

# Clinical Testing and Guidelines

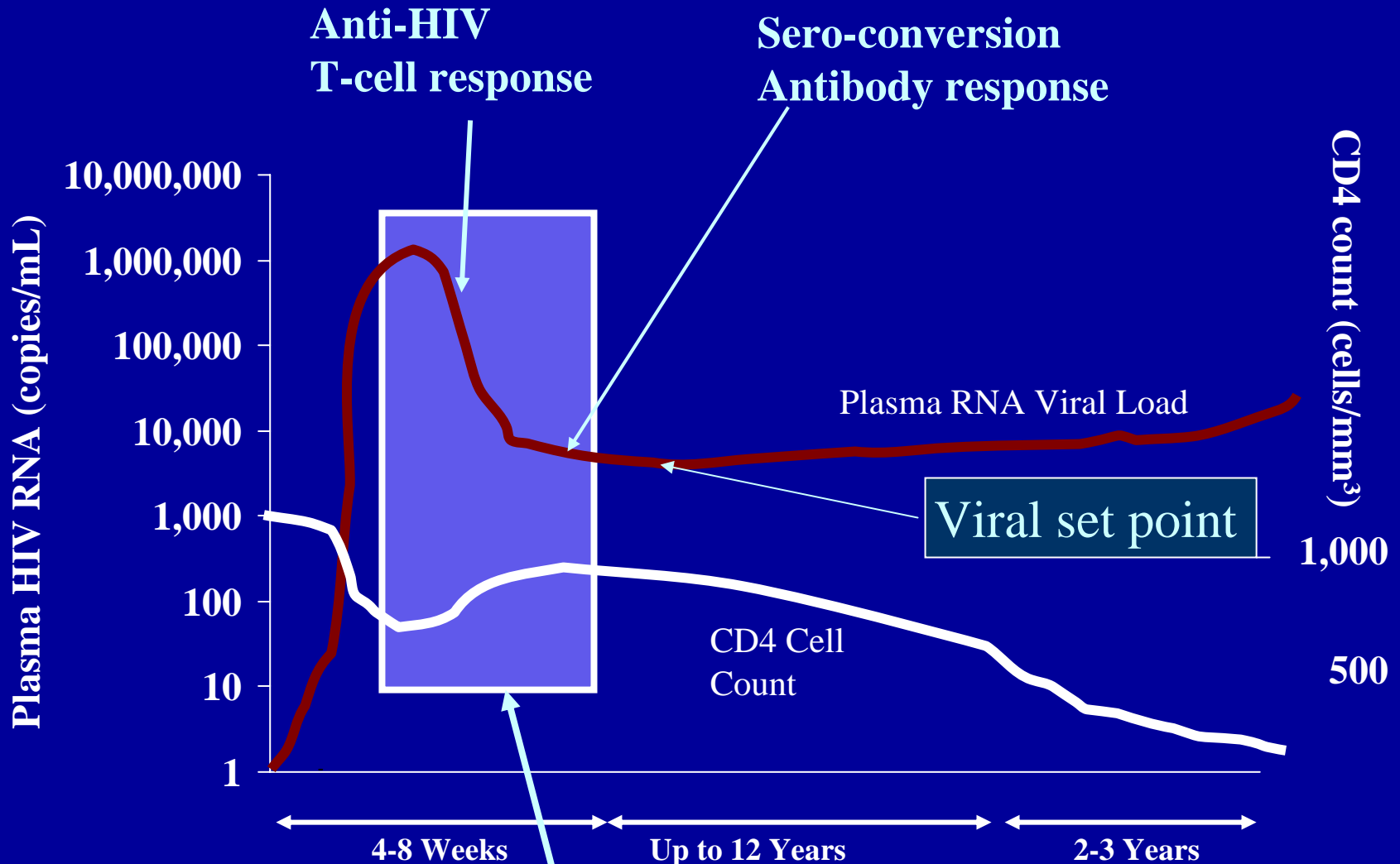
Mark Holodniy, MD, FACP

Stanford University

# Diagnostic Tests for HIV Infection

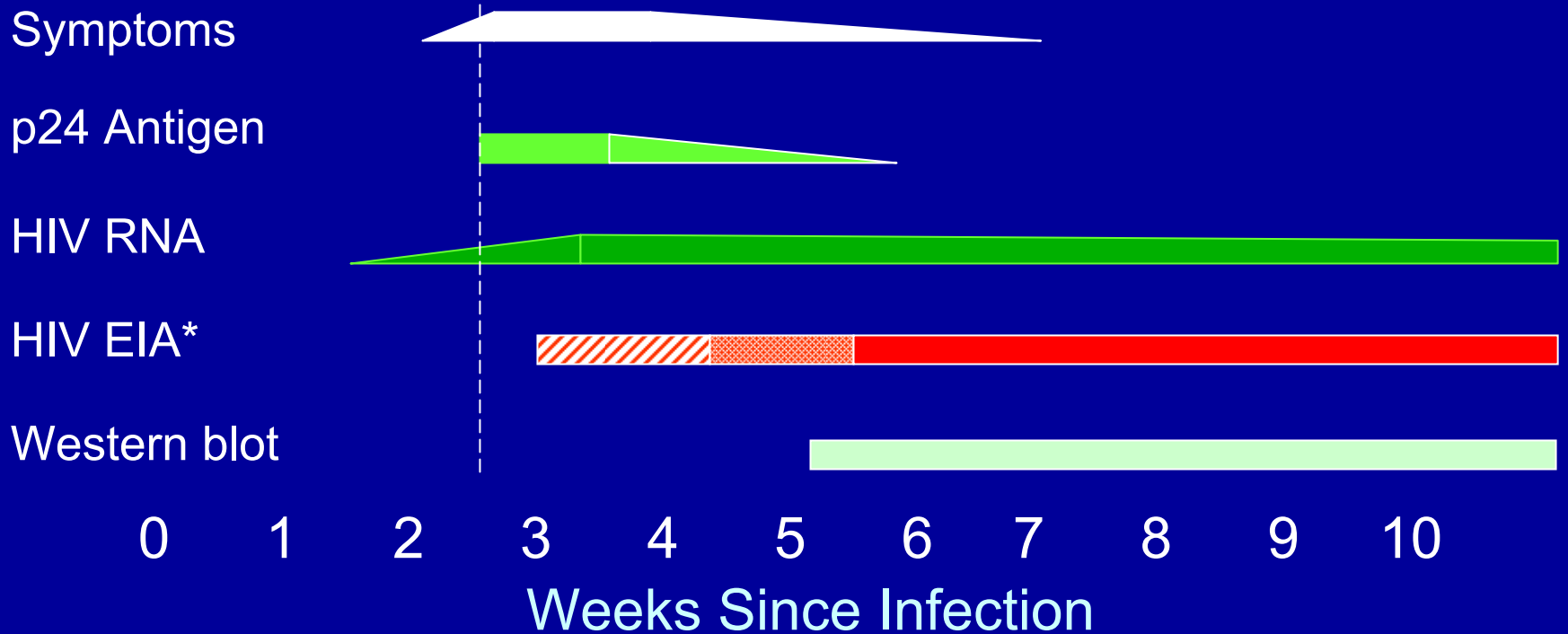
- Antibody testing
  - ELISA (screening)/Western Blot (confirm)
  - Saliva, Urine EIA/Western Blot
  - Rapid tests
    - Blood
    - Oral
  - Consumer products (HomeAccess system)
- Viral Load (plasma HIV-1 RNA)
- PBMC DNA PCR
- Serum p24 antigen




