Ortho Clinical Diagnostics

Current Concepts in HIV Diagnostics

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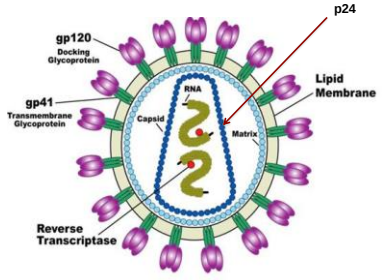
Agenda

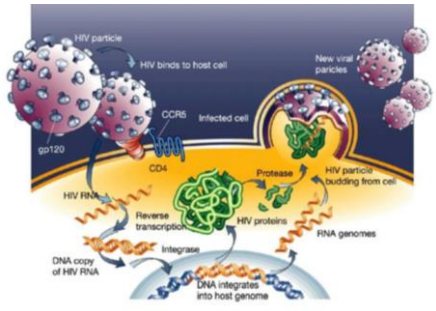
- HIV Infection
- Testing Overview
- CDC's HIV Testing Algorithm
- HIV 4th Generation Combo Tests
- POC HIV Testing
- Summary

Human Immunodeficiency Virus (HIV)



Source: DOI: 10.13140/RG.2.1.4923.8803 -Available from: Jef Hens, Mar 08, 2016

HIV Life Cycle



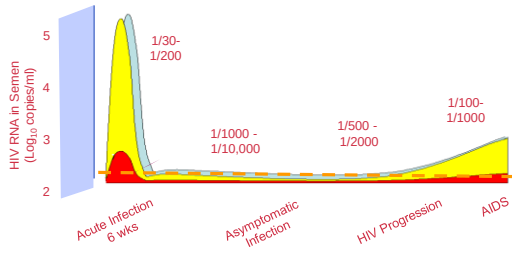
Source: DOI: 10.13140/RG.2.1.4923.8803 -Available from: Jef Hens, Mar 08, 2016

Transmission of HIV

- People get or transmit HIV through sexual behaviors and injection drug use
- Mother to child during pregnancy, birth or breastfeeding
- Certain body fluids can transmit HIV:
  - Blood
  - Semen
  - Pre-seminal fluid
  - Rectal fluids
  - Vaginal fluids
  - Breast milk

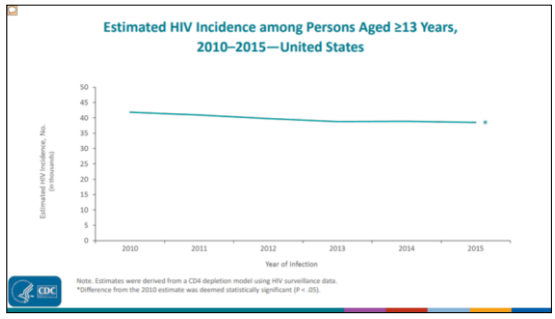
https://www.cdc.gov/hiv/basics/transmission.html

Risk of Sexual Transmission

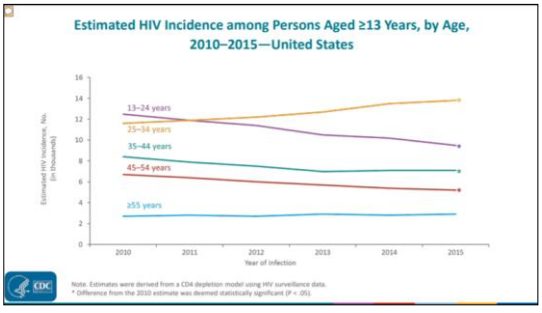


Cohen MS, et al. *Journal of Infectious Diseases*. 2005; 191:1391-3.

