



Comparison of 6 HIV Incidence Assays with the Less Sensitive Vironostika HIV Antibody Assay for Screening Subjects for A5217

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Introduction

Detuned HIV-1 antibody assays were developed to estimate incidence of HIV among populations. The first detuned assay used the Abbott 3A11 HIV-1 EIA, but manufacture of this kit subsequently ceased. A less sensitive (LS) version of the bioMérieux Vironostika HIV-1 Antibody assay and the BED assay took its place. These detuned antibody assays were never designed to be used to determine an individual's stage of disease. Despite this, the Vironostika LS-EIA (V-LS-EIA) was widely used not only to estimate HIV-1 incidence, but also to recruit into early HIV infection clinical trials, including ACTG study A5217. However, production of V-LS-EIA was also phased out and the A5217 team had to find an alternative incidence assay to screen prospective subjects for the study.

Several alternative incidence assays have been developed. These include: BED, Avidity Index (AI), and detuned Vitros and Avioq EIAs, and detuned Determine and Ora-Quick rapid antibody tests. We evaluated these 6 alternative incidence assays as a replacement for enrolling subjects into A5217.

Methods

Samples: Excess serum from 99 subjects screened for enrollment in A5217 using the V-LS-EIA assay were used to evaluate the alternative assays. The protocol defined cut-off was an SOD of <0.75 in the V-LS-EIA assay. Anyone with an SOD less than 0.75 was considered recently infected and eligible for the study. Vironostika SODs ranged from -0.345 to 14.595. 29 samples were non-reactive (i.e. incident infection) in the V-LS-EIA, while 70 were reactive (chronic infection).

BED: The BED assay (Trinity) uses a branched peptide that includes gp41 immunodominant sequences from HIV-1 subtypes B, E, and D. Because of the competitive nature of the capture EIA, a gradual increase in the proportion of HIV-1-specific IgG in total IgG is observed for 2 years after seroconversion. Sera (100 ml of 1:100 dilution in diluent buffer) were added to the BED plates and incubated for 1 hr at 37°C. After washing the BED-biotin peptide antigen was added to each well, and the plates were incubated for 1 hr at 37°C. After washing streptavidin-peroxidase reagent was added and incubated for 90 min at 37°C, followed by washing. TMB substrate was added to each well, and the plates were incubated for 15 min at 25°C. Color development was stopped with 1N H₂SO₄. The OD values were read at 450/650. Specimens with a normalized optical density (ODn) below a cutoff value of 0.800 were classified as "recent."

Vitros (VT)- The Ortho Vitros ECI HIV-1/2 contains four recombinant antigens (HIV-1 Env 13, HIV-1 Env 10, HIV-1 p24, and HIV-2 Env AL). The assay uses a two stage reaction, first HIV antibody present in the sample binds with HIV recombinant antigen coated on the wells, unbound sample is removed by washing and horseradish peroxidase (HRP)-labeled recombinant HIV antigens are added in the conjugate reagent. The conjugate binds specifically to any human anti-HIV-1 or anti-HIV-2 (IgG and IgM) captured on the well in the first stage. The bound HRP conjugate is measured by a luminescent reaction which is indicative of the level of anti-HIV-1 and anti-HIV-2 present. The LS assay is done using a 2 step 1:400 (1:20 and 1:20) dilution of HIV positive sample in normal human serum. After the sample is diluted, it is run on the fully automated Vitros ECI, the assay takes 49 minutes and the results are reported as signal to cutoff. 20 S/C is used as the cut-off that discriminates between recent and established HIV infection.

Methods

Avioq (AQ) - This test is a modification of Avioq HIV-1 microelisa system, Avioq Inc, Rockville Maryland. Samples are diluted in three steps, to reach final dilution of 1:20,000. Diluted samples are added to antibody coated wells, and incubated for 1 hr at 37°C. After washing, 150 ul of conjugate enzyme is added and incubated for 1 hr at 37°C. Plate is washed after incubation, and substrate is added. The plate is then incubated for 8 min at room temperature. The reactions are stopped, and the plate is read at single wavelength of 405nm. The readings are analyzed using STARHS software provided by CDC.

