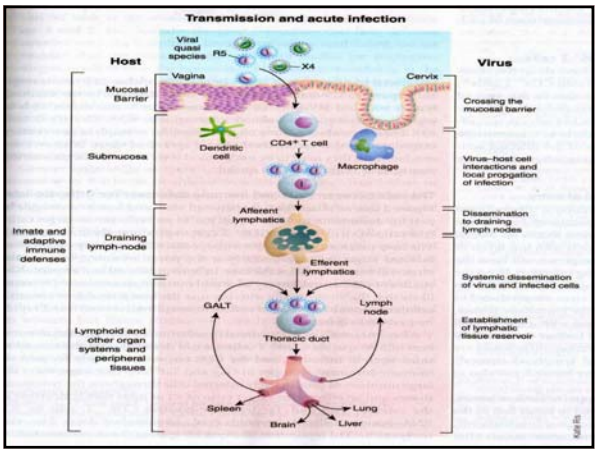
Peter A. Leone,MD
Professor of Medicine
University of North Carolina
Medical Director
North Carolina HIV/STD Prevention and Care Branch



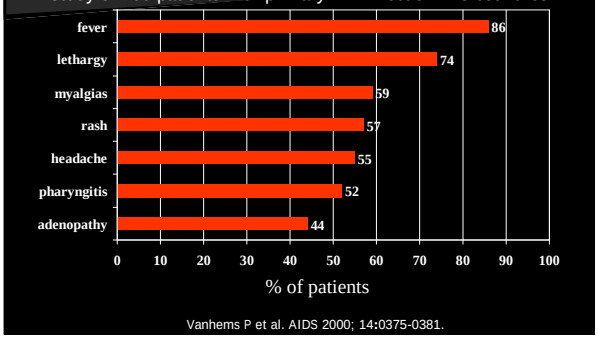
Acute Retroviral Syndrome

- 40-90% of new HIV infections are symptomatic
- Signs and symptoms typically begin 1-4 weeks following the exposure
- Symptoms can last from days to several weeks, but usually <14 days

Pilcher C et al. N Engl J Med 2005;352:1873-1883
Kahn JO, Walker BD. N Engl J Med. 1998;339:33-39
Schacker T, et al. Ann Intern Med. 1996;125:257-264

Common Signs & Symptoms

Study of 160 patients with primary HIV infection in 3 countries



Vanhems P et al. AIDS 2000; 14:0375-0381.

Acute HIV and Symptoms

	Schacker	Kinloch-de Loes	NC STD
Fever	93%	87%	48%
Fatigue	93	26	37
Pharyngitis	70	48	30
Headache	55	39	26
Rash			15
GI Symptoms			37

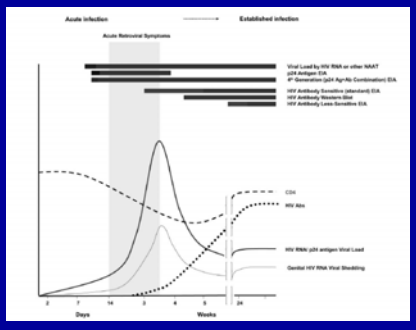
Schacker TW, et al., AIM 1996 125:257-64

Common Mis-diagnoses

- Mononucleosis
- Rocky Mountain Spotted Fever
- Strep throat
- Influenza
- "Viral illness"
- Secondary syphilis

AHI Syndrome and Medical Evaluation

- 78% (25/31) with symptoms 3 mo. Prior to 1st positive test
- 65%(20/31) sought medical evaluation
- 50% (10/20) went to ED or Urgent Care
  20% (4/20) went to private MD
- 30% (6/20) Dx bacterial infection
  30% (6/20) Dx viral syndrome
  15% (3/20) Dx AHI
- 18.8% (6/31) aware of AHI prior to Dx



