La diagnosi dell'infezione da HIV: protocollo standard e principali markers di progressione della malattia

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TORINO

LA STORIA DELLA SCOPERTA DI HIV

1981

- **Primi casi di AIDS (CDC Atlanta, USA)**

1983

1. **Luc Montagnier e Barre-Sinoussi Institut Pasteur a Parigi**

   LAV – Lymphadenopathy virus

2. **Robert Gallo, NIH, Bethesda, USA**

   HTLV I, II – Human T-cell Leukemia viruses

3. **HIV è l'agente che causa l'AIDS:**
   Gallo identifica LAV come HTLV III

1985

- **Primi test sierologici**

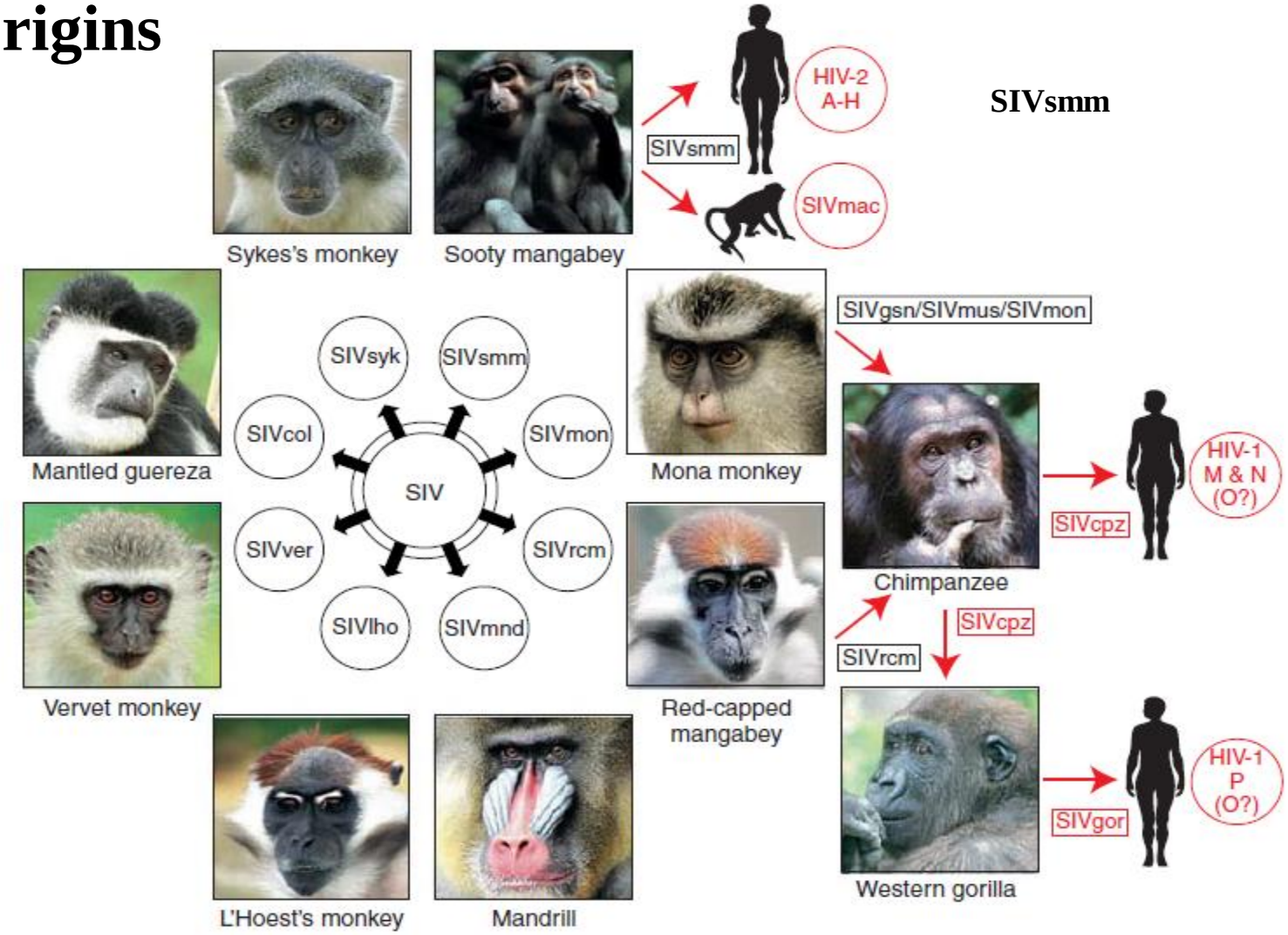
1995

- **HAART** ***Highly Active Antiretroviral therapy***

Nobel Prize for HIV descovery, 2008

Luc Montagnier at Amedeo di Savoia Hospital, 2012

Origins



SIVsmm

SIVcpz (Camerun)

Figure 1. Origins of human AIDS viruses. Old World monkeys are naturally infected with more than 40 different lentiviruses, termed simian immunodeficiency viruses (SIVs) with a suffix to denote their primate species of origin (e.g., SIVsmm from sooty mangabeys). Several of these SIVs have crossed the species barrier to great apes and humans, generating new pathogens (see text for details). Known examples of cross-species transmissions, as well as the resulting viruses, are highlighted in red.

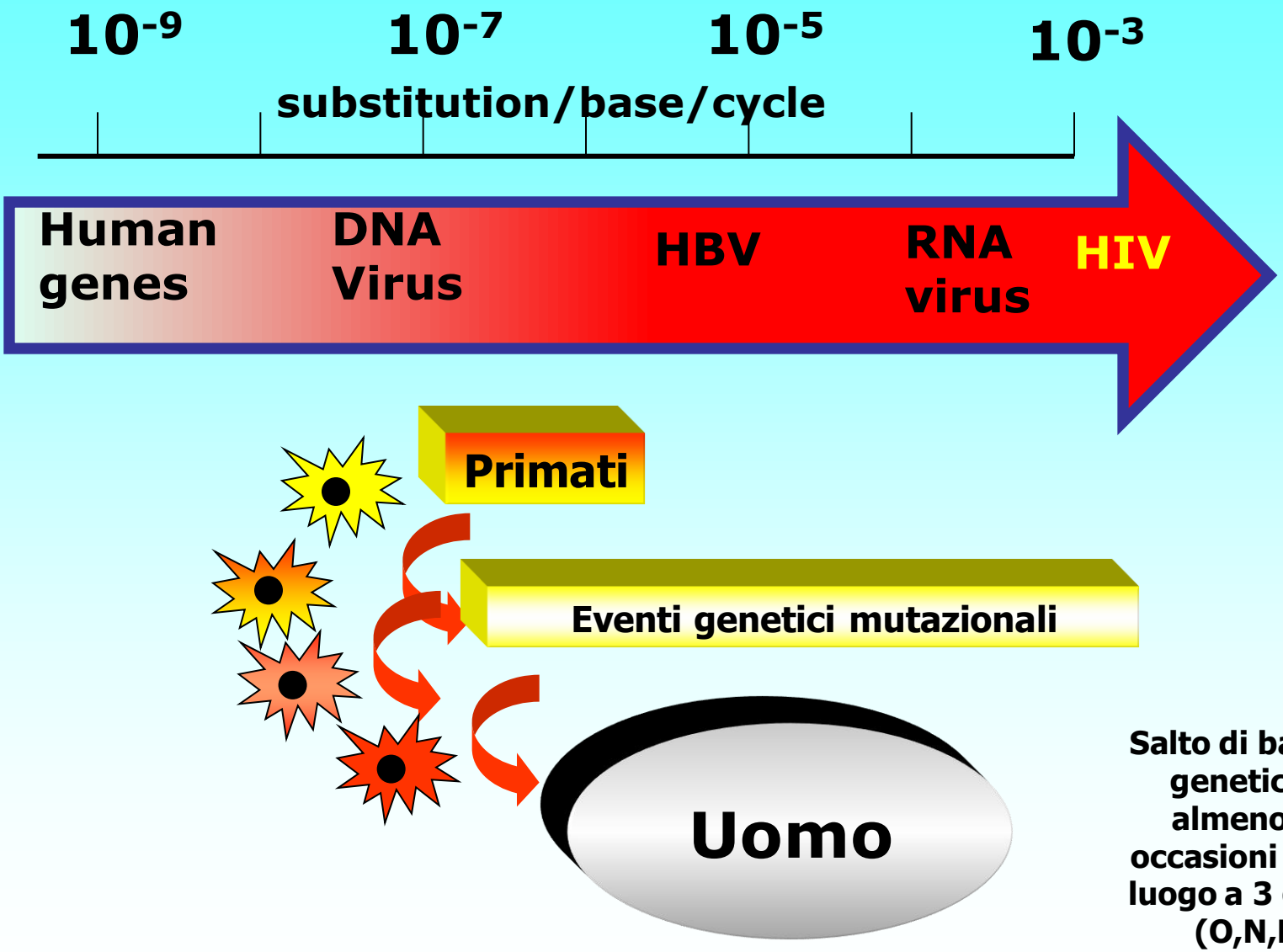
A Tale of Two Monkeys

Rhesus

Sooty Mangabey

- Rhesus (pathogenic SIV infection) – model for HIV infection
  - High-level viremia
  - Circulating CD4 depletion
  - OI risk and death
  - High-level immune activation
- Sooty mangabey (nonpathogenic SIV infection)
  - High-level viremia
  - CD4 depletion extremely rare
  - No OIs
  - Low-level immune activation

