MAYO CLINIC

Rapid HIV Assays

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Heart of America Point of Care Network

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Outline

- HIV in US and other countries
- HIV markers and testing algorithm
- Need for rapid HIV in hospitals
- Performance of rapid HIV assays
- Mayo evaluations of rapid antibody and antibody/antigen tests
- Conclusions

HIV in US

- ~ 1,200,000 HIV infected in US as of 2018
- ~ 14% unaware of HIV status (down from close to 25% a decade ago)
- 15,280 deaths in 2018 (all causes)
- New HIV diagnoses 2018:
  - 66% male to male sexual contact
  - 24% heterosexual contact
  - 7% injection drug use
  - 4% male to male sexual contact and injection drug use
  - 42% Black/African American, 27% Hispanic/Latinos, 25% Whites

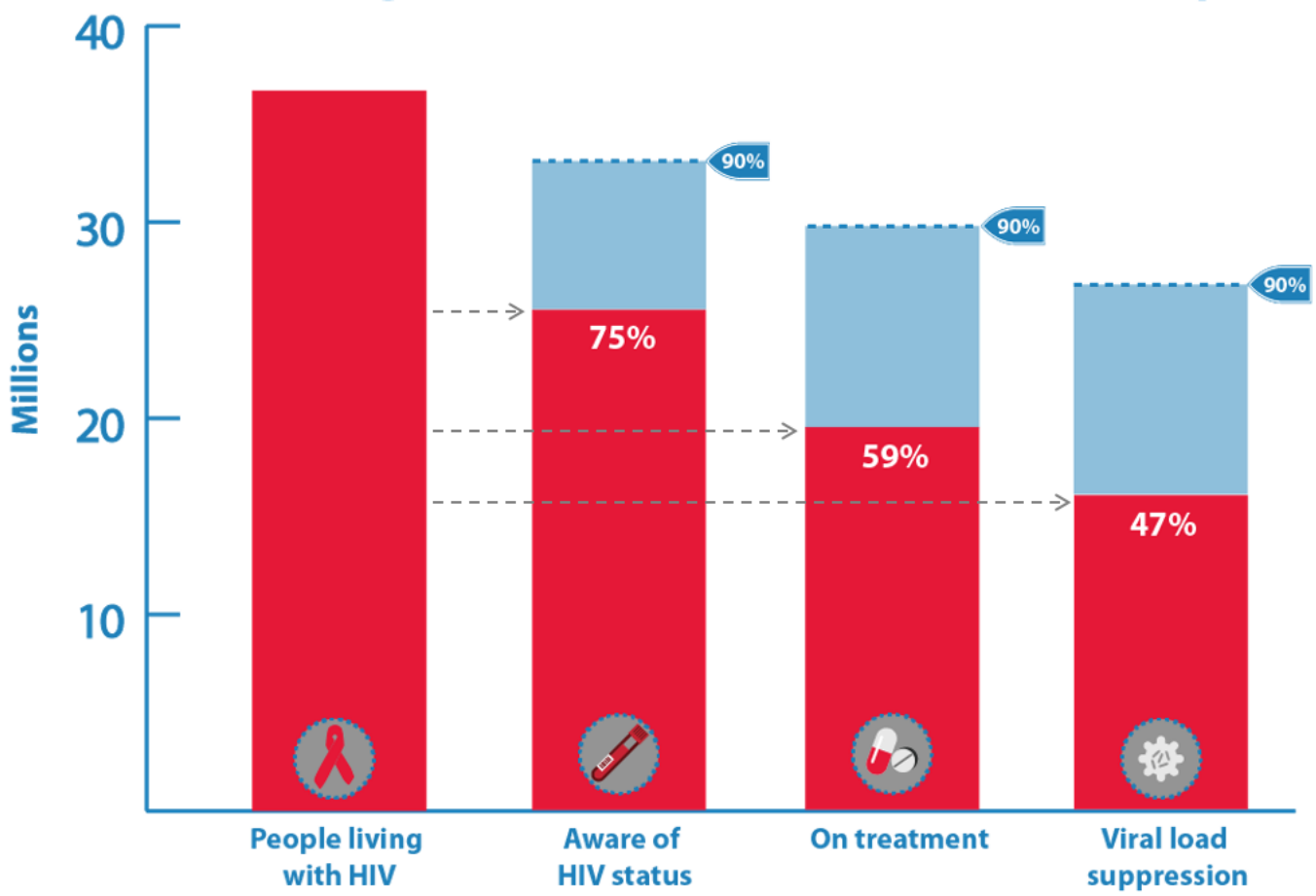
HIV in US

- Screening for HIV antibodies done with combined antigen.antibody EIA test, takes 3-4 hours technical time, confirmation if positive
    - 8-24 hours TAT standard in most labs
- CDC now recommends HIV screening for all adults and adolescents ages 13-64 in healthcare setting (everyone should get tested at least once), once/year for high risk groups
    - How to get testing, counseling done in order to initiate treatment
    - Rapid HIV tests

