Advantages and disadvantages of different types of FDA-approved HIV immunoassays used for screening by generation and platform*

HIV immunoassays grouped by generation, platform, and CLIA complexity [+]	Advantages	Disadvantages
2nd generation lateral-flow rapid HIV antibody tests that are CLIA-waived when used with whole blood or oral fluid[#] 1) **Clearview HIV 1/2 STAT-PAK** 2) **Clearview COMPLETE HIV 1/2** 3) **OraQuick ADVANCE Rapid HIV-1/2 Antibody Test** 4) **Uni-Gold Recombigen HIV 1/2****	1) CLIA-waived tests can be performed by non-laboratorians 2) Quick turnaround time (20 minutes or less) 3) Portable 4) If test performed at point of care, high likelihood that person will receive test result	1) Less sensitive for early infections than flow-through rapid tests, and 3rd or 4th generation tests[##] 2) Rapid tests used with oral fluid, which has lower antibody concentration, are less sensitive and specific than when used with blood[1,2,3]
2nd generation CLIA high complexity manual HIV-1 antibody immunoassay **Avioq HIV-1 Microelisa System**	1) Can be used with dried blood spots or oral fluid collected with the OraSure oral fluid collection device 2) Low cost	1) Less sensitive for early infections than 3rd or 4th generation tests 2) Results from Specimens collected with the OraSure collection device have reduced sensitivity and specificity compared with blood specimens[4] 3) Labor intensive 4) Long turnaround time (> 3 hours); if delivery of test result is delayed there is an increased likelihood person

		tested may not receive results 5) FDA-approved for HIV-1 only
2nd generation flow-through rapid HIV antibody tests[++] 1) **INSTI HIV-1 Antibody Test (CLIA-waived)** 2) **MedMira Reveal G3 Rapid HIV-1 Antibody Test (CLIA-moderate complexity)**	1) More sensitive during early infection than 2nd generation lateral flow rapid HIV antibody tests [**] 2) Quick turnaround time (< 5 minutes). 3) Both tests can be read immediately after adding reagents. 4) Portable 5) If test performed at point of care, high likelihood that person will receive test result	1) MedMira Reveal G3 Rapid HIV-1 Antibody Test is for use only with serum or plasma 2) Less sensitive than 3rd and 4th generation tests 1) FDA-approved for HIV-1 only
2nd generation CLIA-moderate complexity flow-through rapid HIV-1/HIV-2 antibody differentiation test **Multispot HIV-1/HIV-2 Rapid Test**	1) More sensitive during early infection than lateral flow rapid HIV antibody tests 2) Differentiates HIV-1 from HIV-2 3) Quick turnaround time (< 20 minutes) 4) If test performed at point of care, high likelihood that person will receive test result	1) For use only with serum or plasma 2) Less sensitive for early HIV infection than 3rd or 4th generation tests 3) If delivery of test result is delayed there is an increased likelihood person tested may not receive results
2nd generation CLIA-moderate complexity the Dual Path Platform® HIV-1/HIV-2 antibody test **Chembio DPP HIV-1/2 Assay**	1) Quick turnaround time (< 20 minutes) 2) If test performed onsite, high likelihood that person will receive test result 3) Portable 4) Can use venous or finger stick blood, oral fluid, plasma or	1) Less sensitive than 3rd and 4th generation tests