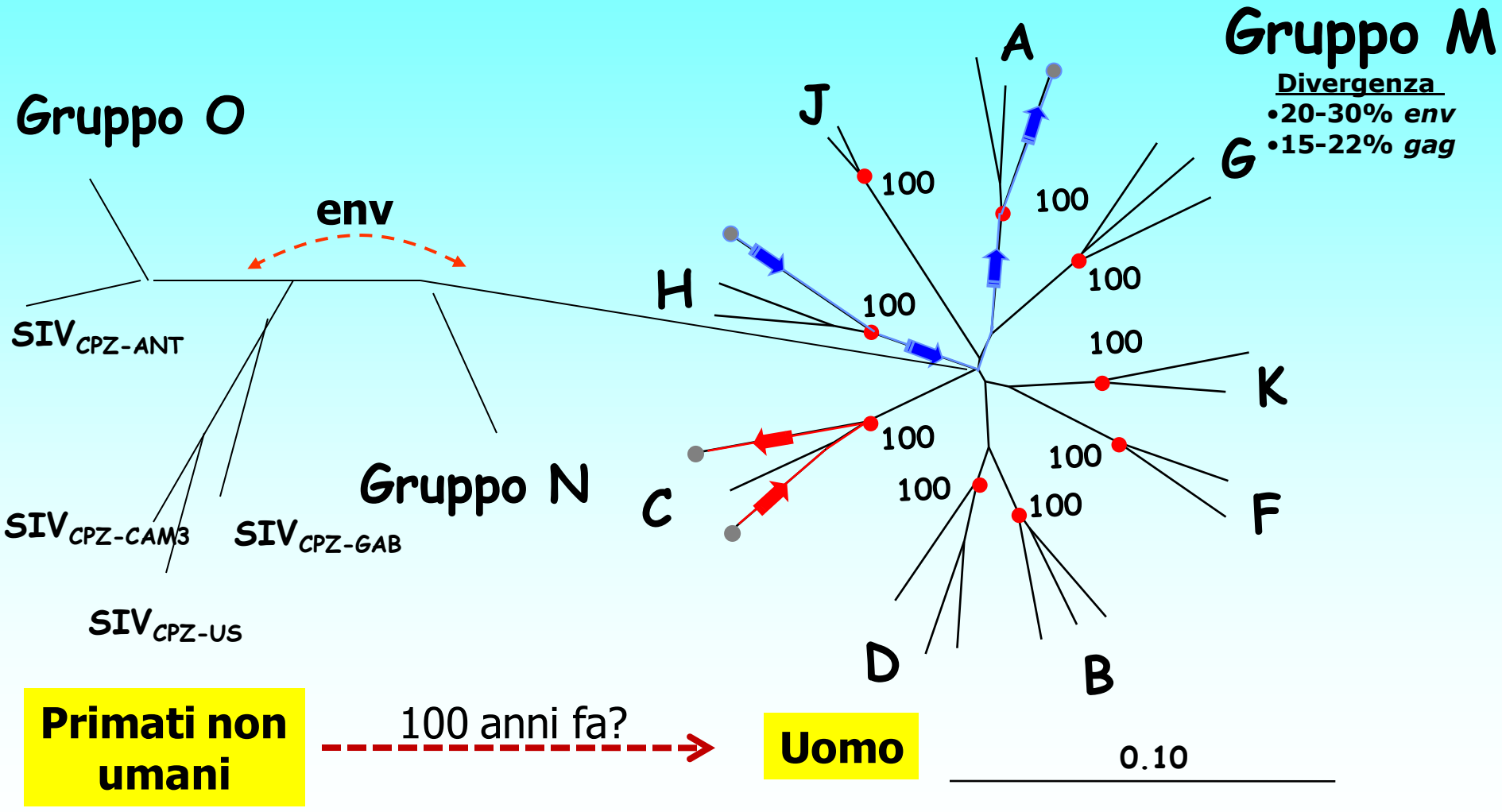
HIV and genetic variability



Salto di barriera genetica in almeno tre occasioni dando luogo a 3 gruppi (O,N,M)

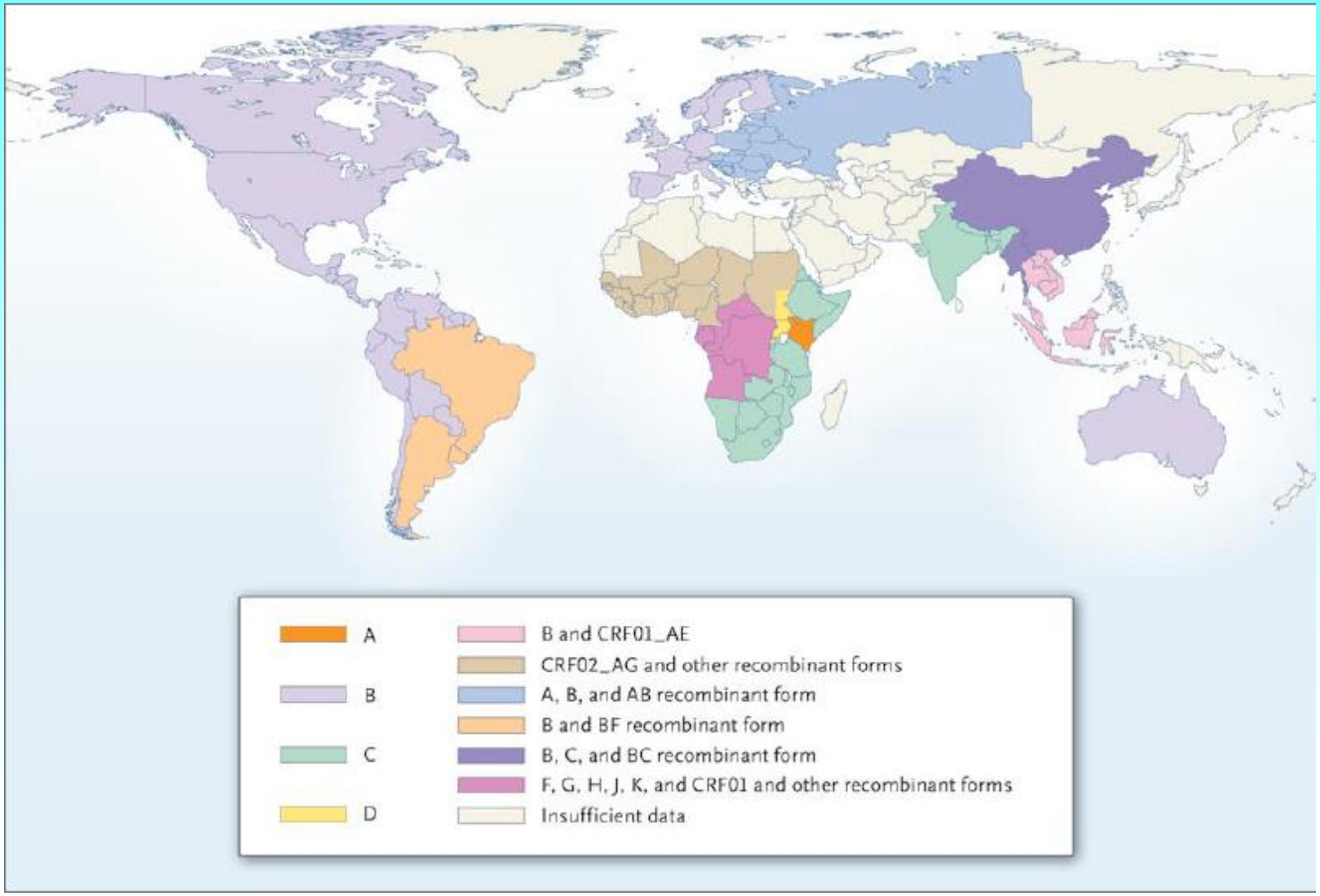
Variabilità genetica di HIV-1 gruppo M, N e O e isolati da primati

Analisi sequenza completa gene



Adattata da Korber B. in Field Virology 4th Ed.2007

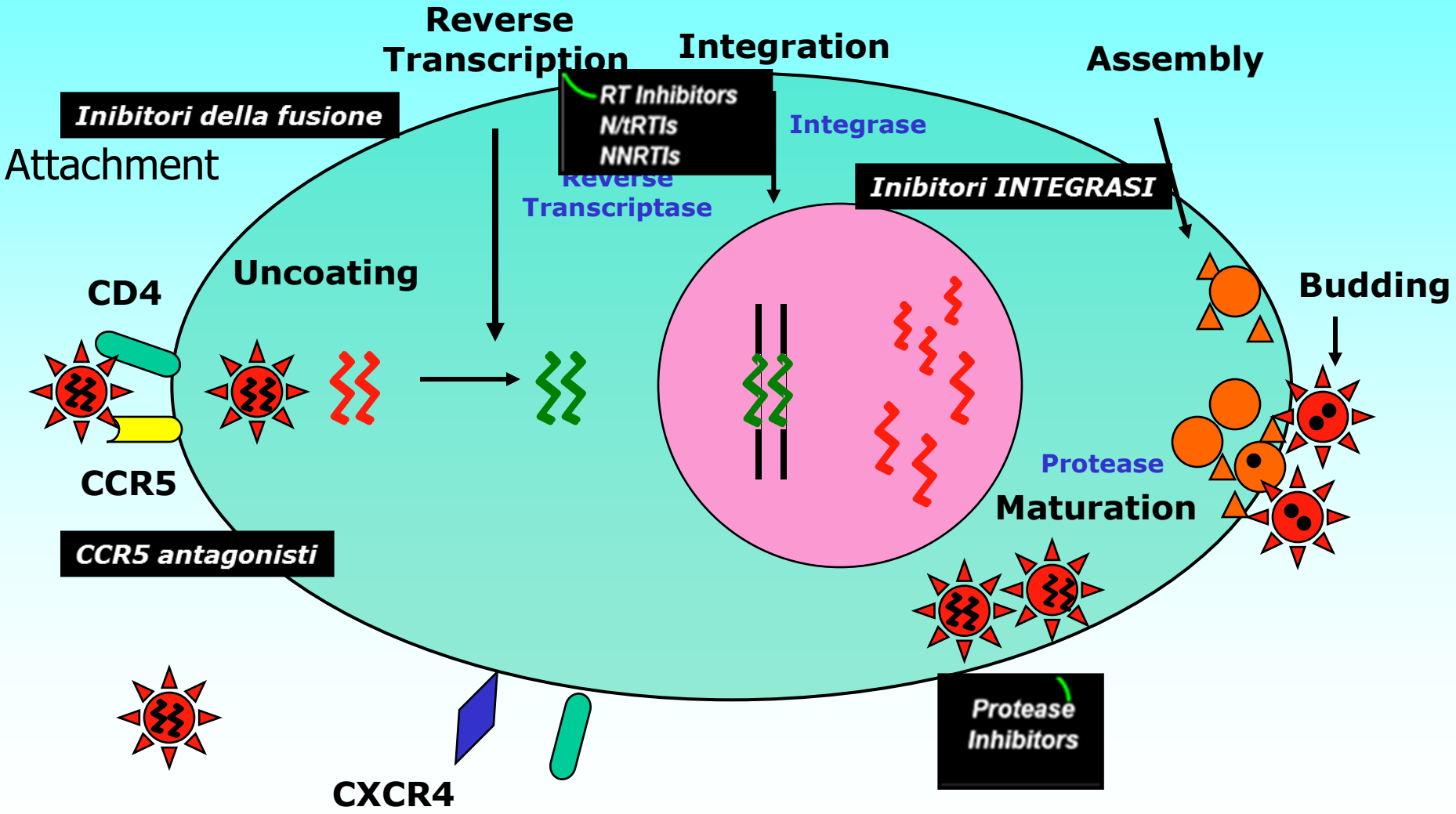
Distribuzione dei sottotipi di HIV e forme ricombinanti



