Identification of recent HIV infections in single serum sample: Comparison between the avidity index method and the serologic testing algorithm for recent HIV seroconversion (STARHS)

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Background

The identification of recent HIV infections using an antibody test is of utmost importance in establishing HIV incidence rates, and characterizing recently infected individuals. Increasing interest on estimating the incidence of HIV-infection using cross-sectional specimens has led to the development of several laboratory methods. Techniques based on the properties of the evolving antibody response (specific antibody titers, avidity or the relative abundance of specific IgG) offer some advantages, and those that can be performed using commercially available HIV serologic tests while including modifications are of particular interest.

A sensitive/less sensitive serologic strategy was developed in 1998 at the Centers for Disease Control and Prevention (CDC) for this purpose, which uses two enzyme-linked immunosorbent assays (ELISA).[1] Specimens from recent seroconvertors, having low antibody titers, are negative in a modified, less-sensitive ELISA in comparison to the standard ELISA. This approach, later on called Serological Testing Algorithm for Recent HIV Seroconversion (STARHS), was optimized with the Abbott 3A11 assay (Abbott Park, Illinois). This product was discontinued after 2002 and replaced by a modified protocol using the 96-well plate Vironostika HIV-1 Microelisa System (bioMerieux, Durham, North Carolina).[2] The CDC established an international STARHS quality assurance program, for which they provide protocols to participating centers, specific software for data analysis, quality control materials, calibration materials, and blinded proficiency testing panels. However, in 2005 the current kit is scheduled to be replaced by the Vironostika Plus O kit (bioMerieux, Durham, North Carolina, USA), which will not be available in the European market. For each new test being used for the STARHS, extensive optimization and standardization are necessary.

On the other hand, antibody avidity has long been used as a marker for recent infection with several pathogens, and is currently being used for Rubella virus, *Toxoplasma gondii*, and human Cytomegalovirus, among others. Antibodies produced in the early phase of infection show a lower avidity for the antigen. In 2002, a quantitative avidity technique was developed which uses a commercialized automated micro-particle immunoassay (MEIA).[3,4]

Aim of the study

The aim of the present study was to compare two methods for the detection of recent HIV infection:

- An **avidity index** method using the AxSYM platform (Abbott Diagnostics).
- The serological testing algorithm for recent HIV seroconversion (**STARHS**).

The methods were compared to assess their ability to detect persons with recent or long-standing HIV infections, and in terms of ease of performance, standardization, automation, accuracy, and to determine the concordance of results.

Materials and Methods

Study specimens.

A total of 160 serum specimens from documented HIV-positive subjects (according to standard HIV testing algorithms including screening ELISA tests and confirmatory Western blot or PCR) were included in the study. Sera specimens where collected at several clinical laboratories in Spain and at an infectious disease clinic in Italy. Before testing, 94 specimens, for which clinical, virological and immunological data were available, were sorted in three groups according to stage of HIV infection:

- **Group 1: recent HIV infection** (n=13 serum specimens), i.e. sera drawn < 6 months after the seroconversion date, which was estimated as the mid point between the last HIV-negative test and the first HIV-positive test, with the two tests less than 6 months apart. The mean time period between the last negative and the first positive test was 3.1 months (standard deviation, 1.2). The mean time elapsed from estimated seroconversion date to the date of specimen collection was 2.9 months (standard deviation, 1.6).
- **Group 2: long-standing infections** (n=57 serum specimens), i.e. sera drawn > 6 months after the diagnosis of HIV infection. Three individuals were on highly active antiretroviral treatment (HAART).
- **Group 3: AIDS** (n=24 sera), from AIDS patients.

Data analysis.

In order to assess overall agreement between the STARHS and avidity index techniques, a Kappa statistic was calculated on the 160 specimens. Besides, the percentage of recent infections detected in each of the three groups of specimens was compared.

Laboratory methods.

All specimens where tested with both methods as shown in **Figure 1**.

- **STARHS** was performed at the Microbiology Service (Hospital Universitari Germans Trias i Pujol), which in collaboration with CEESCAT has been participating in CDC's STARHS IND Testing Program since 1999. The less-sensitive EIA (Vironostika HIV-1 Microelisa System) was performed as previously described.[2]
- The **avidity index method** was carried on at the "Amedeo di Savoia Hospital", Laboratory of Virology, Turin, Italy. The AxSYM HIV1/2gO Test (Abbott Diagnostics Division, Delkenheim, Germany), was performed according to manufacturer instructions. Guanidine hydrochloride 1M (G) (Pierce Chemicals, Dallas, Texas), a dissociating agent that disrupts the weak bonds between antibody and antigen was used. The cutoff was chosen according to previous results which showed a sensitivity of 94.6% and a specificity of 94.4% in discriminating recent (within the previous 6 months) infections.[5]

Results

Assay performance.

- **Analytical characteristics:** inter-assay coefficients of variation for STARHS calculated from runs on four different days ranged from 10% to 30%. The overall correlation coefficient between SOD values obtained in screening vs. confirmatory modes performed on different days was 0.7471. Six specimens had SOD values above and below 1.000 when comparing screening and confirmatory modes. Similarly, a number of specimens were very close to the avidity index cutoff (**Figure 1**), even though the avidity technique showed good intra and inter-day coefficients of variation (<10%).[3]
- **Time needed to perform the assays:** for each method, it takes approximately two hours and 15 minutes to process 90 specimens (45 minutes for the dilutions and 90 minutes for running the test). However, in our study slightly more than one third of the samples needed to be repeated under STARHS confirmatory mode, thus increasing the time required (an additional 2 hours for each 90 specimens). Using AxSYM, new samples can be continuously loaded in the machine saving time for the reading of the controls. Partial automation is possible with STARHS, by using an automated dilutor and an ELISA liquid handling system. However, automation is not routinely adopted by all the laboratories that use or have used STARHS, and the kit availability is limited to US labs participating under an agreement with the CDC and the US FDA.