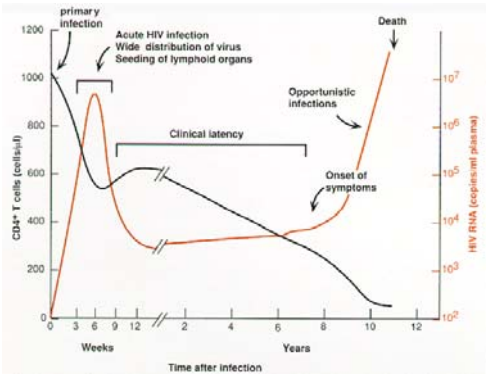
Pilcher, et al **JID 2010:201 (Suppl 1)**

Window Periods for HIV Tests

HIV test	Assay method	"Window period" estimates, weeks*	"Window period" reduction, days†
First-generation EIA	Viral particles used to bind patient HIV Ab, detected by marker conjugated to anti-human Ab	~6	...
Second-generation EIA	Same as first-generation EIA except uses purified HIV Ag or recombinant virus	~4–6	10
Third-generation EIA	"Antigen sandwich": synthetic peptide used to bind patient HIV Ab followed by marker conjugated to additional HIV Ag; able to detect IgM	~3–4	6
Fourth-generation EIA	Uses third-generation EIA methodology plus monoclonal Ab to p24 Ag to detect patient p24 Ag	~2	5
Pooled HIV NAT	First combines multiple individual samples into one common pool, then uses PCR or other amplification techniques to detect patient viral nucleic acids	<1–2	3
Individual HIV NAT	As above, except that samples are tested individually rather than diluted by pooling	<1–2	3

Stekler J. et al CID 2007

Acute HIV Infection



Slide adapted from Fauci A, *Ann Intern Med* 1996;124:654-663.

Rationale for Acute HIV Diagnosis

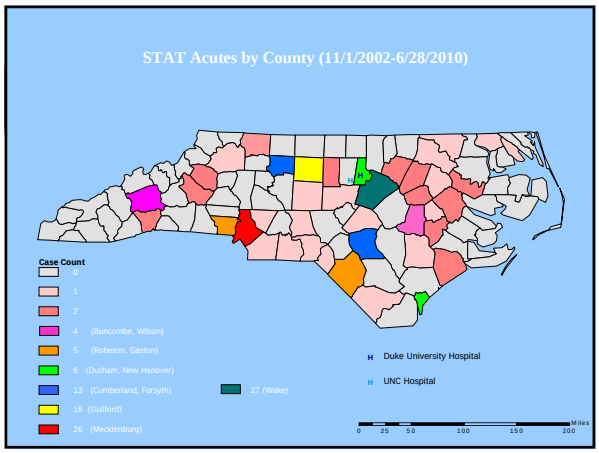
- Most Infectious period and Dx often missed
- Individual Perspective
  - Improve prognosis with acute treatment????
    - Lowering of viral set point
    - Preservation of CD4 T cells
    - Decrease in rate of progression
    - Long-term control of HIV viremia
    - Viral eradication
  - Early entry into care
  - Short-term behavioral change results in large benefit
  - Management of STIs