# Primary HIV Infection: Pathogenesis



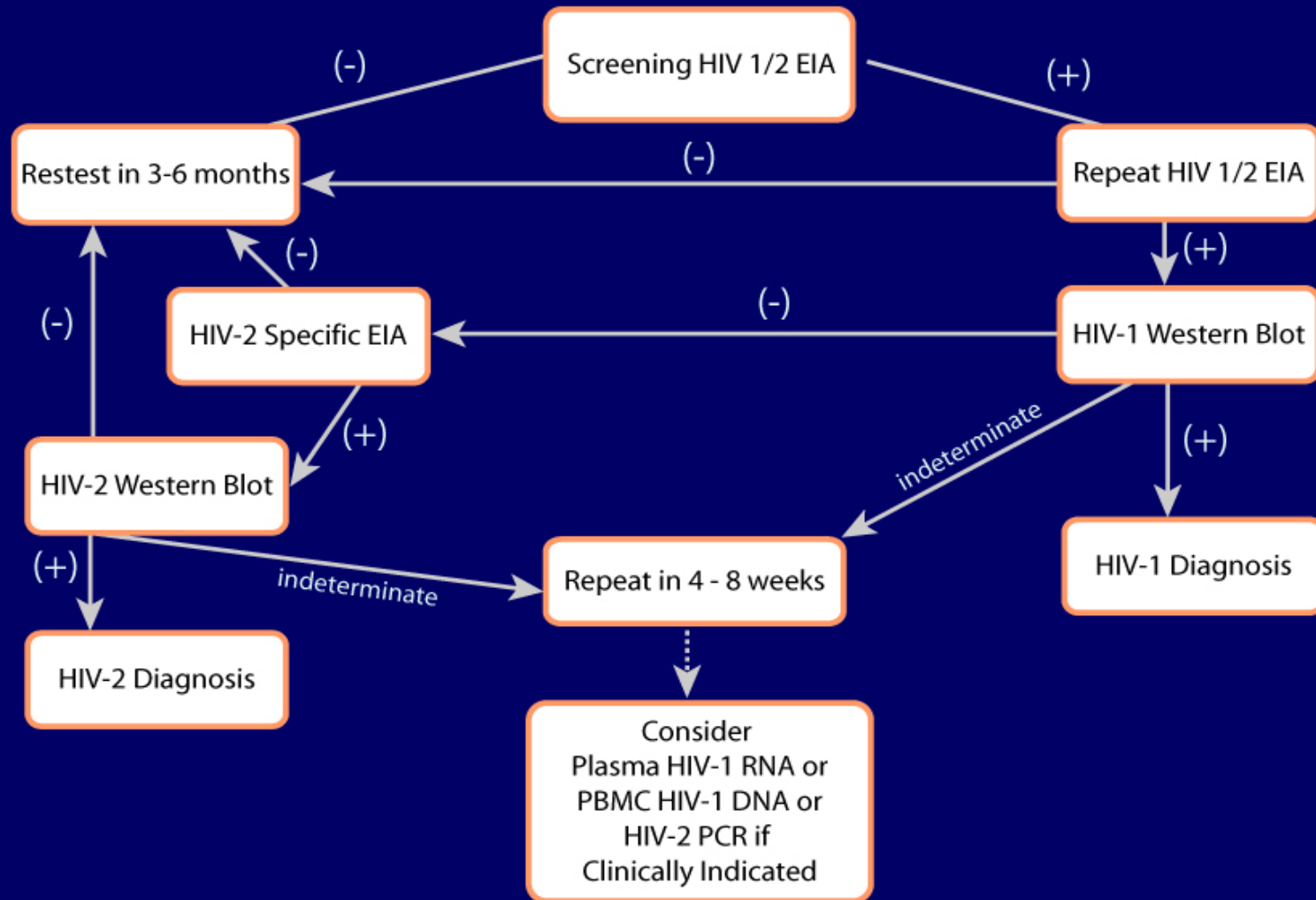
**A lot of important stuff happens here**

# Detection of HIV by Diagnostic Tests



-  \*3<sup>rd</sup> generation, IgM-sensitive EIA
-  \*2<sup>nd</sup> generation EIA
-  \*viral lysate EIA

# Algorithm for Serologic Diagnosis of HIV-1/HIV-2 infection



# **Revised Recommendations for HIV Testing in Adults and Adolescents - I**

- Routine, voluntary HIV screening for all persons 13-64 in health care settings, not based on risk**
- Repeat HIV screening of persons with known risk at least annually**
- Opt-out HIV screening with the opportunity to ask questions and the option to decline**
- Include HIV consent with general consent for care; separate signed informed consent not recommended**
- Prevention counseling in conjunctions with HIV screening in health care settings is not required**

# Revised Recommendations for HIV Testing in Adults and Adolescents - II

- **Intended for all health care settings**
- **Communicate test results in same manner as other diagnostic/screening tests**
- **Provide clinical HIV care or establish reliable referral to qualified providers**
- **Low prevalence settings:**
  - Initiate screening
  - If yield from screening is less than 1 per 1000 (< 0.1%), continued screening is not warranted
- **Know what your local laws are on HIV testing (<http://www.statehealthfacts.org>)**