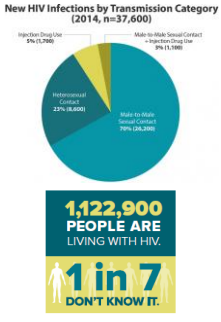
HIV Incidence



HIV Incidence



HIV Infection in the US-CDC Data

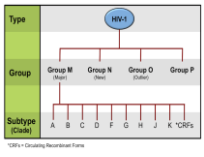


- In 2016, 18,160 people received an AIDS diagnosis. Since the epidemic began in the early 1980s, 1,232,346 people have received an AIDS diagnosis.
- HIV diagnoses are not evenly distributed **geographically**. The population rates (per 100,000 people) of people who received an HIV diagnosis were highest in the South (16.8), followed by the Northeast (11.2), the West (10.2), and the Midwest (7.5)

https://www.cdc.gov/hiv/pdf/statistics/overview/cdc-hiv-us-ataglance.pdf
https://www.cdc.gov/hivbasics/statistics.html
Accessed January 2018

Global HIV-1 and HIV-2 Epidemiology

- Strains of HIV-1 can be classified into four groups: M, O, N and P
  - Group M is responsible for most of the global HIV pandemic
  - Group N has only been found in Cameroon
  - Group O is responsible for some of the infections in West and Central Africa
  - Group P is a new group identified in Cameroon



- 2.7% - 5.4% of the individuals living with HIV worldwide are infected with HIV-2
  - Most persons infected with HIV-2 reside in West Africa or immigrate from West Africa
  - Fewer than 1% of HIV infections in the United States are caused by HIV-2

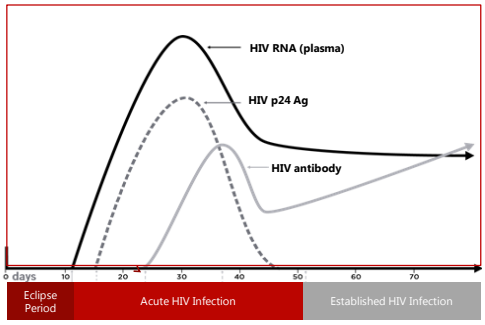
https://www.hiv.uw.edu/go/screening-diagnosis/epidemiology/core-concept/all Accessed on Feb 22 2018

Clinical Manifestations of Acute HIV Infection

- 40-90% develop symptoms of acute HIV[1]
- 50%-90% with symptoms seek medical care
- Of those diagnosed with acute HIV[1], 50% of patients seen at least 3 times before diagnosis[2]
- The symptoms of HIV that are caused by the body's nature response against the virus include:
  - Fever
  - Fatigue
  - Malaise
  - Myalgia
  - Rash
  - Sore throat
  - Lymphadenopathy[3]

1. Kahn, et al. *New England Journal of Medicine*. 1998:339:33-40.
2. Weintrob A, et al. *Archives of Internal Medicine*. 2003; 163:2097-2100.
3. Celum, et al. *Journal of Infectious Diseases*. 2001;183(1):23-35.

Laboratory Stages of HIV Infection



Busch MP, et al. *American Journal of Medicine*. 1997; 102(5B):117-124.

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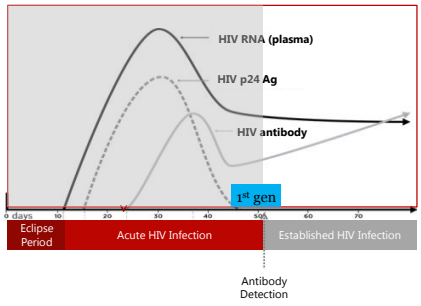

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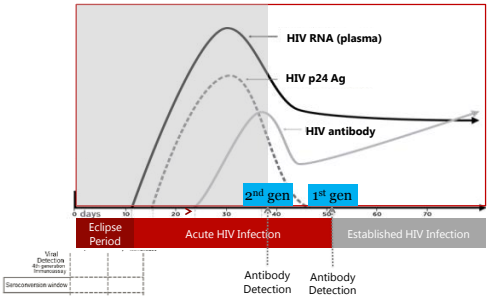

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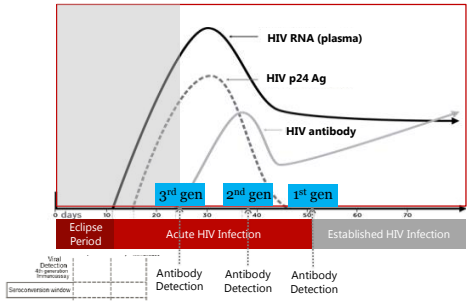

