

# Apparent HIV-1 glycoprotein reactivity on Western blot in uninfected blood donors

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**Objective:** To investigate samples with 'false-positive' reactivity to HIV-1 glycoproteins on Western blot (WB).

**Design:** Samples from 13 blood donors with glycoprotein reactivity were examined for serological evidence of HIV infection and followed-up where possible.

**Methods:** Samples were tested for anti-HIV-1, HIV-1 p24 antigen, anti-HIV-2 and anti-HTLV-I. Reactivity to multimeric, monomeric, and deglycosylated gp41 was determined, as was the ability of recombinant gp160 (rgp160) to inhibit reactivity to multimeric gp41.

**Results:** Serology and follow-up failed to confirm HIV infection in any of the donors. All samples reacted to multimeric gp41, and eight out of the 13 reacted to deglycosylated gp41. Reactivity on a commercial WB was inhibited by rgp160.

**Conclusion:** Apparent reactivity to HIV-1 glycoprotein may occur in individuals with no other serological evidence of HIV infection. Reactivity to different forms of gp41 and inhibition by rgp160 suggested that the observed WB reactivity may be due to cross-reactivity with gp41 rather than to a co-migrating contaminant.

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**Keywords:** HIV-1, Western blot, glycoprotein, indeterminate, multimers, cross-reactivity.

*Bolton is wrong?*

## Introduction

Since the introduction of anti-HIV-1 screening assays that use recombinant HIV-1 envelope glycoprotein antigens, indeterminate Western blot (WB) reactivity to HIV-1 glycoprotein bands has been observed in individuals who do not otherwise appear to be HIV-infected [1]. Furthermore, samples from these individuals may react on some WB assays but not on others [2].

The clarification of these glycoprotein-reactive WB-indeterminate results is important because glycoprotein reactivity plays a crucial role in many WB interpretive criteria. This report describes the investigation of 13 glycoprotein-reactive, WB-indeterminate, blood donor samples found to be reactive in a screening as-

say incorporating recombinant glycoprotein antigens (Abbott rDNA anti-HIV EIA; Abbott Diagnostics, Wiesbaden, Germany).

## Materials and methods

### Blood donor samples

Of a population of blood donors with reactivity to one or more glycoprotein bands on anti-HIV-1 WB (Diagnostic Biotechnology, Singapore), 13 had sufficient sample volume for additional investigation. Two or more samples, collected over intervals ranging from 3 to 21 months, were available for nine of these donors. Samples were obtained at routine blood donation be-

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tween 1988 and 1991, and donors signed declaration forms stating that they had not participated in any specified HIV-related risk behaviour [3].

#### Anti-HIV-1 screening assays

All donors had at least one sample that was repeatedly reactive by the Abbott rDNA anti-HIV enzyme immunoassay (EIA). In addition, at least one sample from each donor was tested for anti-HIV-1 by three other screening assays: Genetic Systems HIV-1 EIA (Genetic Systems Corporation, Redmond, Washington, USA), Wellcome recombinant HIV-1 EIA (Wellcome Diagnostics, Dartford, England, UK) and Serodia-HIV particle agglutination assay (Fujirebio, Tokyo, Japan). All tests were performed as recommended by the manufacturers.

#### Additional testing

Where possible, the samples were also tested for HIV-1 p24 antigen (Genetic Systems or Coulter, Hialeah, Florida, USA), anti-HIV-2 (Genetic Systems) and anti-HTLV-I (Fujirebio Serodia-HTLV-I particle agglutination). Reactivity to peptides representing the major antigenic epitope of the HIV-1 and HIV-2 transmembrane glycoproteins was assessed by the Genetic Systems GENIE HIV-1/HIV-2 assay. All tests were performed as recommended by the manufacturers.

#### Western blot assays

The Diagnostic Biotechnology (DB) HIV-1 immunoglobulin G (IgG) WB and Novapath Immunoblot (BioRad, Richmond, California, USA) assays were performed as recommended by the manufacturers.

An in-house WB was performed as described previously [4]. It exhibits little or no gp120 or gp160, and gp41 is present in monomeric form only. Two modifications of this WB were performed to separate viral gp41 from potentially confounding, co-migrating contaminants, gp41 trimers (120 kD) and tetramers (160 kD) were formed in the first; and gp41 was deglycosylated in the second, reducing its electrophoretic relative molecular weight ( $M_r$ ) to approximately 40 kD.