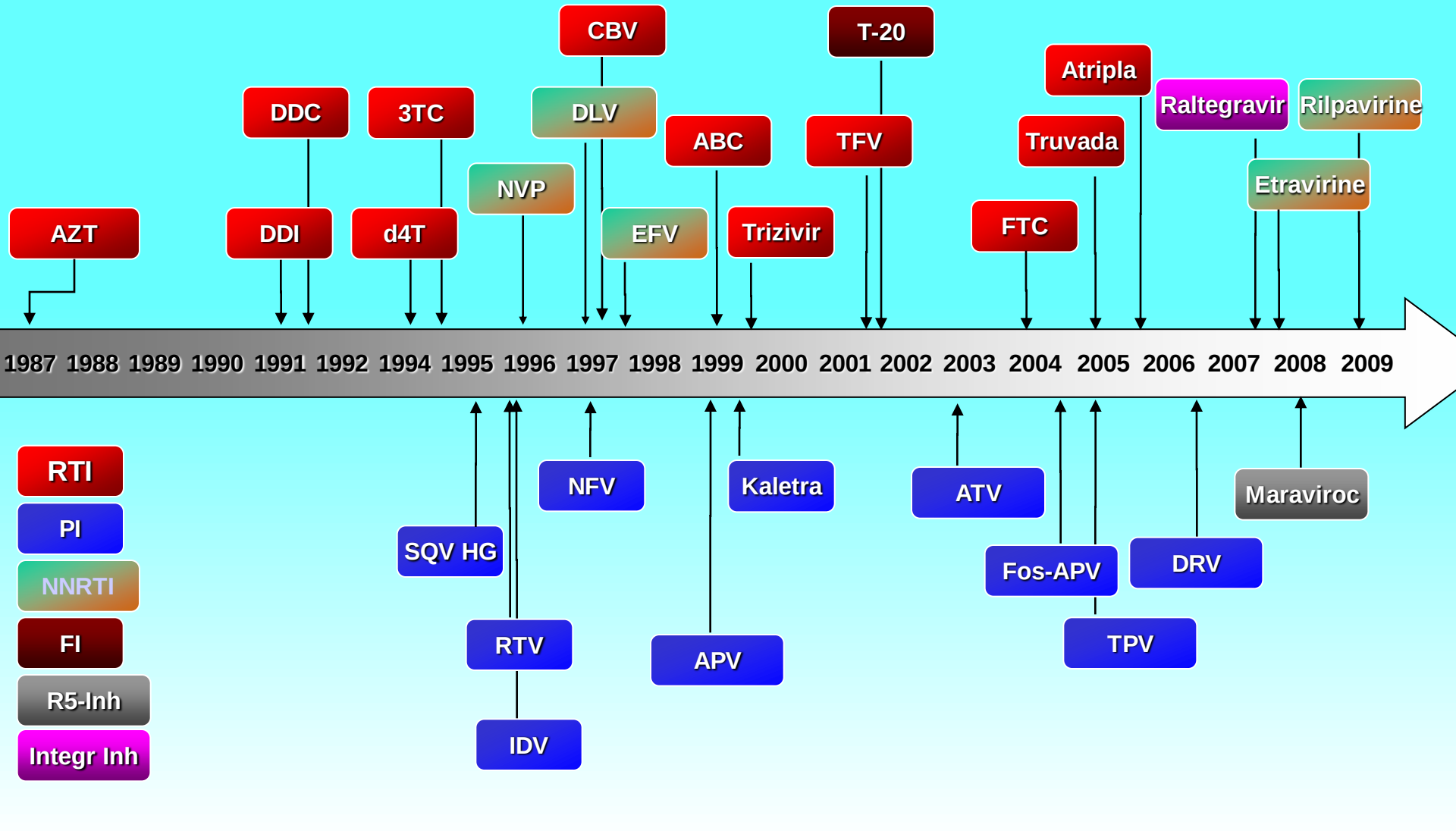
Principale cellula bersaglio del virus: Linfocita T CD4+

(e macrofagi, monociti, cellule della microglia SNC, cellule dendritiche cute e mucosa)