# Revised Recommendations for HIV Testing Pregnant Women - I

- **Universal opt-out HIV screening**
  - Include HIV in routine panel of prenatal screening tests
  - Consent for prenatal care includes HIV testing
  - Notification and option to decline
- **Second test in 3<sup>rd</sup> trimester for pregnant women:**
  - Known to be at risk for HIV
  - In jurisdictions with elevated HIV incidence
  - In high HIV prevalence health care facilities
- **Know what your local laws are on HIV testing**  
**(<http://www.statehealthfacts.org>)**

# Revised Recommendations for HIV Testing Pregnant Women - II

- Opt-out rapid testing with option to decline for women with undocumented HIV status in L&D
  - Initiate ARV prophylaxis on basis of rapid test result
- Rapid testing of newborn recommended if mother's status unknown at delivery
  - Initiate ARV prophylaxis within 12 hours of birth on basis of rapid test result

# Pediatric HIV-1 diagnosis

- Neonates/infants may have maternal HIV-1 antibodies for > 1 yrs
- Infection occurs intrapartum >> *in utero*
- Plasma HIV viral load somewhat more sensitive than PBMC DNA PCR
- *In utero* infection: these tests are positive at birth
- Intrapartum infection: these tests are positive by 1 month of age

# Diagnosis of HIV Infection in Infants

- Criteria for HIV diagnosis:
  - 2 positive HIV virologic tests on separate blood samples (regardless of age)
  - Positive HIV antibody test with confirmatory Western blot (or IFA) at age  $\geq 18$  months
- Criteria to rule out HIV infection:
  - 2 or more negative virologic tests, performed age  $\geq 1$  month, at least once  $\geq$  age 4 months (in non-breast-fed infants)
  - Loss of HIV antibody in child with previously negative virologic tests

# Diagnostic Testing in Infants

- Under 18 months of age: virologic assays (HIV DNA PCR or HIV RNA assays)
  - Virologic diagnostic testing at 3 time points:
    - Birth to 14 days
    - 1-2 months
    - 3-6 months
  - Antibody test at 12-18 months to document seroreversion in HIV-uninfected infants
- Aged  $\geq 18$  months: use HIV antibody assays

# Diagnostic Issues in Infants: Choice of Diagnostic Test

- HIV DNA PCR
  - Sensitivity <40% at <48 hours of age
  - Sensitivity increases to >90% by 2-4 weeks
- HIV RNA assays
  - As sensitive as DNA PCR for early diagnosis
  - Unclear whether sensitivity affected by maternal or infant ARV prophylaxis
- HIV viral culture
  - Sensitivity similar to HIV DNA PCR
  - Complex, expensive, results: 2-4 weeks