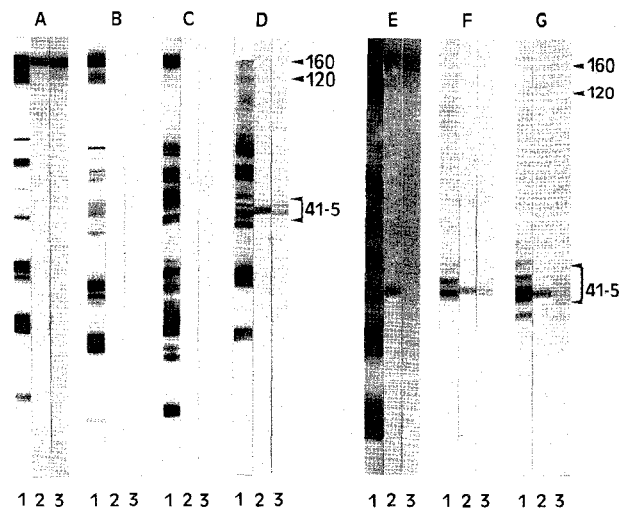
#### Formation of gp41 multimers

Multimers were formed by excluding sodium dodecyl sulphate (SDS) from the polyacrylamide gel, reducing its concentration in the sample buffer from 2% to 0.4%, and in the electrophoresis buffer from 1% to 0.025%. The acrylamide concentration was also reduced from 12% to 10% to improve detection of multimers.

#### Deglycosylation of gp41

Deglycosylation of gp41 was performed by treating viral antigen with endoglycosidase H (Calbiochem, La Jolla, California, USA), which cleaves N-glycans of high mannose content. Briefly, 100 mU lyophilized endoglycosidase H was reconstituted in 300  $\mu$ l phosphate-buffered saline (PBS; pH = 7.4) or acetate buffer (pH = 5.5), containing 0.025% SDS, 0.1 mol/l 2-mercaptoethanol and 0.5 mmol/l phenylmethylsulphonyl fluoride (PMSF). The 300  $\mu$ l of reconstituted enzyme

was added directly to 300  $\mu$ g of HIV-1 antigen and mixed by rotation for 6 h at room temperature and then overnight at 37°C. The treated antigen was used to prepare a WB in which electrophoresis was prolonged to allow better protein separation in the 30–50 kD region. A monoclonal antibody (41-7; Genetic Systems) was used to visualize reduction in  $M_r$  of the gp41 after deglycosylation (Fig. 1).



**Fig. 1.** Examples of each Western blot used to clarify antigenic glycoprotein reactivity. A, Diagnostic Biotechnology; B, Diagnostic Biotechnology inhibited with gp160 (compare with A); C, BioRad; D, in-house; E, in-house multimeric; F, in-house glycosylated; G, in-house deglycosylated. Lane 1, positive control; lanes 2 and 3, samples 3 and 6. In the case of F and G, the positive control was mouse monoclonal anti-gp41 (41-7). The gp41 band is designated gp41-5 because its typical appearance on the in-house Western blot is as a number of bands from 41 to 45 kD (see D, lane 1).

#### Inhibition by recombinant gp160

To determine whether recombinant gp160 (rgp160) could block reactivity at 160 kD on the DB WB, 1  $\mu$ g rgp160 (MicroGeneSys, West Haven, Connecticut, USA) was mixed with 40  $\mu$ l of sample diluted in 100  $\mu$ l DB WB sample buffer. This mixture was incubated with mixing for 1 h at room temperature, then without mixing for a further hour at 37°C. A routine DB WB was then performed on the blocked sample at the final serum dilution specified by the manufacturer. The rgp160 used was a full-length envelope precursor protein (containing gp41), expressed using a baculovirus vector.

#### Results

The results of the HIV-1, HIV-2 and HTLV assays and the length of follow-up are shown in Table 1. Length of follow-up was defined as the maximum time that the blood donor was monitored for any change to the

indeterminant WB profile. None of the blood donors included in this study developed any WB reactivity that indicated seropositivity.

The results of all WB assays are shown in Table 2. Eleven of the 13 samples showed reactivity to gp160 (and more weakly at gp120) on the DB WB. One sample reacted at gp160 alone, and one at gp120 alone. Only two samples reacted on the Biorad WB: one at gp120 alone and one at gp160 alone. These two samples reacted similarly on the DB WB.

All samples showed reactivity to the 41–45kD region on the in-house WB. Six were considered atypical because the reactivity was not consistent with the normal pattern seen in anti-HIV-1-seropositive samples. Sample 1 reacted at gp120 on both commercial WB assays and the in-house WB, despite its lack of gp120.

All samples showed reactivity to multimeric gp41. Five did not react to the deglycosylated gp41, and showed atypical reactivity to gp41 on the in-house WB. Another sample showed this atypical reactivity, but did react to the deglycosylated gp41.

After pre-incubation with rgp160, none of the samples reacted to HIV-1 glycoproteins on the DB WB. Titration of a positive control showed a greater than eight-fold reduction in anti-glycoprotein titre after inhibition with rgp160.