HIV outside US

- ~ 37 million living with HIV worldwide in 2018
- ~ 1.7 million new infections
- Around half become infected before age 25
  - 2nd leading cause of death among 20-24 yo
- Sub-Saharan Africa accounts for 2/3 new HIV infections

HIV Testing and Care Continuum



UNAIDS/WHO Estimates 2017

HIV outside US

- Multiple challenges in identifying HIV infected
  - Resources for screening
  - Test and sample stability
  - Resources for confirmation of pos screens
  - Lab personnel for testing
- Rapid HIV
  - Fingerstick or oral fluid sample types
  - Simple methods require minimal training
  - No lab equipment, low cost
  - Long shelf-life and RT storage

Markers of HIV Infection

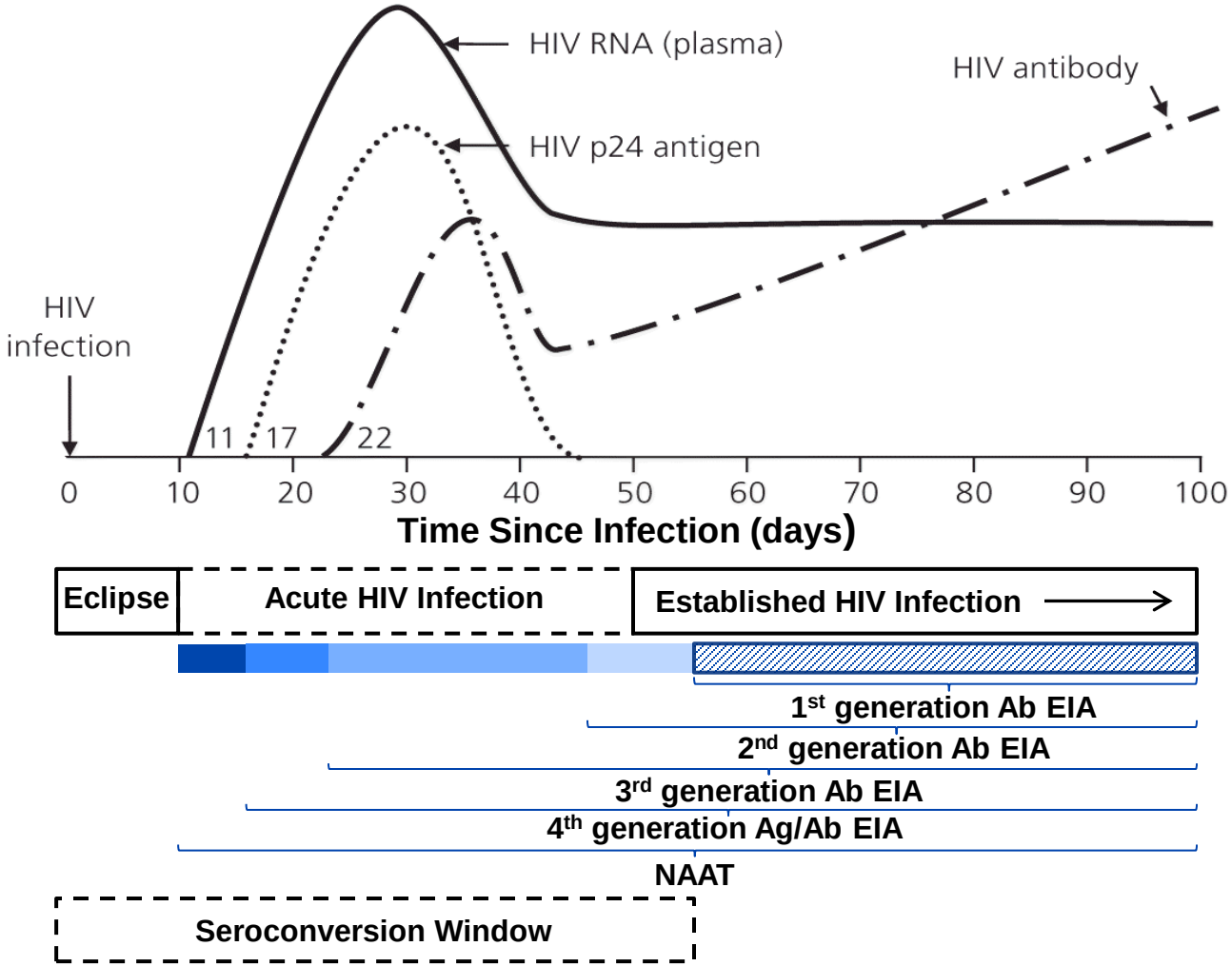
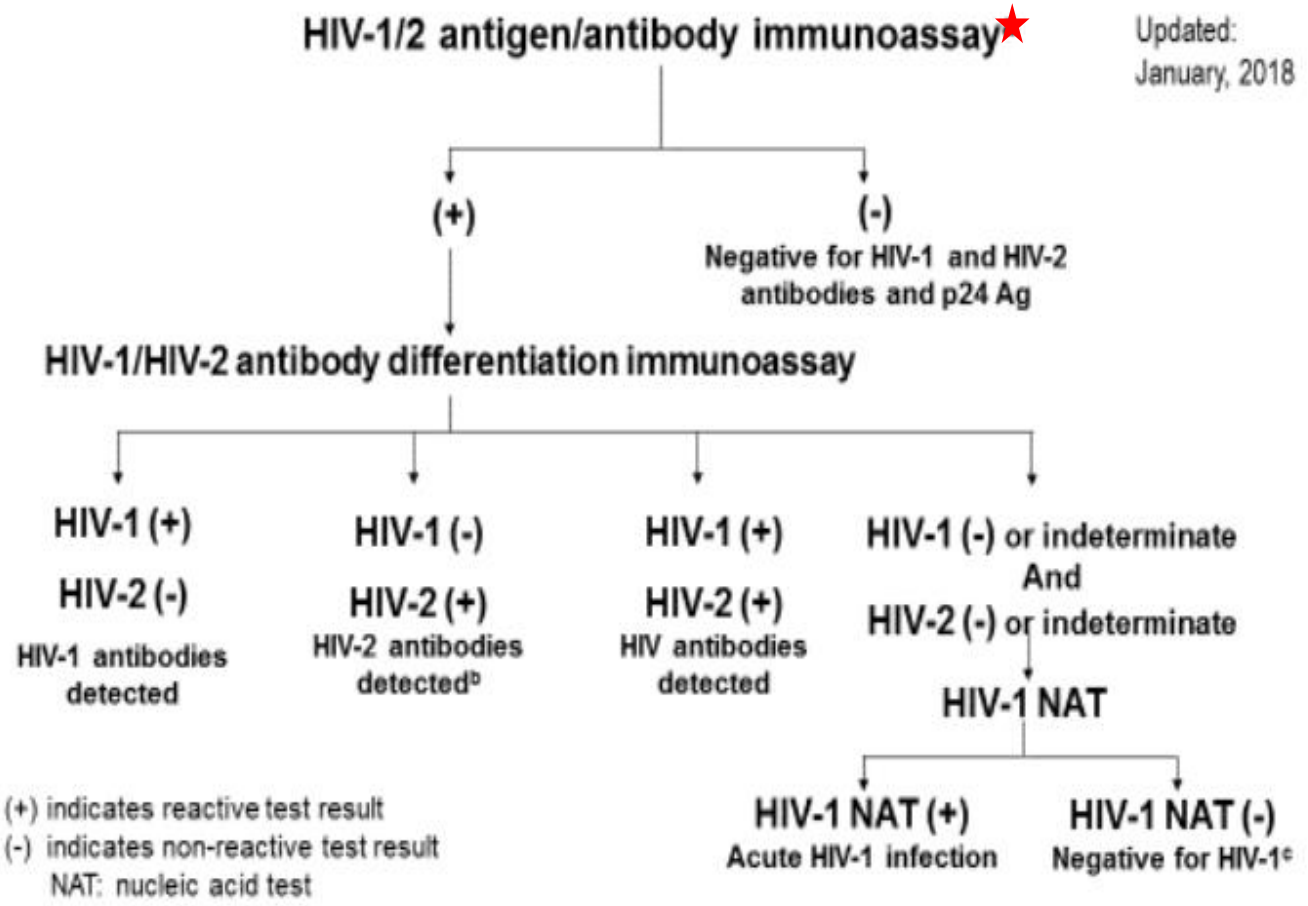