# Diagnostic Issues with Non-Subtype B HIV Infection

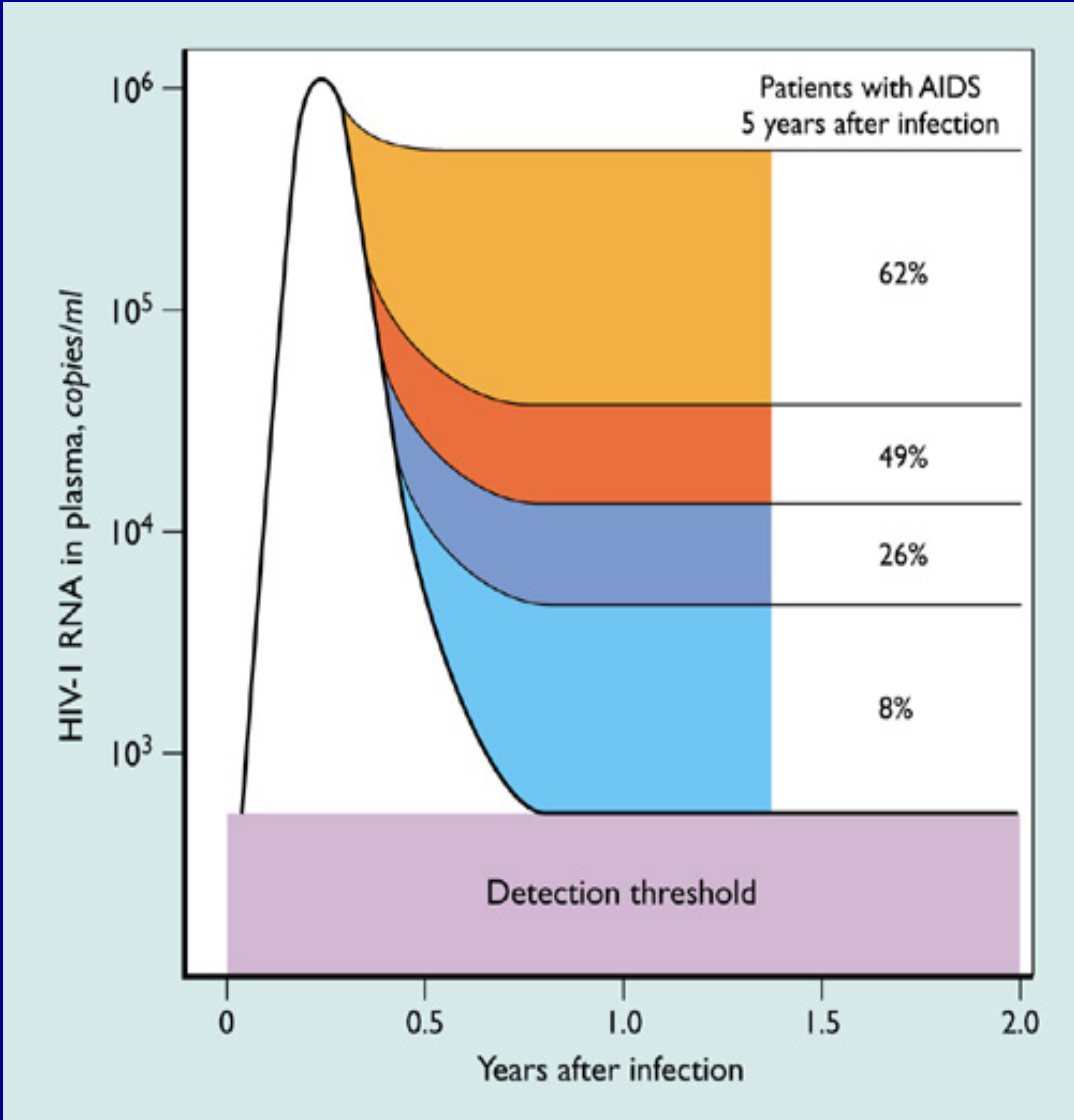
- HIV DNA PCR
  - Less sensitive in non-subtype B
  - False negatives reported
- HIV RNA assays
  - Newer RNA assays more sensitive
- If non-subtype B infection is suspected despite negative tests, repeat serologic testing at 18 months of age; consult with specialist