**Discussion**

This study has shown that reactivity apparently directed at HIV-1 glycoproteins may occur in individuals who show no other evidence of HIV infection. ~~The results suggest that this reactivity may be due to antibody cross-reactivity with gp41 rather than to a co-migrating contaminant. It appears unlikely that a contaminant would occur at the various  $M_r$  values represented by monomeric, multimeric and deglycosylated gp41 on the in-house WB, and that antibody reactivity to it could be blocked by rgp160. Furthermore, gp160 is tetrameric gp41 in some commercial WB assays [5], while gp120 may be trimeric gp41 [6].~~

**Table 1.** Results of testing 13 indeterminate samples using HIV-1, HIV-2 and HTLV assays.

Assay	Sample number												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Anti-HIV-1													
Abbott rDNA	+	+	+	+	+	+	+	+	+	+	+	+	+
Wellcome rDNA	-	-	-	-	-	-	-	-	-	+	-	-	-
Genetic Systems	-	-	-	-	-	-	-	-	-	-	-	-	-
Fujirebio Serodia	+	+	+	-	-	-	-	-	-	+	-	-	+
p24 antigen	-	-	-	NA	NA	-	NA	-	-	-	NA	NA	-
Anti-HIV-1 & HIV-2													
GENIE HIV-1/HIV-2	-	-	-	-	-	-	-	-	-	-	-	-	-
Anti-HIV-2	-	-	-	-	-	-	-	-	-	-	-	-	-
Anti-HTLV-1	-	-	-	-	-	-	-	-	-	-	-	-	-
Follow-up (months)	18	15	18	12	9	21	9	3	NA	NA	NA	12	NA

rDNA, recombinant DNA; NA, not available.

**Table 2.** Results of all Western blot assays performed to clarify glycoprotein reactivity.

Assay	Sample number												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Diagnostic Biotechnology													
gp160	-	+	+	+	+	+	+	+	+	+	+	±	+
gp120	+	±	±	+	±	+	±	+	+	±	±	-	+
gp41	-	-	-	-	+	+	-	+	-	-	+	-	+
BioRad													
gp160	-	-	-	-	-	-	-	-	-	+	-	-	-
gp120	+	-	-	-	-	-	-	-	-	-	-	-	-
In-house													
gp41	+A	+A	+A	+	+A	+	+	+	+	+A	+	+A	+
gp41 trimer	-	-	+	-	+	-	-	+	-	-	-	-	+
gp41 tetramer	+	+	+	+	+	+	+	+	+	+	+	+	+
gp41 deglycosylated	-	-	-	+	±	+	+	+	+	-	±	-	+

+ A, atypical reactivity.

OL160

This would explain reactivity to gp41 producing apparent anti-gp120 and anti-gp160 reactivity.

Reactivity to gp41 may be due to the homology between gp41 and non-viral proteins [7-10], or to anti-carbohydrate antibodies [11]. The spectrum of atypical WB glycoprotein reactivities shown here suggest that there may be various contributing reactivities. Further studies with additional recombinant proteins and overlapping synthetic peptides may confirm and clarify these suspected reactivities.

This study assumed that the investigated samples were from non-HIV-infected individuals. While we were unable to unequivocally prove this, samples were obtained from normal blood donors who signed a declaration form stating that they had not engaged in any HIV-related risk behaviour. Because these individuals were healthy enough to present for blood donation, it is unlikely that their indeterminate WB reactivities could have resulted from the loss of anti-core antibodies, which has been associated with late-stage AIDS [12]. Since none of the donors tested for p24 antigen were reactive (which would be expected if an infected individual lacked anti-p24), it is also unlikely that the atypical WB patterns seen could be due to seroconversion. The nine donors followed for at least 3 months did not show any evolving WB patterns consistent with seroconversion, and while anti-HIV-1 seroconversion may be an extended process in some individuals, it generally occurs 4-6 weeks after infection [13].

Ideally, we would like to have tested these donors for proviral HIV-1 DNA by polymerase chain reaction and to have attempted viral isolation, but were prevented by sampling constraints and an unwillingness to alarm volunteer blood donors without necessity.

This study did not determine the precise nature of the problematic glycoprotein reactivity on the DB WB. It did, however, reinforce the need for careful consideration of HIV-1 glycoprotein reactivity on WB in the absence of the spectrum of reactivity to viral proteins shown by HIV-1-infected individuals. We suggest that HIV-1 WB glycoprotein reactivity of this nature should be evaluated using a comprehensive panel of supplementary assays and interpreted in the light of clinical and epidemiological details. Such an approach is particularly important where the use of WB-interpretive criteria, such as those proposed by the World Health

Organization [14], are contemplated. These criteria allow individuals reactive to two glycoprotein bands to be considered anti-HIV-1-seropositive, and would inappropriately classify 11 out of the 13 samples described in this study as anti-HIV-1-seropositive by the DB WB.

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