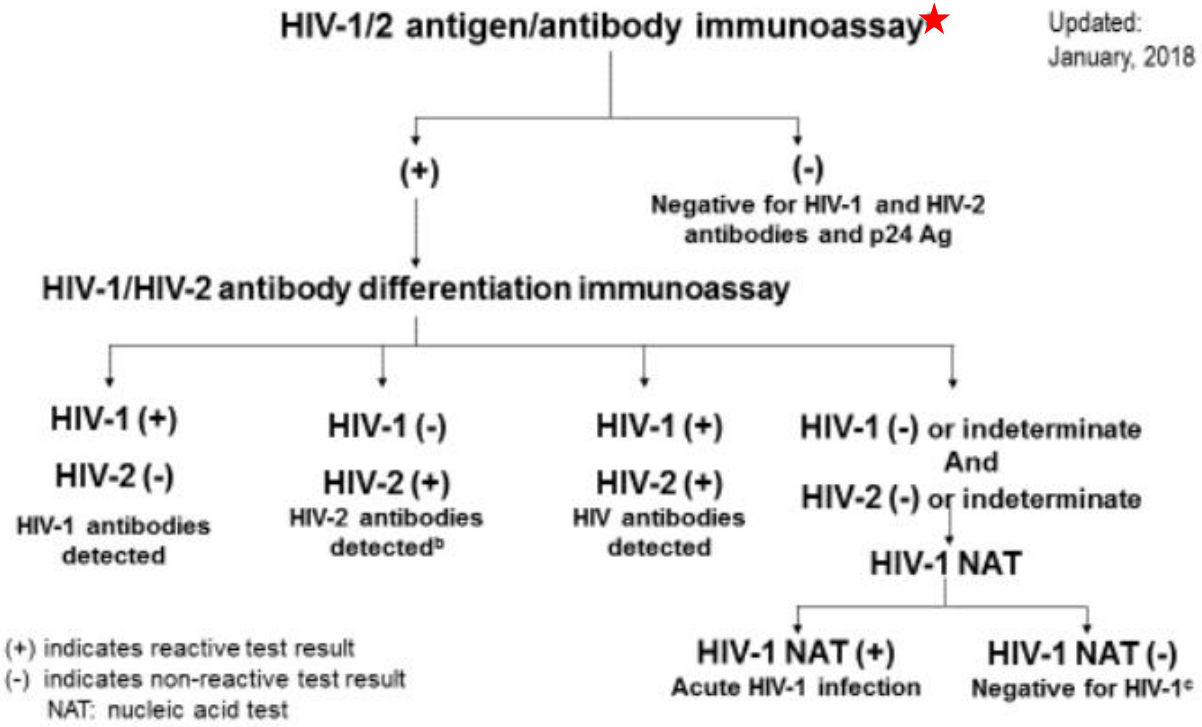
Figure adapted from Laboratory Testing for the Diagnosis of HIV Infection: Updated Recommendations. CDC 2014.

CDC Testing Algorithm



2018 Quick reference guide: Recommended laboratory HIV testing algorithm for serum or plasma specimens. https://stacks.cdc.gov/view/cdc/50872

CDC Testing Algorithm

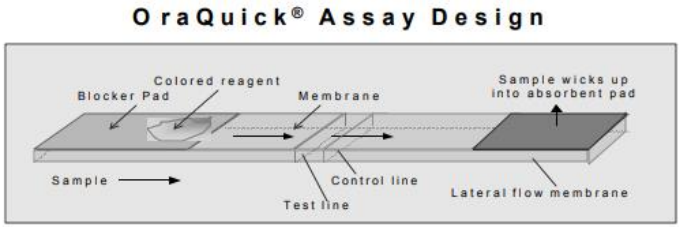


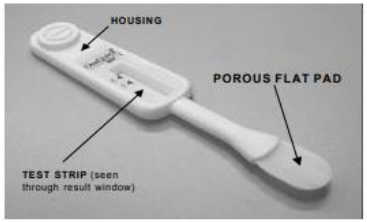
The FDA-approved single-use rapid HIV-1/HIV-2 Ag/Ab immunoassay can be used as the initial assay in the laboratory HIV testing algorithm for serum or plasma. If any instrumented Ag/Ab test is available, it is preferred due to its superior sensitivity for detecting HIV during acute infection. More data needed on whole blood performance.

2018 Quick reference guide: Recommended laboratory HIV testing algorithm for serum or plasma specimens. https://stacks.cdc.gov/view/cdc/50872

What about rapid/POC tests?