# Diagnostic Testing: Viral Load

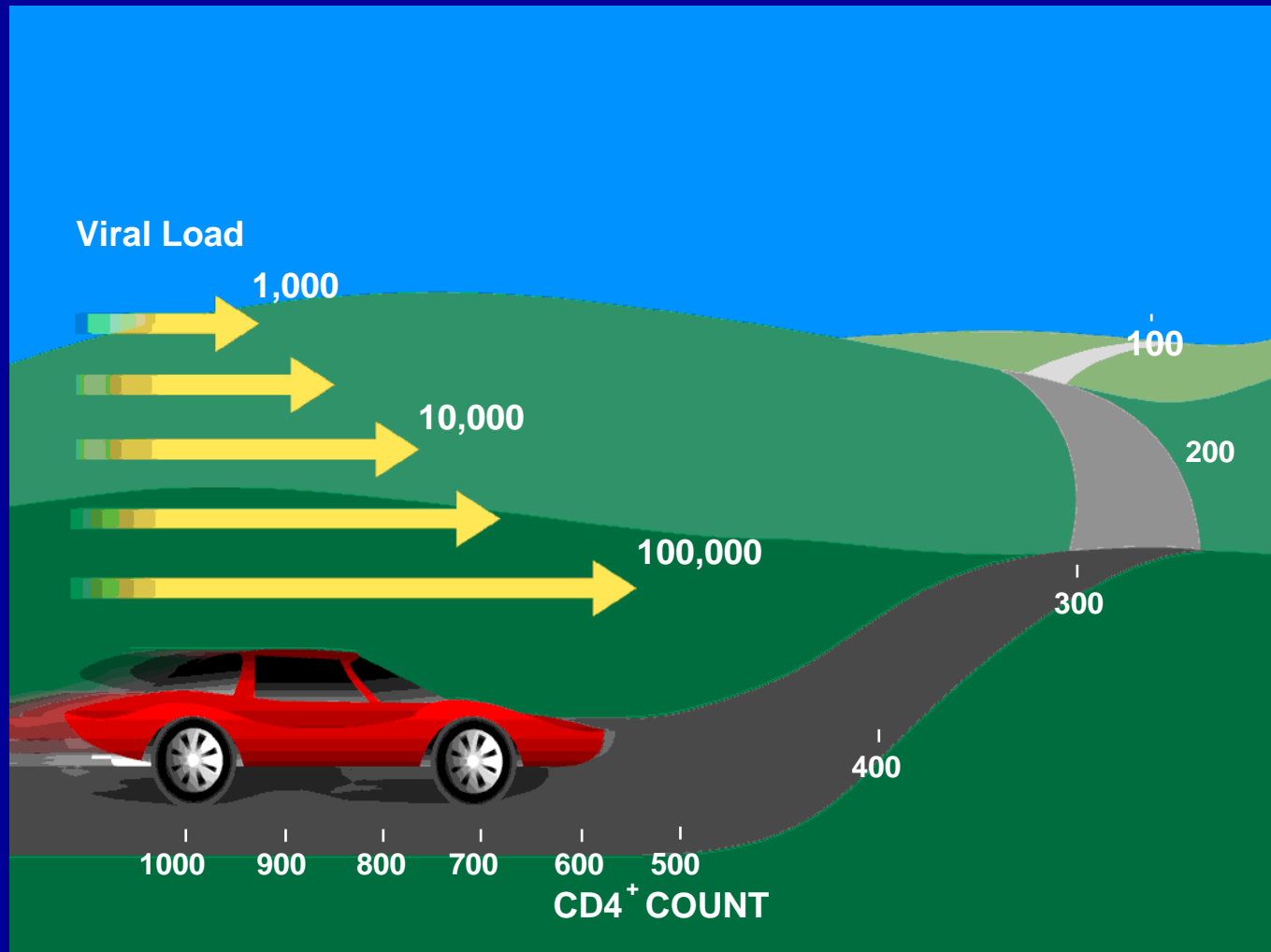
- More sensitive than HIV antibody or p24 Ag test<sup>3</sup>
- Positive one to three weeks before antibody test<sup>1</sup>
- Typically high level, e.g. greater than 50,000-100,000 copies/mL<sup>2,3</sup>
- False positives can occur
  - Most false positives are low level (<10,000 copies/mL)
  - HIV VL <10,000 copies/mL should probably be considered “indeterminate”
- If diagnosis is made by HIV RNA testing, confirmatory serologic testing should be performed subsequently

1. Busch MP, Satten GA. *Am J Med* 1997;102:Suppl 5B:117-24.  
2. Kahn JO, Walker BD. *N Engl J Med*. 1998;339:33-39.  
3. Daar ES et al. *Ann Intern Med*. 2001;134:25-29.

# Prognostic Value of Plasma HIV-1 RNA



# Relating Disease Progression to Plasma HIV-1 RNA Level and CD4 Cell Count



Adapted with permission from Coffin. *AIDS*. 1996;10(suppl 3):S75-S84.

# Guidelines for the Use of Antiretroviral Agents in HIV-Infected Adults and Adolescents

Developed by the Panel on Clinical  
Practices for Treatment of HIV Infection  
convened by the Department of Health and  
Human Services (DHHS)

<http://www.aidsinfo.nih.gov>

# What the Guidelines Address

- Laboratory testing (viral load, CD4+ T cells, resistance)
- When to initiate therapy
- When to change therapy
- Therapeutic options
- Adherence
- ART-associated adverse effects

# What the Guidelines Address

- Treatment of acute HIV infection
- Special considerations in adolescents, pregnant women, injection drug users, and patients coinfecting with HIV and hepatitis B, hepatitis C, or tuberculosis
- Prevention counseling for HIV-infected patients

# Pretreatment Evaluation

- Confirm HIV results
- Complete H&P
- CBC, chemistry profile
- CD4 cell count
- Plasma HIV RNA measurement
- Resistance testing
- Assess “readiness” for treatment and adherence