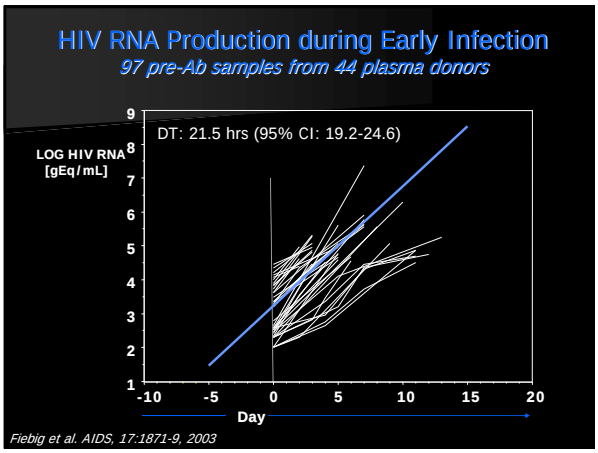
Rational for AHI Diagnosis

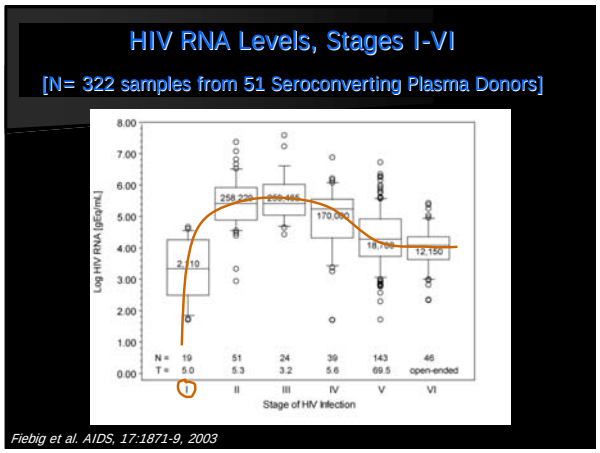
Public Health

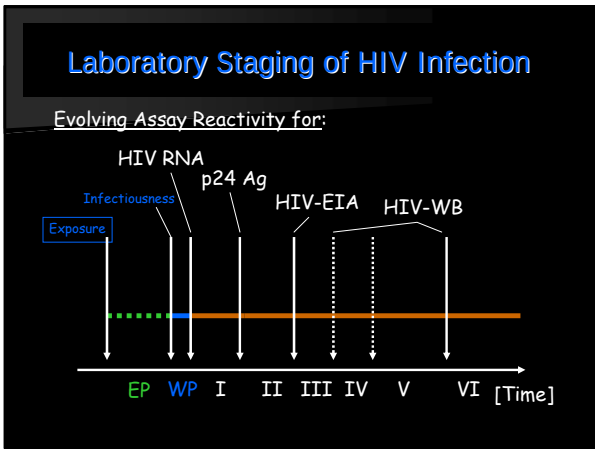
- Recognized previously missed infections
- Avoid transmission to partners with risk reduction
  - 10-100 fold increased transmission risk x 3-6 months
  - May be responsible for 14-50% of all transmission of HIV
  - **Quebec AHI/PHI <10% of infection but ~50% transmission**
- Networks – leading edge of transmission
  - -Identify Transmission networks for intervention
  - -prevent secondary transmission by contact tracing and counseling to modify risk behaviors at risk partners
  - - Geographic focus

Brenner BG, et al, JID 2007:195


STAT Acutes by County (11/1/2002-6/28/2010)
Case Count
1
2
4 (Buncombe, Wilson)
5 (Robeson, Gaston)
6 (Durham, New Hanover)
13 (Cumberland, Forsyth)
16 (Guilford)
26 (Mecklenburg)
27 (Wake)
Duke University Hospital
UNC Hospital
Miles
0 25 50 100 150 200


HIV RNA Production during Early Infection
97 pre-Ab samples from 44 plasma donors
LOG HIV RNA [gEq/mL]
DT: 21.5 hrs (95% CI: 19.2-24.6)
9
8
7
6
5
4
3
2
1
-10 -5 0 5 10 15 20
Day
Fiebig et al. AIDS, 17:1871-9, 2003


HIV RNA Levels, Stages I-VI
[N= 322 samples from 51 Seroconverting Plasma Donors]
Log HIV RNA [gEq/mL]
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
2,110
170,000
18,150
12,150
N = 19 51 24 39 143 46
T = 5.0 5.3 3.2 5.6 69.5 open-ended
I II III IV V VI
Stage of HIV Infection
Fiebig et al. AIDS, 17:1871-9, 2003


Laboratory Staging of HIV Infection
Evolving Assay Reactivity for:
HIV RNA
p24 Ag
Infectiousness
HIV-EIA
HIV-WB
Exposure
EP WP I II III IV V VI [Time]

HIV Stage Progression based on 51 Seroconverting Plasma Donors

Fiebig stage classification for sub-stages of HIV-1 primary infection, and the average and cumulative duration of each phase.