- November 2002: FDA approved first rapid HIV diagnostic test kit for use in the U.S.
- OraQuick Rapid HIV-1 Antibody Test
  - Less than a drop of whole blood
  - Results interpreted in 20 minutes
  - 99.6% sensitivity; 100% specificity
  - Room temperature storage
  - 4-6 weeks after infection most enough Ab to test +





POC Testing Characteristics

ADVANTAGES

- Reduce patient loss to follow-up
- Increase access to therapy
- Decrease transmission
- Access more people
- Patient preference

DISADVANTAGES

- Higher rates of misdiagnosis (false pos)
- Longer window period
- Ambiguous test results
- Subjective variability in reading

Evolution of POC Tests

Test Category	HIV Screening Tests	Manufacturer	Run Time	Detects IgG	Detects IgM	Whole Blood	Oral Fluid
Ab test	DPP HIV-1/2 Assay	Chembio	10 min WB 25 min OF	×		×	×
	HIV 1/2 STAT PAK	Alere	15 min	×		×	
	INSTI HIV-1/HIV-2 Ab Test	BioLytical	<2 min	×	×	×	
	OraQuick ADVANCE Rapid HIV-1/2 Ab Test	OraSure Technologies	20 min	×	×	×	×
	Reveal G4 Rapid HIV-1 Ab Test*	MedMira	<2 min	×		×	
	SURE CHECK HIV 1/2 Assay	Chembio	15 min	×		×	
	Uni-Gold Recombigen HIV-1/2	Trinity Biotech	10 min	×	×	×	
Ag/Ab test	Determine HIV-1/2 Ag/Ab Combo	Alere	20 min	×	×	×	

Advantages and Disadvantages of Different Types of HIV Tests.
https://www.cdc.gov/hiv/testing/laboratorytests.html

Role of POC HIV Testing at Mayo Clinic (use case for hospital-based rapid HIV)

Labor and Delivery

Rapid testing of women whose HIV status is unknown at labor

Occupational Health

Rapid testing for high- or unknown-risk patients after a needlestick, blood, or body fluid exposure has been reported

HIV Clinic

Anonymous and confidential, rapid testing for patients presenting to the clinic

Other use cases: ED (not done at Mayo), school clinics (one site at Mayo), public health screening

Mother-to-Child HIV Transmission

- Risk of HIV transmission is <1% when:
  - Recommended antiretroviral/obstetric interventions are used in women who know of infection early in pregnancy
  - Risk ~25% without intervention
- In 2000, of the 6000-7000 HIV-infected women who gave birth in the U.S., 40% had not been diagnosed with HIV before L&D
- 2006 *CDC's revised Recommendations for HIV Testing of Adults, Adolescents, and Pregnant Women in Health-Care Settings*
  - HIV screening included in routine panel of prenatal screening tests for all pregnant women

Rapid Testing for Labor and Delivery

- Starting treatment during L&D or providing it to the newborn within hours after birth can reduce transmission by 50%
- Critical to rapidly obtain HIV test results for women in labor to begin treatment as soon as possible
- CDC recommends rapid HIV testing either prior to the onset of labor or immediately post-partum
  - 9-13% HIV transmission rates achieved when intervention begins intrapartum or neonatal periods
- “Every delivery unit needs to have access to an HIV test that can be done rapidly (i.e., in <1 hour) 24 hours a day”

Panel on Treatment of Pregnant Women with HIV Infection and Prevention of Perinatal Transmission. Recommendations for Use of Antiretroviral Drugs in Transmission in the United States. http://aidsinfo.nih.gov/contentfiles/lvguidelines/PerinatalGL.pdf.

Rapid Testing for Occupational Exposures

- Occupational transmission of HIV to health care workers is rare
  - As of December 31, 2013, 58 confirmed occupational transmissions of HIV and 150 possible transmissions had been reported in the U.S.
- Post-exposure prophylaxis (PEP) regimens are recommended when occupational exposures to HIV occur
  - Treatment should begin within 2 hours (ideally), but no longer than 72 hours of an exposure
- Rapid testing is critical if HIV status of the source patient is unknown
  - A positive rapid HIV test is preliminarily considered a true positive for the purposes of PEP decision-making

Rapid HIV testing US Public Health

- Overall, sens and spec of waived rapid HIV tests very good in public health setting
  - NYS experience very good except oral fluid testing in one NYS site
  - Some studies found increase false positive with non-lab testing personnel
- Variable effectiveness of screening programs
  - Rapid screening effective in getting HIV results to population that otherwise would not be screened
  - Rates of counseling and confirmation testing vary
  - Rates of entry into medical treatment vary from 47-97%
  - Publicly sponsored programs do better than privately sponsored

