COMPARISON OF ASSAYS USED IN THE SEROLOGICAL TESTING ALGORITHM FOR RECENT HIV SEROCONVERSION (STARHS) IN AN STI CLINIC POPULATION AND A PROPOSED ALGORITHM TO IMPROVE THE ACCURACY OF IDENTIFICATION OF RECENT HIV INFECTION (RHI).

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INTRODUCTION

STARHS assays have the ability to identify those individuals early in their HIV infection.

This may have benefits both for the individual and the wider population.

Many factors can potentially lead to misclassification of specimens as recent HIV infections.

AIMS OF THE STUDY

1) How well do STARHS assays correlate?
2) How do 'confounding' factors effect the different assays?
3) Could the different assays be combined in an algorithm to provide more certainty regarding a result on an individual patient?

METHODS

Specimens were tested in 3 assays using previously published procedures

- bioMérieux Vironostika 'detuned' assay (Kothe).
- Calypte BED assay (Parekh).
- Abbott AxSYM HIV 1/2gO assay modified for determination of antibody avidity (Suligoi).

Recent HIV infection inferred if:

- SOD (detuned) <1.0
- ODn (BED) <0.8
- Avidity Index (Abbott AxSYM) <80%

The characteristics of the 3 assays are summarised in Table 1.

3 sets of specimens were tested by all 3 methods:

- Anti-HIV-1 positive specimens from 89 newly diagnosed individuals attending a STI clinic.
- 34 specimens from patients with documented long-standing HIV infection (without AIDS and treatment naive).
- 95 sequential specimens from 19 patients receiving HAART.

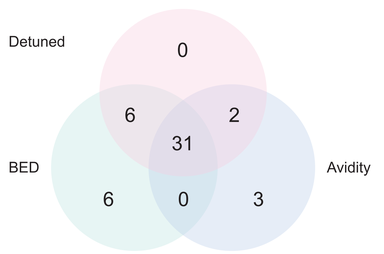
Newly diagnosed specimen results.

- Figure 1 shows the concordance of results of specimens on 3 different STARHS assays.
- The detuned assay is the only assay not to identify a specimen as from a recent HIV infection without agreement from at least one other assay.
- The avidity assay identifies the fewest number of specimens as from recent HIV infections.

Table 1. Comparison of recent infection assay characteristics

Factor	Detuned (bioMérieux Vironostika-LS EIA)	Avidity (Abbott AxSYM HIV 1/2/gO)	BED (Calypte® HIV-1 BED Incidence EIA)
Type of antibody measured	Anti-HIV quantity	Anti-HIV quality (antibody avidity)	Anti-HIV gp41 quantity (as proportion of total IgG)
Assay generation	1st generation viral lysate from subtype B. Assay detects IgG (HIV-1 group M)	3rd generation, Mixture of recombinant and peptide antigens. Detects IgG and IgM (HIV-1 groups M, O & HIV-2)	2nd generation, branched chain peptide from gp41 HIV-1 subtypes B, E and D. Assay detects IgG (HIV-1 group M)
Special equipment required	No	Yes (AxSYM Analyser)	No
Working dilution	1:20,000	1:10	1:101
Reagent storage requirements	4°C	4°C	-20°C; 4°C (depending on reagent)
Automated	Partial automation possible	Yes	Partial automation possible
Assay duration	90 minutes per plate	Minimum of 60 minutes; 2-3 minutes for each additional specimen above 10	245 minutes per plate
Specimens per run	84 in screening mode 28 in confirmatory mode	Minimum of 1. Can be continually loaded on to the machine	85 in screening mode 28 in confirmatory mode
Confirmatory algorithm utilized to confirm initial results	Yes Triplicate retests, each from Individual dilutions	No	Yes Triplicate retests, each from Individual dilutions
Mean window period (WP) defined	Yes (For some subtypes)	No	Yes
WP variations by subtype	Yes	Unknown	Yes
% AIDS cases misclassified as RHI	2.4%	Unknown	2-3%
Assay specificity affected by HAART	Yes	Unknown	Yes
Sensitivity	Unpublished	88%	83%
Sensitivity	Unpublished	87%	98%

Figure 1. Concordance of recent HIV Infection among 89 specimens from patients with newly diagnosed HIV Infection by 3 recent HIV Infection assays

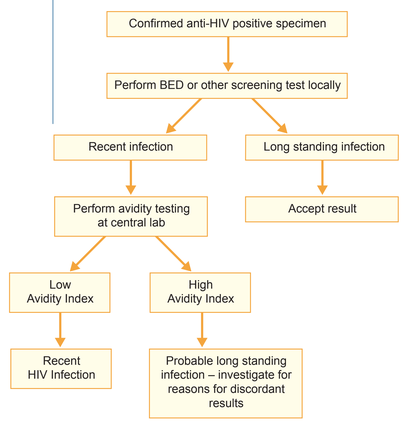


Proportion recent

Detuned 43.8%
BED 48.3%
Avidity 40.4%

41 Concordant Long Standing Infections

Figure 3. Proposed algorithm for recent HIV infection testing



Anti-retroviral treatment specimens' results.