Stage	Duration of each phase (days)	Cumulative duration (days)
Eclipse	10 (7,21)	10 (7,21)
I (vRNA+)	7 (5,10)	17 (13,28)
II (p24Ag+)	5 (4,8)	22 (18,34)
III (ELISA+)	3 (2,5)	25 (22,37)
IV (Western Blot ±)	6 (4,8)	31 (27,43)
V (Western Blot +, p31−)	70 (40,122)	101 (71,154)
VI (Western Blot +, p31+)	Open-ended	

Fiebig et al. AIDS, 17:1871-9, 2003
Lee et al. J Theor Biol, 2009

Incidence Rate / Window Period (WP) Model Allows Prediction of Test Yields for Direct HIV (p24 Ag, HIV RNA) vs Antibody Assays

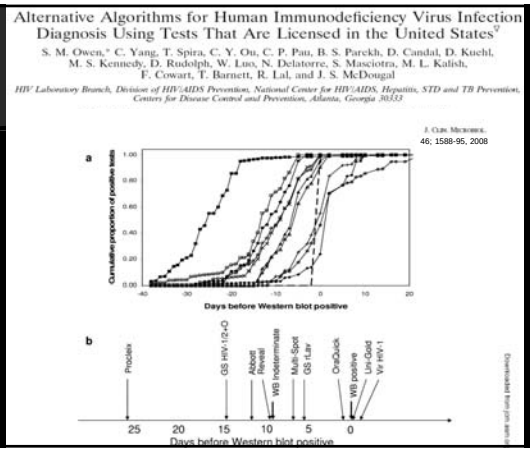
Test Yield (per unit) =

Incidence Rate (person-years)
x Decrease in WP (fraction of year)

Projected WP Closure and Yield of p24 Ag, MP and ID NAT Assays Relative to a Sensitive HIV-1/2 EIA Antibody Test in the Detection of WP HIV Infection

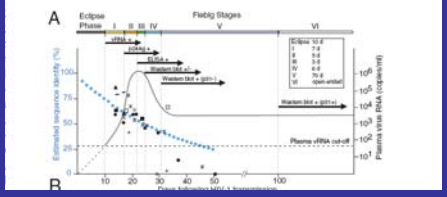
Assay	Sensitivity [gEq / mL]	WP Closure [days]	Yield, WP HIV Infections per 1,000 Persons Tested in Various Screening Settings [Representative Incidence Rate / Person-Years]		
			Blood Donors [2 / 100,000 = 0.002%]	STD Clinic [1 / 1,000 = 0.1%]	High Risk Clinic [1 / 10 = 10%]
p24 Ag	10,000	6	0.00033	0.016	1.6
MP NAT	1,000	9	0.00049	0.025	2.5
ID NAT	50	13	0.00071	0.036	3.6

Fiebig et al. AIDS, 17:1871-9, 2003



PNAS

Identification and characterization of transmitted and early founder virus envelopes in primary HIV-1 infection



102 acutely infected plasma donor panels

3476 complete *env* sequences from single genome amplifications

Inferred consensus sequence at estimated time of virus transmission

78 donors infected by single virion; 24 by 2-5 virions

Macaque/SIV model

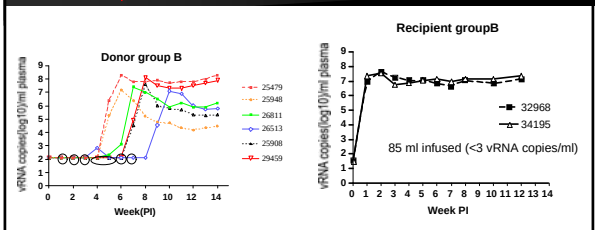
- SIV infection in macaques considered excellent model for HIV in humans for both transmission and pathogenesis research
- "Donor" monkeys repeatedly exposed by intravaginal inoculation of infectious plasma
- Samples taken pre-infection, "blip". Immediately pre-ramp-up, ramp-up and set-point
- Samples "transfused" into naïve "recipient" monkeys

High Specific Infectivity of Plasma Virus from the Pre-Ramp-Up and Ramp-Up Stages of Acute Simian Immunodeficiency Virus Infection