Assays/Technologies available

- Immunochromatographic (lateral flow) immunoassay
  - HIV Ag applied to line on nitrocellulose strip
  - Blood diluted in buffer, added to well
  - Lateral flow pulls blood past indicator and over to test line (Ag), then to control line (indicator)

Assays/Technologies available

- Immunochromatographic (lateral flow) immunoassay
- Advantages:
  - Fast (10-20 minutes), few steps, minimal sample processing, varied sample types (whole blood, serum, plasma, oral fluid), many CLIA waived
- Disadvantages:
  - Cannot distinguish HIV-1 and HIV-2, need to dilute sample in buffer, interpretation of lines

Performance of rapid HIV assays

	Sensitivity (95% C.I.)	Specificity (95% C.I.)
OraQuick Advance		
- oral fluid	99.3%(98.4-99.7)	99.8%(99.6-99.9)
- whole blood	99.6%(98.5-99.9)	100%(99.7-100)
- plasma	99.6%(98.9-99.8)	99.9%(99.6-99.9)
Unigold Recombigen	100.0%(99.5-100)	99.8%(98.3-100)

Performance of rapid HIV assays

- Rapid HIV Ab tests use only one (gp41) or two (gp120 and gp41) antigens as targets
- Despite smaller number Ag targets sensitivity compares well to lab EIA
- One limitation patients treated with high dose antiviral agents (anti gp 41 decreased with therapy)
  - Treated patients unlikely to be tested with rapid HIV test

Performance of rapid HIV assays

- Some studies found poorer detection of HIV Ab early in infection with rapid tests
  - Ab EIA longer window period than Ag/Ab or NAT test (22 vs 17 days after infection)
  - Fewer antigen targets in rapid tests may lead to fewer early pos results compared to lab EIA
  - Some data suggests variability among rapid tests (some may detect IGM Ab better)
  - With non-lab users false positives can be a problem

Performance of rapid HIV assays

- Oral fluid testing
- NYC public screening program 2005-08
- Higher rate of false pos Oraquick results with oral fluid compared to WB
- Many false pos seen in one site, no cause determined
- Still within stated 98% specificity
- CDC now warns that oral fluid testing less sensitive and specific than WB or serum/plasma

Impact of new guidelines for HIV testing

- Start with combined antibody/antigen test
- Only one FDA-approved rapid antigen/antibody combo test available
- Alere Determine™ HIV-1/2 Ag/Ab Combo
- Simultaneous detection of HIV-1 p24 Ag and Ab to HIV-1 and HIV-2
  - Capillary whole blood (waived), serum or plasma
- Rapid differentiation of HIV-1 and HIV-2 antibodies (Multispot no longer manufactured)

Mayo Clinic Evaluation-2005

- Study Design
  - Oraquick, Uni-Gold and Multi-spot evaluated
  - 50 blood bank samples (HIV negative)
  - 20 HIV positive samples (viral load positive)
  - 20 cross-reactive samples (Hep A or B, EBV)
  - 10 EIA positive, WB negative samples
  - Background clarity and line intensity graded
  - 0-3 (0 background best, 3 line intensity best)

Mayo Clinic Evaluation-2005

- HIV negative samples
  - Oraquick: 50/50 negative
  - Uni-Gold: 49/50 negative (1 false positive)
  - Multi-spot: 48/50 negative (2 false positives, undifferentiated)
  - Background clarity:
  - At 10 min, all had 0 background except one sample on Uni-Gold (hemolyzed)
  - At 20 min, Uni-Gold and Multi-spot had higher background than Oraquick

Mayo Clinic Evaluation-2005

- Positive samples
- All 3 methods had 20/20 positive
- All samples resulted in 2-3+ line intensity
- All 3 methods have excellent sensitivity

Mayo Clinic Evaluation-2005

- Crossreactive samples
- 1 sample positive by all 3 methods, record reviewed and re-classified as HIV positive based on history HIV infection
- 19/19 crossreactive samples negative by all 3 methods

Mayo Clinic Evaluation-2005

- EIA Positive, WB negative samples
- 10/10 negative by both Oraquick and Uni-Gold methods
- 6/6 negative by Multi-spot (insufficient volume 4 samples)

Mayo Clinic Evaluation-2005

	OraQuick % (95% CI)*	Uni-Gold % (95% CI)	Multispot % (95% CI)
Specificity	100 (95-100)	98.7 (93-100)	97.3 (91-100)
Sensitivity	100 (84-100)	100 (84-100)	100 (84-100)
Positive Predictive Value	100 (84-100)	95.5 (77-100)	91.3 (72-99)
Negative Predictive Value	100 (95-100)	100 (95-100)	100 (95-100)

