

Sensitivity of a rapid point of care assay for early HIV antibody detection is enhanced by its affinity for HIV gp41 IgM antibodies

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Abstract

Anti-HIV-1 IgM antibody is an important immunoassay target for early HIV antibody detection. The objective of this study is to determine if the early HIV antibody sensitivity of the 60 second INSTI™ HIV-1/HIV-2 Antibody Test is due to detection of anti-HIV-1 IgM in addition to IgG. This study demonstrates that both the recombinant gp41 antigen and protein-A in the INSTI test have affinity to human IgM. Removing IgM from early seroconversion samples that were reactive with INSTI, renders them non-reactive or barely reactive. Thus, INSTI can detect anti-HIV-1 IgM antibodies which enhances its utility in early HIV diagnosis.

Introduction

Early detection of HIV is an important component of HIV prevention and control programs. In previous studies (Masciotra et al.), INSTI has shown an ability to detect HIV-1 seroconversion earlier in comparison to other rapid HIV antibody tests. We hypothesized that this capacity for early HIV antibody detection with INSTI is due to its ability to detect the presence of anti-HIV-1 IgM.

Methods

Anti-HIV-1 IgM ELISA. To determine gp41 recombinant protein affinity to IgM, the antigen was spotted onto nitrocellulose (NC) membranes. Commercial seroconversion panel samples with different levels of HIV-IgM antibody were used as primary antibodies. Mouse monoclonal anti-human IgM, horseradish peroxidase-labelled rabbit polyclonal anti mouse IgG, and chemiluminescence reagent were used as a secondary antibody, conjugate antibody, and a developer reagent respectively (see Figure 1).

Detection of human IgM by INSTI

Colour Developer: The affinity of INSTI colour developer to five different classes of purified human immunoglobulin was tested by reacting colour developer to NC-fixed purified immunoglobulins and observing for any visible test spots.

Detection of anti-HIV-1 IgM antibody by the INSTI HIV-1/HIV-2 Antibody Test:

Total IgM was removed from INSTI positive seroconversion samples (Anti-IgM Microbeads) that were WB (IgG) negative or indeterminate to observe for significant reduction or disappearance of spot intensity following INSTI testing.