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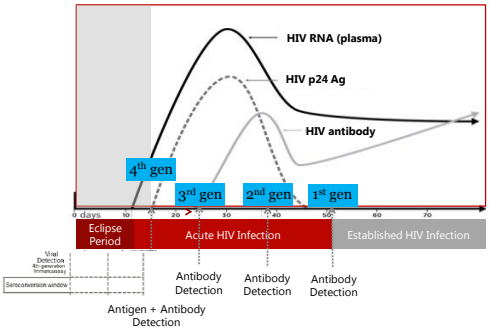
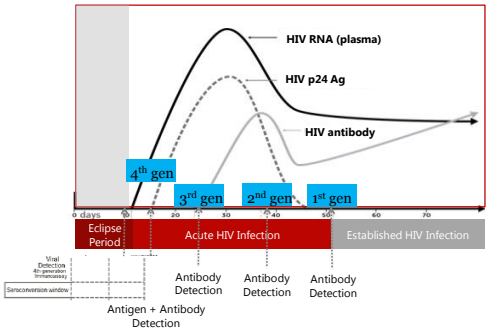
Laboratory Stages of HIV Infection



Busch MP, et al. *American Journal of Medicine*. 1997; 102(5B):117-124.

Laboratory Stages of HIV Infection



Busch MP, et al. *American Journal of Medicine*. 1997; 102(5B):117-124.

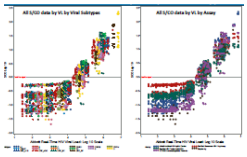
Comparison of Detection Limits of 4th and 5th Generation Combination HIV assays

19

A study supported by NIH, evaluated the sensitivity of HIV clinical diagnostic blood screening and next generation P24 assays :

- Comparison study performed on 20 geographically diverse HIV isolate samples.
- Completed in EQAPOL Laboratories.

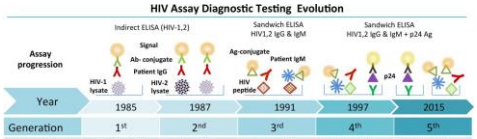
"Similar performance" (sensitivity) across sub-types between 4th gen and stand alone p24 Ag assays and Bio-Rad's HIV Ag-Ab assay



- Demonstrated enhanced sensitivity of next generation p24 digital detection platforms, and observed poor performance of POC assays

Stone M, Bainbridge J, Sanchez A et al. Comparison of Detection Limits of 4th and 5th Generation Combination HIV.. Presented at Conference on Retroviruses and Opportunistic Infections. , March 2018. , Poster #567.

HIV Assay Diagnostic Testing Evolution



Year	1985	1987	1991	1997	2015
Generation	1st	2nd	3rd	4th	5th
Antigen (Ag) Source	Virus Infected Cell Lysate	Lysate & Recombinant	Recombinant & Synthetic peptides	Recombinant & Synthetic peptides	Recombinant & Synthetic peptides
Specificity	95-98%	>99%	>99.5%	99.5%	99.5%
Sensitivity	99%	>99.5%	>99.5%	>99.8%	100%
Negative Window	8-10 weeks	4-6 weeks	2-3 weeks	2 weeks	2 weeks
Detects Antibody (Ab) and Ag	IgG Anti HIV-1	IgG anti HIV-1 and IgG anti HIV-2	IgG and IgM anti HIV-1, HIV-2 and Group O	IgG and IgM anti HIV-1, HIV-2 and Group O. Also detects HIV-1 p24 Ag	IgG and IgM anti HIV-1, HIV-2 and Group O. Also detects HIV-1 p24 Ag
Results	Single result	Single result	Single result	Single result; does not differentiate Ab from Ag positivity	Separate HIV-1 and HIV 2 Ab and Ag results
Confirming Tests	HIV-1 western blot (WB) or immunofluorescence (IFA)	HIV-1 WB or IFA, HIV-2 ELISA and WB if HIV-1 confirm is negative	HIV-1 WB or IFA, HIV-2 ELISA and WB if HIV-1 confirm is negative	HIV-1,2 differentiation Assay followed by qualitative HIV-1 RNA PCR if differentiation assay is negative	Not determined at the time of this writing

Alexander, T. S. (2016). Human immunodeficiency virus diagnostic testing: 30 years of evolution. Clinical and Vaccine Immunology 23:249 – 253. doi:10.1128/CVI.00053-16.

