

## NON-INSTRUMENTAL IMMUNOASSAY FOR ANTIBODIES TO HIV-1 AND HIV-2

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### Introduction

Human immunodeficiency virus (HIV) infection and AIDS have become a global health problem in the two last decades. From the beginning of the pandemic in 1980 until mid-1996, about 27.9 million people have been infected worldwide with HIV and more than six million adults have developed AIDS. In mid-July 1996, an estimated 21.8 million adults and children worldwide were living with HIV-AIDS, 94 % of them in the developing world (1). Since the identification of the human immunodeficiency viruses, substantial progress has been made in the development of diagnostic methods for the detection of HIV-1/2 infection and blood screening for antibodies to HIV-1/2 has been established as mandatory in most countries. The ELISA type systems are the traditionally used assays for this purpose. However, in the course of the last years, different non-ELISA tests for antibodies to HIV-1/2 have been reported. They include latex agglutination, hemagglutination, chromatographic, flow-through dot- and line-blots formats (2-5). These assays are highly recommended in situations where rapid results are needed such as doctor offices, emergency posts, and dental clinics. In such situations the use of blood collected by digital extraction instead of serum constitutes an additional advantage. Moreover, in developing countries, where sophisticated equipment and automated instruments are not always available and the supply of electricity is inconsistent, there is an urgent need for simple, fast tests which require semiskilled personnel, and can give unambiguous results (6).

The aim of this study was to develop a simple visual immunoassay for antibodies to HIV-1 and HIV-2 using the proprietary AuBioDOT™ technology, and a combination of two HIV-1 (p24r and gp41r) recombinant antigens and a HIV-2 synthetic peptide (pep36) as coating. The sequential incubations of the coated AuBioDOT™ slides with serum, a protein A-colloidal gold conjugate, and a silver ion enhancer result in dark color metallic deposits in the reaction areas incubated with the positive samples. The whole procedure takes 40 min and needs no incubation equipment.

### Materials and Methods

#### Antigens

The recombinant antigens were expressed in *Escherichia coli* using a system described in our European Patent Office Application No. 90202108.8. Briefly, genes were cloned in vector pFP-15, bearing the tryptophan promoter and T4 terminator. Using this vector the antigens are expressed as fusion proteins with a 58-aminoacid fragment of the human interleukin 2 at their amino terminal. This fragment increases expression by stabilizing messenger RNA. In our cloning strategies, specific aminoacids have been incorporated at the carboxyl terminus of the antigens in order to use affinity purification procedures. Recombinant proteins were expressed in *E. coli* as inclusion bodies. While the recombinant p24r comprises the whole sequence of the natural HIV-1 (subtype B) protein, the gp41r contains the N-terminal segment of the transmembrane HIV-1 (subtype B) glycoprotein (7, 8). The specific detection of antibodies to HIV-2 was enhanced with the inclusion of a synthetic peptide (pep36) that comprises aminoacids 595-612 of the HIV-2<sub>ROD</sub> transmembrane protein. This peptide was synthesized by the tea bag method and subsequently purified by reverse-phase-high-performance liquid chromatography (HPLC) on a C18 column. Purity was around 95 % according to analytical HPLC procedure.

The purification of the recombinant antigens was performed mainly by washed pellet cells and selective precipitation procedures. A final purification step using ion metal affinity chromatography (IMAC) was included. The use of IMAC for the purification of recombinant proteins allowed us to achieve more than 85-90 % of purity in one chromatographic step.

#### Samples

In a first approach we studied a panel of 702 serum samples collected from blood donors, 507 HIV-1 and 91 HIV-2 seropositive individuals. This panel included HBsAg positive samples as well as sera

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reactive to the Venereal Disease Research Laboratory assay (VDRL) and to hepatitis C and HTLV antibody tests.