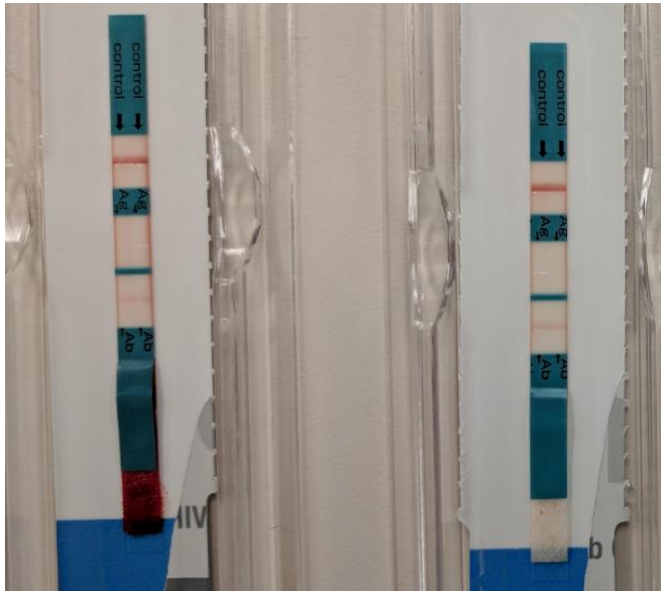
Implementation decision (2006-7)

- Implement Oraquick rapid HIV testing using EDTA plasma (ease of use, ease of reading)
- Later changed to EDTA whole blood to standardize between waived and non-waived sites performing testing

Evaluation of Determine™ Ag/Ab Combo Test-2018

- Sample Type: compared 6 waste EDTA whole blood and plasma samples
  - Spiked with positive control material
  - Read in duplicate

	EDTA Whole Blood		EDTA Plasma	
	Reader 1	Reader 2	Reader 1	Reader 2
Clear	3	2	5	6
Difficult	3	4	1	0
Not Clear	0	0	0	0



Evaluation of Determine™ Ag/Ab Combo Test

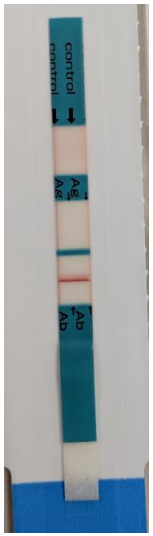
- Precision: 5 replicates x 5 days using 4 different controls
  - HIV-1 reactive, HIV-2 reactive, p24 reactive, nonreactive
  - Two techs blinded to results read strips
    - 100% concordance with control type
    - 100% concordance between techs

Evaluation of Determine™ Ag/Ab Combo Test

- Accuracy:
  1. Spiked EDTA plasma samples (n = 10) with SeroDetect HIV-1/HIV-2 Ag/Ab Combo Verification Panel
     - Used as reference for testing the Determine™ Ag reaction line
     - 100% concordance (8 Ag reactive/2 Ag non-reactive)
  2. Obtained samples (n = 60) from Hepatitis/HIV Serology Lab with HIV testing performed on Geenius HIV 1/2 Supplemental Assay (Bio-Rad)

Accuracy Results

- Ab Accuracy Compared to Reference Method:
  - 30 positive and 30 negative (reference method)
  - 98% concordance (59/60)
    - Discordant sample was Ab positive by reference method and indeterminate on the Determine™ (invalid control)



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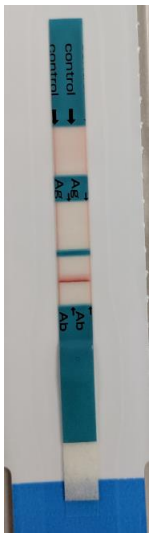
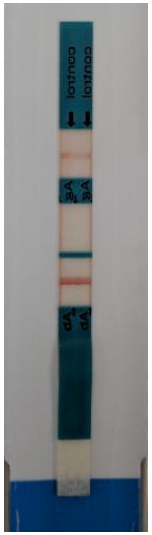
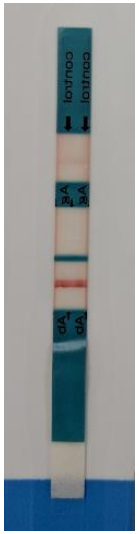