Key Dates in the History of HIV Testing

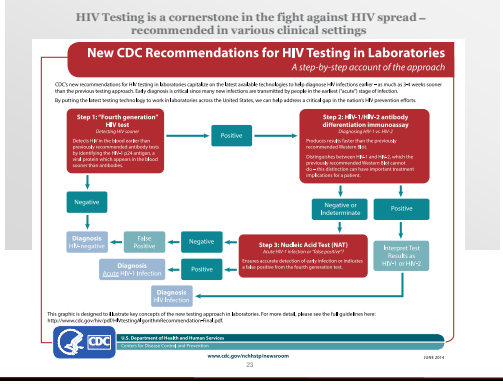
1981	First AIDS case reported
1984	Human immunodeficiency Virus (HIV) identified
1985	First test for HIV licensed (ELISA)
1987	First Western Blot blood test kit
1992	First rapid test
1994	First oral fluid test
1996	First home and urine tests
2002	First rapid test using finger prick
2003	Rapid finger prick test granted CLIA (Clinical Laboratory Improvement Amendments) waiver
2004	First rapid oral fluid test (also granted CLIA waiver)
2006	CDC recommends routine HIV screening in U.S. health care settings
2007	CDC launches Expanded HIV Testing Initiative in U.S. WHO/UNAIDS global guidelines recommend routine HIV screening in health care settings
2010	First test approved that detects both antigen and antibodies
2012	First rapid oral fluid home test
2013	USPSTF gives routine HIV screening an "A" rating First rapid test approved that detects both antigen and antibodies, and distinguishes between acute and established HIV-1 infection
2015	Centers for Medicare and Medicaid Services announces Medicare coverage of annual HIV screening for all beneficiaries 15-65, and for those older and younger beneficiaries at "increased risk" for HIV

https://www.kff.org/hivaids/fact-sheet/hiv-testing-in-the-united-states/

General Approach to HIV Diagnostic Testing

- Screening assays
  - Optimized for sensitivity
  - Define samples as negative
- Supplemental/confirmatory assays
  - Optimized for specificity

HIV Diagnostic Testing Algorithm



FDA-Approved Tests Applicable to the CDC Recommended HIV Testing Algorithm

TEST KIT NAME	MANUFACTURER
Step 1. HIV-1/HIV-2 Ag/Ab combo immunoassay (screening assay)	
Abbott Architect® HIV Ag/Ab Combo Assay	Abbott Laboratories
GS HIV Ag/Ab Combo EIA	Bio-Rad Laboratories
ADVIA Centaur® HIV Ag/Ab Combo	Siemens HealthCare Diagnostics
VITROS® HIV Combo Test	Ortho Clinical Diagnostics
BioPlex® 2200 HIV Ag-Ab Assay	Bio-Rad Laboratories
Roche Elecsys® HIV Combi PT 4th Gen	Roche Diagnostics
Step 2. HIV-1/HIV-2 antibody differentiation immunoassay (supplemental Ab assay)	
Multispot HIV-1/HIV-2 Rapid Immunoassay	Bio-Rad Laboratories
Geenius™ HIV 1/2 Supplemental Assay	Bio-Rad Laboratories
Step 3. HIV nucleic acid test (supplemental RNA assay)	
Aptima® HIV-1 RNA Qualitative Assay	Hologic Gen-Probe

https://www.health.ny.gov/diseases/aids/providers/testing/docs/guidelines.pdf

Guidance for Reporting Results of HIV Diagnostic Testing Algorithm

Step 1 HIV Ag-Ab Screening Assay	Step 2 HIV-1/2 Ab Differentiation Assay	Step 3 HIV-1 RNA Assay	Interpretation for Laboratory Report returned to Health Care Provider	Results Reported
NR	N/A	N/A	Negative for HIV-1 antigen and HIV-1/HIV-2 antibodies. No laboratory evidence of HIV infection.	NOT reportable
R	HIV-1 Pos	N/A	Positive for HIV-1 antibodies. Laboratory evidence of HIV-1 infection is present.	Report all results
R	HIV-2 Pos	N/A	Positive for HIV-2 antibodies. Laboratory evidence of HIV-2 infection is present.	Report all results
R	NR or Any IND (HIV-1,HIV-2 or HIV)	Detected	Positive for HIV-1. Laboratory evidence consistent with acute or early HIV-1 infection is present.	Report all results
R	NR or Any IND (HIV-1,HIV-2 or HIV)	Not Detected	HIV antibodies were not confirmed and HIV-1 RNA was not detected. Possible false positive. Further testing is recommended if warranted by clinical evaluation or risk factors.	Report all results
R	HIV Pos Untypable	N/A	Positive for HIV antibodies. Laboratory evidence of HIV infection is present. Antibodies not differentiated as HIV-1 or HIV-2. HIV-1 RNA and HIV-2 RNA or DNA testing is recommended.	Report all results