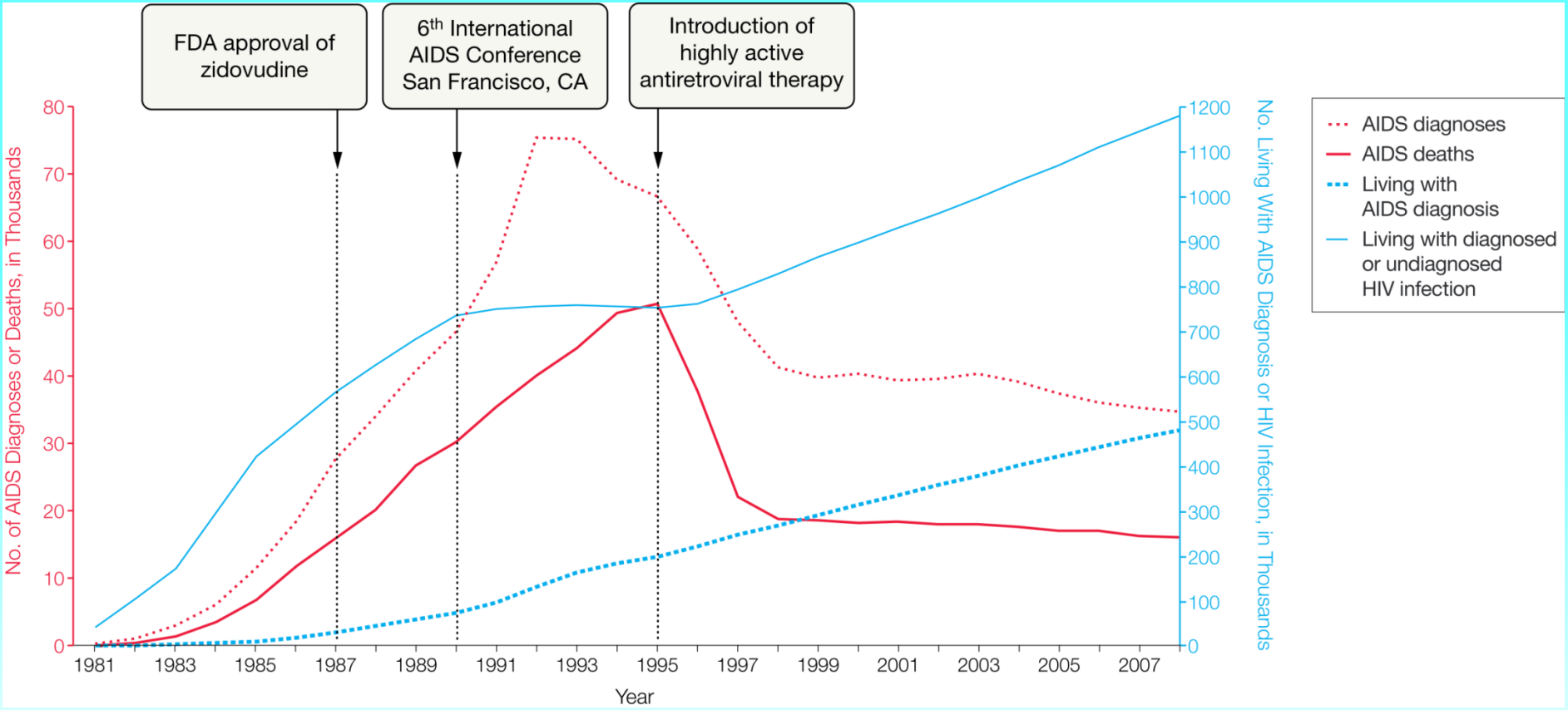
HIV-1 Replication Cycle



Approved Antiretrovirals



HIV/AIDS from 1990 to 2012
From San Francisco, 1990 to Washington, DC, 2012



Steinbrook, et al. JAMA. 2012;308(4):345-346

HIV Research Network

32 483 patients received care at 12 clinics between 2001 and 2010

Table 1. Demographics of Study Sample by Calendar Year

	No. (%) of Patients by Calendar Year[a]									
	2001 (n = 5445)	2002 (n = 6433)	2003 (n = 8287)	2004 (n = 9537)	2005 (n = 10137)	2006 (n = 11030)	2007 (n = 12194)	2008 (n = 13719)	2009 (n = 14684)	2010 (n = 15944)
CD4 T-cell count, /μL										
≤200	1653 (30.4)	1711 (26.6)	2062 (24.9)	2271 (23.8)	2357 (23.3)	2382 (21.6)	2515 (20.6)	2767 (20.2)	2728 (18.6)	2674 (16.8)
>200	3792 (69.6)	4722 (73.4)	6224 (75.1)	7266 (76.2)	7780 (76.8)	8648 (78.4)	9679 (79.4)	10952 (79.8)	11956 (81.4)	13270 (83.2)
Receiving ART										
Recommended[c]	1596 (77.6)	1752 (79.2)	2166 (77.2)	2280 (77.5)	2475 (80.9)	2498 (81.7)	4956 (80.9)	5905 (85.5)	6320 (89.9)	6313 (91.3)[b]
All	3597 (66.1)	4340 (67.5)	5490 (66.3)	6254 (66.0)	6887 (69.2)	7769 (72.0)	8773 (74.9)	10609 (79.3)	11980 (83.7)	13372 (86.3)[b]
HIV RNA ≤400 copies/mL[d]	1008 (44.7)	1432 (44.9)	1895 (45.8)	2543 (51.5)	3146 (55.2)	3831 (58.6)	4358 (60.5)	5591 (63.8)	6852 (68.6)	8061 (72.2)[b]

[c] Among patients recommended to receive ART: CD4 T-cell count of 200/μL or less for 2001-2006; CD4 T-cell count of 350/μL or less for 2007-2010.

Yehai, et al. JAMA, July 25, 2012—Vol 308 (4):339-341

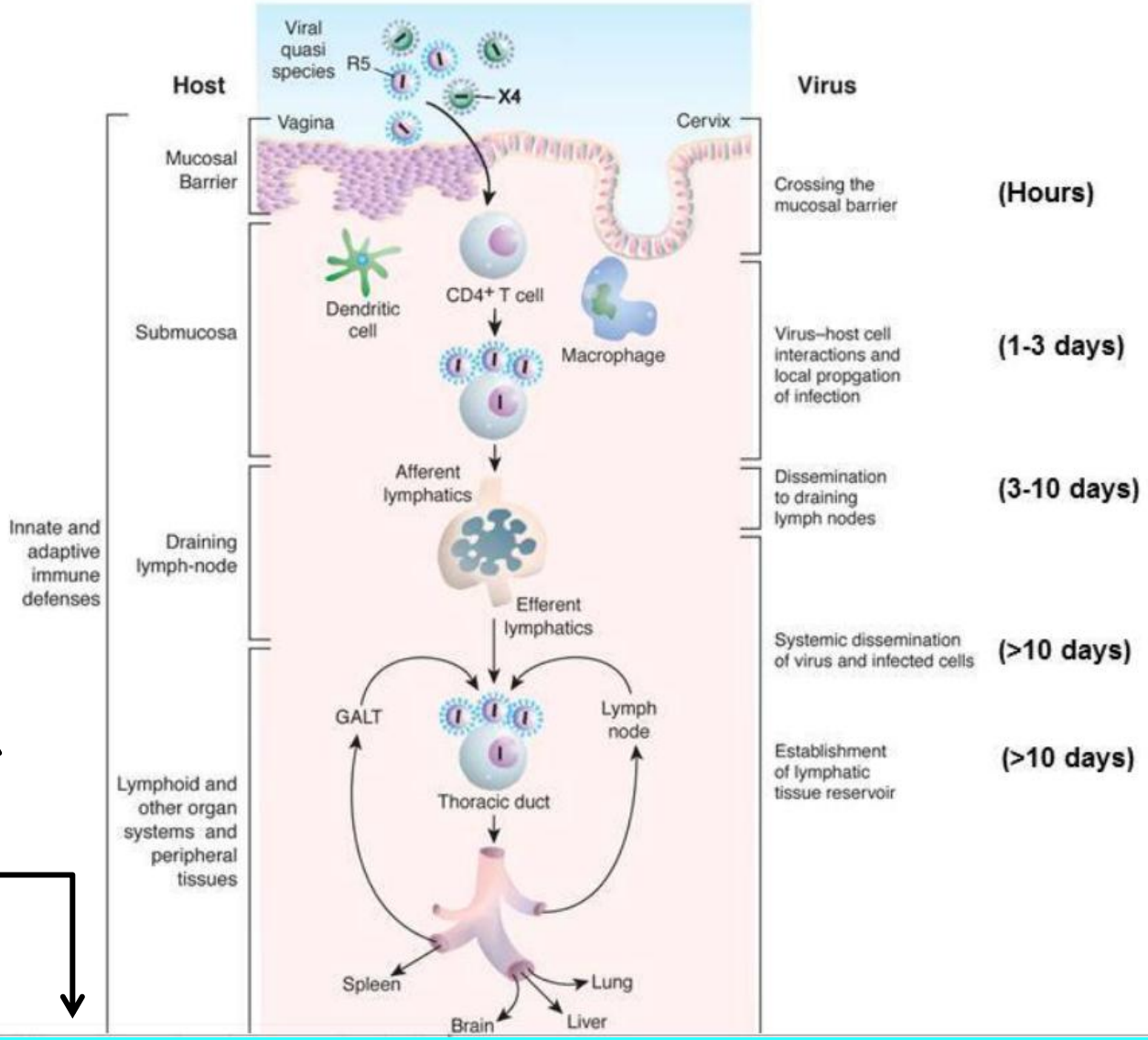
Metodi di Laboratorio per l'identificazione di HIV

Murray, 9th Ed, 2007

Ruolo nella diagnosi di infezione da HIV			
Isolamento in coltura *Only for research*	**Ricerca Antigene**	**Ricerca di HIV RNA Test molecolari**	**Ricerca anticorpi Test sierologici**
↑	↑	↑↑↑	↑↑↑

Serology is the primary diagnostic methods
HIV RNA is to confirm the diagnosis of HIV infection in the early stages or when serology is negative (window period)