# Pretreatment Evaluation: Additional Tests

- RPR or VDRL
- PPD
- Chest X ray
- Hepatitis A,B,C serology
- Toxoplasma IgG
- Fasting glucose and lipids
- Gynecologic exam with pap smear
- Testing for chlamydia and gonorrhea
- Ophthalmology exam (CD4+ T cell count <100 cells/ $\mu$ L)

# When Should an Adult Be Treated?

Clinical Category	CD4 Count	HIV RNA	Recommendations
AIDS-defining illness or severe symptoms	Any value	Any value	Treat
Asymptomatic, AIDS	< 200/mm <sup>3</sup>	Any value	Treat
Asymptomatic	< 200/mm <sup>3</sup> but < 350/mm <sup>3</sup>	Any value	Treatment should generally be offered, following full discussion of pros and cons with each patient
Asymptomatic	> 350/mm <sup>3</sup>	> 100,000 copies/mL	Most clinicians recommend deferring therapy; some will treat
Asymptomatic	> 350/mm <sup>3</sup>	< 100,000 copies/mL	Defer therapy

# Use of HIV RNA & CD4+ T Cell Levels to Guide Therapy Decisions

- Syndrome consistent with acute HIV infection
- Initial evaluation of new HIV diagnosis
- Every 3-4 months in the untreated patient
- Immediately prior to initiating therapy
- 2-8 weeks after initiating therapy
- Every 3-4 months in patients on therapy
- As clinically indicated

# Additional Periodic Testing

- **Q 3-4 months**
  - Viral load, CD4/CD8, Comprehensive Chemistry, CBC with Differential
- **Q 6-12 months**
  - Lipids (fasting), PPD, RPR (previously positive or sex active)
  - Females- PAPS w/ HPV, Gonorrhea Chlamydia
  - Males- Gonorrhea and Chlamydia

# Immunologic Parameters in Children

- Absolute CD4 counts in healthy children are much higher than in adults
- Normal absolute CD4 counts slowly decline to adult levels by age 6
- If using CD4 count for ARV decision, use age-appropriate levels
- Age should be considered when interpreting risk of disease progression based on CD4 percentage or count & HIV RNA

# Laboratory Monitoring of Pediatric HIV Infection

- Use CD4% for children <6 years of age
  - CD4% less variable with age than absolute CD4
  - Must be interpreted according to child's age
- Monitor CD4% or count every 3-4 months after initial diagnosis
- Monitor HIV RNA every 3-4 months after diagnosis

# HIV RNA in Children: Clinical Considerations

- Low levels at birth, rise to >100,000 to several million copies/mL within the first 1-2 months of life
- Without treatment, very slow decline over several years to reach “set point”
- High HIV RNA associated with higher risk for disease progression and death
  - HIV RNA levels difficult to interpret in first year of life
  - Predictive value of HIV RNA in infants <12 months of age is lower than in older children
  - In infants, HIV RNA levels overlap in rapid and nonrapid progressors
  - CD4 counts/percentages may be more useful than HIV RNA in evaluating risk in infants <12 months; in older children both parameters are useful

# HIV RNA in Children: Clinical Considerations

- HIV RNA and CD4 assays are independently predictive of risk of disease progression
- Both help determine when to start & when to change ART
- A 5-fold ( $.7 \log_{10}$ ) change in HIV RNA copies/mL in infants or 3-fold ( $.5 \log_{10}$ ) change in children age  $\geq 2$  years is biologically & clinically significant
- Obtain 2 baseline HIV RNA tests when child is clinically stable
- Confirm HIV RNA changes with a 2nd test before changing therapy
- Consult pediatric HIV specialist when making clinical decisions based on HIV RNA test results

# Indications for Initiation of ART in Children 1 to <4 Years of Age

Criteria	Recommendation
AIDS/significant HIV-related symptoms	<i>Treat</i>
Asymptomatic or mild symptoms <u>and</u> CD4 <20%	<i>Treat</i>
Asymptomatic or mild symptoms <u>and</u> CD4 20-24% <u>or</u> HIV RNA ≥100,000 copies/mL	<i>Consider</i>
Asymptomatic <u>and</u> CD4 ≥25% <u>and</u> HIV RNA <100,000 copies/mL	<i>Defer</i>