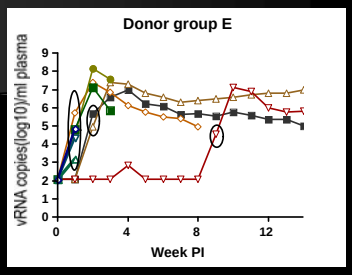
Zhong-Min Ma,[1,2] Mars Stone,[1,2] Mike Piatak, Jr.,[3] Becky Schweighardt,[4] Nancy L. Haigwood,[5] David Montefiori,[6] Jeffrey D. Lifson,[3] Michael P. Busch,[7,8] and Christopher J. Miller[1,2,9]*

Journal of Virology 83; 3288-3297, 20

Plasma transfusion from "SIV exposed-eclipse phase" monkeys



Ma et al. Journal of Virology 83; 3288-3297, 2009



Ramp up plasma pool

- 1.3 x10^5 vRNA copies/ml (6.5 x10^4 SIV particles/ml)
- serial dilute to 1-10, 10-100, 100-1000 copies
- 0 $TCID_{50}$/ml (CEMx174 cells, SEA-stimulated rhPBMC)

Ma et al. Journal of Virology 83; 3288-3297, 2009

Slide 22

AS1 Reference?
Adonis Stassinopoulos, 3/20/2009

Ramp-up virus is highly infectious

AS2

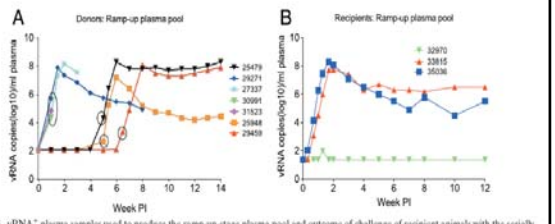
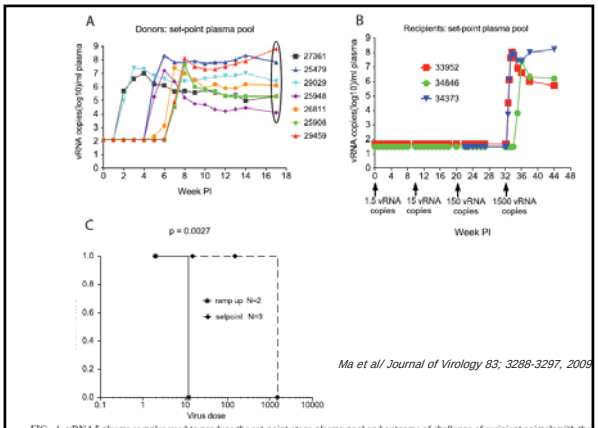


FIG. 3. vRNA* plasma samples used to produce the ramp-up-stage plasma pool and outcome of challenge of recipient animals with the serially diluted ramp-up-stage plasma pool. (A) Plasma vRNA levels in donor animals that were vaginally inoculated twice in one day with 10^5 $TCID_{50}$ of SIVmac251 or weekly from 0 to 13 weeks with 10^5 $TCID_{50}$ of SIVmac251 until infection was detected. Each sample used to make up the ramp-up-stage pool is circled. (B) Plasma vRNA levels in SIV-naive recipient animals after i.v. infusion of the ramp-up-stage plasma pool. While 1 animal inoculated i.v. with 2 SIV RNA copies (animal 32970) did not become infected, 2 of 2 animals inoculated i.v. with 20 SIV RNA copies (animals 33815 and 35036) did become infected. These two animals had a typical pattern of viremia after the plasma transfer.

Macaque ID_{50} = 1-10 SIV virions / inocula

Ma et al. Journal of Virology 83; 3288-3297, 2009



Ma et al/ Journal of Virology 83; 3288-3297, 2009

FIG. 4. vRNA* plasma samples used to produce the set-point-stage plasma pool and outcome of challenge of recipient animals with the diluted set-point-stage plasma pool. (A) Plasma vRNA levels in donor animals that were vaginally inoculated weekly with 10^5 $TCID_{50}$

Amplified transmission of HIV-1: comparison of HIV-1 concentrations in semen and blood during acute and chronic infection