Agreement between STARHS and the avidity index assay.

The overall agreement was good (kappa = 0.67, p<0.001). Among the 160 tested specimens, both techniques agreed in classifying 25 specimens as recent infections and 117 as long-standing infections. On the other hand, 14 specimens only and 4 specimens only were considered as recent infections by STARHS and the avidity index, respectively. Results according to groups based on clinical information are shown in **Table 1**, and **Figure 2**.

Discussion

Ideally, a serologic strategy that allows for the identification of recently infected individuals by using a relatively easy laboratory method should have high accuracy and reproducibility, perform similarly for the different HIV subtypes, and be widely available to laboratories around the world.

While STARHS has a proven value, several drawbacks limit its use:

- It involves testing of samples in triplicate after a predilution, and >20% variability of the replicates requires additional testing.
- It needs a calibrator and quality control material, along with a dedicated software package, which must be provided by the CDC.
- Problems with the availability of the commercialized test used have occurred in the past and will arise again in the near future.
- Assay results vary according to HIV subtype, requiring the adoption of different cutoffs[5] (sample genotyping should be performed before interpreting results).

As specified in **Table 2**, the avidity index method has shown several advantages over the STARHS, as previously suggested:[4]

- After a single 1:10 dilution, the technique can be fully automated with the AxSYM platform (better reproducibility). This instrument is widely available among clinical laboratories in Europe and in most countries worldwide.
- The assay used is a third-generation MEIA that is more sensitive and detects antibodies earlier (able to recognize antibodies of all classes), and allows for the detection of HIV-1 (including group M and O) and HIV-2.
- It provides quantitative results and the selection of different cutoffs can be adjusted to give different sensitivity and specificity, depending on the study objectives.
- The method is easily exportable and does not require any specific training (although inter-laboratory variability when using an automated system is expected to be low, an external quality control would be useful to verify reproducibility).
- There is no need for an official approval by the regulatory authority in any country because it is not a new assay or a modification of an already existing assay (only a preparation of the sample is needed).

Furthermore, the avidity index method showed better results than STARHS among the group of individuals with long-standing HIV infection, and also among AIDS patients.

- CD4 count: neither of the two tests seemed to be affected by a low CD4 count.
- HAART: in our study there were too few subjects on HAART to evaluate its effect on the performance of both techniques. However, several studies have noted that STARHS tends to misclassify the subjects on therapy as being recently infected, due to a fall in antibody titers. Recently, a longitudinal study that compared STARHS and the avidity index assay on patients who had started antiretroviral therapy, concluded that this therapy had a marked effect on STARHS but had only minor effects on the avidity assay.[7]

❑ It is appropriate to stress that at the present moment the results of both these tests cannot be interpreted at an individual level to give information to patients. In fact, the discrimination between recent and long-standing HIV infection is based on a cutoff which defines the time period elapsed from seroconversion to either high antibody titers (STARHS) or antibody maturation (avidity index). This time period may vary among individuals causing misclassification in some cases, while at the population level the false-negative and false-positive results balance each other.

Conclusions

☑ **In comparison to STARHS, the avidity index method is easier to perform, less expensive, automated, exportable, time-saving, and does not need sophisticated laboratory requirements or personnel training.**

☑ **Our results show that both techniques perform similarly in identifying recent infections, although STARHS tended to misclassify a few more individuals that have a longstanding infection or AIDS as being recently infected.**

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Figure 1

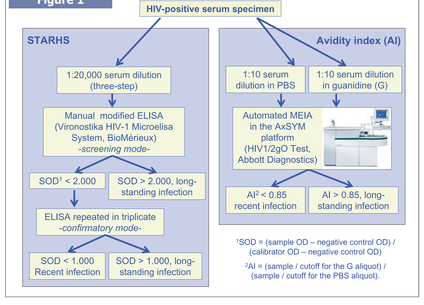


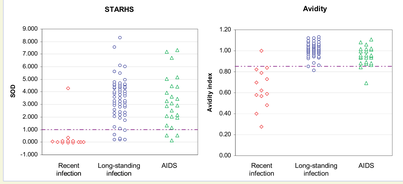
Table 1

Characteristics of study specimens according to clinical and laboratory information.

Type of infection	N	Nº of recent infections according to: STARHS	Avidity Index	Both methods
Group 1: known recent infection	13	**12**	**12**	**11**
Group 2: known long-standing infection	57	**6**	**1**	**1**
Group 3: AIDS patients	24	**3**	**2**	**2**

Figure 2

Distribution of standardized optical density (SOD) results for STARHS and avidity indexes according to the three groups of specimens.



Cutoff: dashed line

Table 2

Performance characteristics of STARHS and the avidity index method

Issue	STARHS	Avidity Index
Volume of specimen needed	10 µl (40 µl for recent infections)	40 µl
Working specimen dilution	1:20,000	1:10
Assay format	96-well plate ELISA	MEIA
Automation	Partial	Yes
Assay generation	2nd generation	3rd generation
Antigens in the test	HIV-1	HIV-1 (groups M and O), and HIV-2
Availability of the test	United States (not CE marked)	Europe, rest of the world