# Indications for Initiation of ART in Children $\geq 4$ to 12 Years of Age

Criteria	Recommendation
AIDS/significant HIV-related symptoms	<i>Treat</i>
Asymptomatic/mild symptoms <u>and</u> CD4 <15%	<i>Treat</i>
Asymptomatic/mild symptoms <u>and</u> CD4 15-24% <u>or</u> HIV RNA $\geq 100,000$ copies/mL	<i>Consider</i>
Asymptomatic <u>and</u> CD4 $\geq 25\%$ <u>and</u> HIV RNA <100,000 copies/mL	<i>Defer</i>

# Indications for Initiation of ART in Children >13 Years of Age

Criteria	Recommendation
AIDS/significant HIV-related symptoms	<i>Treat</i>
Asymptomatic/mild symptoms <u>and</u> CD4 <200 cells/ $\mu$ L	<i>Treat</i>
Asymptomatic/mild symptoms <u>and</u> CD4 201-350 cells/ $\mu$ L <u>or</u> HIV RNA $\geq$ 100,000 copies/mL	<i>Consider</i>
Asymptomatic <u>and</u> CD4 $\geq$ 350 cells/ $\mu$ L <u>and</u> HIV RNA <100,000 copies/mL	<i>Defer</i>

# Periodic Testing in HIV Infected Children

- Q 3-4 months
  - HIV Viral Load
  - CD4/ CD8
  - Comprehensive chemistry
  - CBC w/ Differential
- Q6- 12 months
  - Lipids (Elevations with HAART/ PI's)
  - Amylase
  - Lipase
- Q12 months
  - PPD or TB evaluation if Hx of (+) PPD

# Testing for Antiretroviral Drug Resistance

- Adjunct to guide antiretroviral therapy
- Combine with obtaining a drug history and maximizing drug adherence
- Research supports use in certain settings
- Genotyping vs. phenotyping
- Limitations of resistance testing and specific indications

# HIV Resistance Testing Assays

## RESISTANCE

- The (in)ability of HIV to replicate in the presence of antiretroviral drugs
- Caused by changes in relevant parts of the virus' genome (mutations)

### Phenotyping Assay

- Direct measure of the ability of the virus to grow in the presence of antiretroviral drugs
- Compared to laboratory reference strain
- *In vitro* assay

### Genotyping Assay

- Indirect measure of the virus' susceptibility to antiretroviral drugs
- Based on sequence (mutations) of relevant parts of the viral genome
- Requires analysis/interpretation of sequence information

### *VirtualPhenotype*<sup>™</sup>

- Interrogation and matching of the patient genotype sample to a large phenotype database
- A quantitative prediction of HIV drug resistance that provides both a genotype plus a phenotypic analysis of the patient's virus

# Guidelines for the Use of Resistance Testing

	DHHS <sup>1</sup>	IAS-USA <sup>2</sup>
Primary/acute infection	Recommended	Recommended* Consider <sup>†</sup>
Chronic infection	Recommended	Recommended* Consider <sup>†</sup>
Virologic failure during therapy	Recommended	Recommended
Suboptimal suppression of HIV RNA	Recommended	Consider
Discontinuation of drugs	Not recommended	--
HIV RNA <1000 copies/mL	Not recommended	Not recommended

\*If the prevalence of transmitted HIV drug resistance is >5%.

<sup>†</sup>If the prevalence of transmitted HIV drug resistance is unknown, but considered likely due to high ART use in the population.

<sup>1</sup>Available at: <http://aidsinfo.nih.gov/Default.aspx>. Revision October 10, 2006.

<sup>2</sup>Hammer S, et al. *JAMA* 2006;296:827-843.

# Antiretroviral Drug Resistance Testing in Pregnancy

Recommended for all pregnant women:

- Before starting ART or prophylaxis, if not done previously
- Before ART or prophylaxis in women who received prophylaxis in previous pregnancies
- If HIV RNA is detectable while on ART (virologic failure or suboptimal viral suppression)
- In some situations ART or prophylaxis may be started empirically before resistance test results are available (eg, women admitted for delivery with no prior ART therapy)

# ARV Drug Resistance Testing in Pediatrics

- Rules for who should be tested generally follow those for adult patients
- Presence of viral resistance to a particular drug suggests the drug is unlikely to suppress viral replication
- Absence of resistance to a drug does not ensure that its use will be successful, particularly if it shares cross-resistance with drugs previously used
- Consult pediatric HIV specialist for interpretation of resistance assays