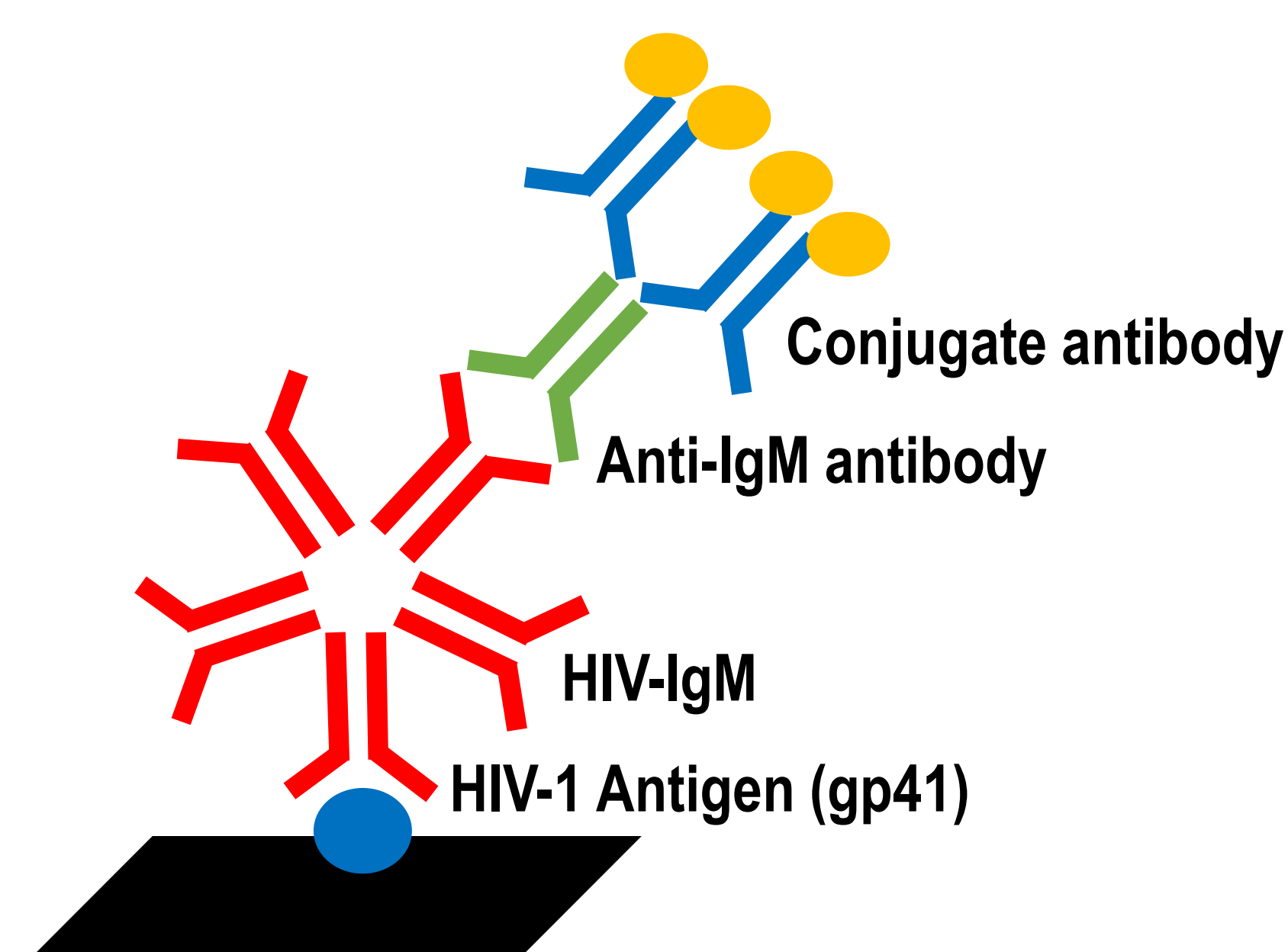


Figure 1: ELISA method for detecting anti-HIV-1 IgM antibody captured by membrane-bound recombinant gp41.

Results

The ability of INSTI HIV-1 gp41 antigens to bind anti-HIV-1 IgM antibody: Anti-HIV-1 IgM antibody ELISA results for HIV positive seroconversion samples with different amount of HIV-IgM.

Panel # PRB944	1	2	3	4	5	6
Day since first bleed	0	2	7	9	14	16
INSTI HIV1/HIV2	N	N	N	N	P	P
In-House IgM (S/CO)	N-0.3	N-0.3	N-0.4	N-0.9	P-12.9	P-15.2

Panel# PRB943	1	2	3	4	5	6	7
Day since first bleed	0	5	7	12	14	19	21
INSTI HIV1/HIV2	N	N	N	N	N	P	P
In-House IgM (S/CO)	N-0.2	N-0.3	N-0.4	N-0.3	P-1.0	P-11.7	P-12.4

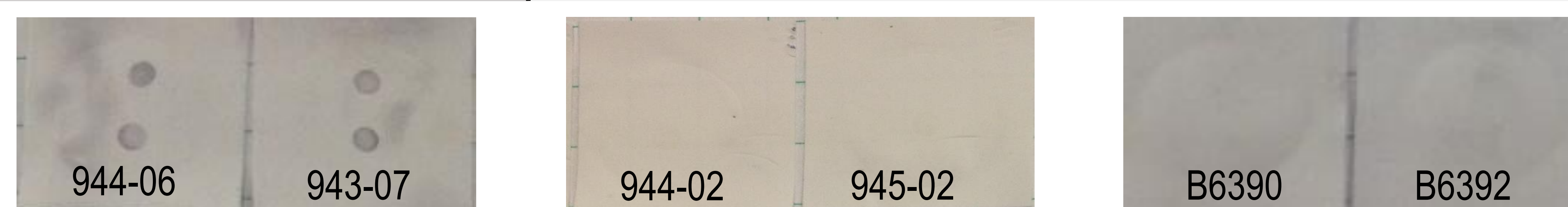


Figure 2: ELISA on seroconversion samples with high level of HIV-IgM (944-06, 943-07), and HIV antibody negative plasma samples from seroconversion panels (944-02, 945-02), and HIV negative serum (B6390, B6392) are presented below. N: Negative; P: Positive; S/CO: Signal/Cutoff.

The affinity of INSTI solution 2 (solution 2-ProA) to bind to human immunoglobulin classes.



Figure 3: INSTI protein-A/indigo solution produces visible reactions to human IgA, IgG, IgM, but not to IgD, IgE.

Summary of INSTI test results on HIV-1 Seroconversion Samples before and after IgM removal.

Sample #	Western Blot (IgG) for INSTI™ positive samples	In house-IgM	Test dot disappeared after IgM removal in %
914-01	IND	ND	100%
914-02	IND	ND	>50%
914-03	IND	ND	100%
924-06*	N	P	100%
924-07	IND	P	>50%
924-08	IND	P	>50%
925-05	IND	P	100%
925-06	IND	P	>50%
927-03*	N	P	>50%
927-04	IND	P	100%
928-02*	N	P	100%
934-02	IND	ND	>50%
934-03	IND	P	>50%
938-03	IND	P	100%
940-03	IND	P	100%
940-04	IND	P	>50%
940-05	IND	ND	<50%
941-04	IND	P	>50%
943-06*	N	P	100%
944-05	IND	P	100%
950-04	N	ND	>50%



Figure 4: A 100% reduction in INSTI intensity is interpreted as non-reactive. P: Positive; N: Negative; IND: Indeterminate; ND: in-house IgM assay not determined; *: meets Fiebig class III staging definition.

Conclusion

The INSTI HIV-1/HIV-2 Antibody Test is shown to detect anti-HIV-1 IgM antibodies in early HIV infection which enhances its utility in early HIV diagnosis.