https://www.health.ny.gov/diseases/aids/providers/testing/docs/guidelines.pdf

HIV Testing Algorithm Proposed by CDC/APHL

- CDC group tested 228 plasma specimens collected during seroconversion from 26 plasma donors
- Results were analyzed by assay based on current algorithm (WB confirmation) or proposed algorithm (Multispot + NAT confirmation)
- Multispot offered greater sensitivity versus WB
- Combo assay offered higher sensitivity, but only if NAT confirmation was employed

Screening Assay	3rd Generation HIV Antibody Assay	4th Generation HIV Antigen/Antibody Assay
Number of screen positive results	110	135
Western Blot positive	56	56
Multispot positive	90	90
Multispot- or NAT positive	109	134

Source: S Masciotra *et al.* J Clin Virology 2011; 52S: S17-S22.

HIV 4th Generation Testing

4th Generation HIV Assay

- **Abbott Architect® Ag/Ab Combo**
- **Bio-Rad BioPlex® Ag/Ab Combo**
- **Ortho Clinical Diagnostics VITROS® HIV Combo test**
- **Roche Elecsys® HIV combi PT cobas®**
- **Siemens Advia Centaur® CHIV**

How to Assess an HIV Test

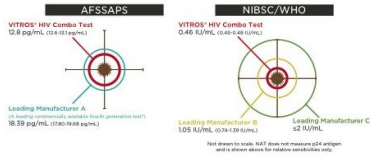
- Sensitivity: capability of a test to correctly identify people infected with HIV
- Specificity: capability of a test to correctly identify people that are not infected with HIV
- Positive Predictive Value is the probability that a person who tests reactive is indeed infected with HIV
- Negative Predictive Value is the probability that a person who tests negative is not infected with HIV

Antigen Sensitivity

** based on NIBSC/AFSSAPS standards data from three other 4th generation manufacturers assay Instructions for Use.

Analytical Sensitivity
NIBSC/WHO: 0.46 IU/mL
AFSSAPS: 12.8 pg/mL

Helps provide assurance in detecting infection



Detection of HIV-1 viral nucleic acid with Nucleic Acid Test (NAT) remains the most sensitive method in identifying acute HIV-1 infection but its use is not widespread due to associated cost, time and labor.

*VITROS HIV Combo test Instructions for Use, GEM1256_US_EN, Version 1.0..

VITROS® HIV Combo Test – Clinical Sensitivity*

Clinical Sensitivity
100% in Adult High Risk
95% Exact Confidence Intervals
94.94% - 100.00%

100% in Adult Low Risk
95% Exact Confidence Intervals
91.96% - 100.00%

Helps Provide Assurance In Detecting Infection

VITROS HIV Combo test has a clinical sensitivity of 100% in both Adult High and Adult Low Risk Populations

*VITROS HIV Combo test Instructions for Use, GEM1256_US_EN, Version 1.0..

VITROS ® HIV Combo Test – Competitive Performance Uncompromised Specificity*

Clinical Specificity
Adult Low Risk 99.58% (CI 99.38%-99.73%) Adult High Risk 99.68% (CI 99.06% - 99.93%)

Fewer false positives can save time, cost and labor in repeat and confirmatory tests

Testing performed at three external trial sites.

Assay specificity assessed using **6003 low risk patients and 1004 high risk patients.**

High sensitivity while maintaining specificity.

*VITROS HIV Combo test Instructions for Use, GEM1256_US_EN, Version 1.0..

