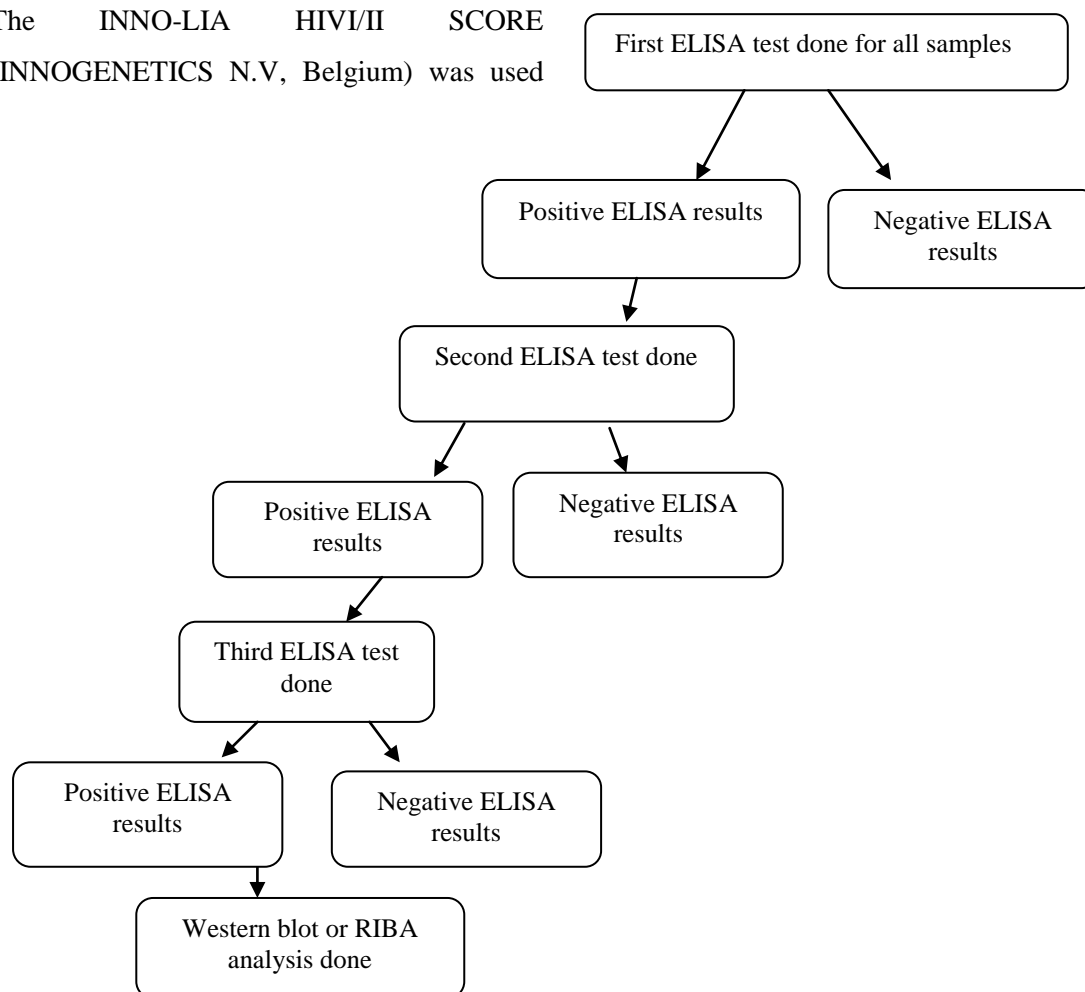


Fig. 1. Flowchart of design and protocol of the study.

Samples with negative ELISA result were excluded from the experimental.

Among 1055 samples with positive ELISA result, 395 belonged to blood donors. Infection was determined in 95 cases by RIBA test and in 297 others by western blot assay. The rest which belonged to high risk group were examined by WB and RIBA methods (463 and 207 cases, respectively).

## Results

Frequency distribution of the results of the two methods is shown in table 1. The

indeterminate results of WB and RIBA for both groups are shown in tables 2 and 3, respectively. In this cross-sectional study which was conducted from October 2009 to March 2014, 1.26% of blood donors and 39.55% of the high risk group referring to the health centers for HIV infection in the northeast of Iran were included. P24 was reacting in most cases. Their bands appeared in 76.17 % of WB analysis and 64.28% of RIBA tests. A sample of HIV glycoproteins reaction in western blot is shown in Fig. 2.