HIV-1 Infection Events and Time Course



Diagnosi di laboratorio

HIV-RNA

⬇

Antigene p24

⬇

Anticorpi

Diagnosi di Laboratorio di infezione da HIV

Anticorpi anti-HIV 1/2

Test di screening

Sensibilità e specificità

molto elevate

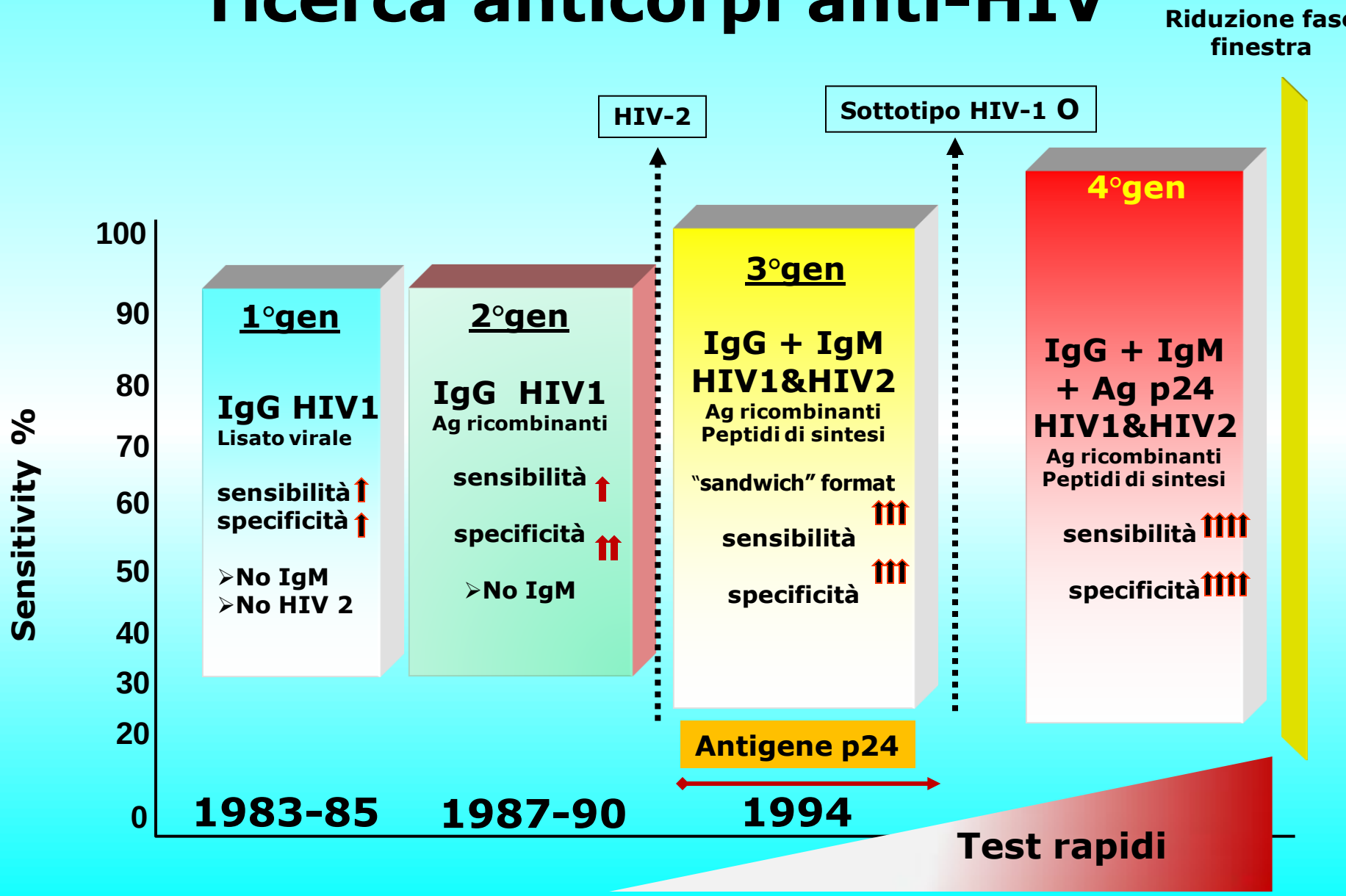
Test di conferma

Western blot

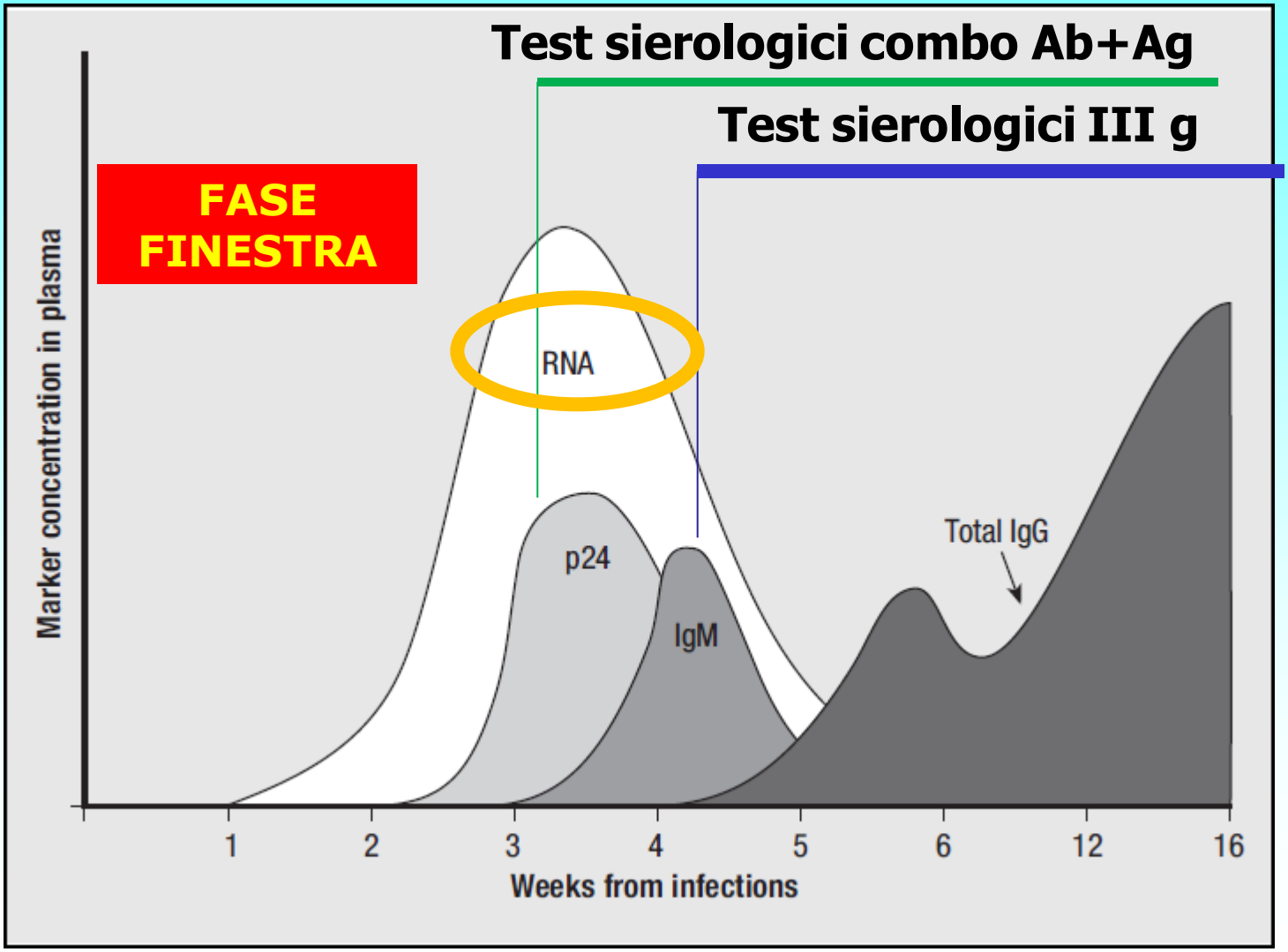
Test per la ricerca del virus

HIV RNA

Evoluzione tecniche sierologiche per ricerca anticorpi anti-HIV



Virological markers during the first weeks following infection with HIV



Fase finestra

- **Un buon numero di casi è già sieropositivo a 2-3 settimane dal primo contatto**
- **La maggior parte positivizza entro 3 mesi.**
- **Un test a 6 mesi è risolutivo.**