Excellent Precision Can Help Ensure Consistency of Test Results

Panel Member	Mean S/C (Signal to Cut Off)	Within-run CV (%)	Within-lab CV (%)
Anti-HIV-1	0.76	6.6	8.9
Anti-HIV-1	1.28	6.2	7.6
Anti-HIV-1	2.70	5.0	5.8
Anti-HIV-2	0.95	6.6	8.6
Anti-HIV-2	1.30	5.2	7.7
Anti-HIV-2	2.94	5.1	5.8
Anti-HIV-1 Group O	1.14	6.1	7.7
Anti-HIV-1 Group O	1.43	6.8	8.5
Anti-HIV-1 Group O	2.93	5.7	6.6
HIV p24 Ag	0.90	9.1	13.4
HIV p24 Ag	1.58	7.2	10.0
HIV p24 Ag	3.63	6.0	9.2

*VITROS HIV Combo Instructions for Use, 6842781 and 684 2782, GEM1256_US_EN, Version 1.0.
†Clinical and Laboratory Standards Institute

Comparison Between the 4th Gen With or Without Results Differentiation

Seroconversion Panels Data Comparison VITROS® HIV Combo Test vs. BioPlex® 2200

The testing of bleeds from 17 seroconversion panels with both assays shows 100% agreement

DAYS TO DETECT SEROCONVERSION*

PANEL ID	VITROS HIV Combo test[2] Post bleed day of first reactive result	BioPlex HIV Ag-Ab[4] Post bleed day of first reactive result	DIFFERENCE
PRB930	0	0	Agreement
PRB969	63	63	Agreement
HIV6244	27	27	Agreement
HIV6248	18	18	Agreement
HIV9013	23	23	Agreement
HIV9014	0	0	Agreement
HIV9015	30	30	Agreement
HIV9016	30	30	Agreement
HV9020	89	89	Agreement
HIV9021	47	47	Agreement
HIV9028	53	53	Agreement
HIV9032	22	22	Agreement
HIV9075	22	22	Agreement
HIV9077	42	42	Agreement
HIV9079	40	40	Agreement
HIV9089	16	16	Agreement
HIV12008	28	28	Agreement

*Seroconversion panels are a group of serial bleeds from plasma donors during seroconversion. They are intended for use by manufacturers and clinical laboratories to evaluate assay sensitivity. Data is from IFU comparison-NOT from a head to head study.
VITROS® HIV Combo Instructions For Use IFU_HIVc_Gem1256_US_EN
Bio-Rad BioPlex 2200 System HIV Ag-Ab IFU Ref 665-3455 505730D July 2015
Trademarks not owned by Ortho Clinical Diagnostics are the property of their respective owners

Florida Department of Health Study

52,000 subjects→2 cases acute infections confirmed only by nucleic acid testing (Multispot ½ nonreactive, HIV-1 nucleic acid reactive)

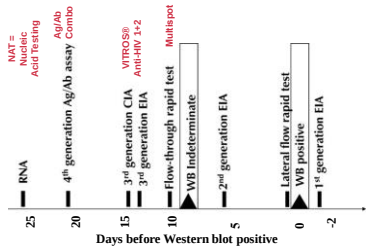


FIGURE 2. Reactivity of FDA-approved assays for HIV-1 compared with Western blot.

BM Branson. J Acquir Immune Defic Syndr 2010;55: S102–S105.

Clinical Data Comparison VITROS® Systems vs. BioPlex®

- BioPlex 2200 HIV Ag-Ab assay does not shorten window period of HIV infection detection compared to VITROS Systems
- There is no published clinical study that demonstrates the superiority in clinical performance of BioPlex 2200 HIV Ag-Ab assay to 4th Gen assays
- Per the current CDC HIV diagnostic testing guidelines, although with separate reporting of antibodies and antigen, BioPlex 2200 HIV Ag-Ab assay is regarded as the 1st step assay, and initial reactive samples still require confirmation in the following steps

https://www.health.ny.gov/diseases/aids/providers/testing/docs/guidelines.pdf

Point-of-Care Testing

HIV Point-of-Care Testing Facts

- What are HIV Point of Care testing (POCT)?
  - POCTs are screening tests which can be performed on-site while the patient waits and provide results within minutes.
- What is the specimen type for HIV POCT?
  - Oral fluid (saliva),whole blood (finger prick)
- What does an HIV POCT detect?
  - Most HIV POCTs can detect antibodies only; some can detect antibody/antigen
- How long does it take to get a result?
  - The length of time required to get a result will depend on the brand of test used. All HIV POCTs can provide a result in 20 minutes or less