**Table 1.** Frequency distribution of the western blot and RIBA method results in studied groups

	Western Blot			RIBA		
	Positive	Indeterminate	Negative	Positive	Indeterminate	Negative
<b>Blood donors group</b>	0	142	155	5	5	78
<b>High risk group</b>	166	88	209	99	12	96

**Table 2.** HIV glycoprotein reactivity in indeterminate results of western blot in the two groups

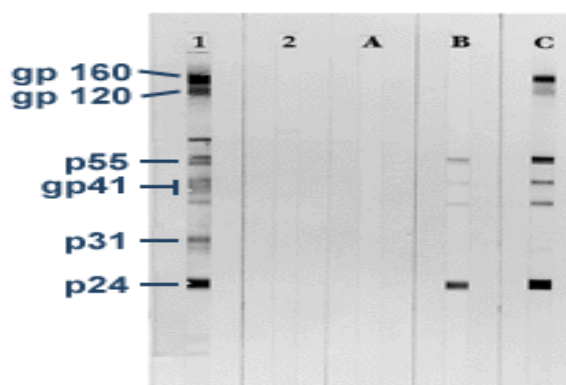
HIV Glycoprotein	P24	P17	Gp120	P31	P39	P41	P51	P55	P66	Gp160
<b>Blood donors group</b>	86.84	5.26	3.86	0.00	0.00	0.00	0.00	0.00	0.00	3.86
<b>High risk group</b>	65.51	4.59	2.29	3.44	0.00	3.44	3.44	1.14	8.05	8.05

Data are presented as percent

**Table 3.** HIV glycoprotein reactivity in indeterminate results of RIBA in the two groups

HIV Glycoprotein	P24	P17	Gp120	P31	P41	P105	P36
Blood donors group	100	0.00	0.00	0.00	0.00	0.00	0.00
High risk group	28.27	21.42	14.28	7.14	28.57	0.00	0.00

Data are presented as percent



**Fig.2.** HIV glycoproteins reaction in western blot

## Discussion

In this study, we evaluated the frequency of HIV indeterminate results and glycoprotein reactivity in two groups by two supplementary tests. As it is clear, frequency of indeterminate results are varied in different groups and different manners [14]. It is very important to approve or disprove HIV infection in suspected patients with indeterminate results of supplementary tests as false positive results may have unwanted consequences [11].

Just as Mas et al. have expressed, comparing redundancy of confirmatory trials exerts low plenty in RIBA assay and decreases the

number of indeterminate conclusions; In addition, high sensitivity and specificity for RIBA outcomes is indicated [10]. P24 band is the most frequently appeared band in both confirmatory tests as has been discovered by Carneiro-Proietti et al. study [25]. In Huang et al. study in the area of serologic tests for indeterminate results of western blot assay, 22.5% of results were indeterminate. The value is very similar to the obtained data in this study [7]. Just as Dodd and associates report, frequency of indistinctive results of WB assay is 6-60% [15].

Comparison of the results of confirmatory tests in both groups revealed high frequency of

indeterminate results in blood donors known as low risk group. This finding has been approved by Cremonesi et al. on the prevalence of indeterminate results in Brazil in 2005 [1], and Dodd in 2000 [15]. As has been discovered by Guan, WB indeterminate patterns occur more commonly for core antigens such as P24, P17 and P55. P24 band was the most abundant band which appeared in indistinctive patterns of WB and RIBA assay. It is necessary to know that P24 is a non-permanent viral protein which will disclose at the advanced stage of the disease [11]. Interaction with P17 antibody band, the most frequently-appeared band after P24, was 21.42% in the high risk group. It, however, failed to appear in the blood donors. Indeed, it is evident that incidence of different bands in both confirmatory tests is dependent on the stage of the disease.

We found that in WB analysis, reactivity with all bands was the dominant pattern. Reactivity with all bands is also reported by Sudha et al. in 92.91% of WB assays. P31 was the most

missing band in their positive templates report, while this did not appear in most of the indeterminate results of this study [26].

## Conclusion

Due to the high incidence of indeterminate results, it seems necessary to use other supplementary tests with less indistinctive results. Most diagnostic laboratories use complex tests to ensure infection. It is important to pay attention to HIV glycoprotein reactivity in some methods such as WB and RIBA as a criterion to determine the stage of the disease. Further studies are suggested to follow up patients with indeterminate patterns to specify which samples are more possible to turn positive.

## Conflict of Interest

The authors declare that they have no conflict of interest

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