Christopher D. Pilcher[a,b], George Joaki[c], Irving F. Hoffman[a,c],

AIDS 2007, Vol 21 No 13

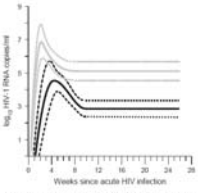


Fig. 3. Viral dynamics in acute HIV infection. To describe the magnitude and timing of trends in the observed data, expected concentration versus time curves for mean HIV-1 RNA level were generated using a statistical model. Shown here are mean estimates for blood (grey) and semen (black) data along with their 95% confidence intervals (dotted lines)

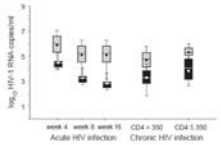


Fig. 4. HIV viremia and shedding, measured over the natural course of HIV infection. Box-and-whisker plots represent HIV-1 RNA levels in blood (grey) and semen (black), estimated for individual study subjects by disease stage. Numerical values at each time-point were estimated for each individual subject by empirical best linear unbiased prediction (eBLUP) making use of the final, unified best fit model including all observed data. Boxes and whiskers denote the 25th, 75th quartiles and total range of values. Internal circles

HIV-1 Transmission, by Stage of Infection

T. Déirdre Hollingsworth, Roy M. Anderson, and Christophe Fraser

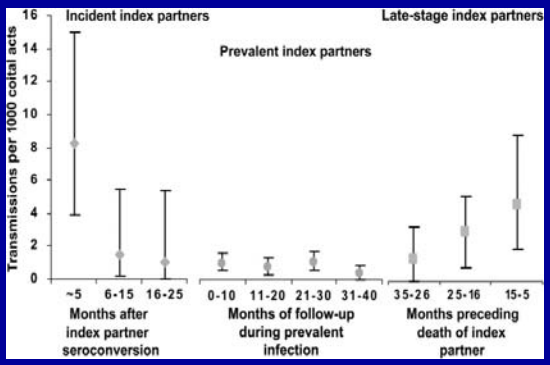
Table 2. Calculation of the basic reproduction number (R_0), according to the contribution from each stage of HIV-1 infection, under 2 extremes of sexual behavior.

Infection stage	Hazard of transmission (β) per person-year	Duration of high infectiousness (d)/interval between seroconversion and death[a] (%), mean, years	No. (%) of new transmissions, by sexual behavior[b]	
			Serial monogamy	Random mixing
Primary	2.76	0.24/10.2 (2)	0.10 (9)	0.67 (31)
Asymptomatic	0.106	8.38/10.2 (82)	0.77 (71)	0.91 (42)
AIDS	0.760	0.75/10.2 (16)	0.21 (20)	0.57 (27)
R_0	...	...	1.09 (100)	2.15 (100)

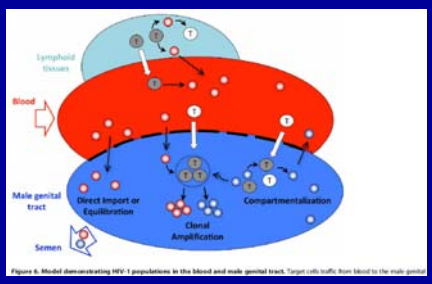
[a] The mean interval between seroconversion and death (10.2 years) was adopted from the report by Morgan et al. [48].
[b] The formula for calculating the number of new transmissions in a scenario of serial monogamy is $\beta cd/(\beta + c + 1/d)$, where c is 1.25 partner changes/year. The formula in a scenario of random mixing is βd (appendix).
[c] d was calculated by subtracting the mean durations of the periods of high transmissibility during primary infection (0.24 years) and AIDS (0.75 years) and the mean duration of zero transmission risk before death (0.83 years) from the mean interval between seroconversion and death (10.2 years).
[d] d corresponds to the period 10–19 months before death during which β was greatest for this infection stage. β was zero during the 10-month period immediately before death.