Sources: https://www.health.ny.gov/diseases/aids/providers/testing/rapid/workbook.htm
http://conditions.health.qld.gov.au/HealthCondition/condition/14/116/812/hiv-point-of-care-testing

HIV Point-of-Care Testing Facts

- What will be the next step if the result is reactive?
  - The reactive result is only preliminary and must be followed-up by confirmatory tests
- What happens if the result is non reactive?
  - No further testing is required. If the patient has a history of recent high-risk activity, repeat testing is recommended

https://www.health.ny.gov/diseases/aids/providers/testing/rapid/workbook.htm
http://conditions.health.qld.gov.au/HealthCondition/condition/14/116/812/hiv-point-of-care-testing

The Clinical Performance of Different HIV POCTs in High Risk Population

- A 3rd Gen EIA assay and pooled NAAT were used to provide final results
- Although the performance of different POCTs vary, they are all less sensitive than EIA assay, including the combo rapid assay
- Testing on oral fluids is less accurate than on whole blood
- Currently there is no adequate POC substitute for laboratory-based 4th gen testing or HIV NAAT I high-incidence populations

	# tests	Sensitivity (95% CI) compared to all cases	Sensitivity (95% CI) compared to EIA+ cases	Specificity (95% CI)
OraQuick® (oral fluid)	2180	51/68 = 75.0% (63.0–84.7)	51/60 = 85.0% (73.4–92.9)	2109/2112 = 99.86% (99.59–99.97)
OraQuick (fingerstick)	2175	53/68 = 77.9% (66.2–87.1)	53/60 = 88.3% (77.4–95.2)	2107/2107 = 100% (99.82–100)
Uni-Gold™	1614	45/53 = 84.9% (72.4–93.3)	45/47 = 95.7% (85.5–99.5)	1561/1561 = 100% (99.76–100)
INSTI®	559	11/15 = 73.3% (44.9–92.2)	11/13 = 84.6% (54.6–98.1)	543/544 = 99.82% (98.98–100)
Determine™ Combo	1523	34/40 = 84.6% (70.2–94.3)	33/36* = 91.7% (77.5–98.2)	1468/1483 = 98.99% (98.34–99.43)
GS™ HIV-1/HIV-2 Plus O antibody (EIA)	2161	58/66 = 87.9% (77.5–94.6)		2091/2095 = 99.81% (99.51–99.95)

* This numerator and denominator do not include the participant who tested EIA-negative but p24 Ag-positive on Determine Combo.

Note: estimates cannot be directly compared, as not all POC tests were used on all participants.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4762745/pdf/nihms750685.pdf

Considerations for HIV Rapid Tests in New Algorithm

Current CDC Recommendation

- FDA approved rapid tests can be used as initial screening test. If any instrumented antigen/antibody test is available, it is preferred due to their superior sensitivity for detecting HIV during acute infection. https://stacks.cdc.gov/view/cdc/48472
- For reactive (preliminary positive) result from any rapid HIV test, laboratories should begin confirmation by testing with one of the laboratory-based HIV Ag/Ab Combo immunoassays and follow the steps in the Recommended HIV Laboratory Testing Algorithm

Summary

Summary

- A fourth generation HIV test detects simultaneously both HIV-1 and HIV-2 antibodies and the HIV p24 antigen
- Provides earlier detection that can help lead to improved diagnosis and prevention
- Current CDC HIV laboratory algorithm recommends the 4th Gen HIV test as the initial screening assay
- Although the performance for rapid tests has improved, they cannot replace the initial screening assay in the recommended algorithm

PR-03662

47

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HIV/AIDs : A manageable chronic disease with advances in treatment

48

- **Advances in Antiretroviral therapy renders AIDs a chronic and treatable disease more like diabetes…..**

"People living with HIV but benefiting from the latest medical developments can hope to lead normal lives in many respects…"
The World Health Report, 2003, The World Health Organization

As laboratorians we can play an important role in helping to connect patients to therapy

Source: http://www.who.int/whr/2003/chapter3/en/\
http://www.who.int/publications/10-year-review/hiv/en/index5.html

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Questions?

Thank You

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