	serum	
3rd generation fully automated immunoassays 1) **ADVIA Centaur HIV 1/O/2 Enhanced (EHIV) (CLIA-moderate complexity)** 2) **Ortho Vitros ECi/ECiQ Anti-HIV 1+2 (CLIA-high complexity)**	1) Turnaround time for initial result is < 1 hour 2) Requires minimal technician time to process specimens 3) More sensitive for early infection than rapid antibody HIV tests and 2nd generation tests 4) Ortho (per product insert): only borderline reactive specimens need to be repeated, and quality control is run once daily	1) ADVIA (per product insert): specimens must be bracketed with quality controls 2) Not as sensitive for early infection as 4th generation tests 3) Requires specialized equipment and trained technicians to conduct testing
3rd generation CLIA-high complexity manual or semi-automated HIV immunoassay **Bio-Rad GS HIV-1/2 Plus O**	1) More sensitive than rapid antibody HIV tests and 2nd generation tests	1) Labor intensive 2) Not as sensitive for early HIV infection as 4th generation HIV tests 3) Long turnaround time (> 3 hours); if delivery of test result is delayed there is an increased likelihood person tested may not receive results
4th generation CLIA-moderate complexity fully automated HIV test **Abbott Architect HIV Ag/Ab** **Combo Assay**	1) Highly sensitive during early HIV infection [##] 2) Fast turnaround time for initial result (<30 minutes) 3) Requires minimal technician time to process specimens 4) Quality control is run once daily	1) Requires specialized equipment and trained technicians to conduct testing 2) Does not differentiate the p24 antigen from the HIV-1/2 antibody results
4th generation CLIA-high complexity semi-automated HIV	1) Highly sensitive during early infection detection 2) Quality Control is included in	1) Labor intensive 2) Requires specialized equipment and trained technicians to conduct

test	each run.	testing
Bio-Rad GS HIV Combo Ag/Ab EIA		3) Does not differentiate the p24 antigen from the HIV-1/2 antibody results
		4) Long turnaround time (> 3 hours); if delivery of test result is delayed there is an increased likelihood person tested may not receive results

[*]1st generation HIV immunoassays (IA) use virus particle protein antigens and detect IgG antibodies in an indirect IA format. 2nd generation IAs use synthetic peptides or recombinant protein antigens and detect IgG in an indirect IA format. The use of synthetic peptides and recombinant protein antigens improve specificity by eliminating cellular proteins that are contained in viral particles, and thus increase assay specificity by avoiding detection of antibodies to cellular proteins. 3rd generation IAs are constructed in the direct IA format (antigen sandwich) which allows for detection of IgG and IgM antibodies (generally made early after infection). Sensitivity is increased by allowing for the detection of IgM (first class of immunoglobulin made after infection) in addition to IgG and by increased sample volume input. 4th generation IAs use synthetic peptides and recombinant protein antigens allowing for detection of IgM and IgG antibodies in the direct IA format (antigen sandwich). A direct IA (antibody sandwich) component for detecting viral p24 antigen is also incorporated. The 4th generation IA format maximizes specificity by using recombinant protein and peptide antigens for detection of HIV antibody and maximizes sensitivity by using increased sample volumes, allowing detection of IgG and IgM antibodies and viral p24 protein which is known to be present in blood prior to detectable HIV antibodies.

+ Under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) tests are categorized by the complexity of the test. The more procedural steps and requirements for user interpretation, the more restrictions are placed on who can perform the test. CLIA-waived tests are simple laboratory tests where the likelihood of erroneous test results is negligible.

Lateral flow rapid tests: the sample is placed in a sample area followed by a buffer which assists the sample in migrating across the strip in which all of the reactants and detectors are embedded.

** Unigold, used a 3rd generation format but its sensitivity is similar to that of 2nd generation tests.

++ Flow-through rapid tests: Specimen, buffer and wash solution flow through a porous membrane in which the antigens are embedded and then onto an absorbent pad. A second layer inhibits the backflow of fluids, which can obscure results. Once the test is started, attention is required until the addition of the final wash buffer, but after that is added; the test can be read immediately.