Avidity Index (AI) - This is a modification of the Bio-Rad, Genetic Systems HIV-1/HIV-2 plus O assay. Specimens are diluted 1:11 in kit diluent, added to duplicate wells of the plate, and incubated for 1 hr at 4°C. After washing 100 ul of wash buffer is added to one of the wells and 100 ul of avidity reagent (0.1M diethylamine diluted in wash buffer) and incubated at 37°C for 30 min. Wash the plate, add conjugate and incubate 30 min at 37°C. Wash, add substrate, and incubate 30 min at 37°C. Add stop solution and read the plate. The avidity index is calculated as follows: AI = (OD of DEA treated sample/OD wash buffer sample) x 100%. Previous data from samples with known periods of HIV infection suggest that AIs less than 40% are indicative of recent infection.

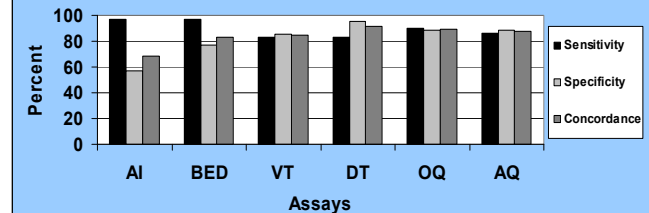
Detuned Determine (DT) and Ora-Quick (OQ) Rapid Tests - Both RTs are manually performed, visually read, qualitative immunoassays for the detection of antibodies to HIV-1 and HIV-2. For Determine-LS, the samples are diluted in two steps: 195 µl of NHP + 5 µl of sample (1:40), followed by a second dilution of 10 µl into 240 µl of NHP for a final dilution of 1:1000. A 50 µl aliquot of this final dilution is applied directly to the fibrous pad of the test strip which is incubated at room temperature and the test is visually read at 15 min and 60 min. For Ora-Quick-LS, the pre-dilution is achieved by adding 5 µl of specimen to 195 µl of NHP (1:40). 20 µl of the pre-diluted specimen is then added to 800 µl of OraQuick buffer (remove 200 µl from 1ml buffer and add 20 µl of pre-diluted sample to achieve final dilution of 1:1,640). The Flat-Pad device from the kit is then inserted into the vial and the test is read after 20 min and 40 min of incubation at room temperature.

Table 1. Sensitivity, specificity, and concordance [95% Confidence Intervals (CI)] with the V-LS-EIA using a 0.75 cut-off.

	Sensitivity N=29 (95% CI)	Specificity N=70 (95% CI)	Concordance N=99 (95% CI)	P
Avidity Index	96.6% (82.2%, 99.9%)	57.1% (44.7%, 68.9%)	68.7% (58.6%, 77.6%)	<0.001
BED	96.6% (82.2%, 99.9%)	77.1% (65.6%, 86.3%)	82.8% (73.9%, 89.7%)	<0.001
Vitros	82.8% (64.2%, 94.2%)	85.7% (75.3%, 92.9%)	84.8% (76.2%, 91.3%)	0.20
Determine	82.8% (64.2%, 94.2%)	95.7% (88.0%, 99.1%)	91.9% (84.7%, 96.4%)	0.48
Ora-Quick	89.7% (72.6%, 97.8%)	88.6% (78.7%, 94.9%)	88.9% (81.0%, 94.3%)	0.13
Avioq	86.2% (68.3%, 96.1%)	88.6% (78.7%, 94.9%)	87.9% (79.8%, 93.6%)	0.20

Results

Performance of Incidence Assays compared to V-LS-EIA



55 specimens demonstrated total agreement on all 6 assays. 20 specimens had a discordant result in only one assay, while the other 5 assays agreed. In 12 of these it was the Avidity assay that was discordant, in 3 the Vitros and Avioq assays, and in 2 the BED assay. 7, 9, 7 and 1 specimens had 2, 3, 4, or 5 assays that disagreed with V-LS-EIA. 87% (20/23) of samples with SOD <0.5 and 84% (27/32) of samples with SOD >3.0 agreed on all assays.

Conclusions

1. Although the detuned Determine assay provides better concordance with the V-LS-EIA using the 0.75 cutoff employed in A5217, it is not available in the US.
2. The assay with the next most concordant results was the detuned OraQuick assay, which is sold in the US.
3. There was no significant difference between Determine, Ora-Quick, Avioq, and Vitros, in comparison to V-LS-EIA (all P>0.13).
4. Limitation: The specimens used in this study were from subjects screened for entry into A5217. These individuals did NOT have known dates of seroconversion. Comparisons using samples from known seroconverters should be used to truly evaluate these assays.
5. Limitation: It is assumed that most if not all of these subjects were infected with subtype B. Comparisons using samples from people with non-B HIV should be made.

Acknowledgements: P30 AI50410 and AI69423