x5 and x10 dilutions with serum and saline



x5 serum



x5 saline

Accuracy Results

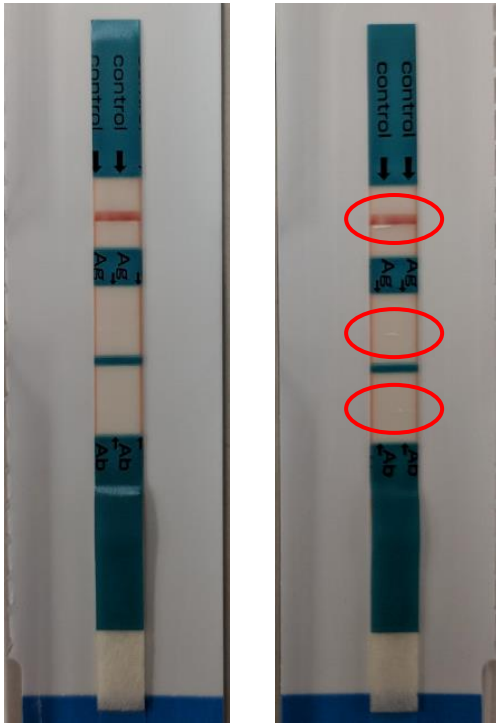
- Control line is formed from same components as test lines; consumption of those components by a high titer sample can limit control line intensity—
- High Dose Hook Effect

Ambiguity in Lines

- Control lines
  - Any visible pink/red color in control area, regardless of intensity, is considered “valid”
  - If there is no pink/red control line in the control area, even in a pink/red line appears in Ab or Ag area, the result is invalid
    - Test should be repeated
  - 8/81 (10%) test have weak/no control lines
    - May result in increase in repeat testing, potential to not release reactive results

Ambiguity in Lines

- “Ghost” Lines
  - White abnormalities observed after running strips
  - Predominantly found in a single lot of strips, but present in others
  - Potential to obscure result reading
  - Manufacturing defect (rare)



Non-specific Reactivity

Table 5: Alere Determine™ HIV-1/2 Ag/Ab Combo Reactivity with Specimens from Individuals with Unrelated Medical Conditions and Specimens with Potentially Interfering Substances

Specimen Description	Alere Determine™ HIV-1/2 Ag/Ab Combo (# Reactive/Total Tested)		
	Specificity Testing: Unspiked Samples	Sensitivity Testing: HIV-1 Samples (Weak Reactive)	Sensitivity Testing: p24 Antigen Samples
Human T-cell Lymphotropic Virus (HTLV)	0/10	10/10	10/10
Epstein Barr Virus (EBV)	0/20	10/10	NT
Cytomegalovirus (CMV)	0/20	10/10	10/10
Hepatitis C Virus (HCV)	0/30	10/10	10/10
HBsAg	0/8	NT	NT
Herpes Simplex Virus (HSV)	1/55	20/20	20/20
Syphilis	0/20	10/10	10/10
Toxo IgG	1/55	20/20	20/20
Cancer	2/55	20/20	20/20
Alcoholic Cirrhosis	0/10	10/10	10/10
Flu Vaccine	0/10	10/10	9/9
Anti-HBc	0/10	NT	NT
Multiparous Females	0/10	NT	NT
Drugs	0/10	NT	NT
Hospitalized patients	8/560	55/55	56/56
HAMA	2/54	20/20	20/20
RF	4/150	21/21	21/21
Triglycerides*	3/55	21/21	21/21
Hemoglobin**	0/21	21/21	21/21
Bilirubin**	0/21	21/21	21/21
High Serum Protein**	0/21	21/21	21/21

**Naturally occurring specimens containing more than 500 mg/dL*
***Specimens artificially created by adding the potentially interfering substance to normal human serum (Lyophilized hemoglobin: 5 mg/mL; Bilirubin: 0.25 mg/mL; Protein: 0.05 g/mL).*

Alere Determine HIV-1/2 Ag/Ab Combo Package Insert Rev. 6 2016/11.

Implementation Decision…

- Agreed to hold off on implementing the Alere Determine™ Ag/Ab Combo Test
  - Challenges and issues observed during the study were concerning
- Continue testing with OraQuick ADVANCE Rapid HIV-1/2 Ab Test
  - Automatic reflex to lab-based Ag/Ab immunoassay
- For rapid testing, means window period longer than lab testing (22 vs 11 days)
- Will need to re-evaluate in future as technologies improve

Conclusions

- HIV still public health threat in US
  - 25% infected globally are still undiagnosed
- Rapid HIV tests have excellent sensitivity for the detection of HIV antibodies
- Rapid HIV testing may be useful for hospital obstetrics, clinic/ED settings, or as part of employee exposure protocol
- Evaluation of devices with intended user group important, potential for false positives greater than with use of automated laboratory tests, as with all POC consider usability and performance data