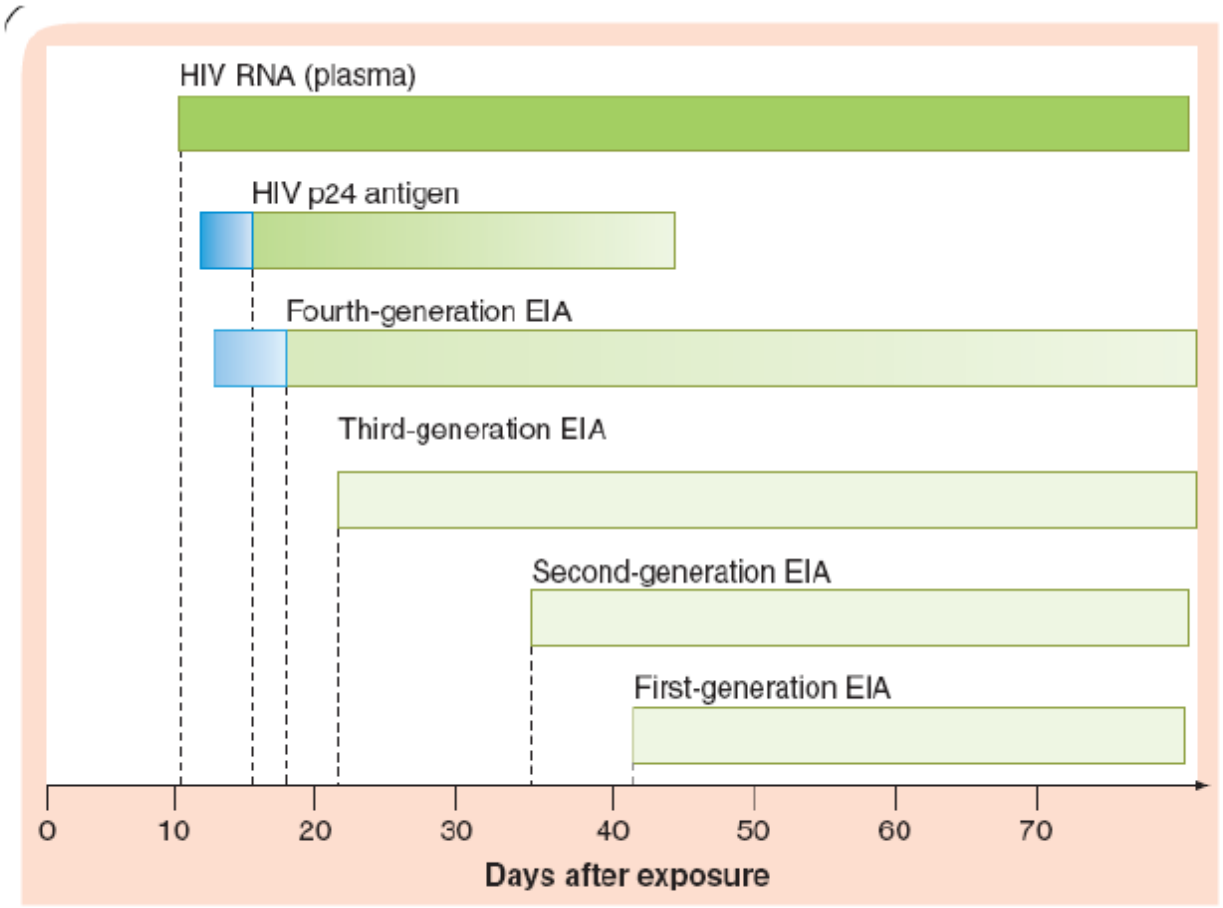


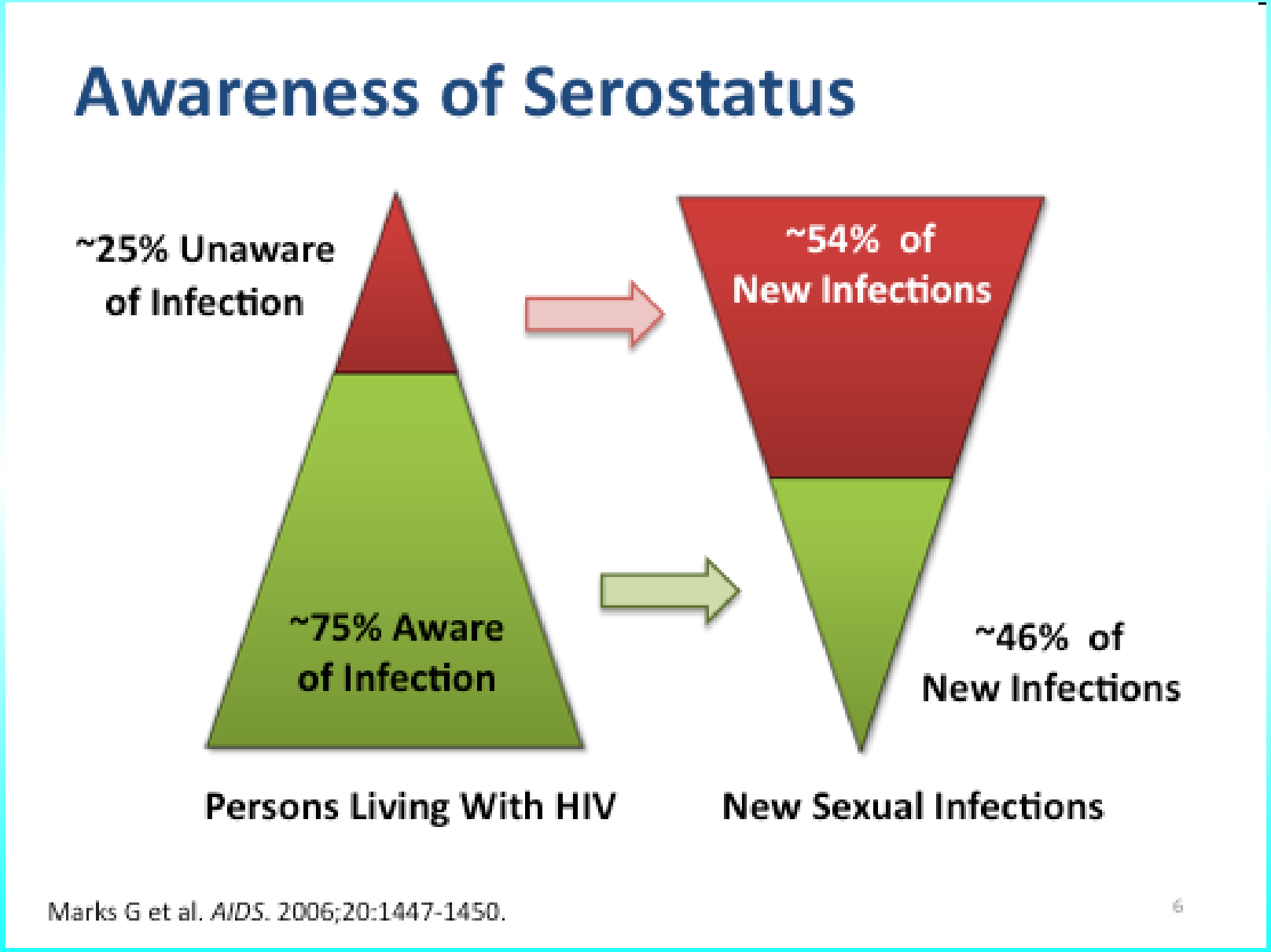
Figure 1. Diagnostic window of specific markers of HIV infection. The blue sections at the beginning of the bars for HIV p24 antigen and fourth-generation antigen EIA indicate the respective reduction of the diagnostic window, which can be achieved with the most sensitive assays.

Test rapidi per anticorpi anti-HIV

I risultati reattivi vanno sempre confermati da un Laboratorio di Riferimento e in tempi rapidi

www.cdc.gov 2008

Perché è importante il test anti-HIV

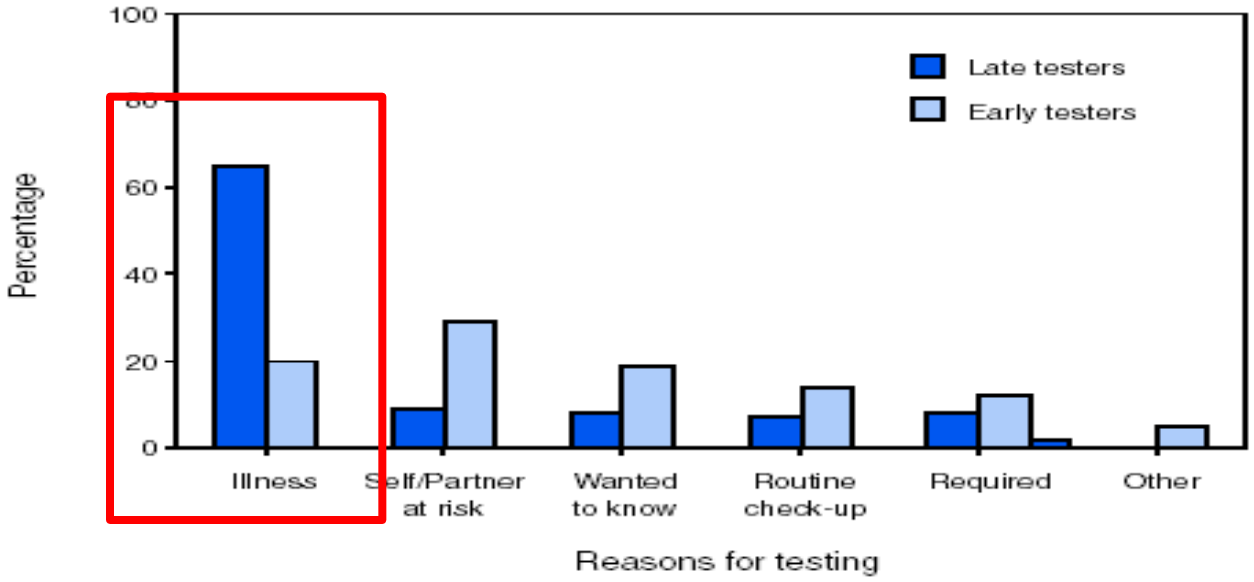


Perché è importante il test rapido

MMWR
Weekly
June 27, 2003 / 52(25);581-586

Figure

FIGURE. Percentage of late and early testers*, by [illegible] testing — 16 sites,† United States, 2000–2003



* Late testers were defined as persons who had their first positive HIV test ≤1 year of diagnosis of AIDS; early testers were defined as persons who either had their first positive HIV test ≥5 years before the diagnosis of AIDS or had ≥5 years without a diagnosis of AIDS after their first positive HIV test.

† Arizona, California, Colorado, Connecticut, Delaware, Florida, Georgia, Kansas, Maryland, Michigan, Minnesota, New Jersey, New Mexico, South Carolina, Texas, and Washington.

Il test rapido ideale
criteri ASSURED

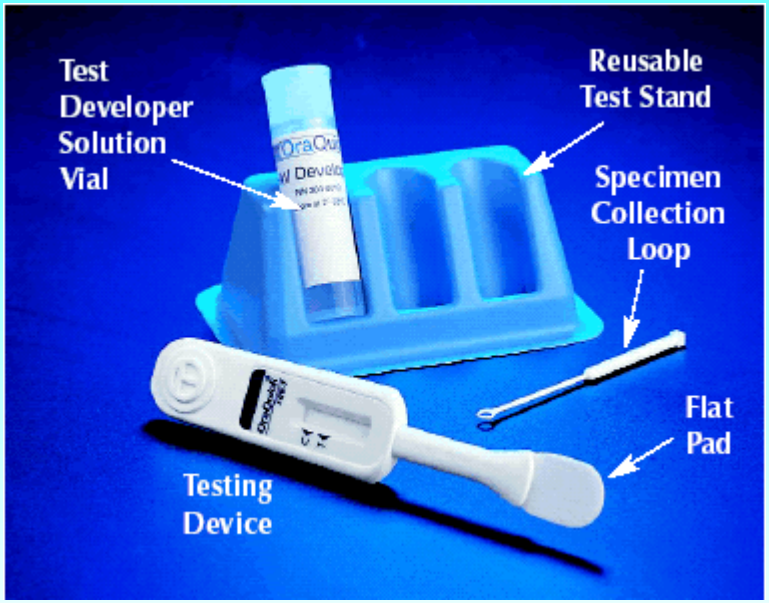
• A= accessibile	*Affordable*
• S= sensibile	*Sensitive*
• S= specifico	*Specific*
• U= user-friendly	*U*
• R= robusto	*Robust*
• E= equipment-free	*E*
• D= disponibile	*Deliverable*

Low cost: 5$

Peeling, Clin Microbiol Infect 2010

Reveal G3 HIV 1

ClearView HIV 1/2 Stat Pak



Ora Quick Advance HIV 1/2

Uni-Gold Recombigen HIV 1

ClearView Complete HIV 1/2

CDC

Test rapidi marcati CE

Nom	Matrice	Technique	Performances annoncées Sensibilité	Spécificité
INSTI HIV 1/2	Sérum, plasma, sang total	Immunofiltration	99,2 (sérum)	99,9
			99,6 (plasma)	99,9
			99,6 (sang total)	99,4
VIKIA HIV 1/2	Sérum, plasma, sang total	Flux latéral	99,7 (sérum, plasma)	99,6
			99,9 (sang total)	99,9
Determine HIV 1-2	Sérum, plasma, sang total*	Flux latéral	100,0 (sérum)	99,75
			100,0 (plasma)	
			100,0 (sang total)	
Core HIV 1/2	Sérum	Flux latéral	100,0	99,75
Immunoflow HIV1-HIV2	Sérum	Flux latéral	100,0	100,0
ImmunoComb II HIV 1+2 BiSpot	Sérum, plasma	EIA phase solide	100,0	99,4
DoubleCheck II HIV 1/2	Sérum, plasma	Immunofiltration	100,0	99,3
Miracare rapid HIV antibody test	Sérum, plasma, sang total	Immunofiltration	99,7	99,7
ORAQUICK Advance	Sérum, plasma, sang total	Flux latéral	99,6 (plasma)	99,9
FDA approved	Salive		99,6 (sang total)	100,0
			99,3 (salive)	99,8
Retrocheck HIV	Sérum	Flux latéral	100,0	99,75
Retroscreen HIV	Sérum, plasma	Flux latéral	100,0	99,8