Slide 25

AS2 Reference?
Adonis Stassinopoulos, 3/20/2009



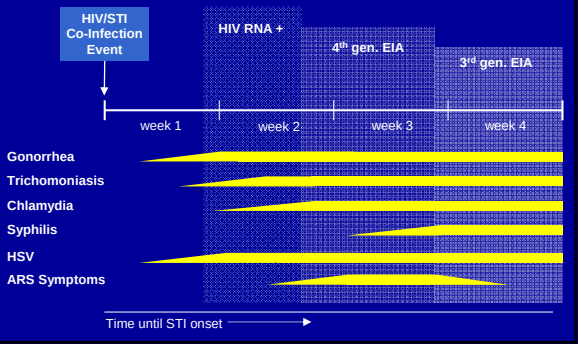
Wawer, et al, JID 2005, 191:1403



target CD4+ T cells and macrophages are likely to be in an activated state in this environment, enhancing their ability to support viral replication

Anderson et al; PLoS 2010

Potential impact of STI co-infection on detection of AHI



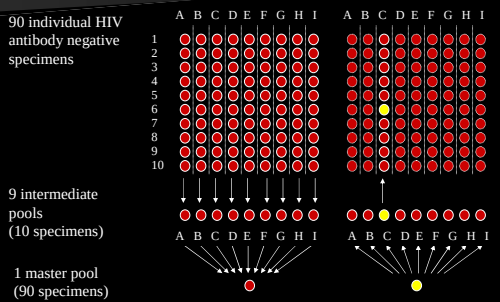
PCR Testing of Pooled Sera to Identify Acute HIV Infection (seronegative, PCR positive)

Pooled HIV RNA Testing: Yields

Program	Population	Prevalence HIV RNA+/EIA-	Increase in Testing Yield
New York City	NYC 3 STD Clinics		15%*
North Carolina	All persons tested for HIV via North Carolina DOH	23/109,250 (0.02%)	4%
Public-Health Seattle & King County	Men who have sex with men tested through PHSKC	21/5995 (0.35%)	13.5%
San Diego	Community based testing	15/3151 (0.48%)	23%
San Francisco	SF STD Clinic Patients	11/2722 (0.40%)	10.5%
Los Angeles	Men tested in 3 STD Clinics	1/1698 (0.06%)	7.1%
Maryland (not Baltimore)	STD clinics	0/15000	0
Atlanta	STD clinics, community testing and drug treatment	4/2128 (0.19%)	5%

Source: ISSTDR, 2007 25% in the Chelsea STD clinic; Morris, et al. 2010

Pooling and HIV RNA testing

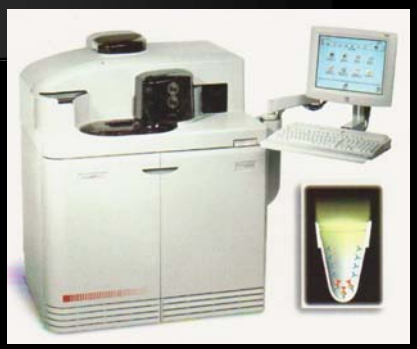


NAT Specimen pooling

- Advantages
  - Seamless (almost) incorporation into HIV testing
  - Reduced cost
  - No real change in specificity
  - Universal application
- Disadvantages
  - Requires large testing volume
  - Small loss in sensitivity
  - Logistics
  - Time to Dx and locating patient

Advantages of p24 Ag and 4th generation EIAs

- Current '4th generation' EIAs can detect both p24 Ag and antibody on a single assay
- Could be used as the initial HIV screening test
- p24 Ag EIAs nearly as sensitive as HIV RNA testing for acute HIV infeciton
- Sensitivity of 4th generation EIAs is now equivalent to heat p24 assays



4th gen HIV Ag/Ab Combo considerations / conclusions

- Can detect infection in antibody-negative individuals
- Viral load cutoff may be about 14,000 – 31,000 RNA copies / ml
- Can be used as a replacement for RNA testing, would detect ~90% of Ab-/RNA+ detected by RNA pooling
- Shorter time to Dx , potential for better PPV, and lower cost than RNA pooling

How does a 4th Generation IA (HIV Ag/Ab Combo) perform on the recent / acute infection panel ?