- Figures 2, 3 and 4 show the change in SOD, ODn and Avidity respectively for 2 specimens each from 19 individuals.
- 1st specimen taken prior to the commencement of HAART, the 2nd 2 years later.
- At the commencement of the study all individuals were treatment naive, HIV positive for >12 months and not diagnosed with AIDS.
- Both the Detuned and BED showed a tendency to decline in SOD or ODn over time.
- Avidity indices remain stable.
- For simplicity only overall change reactivity is shown.
- Largest change in reactivity occured in 1st 6 months following initiation of HAART (data not shown).

Figure 1. Standardised optical density of specimens pre-antiretroviral therapy and after two years of therapy

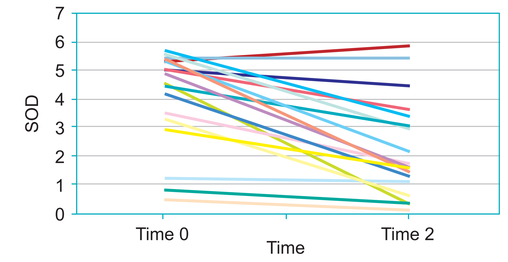


Figure 2. Normalised optical density of specimens pre-antiretroviral therapy and after two years of therapy

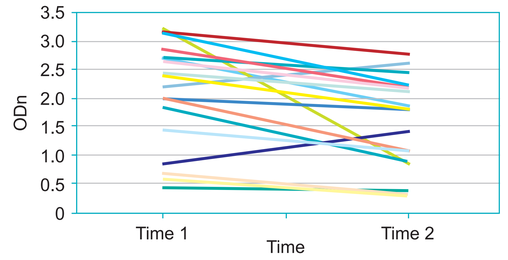
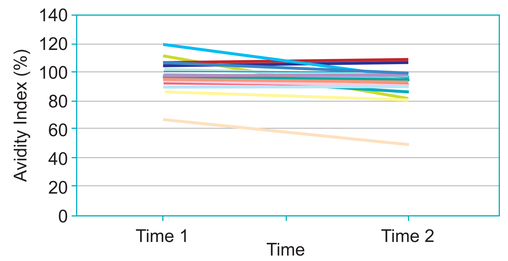


Figure 3. Avidity index of specimens before pre-antiretroviral therapy and after two years of therapy



Long standing infection results

- Table 2 shows the SOD, ODn and avidity index of 34 specimens from patients infected for greater than 12 months who are treatment naïve and not diagnosed with AIDS.
- 1 specimen was misclassified as recent by Avidity, 2 by detuned and 3 by BED.

Table 2. STARHS assay findings on 34 patients with longstanding HIV infection

Patient ID	SOD bioMérieux	ODn BED Assay	HIV 1 2gO Avidity assay – Avidity Index (%)
C1	5.541	3.576	101
C2	7.24	3.684	96
C3	4.733	1.114	92
C4	3.087	2.936	99
C5	5.014	3.541	100
C6	5.282	2.669	92
C7	4.616	2.713	104
C8	6.212	2.835	111
C9	2.043	1.158	89
C10	4.78	2.039	112
C11	5.246	1.641	104
C12	1.873	1.75	106
C13	3.148	0.963	101
C14	3.383	2.974	112
C15	3.222	2.182	107
T1	4.216	1.845	105
T2	5.038	2.874	91
T3	2.987	2.423	90
T4	5.767	2.878	94
T5	5.377	3.198	87
T6	5.51	2.005	107
T7	**0.871**	**0.454**	96
T8	5.065	2.723	104
T9	4.488	2.013	107
T10	1.24	1.494	90
T11	5.605	2.488	105
T12	3.339	**0.629**	88
T13	5.327	2.679	119
T14	3.51	2.657	98
T15	4.794	3.105	98
T16	**0.512**	**0.7**	**68**
T17	4.254	0.904	101
T18	5.508	2.229	86
T19	4.556	3.24	111

% long standing infections not recognised as such by:

Detuned	6%
BED	8.8%
Avidity	2.9%

Potential confounding factors identified in patients who did not reach cut off for LS infections in incidence assays included:

Falling CD4 before initiation into study

Previous pregnancy

CD4 count in long standing infection patients varied between 33 and 900 c/µl

In specimens not classified as long standing the CD4 counts were 321 (T-16), 211 (T-7) and 279 (T-12)

CONCLUSIONS

- Assays show good correlation on individual specimens despite different sensitivities and specificities and modes of operation.
- Avidity assay has a shorter window period than BED or Detuned but is less affected by confounding factors such as ART.
- Avidity assay correctly identify more long standing infections than the detuned or BED assays.
- Avidity assay employed in this study is not conducive to use in the field.
- Screening of specimens in local laboratories by BED may produce more recent infections than the other assays, but many of these may be false.
- Retesting the specimens deemed by BED to be recent with the AXSYM avidity assay reduced the number of false reactions and could be performed in a central lab.
- Low ODn and Low Avidity index is likely to reflect 'true' recent infection.

Ref: K641 JL