Body Fluids Used for HIV Rapid Testing

Serum

Plasma

Whole blood

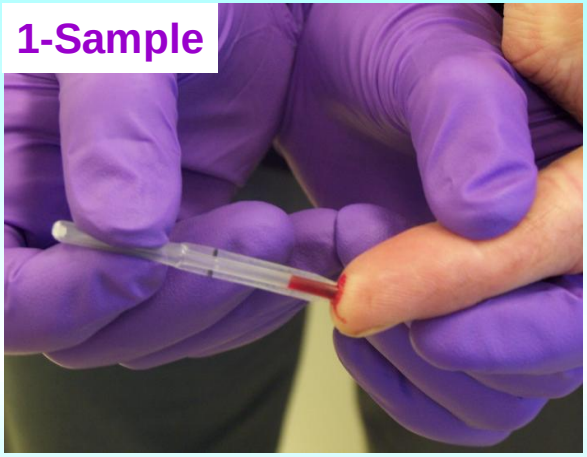
Oral fluids

Dried Blood spot

Easy to perform
Capillary blood

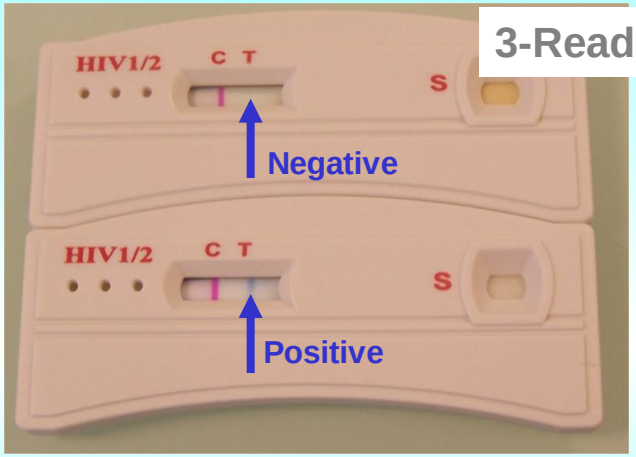
HIV 1/2

1-Sample



15 min

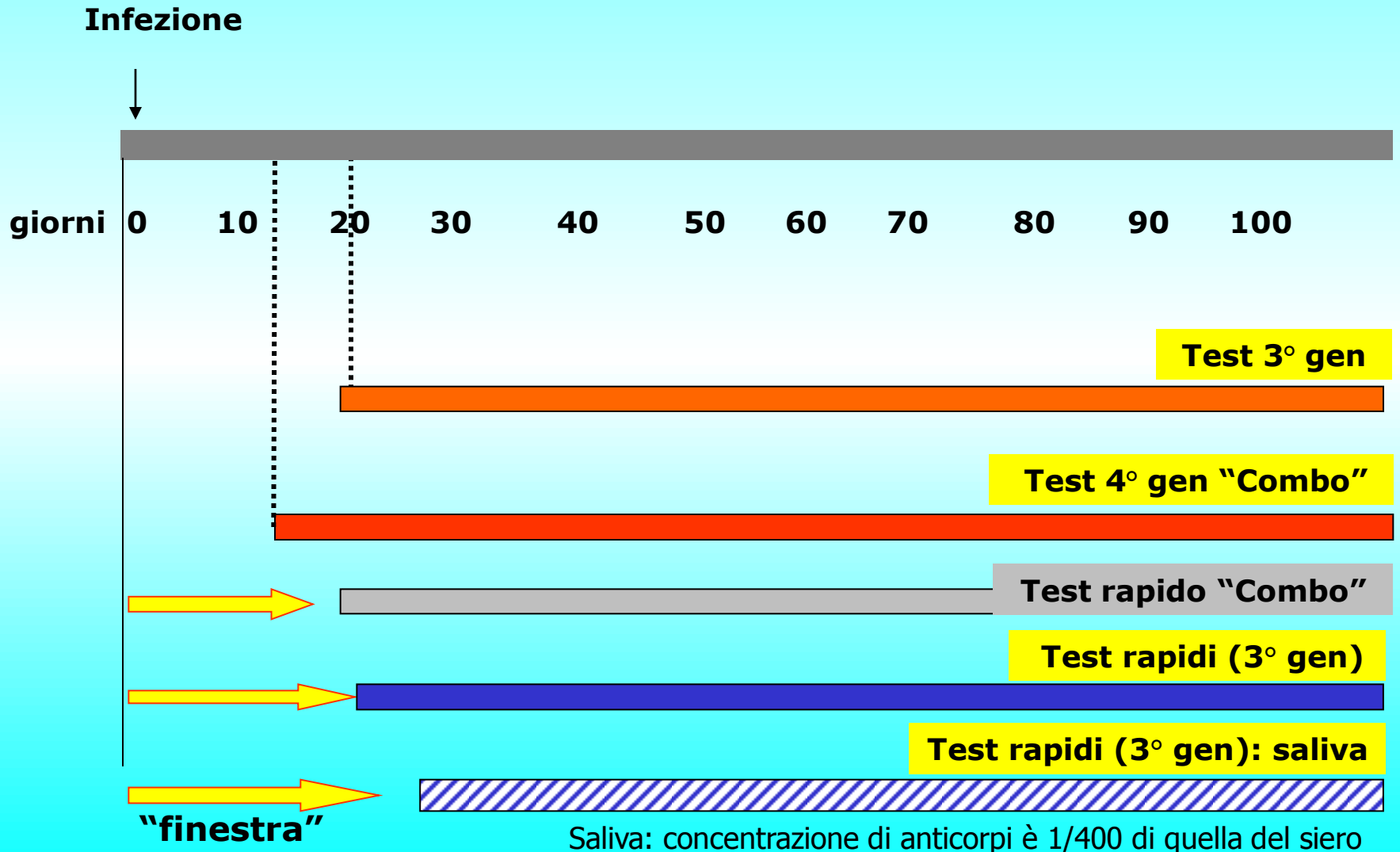
3-Read



Dispense: 2 drops of blood + 1 drop of buffer

Oral Fluid Specimen

Criticità dei Test rapidi: sensibilità



Saliva: concentrazione di anticorpi è 1/400 di quella del siero

Multi-test algorithms, CDC 2009

- **I risultati NON REATTIVI possono essere riportati come NEGATIVI**
  - **Se esposizione recente ➡ test di 4ª GENERAZIONE**
- **I risultati REATTIVI richiedono conferma "IMMEDIATA" con Western blot o HIV RNA**
- **I risultati INVALIDI vanno ripetuti con test di 4ª GENERAZIONE**

I risultati reattivi/positivi al test di screening per anti-HIV vanno sempre confermati con:

Western Blot e/o HIV RNA

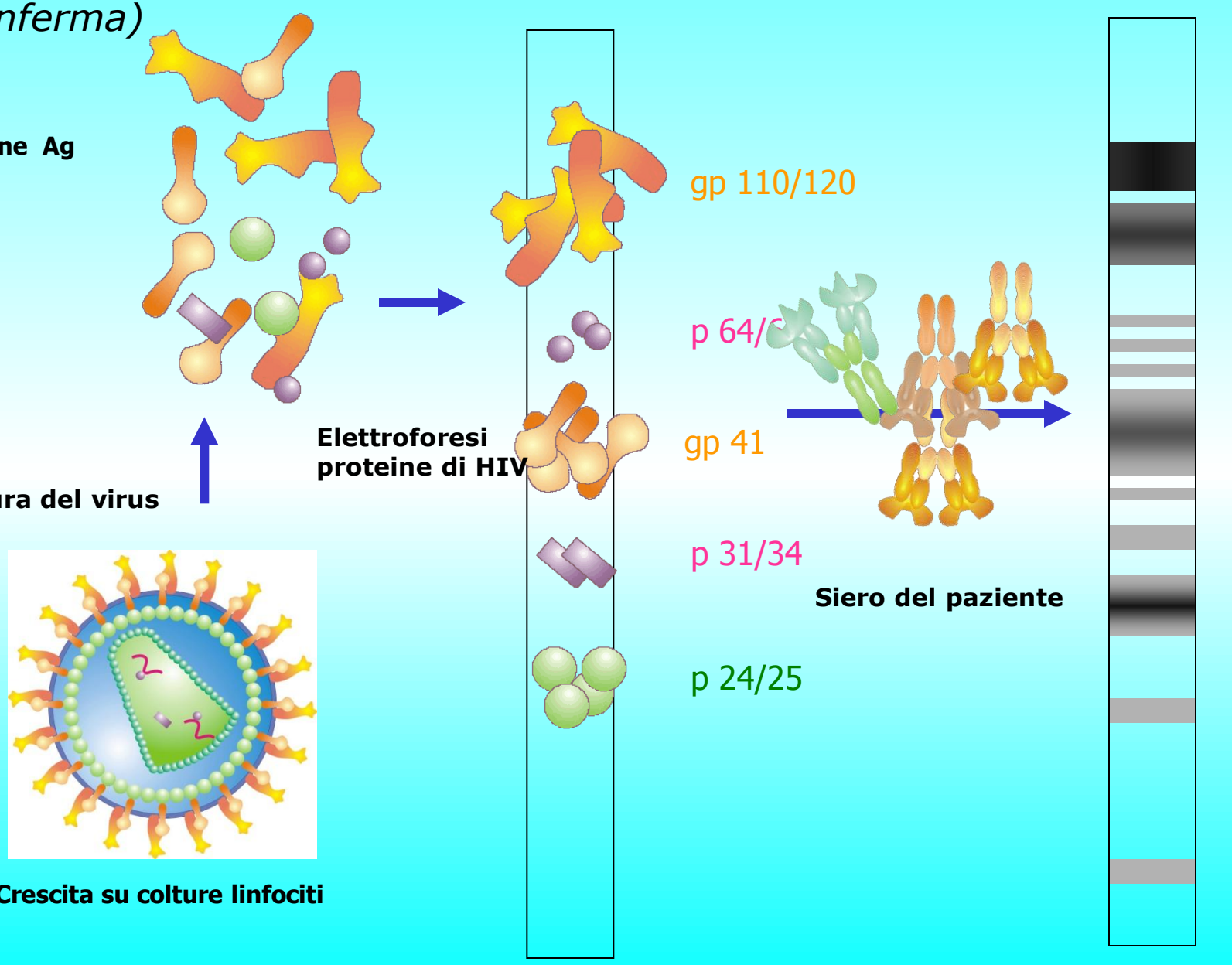
Linee guida WHO, CDC e Ministero della salute

www.cdc.gov 2006, 2008 e 2009 - FDA, 2007

Western blot

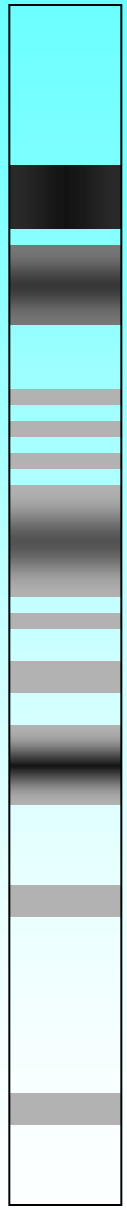
- E' il classico test di conferma
- Viene eseguito su tutti i campioni **non negativi**