- Detects 57 / 64 positively (89%)
  - (3rd gen detected 42%)
- Of the 29 "recently infected" specimens: 29/29 (100%)
  - (3rd gen detected 93%)
  - (Uni-gold Rapid: 76%)
- Of the 35 "acute" specimens (RNA pos, completely Ab negative: 28/35 (80%)

HIV Testing Algorithms

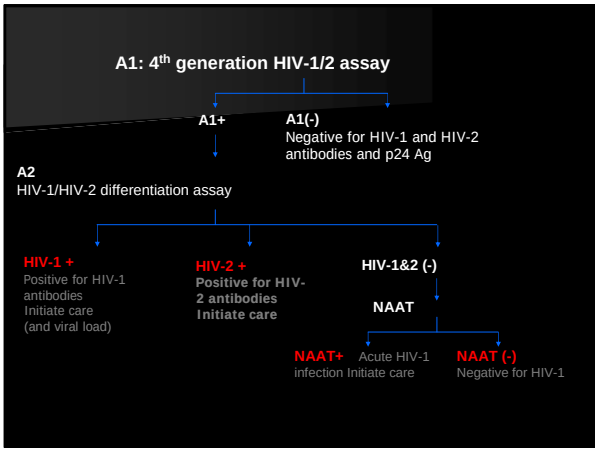
A Status Report

A publication from The Association of Public Health Laboratories and The Centers for Disease Control & Prevention

APRIL 2009

Berry Bennett, MPH, Florida Bureau of Laboratories
Bernard Branson, MD, Centers for Disease Control and Prevention
Kevin Delaney, MPH, Centers for Disease Control and Prevention
Michele Owen, PhD, Centers for Disease Control and Prevention
Michael Pentella, PhD, D(ABMM), University of Iowa
Barbara Werner, PhD, Massachusetts Department of Public Health

The Association of Public Health Laboratories
8515 Georgia Avenue Suite 700 Silver Spring, MD 20910 | Phone: 240.485.2745 | Fax: 240.485.2700 | www.aphl.org



Confirmatory Testing

- Confirmatory test is essential (not just a single EIA)
- For Western blot:
  - Venipuncture for whole blood
  - Oral fluid specimen
- Follow-up testing of persons with negative or indeterminate Western blot results after 4 weeks
- HIV RNA or 4th gen test for suspected acute HIV
- A single positive EIA test is not reportable but confirmation is covered under Ryan White for billing

Rapid HIV Antibody Tests

- **Advantages**
  - **Results in 10 – 20 minutes**
  - **"Preliminary positive"**
  - **Better linkage to care**
  - **Less labor ?, no instrument maintenance**
- **Disadvantages**
  - **False positives – especially pregnant women**
  - **Through put**
  - **Setting for Confedentiality**
  - **Cost?**

Rapid HIV Antibody Tests

- **Other important issues**
  - **Specimen type – oral fluid, serum, plasma, whole blood, dried blood spot**
  - **Who will perform rapid test? POCT by nursing staff? Physicians?**
  - **Waived testing?**
  - **Laboratory-based testing**
  - **How meet licensing and accrediting agency requirements?**
  - **ED "fix what is broken"**

Cost Rapid HIV tests

The mean per-test cost of rapid HIV testing

and counseling:

- $48.07 for an HIV-neg. test
- $64.17 for a preliminary-positive test
- Pre- and posttest counseling costs accounted for 38.4% of the total cost

Pinkerton et al. AIDS and Patient Care and STDS 2010

Rapid HIV Antibody Tests

- Ability to detect acute infection (n=42)
- 3rd generation EIA detected 34% of RNA positive specimens
- Unigold 26%
- Multispot 17%
- OraQuick 2.3%
- Clearview 2.3%
- Western Blot 0%
- Combo assay 80% (n=35)

Detection of Acute HIV Infection

- Important public health issue
- Identifying AHI may decrease HIV transmission
- Earlier treatment with HAART
- Earlier linkage to care
- Most useful in high risk setting i.e. STD clinic, EDs and MSM populations

What to consider

- AHI important at individual and population level
- Consider panels for acute viral illness that would include testing for AHI
- 4th generation assays provide faster alternative for Dx AHI
- Important to screen for AHI in STD clinics and with MSM populations