Table of intertest intervals between HIV-1 RNA nucleic acid test reactivity and reactivity for 14 FDA-approved HIV immunoassays

HIV Test	Median (95% CL)*
4th Generation Laboratory Tests	
Architect HIV Ag/Ab Combo	6.2 (3.5, 8.5)
GS HIV Combo Ag/Ab EIA	7.4 (3.8, 11.0)
3rd Generation Laboratory Tests	
ADVIA Centaur HIV 1/O/2	9.9 (7.7, 12.0)
Vitros anti-HIV 1+2	10.6 (8.7, 12.1)
GS HIV-1/HIV-2 Plus O EIA	13.7 (11.3, 16.1)
3rd Generation Rapid Test	
UniGold Recombigen HIV	21.6 (17.5, 27.8)
2nd Generation Rapid Tests	
INSTI HIV-1 Antibody	13.5 (11.3, 14.8)
Multispot HIV-1/2	16.8 (14.5, 18.9)
DPP HIV-1/2	17.5 (14.0, 21.5)
Reveal G2 HIV-1	19.0 (16.5, 20.0)
Clearview Complete HIV-1/2	19.7 (17.5, 23.4)
Clearview HIV-1/2 STATPAK	20.3 (17.4, 25.4)
Oraquick Advance HIV-1/2	23.7 (18.2, 29.9)
Class Interval (all tests)	
4th-Generation Laboratory Tests	6.8 (3.7, 9.7)
3rd-Generation Laboratory Tests	11.4 (9.7, 13.4)
2nd-Generation Rapid Tests	18.5 (16.0, 21.6)
Western Blot Laboratory Test	24.3 (18.8, 31.0)

*This table lists the 95% confidence intervals representing the estimated range of days that the HIV-1 test begins to detect HIV-1 infection after HIV-1 RNA is detectable. The interval between HIV infection and the appearance of HIV-1 RNA is estimated to be around 10 days, but the absolute range is not yet known. **For more information about these estimates click here for technical notes.**

References

1. Mortimer PP and Parry JV. Non-invasive virological diagnosis: are saliva and urine specimens adequate substitutes for blood? Reviews in Medical Virology 1991; 1:73-78.

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3. CDC. False-Positive Oral Fluid Rapid HIV Tests --- New York City, 2005—2008. MMWR 2008 / 57 (Early Release);1-5.

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Technical notes for table of Intertest intervals between HIV-1 RNA nucleic acid test reactivity and reactivity for 14 FDA-approved HIV immunoassays

The intertest intervals between RNA nucleic acid test reactivity and reactivity for 14 FDA-approved HIV immunoassays were evaluated using longitudinally collected plasma specimens from 25 donors from the United States who were later found to be recently infected with HIV-1.

We calculated the time interval between when an HIV-1 RNA (NAAT) test first detects HIV infection and an immunoassay first becomes reactive using data for which neither event was directly observed, but was known to occur within an interval of time between acquisition of two specimens or within a partially observed interval period if either a last negative or a first positive result was not observed. The earliest possible detection of viral RNA occurs at some unobserved time during the interval between the last negative NAAT result and the first positive NAAT result. Similarly, the earliest possible detection of antigen or antibodies occurs at some unobserved time between the last negative and the first positive immunoassay results. If a negative NAAT test result was not observed, the data were left-censored; if a positive immunoassay result was not observed, the data were right-censored. Reduction of this doubly interval-censored data to singly interval-censored data was calculated as the shortest possible time between first positive NAAT result and last negative immunoassay result and the longest possible time between last negative NAAT and first positive immunoassay result. We used a standard statistical assumption that the timing of an event (such as the earliest possible reactive immunoassay or NAAT result) is uniformly distributed across the observed interval between last negative and first positive results.

A parametric maximum likelihood procedure assuming normal distribution of intertest times was implemented to estimate the median intertest intervals, and other percentiles of the cumulative probability distribution, from singly interval- or right-censored event time data. We assumed all intertest intervals were positive. Bootstrap methods were used to estimate confidence limits for the intertest intervals. For bootstrap estimates, 25 subjects from the donor population were randomly selected with replacement; 1000 replicates were computed. To estimate the median time between infection, as measured by NAAT testing, and immunoassay reactivity by an all-inclusive generation of approved antibody or antigen/antibody tests, we included all repeated measurements from singly interval- or right-censored event time data with equal weighting given to each immunoassay.