#### Immunoassay procedure

The assay was developed following the principles of the AuBioDOT™ indirect immunoassay technology (Heber Biotec S.A., Havana). For the anti-HIV-1/2 visual assay, the AuBioDOT™ slides were coated in 0.05 mol/L bicarbonate coating buffer (pH 9.6) for 3 h at 37 °C with a mixture of purified recombinant gp41, p24 (HIV-1) and gp36 (HIV-2) (5 µg/mL). After removing the unbound material by washing with PBS-T sodium phosphate buffer (20 mmol/L phosphate buffer pH 7.2; 0.13 mol/L NaCl; 0.1 % (v/v) Tween-20) for 5 min, the slides were vacuum dried, sealed and stored at 4 °C until use. The samples were diluted 1:100 with PBS-T and incubated in the reaction areas (20 µL/area), for 20 min at room temperature. After washing with PBS-T for 5 min, the slides were incubated for 20 min at room temperature with 20 µL/area of a protein A- colloidal gold conjugate (Heber Biotec S.A., Havana, main particle of 18 nanometers) diluted at 2 optical densities in water. After a similar washing the reactions were amplified for 10 min with 20 µL/area of the silver enhancer solution (IntenSETM BL, Amersham, code RPN492). Positive results are seen as dark metallic deposits in the reaction areas. Color intensity is proportional to the amount of antibodies contained in the sample. Total assay time is approximately 50 min. A sample was considered positive when a color, darker than those of the negative controls was obtained in the reaction areas. Negative samples developed none or a very weak background color. The results were read directly by simple visual inspection. Doubtful samples were repeated. The slides can be stored for a permanent record of results.

## Results and Discussion

### Assay standardization

Best results were achieved when the AuBioDOT™ high binding surface was coated with 5 µg/mL of each gp41r, p24r and pep36. We did not find any differences when the proteins were coated in carbonate/bicarbonate buffer or in PBS. Full satisfying results in terms of sensitivity, specificity and uniformity of the color were obtained when non-fat milk was used as blocking agent.

### Performance

The results obtained from the evaluation of blood donors and HIV seropositive individuals using serum are shown in the Table 1. While all the 507 HIV-1 and 91 HIV-2 positive sera were reactive (100 % of sensitivity), seven out of 702 blood donors were positive in the AuBioDOT™ anti-HIV-1/2 assay (99.0 % of specificity). Two of these sera were confirmed as HIV positive or indeterminate and therefore excluded from the further analysis. Only two out of 176 sera that were reactive to any other test (HTLV, syphilis, HBsAg, HCV) were false positive (0.02 %) in the ELISA assay.

### Analytical sensitivity

The analytical sensitivity (AS) of a diagnostic test is important for the correct identification of low titer or seroconversion samples. As the AS varies from panel to panel the absolute value is meaningless, and the relative AS compared to a reference assay is usually provided. We evaluated the AS with a panel composed of 20 HIV-1 and 20 HIV-2 positive samples in comparison to the BioScreen recVIH 1/2 ELISA. We were not able to find any significant

Table 1. Cross-tabulated results for the blood donor and HIV positive sera.

	AuBioDOT™ anti-HIV-1/2 +	ELISA + (BioScreen recVIH 1/2)	WB + (David Blot HIV-1)
Blood donors (n = 702)	7	4	2*
HBsAg + (n = 38)	0	0	-
HCV + (n = 82)	0	0	-
VDRL + (n = 51)	1	1	0
HTLV -I (n = 5)	0	0	-
HIV-1 positive (n = 507)	507	507	507
HIV-2 positive (n = 91)	91	91	91

\*One of these sera was WB indeterminate (p24 positive).

differences in the AS values obtained for both assays. Therefore we conclude that the AuBioDOT™ anti-HIV-1/2 assay has an AS similar to the recombinant protein based ELISA used as reference.

#### HIV-2 pep36

HIV-2 positive sera were evaluated with and without the specific HIV-2 peptide. When the AuBioDOT™ slides were coated with gp41r and p24r only 68 % (62/91) of the HIV-2 sera were detected. Different gp41r/p24r ratios did not increase the sensitivity for HIV-2 antibodies. This problem was avoided with the inclusion of the pep36 synthetic peptide. These results are in

concordance with other reports where different peptides from the same region of the transmembrane HIV-2 protein have been found to be extremely sensitive for detecting anti-HIV-2 antibodies (9, 10).

#### Conclusion

The high values obtained for the sensitivity, specificity and AS in addition to the autonomy of the AuBioDOT™ anti-HIV-1/2 with respect to the presence of sophisticated equipment and the supply of electricity makes this assay an alternative to the ELISAs in the conditions of both rural and urban areas of many developing countries.

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