(test di conferma)

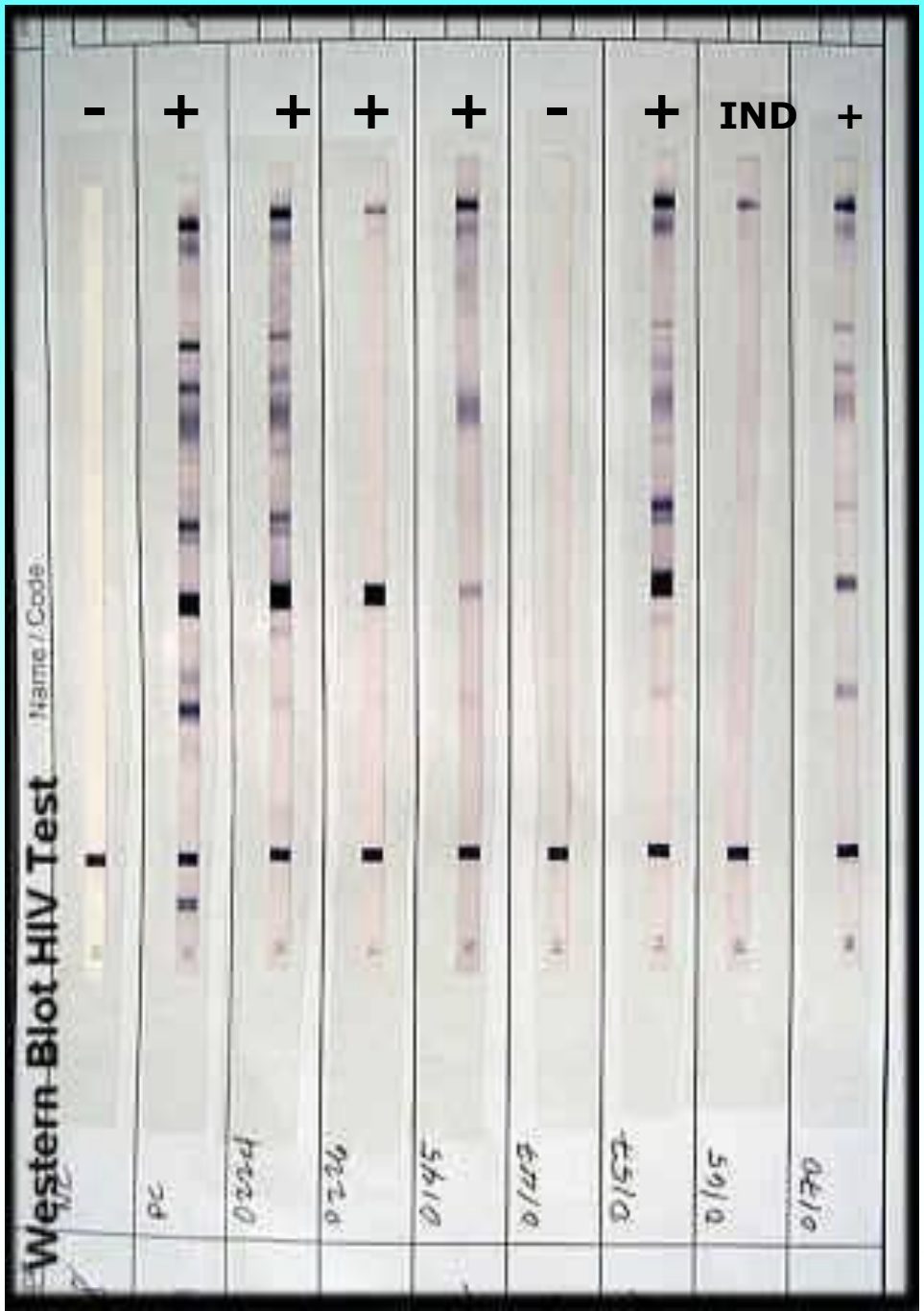


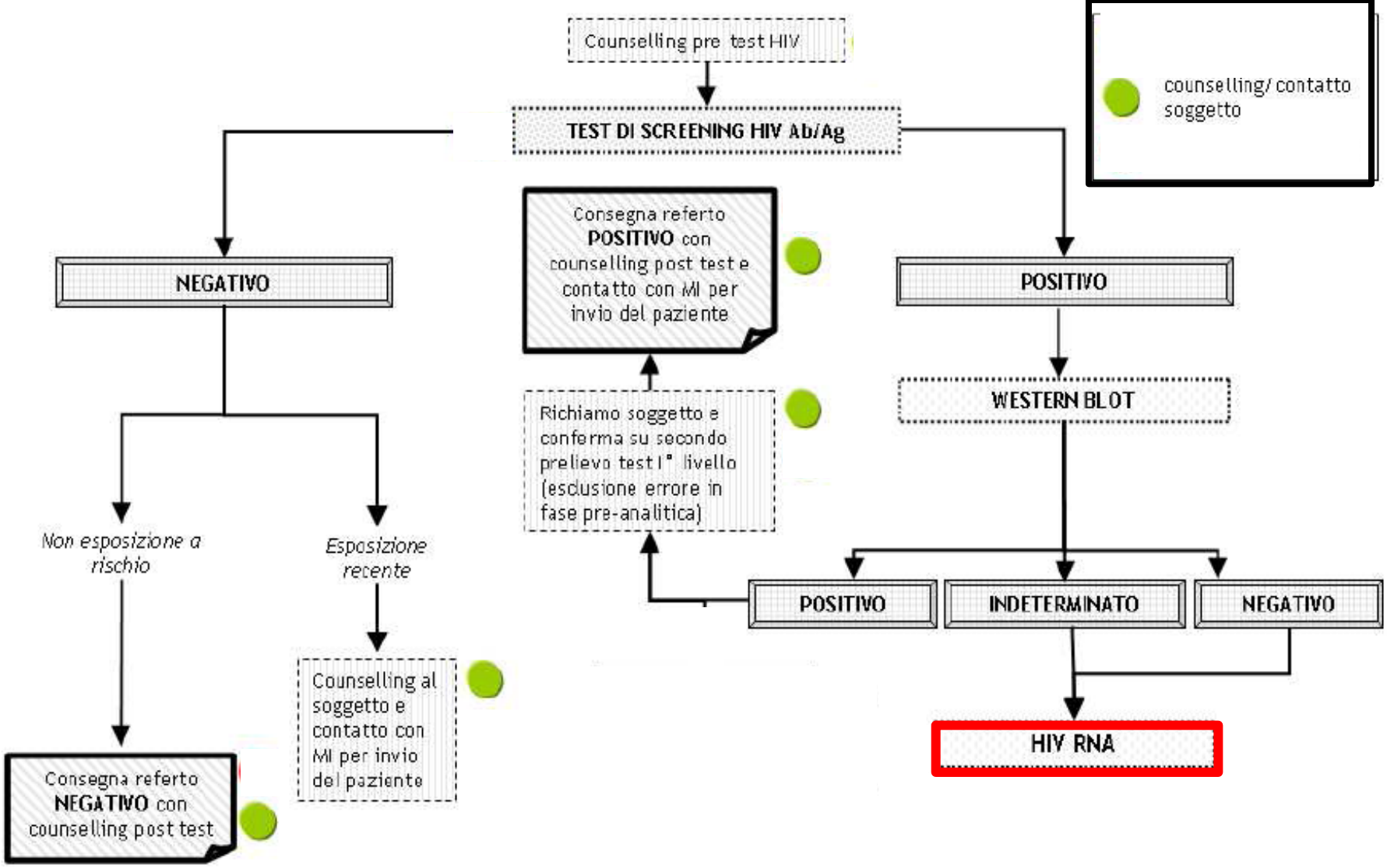
Positività

CDC	2 bande tra p24, gp41, gp 110-160
FDA	p24, p31, gp 41 o gp 110-160
OMS	2 bande ENV e/o GAG e/o POL
Croce Rossa (USA)	1 ENV, 1 POL e 1 GAG
P. Ehrlich Institute	2 bande, di cui una ENV



W. blot


Western Blot HIV Test
Name / Code
NC
PC
-
+
+
+
+
-
+
IND
+
gp 160
gp 110/120
p 64/68
p 52/55
gp 41
p 31/34
p 24
p 17/18



Laboratorio di Microbiologia e Virologia
Ospedale Amedeo di Savoia
Algoritmo diagnostico per accertare infezione da HIV

Diagnosi di Laboratorio di infezione da HIV

Test di screening

Anticorpi anti-HIV

Test di conferma

Western blot

Test per la ricerca del virus

HIV RNA

HIV RNA

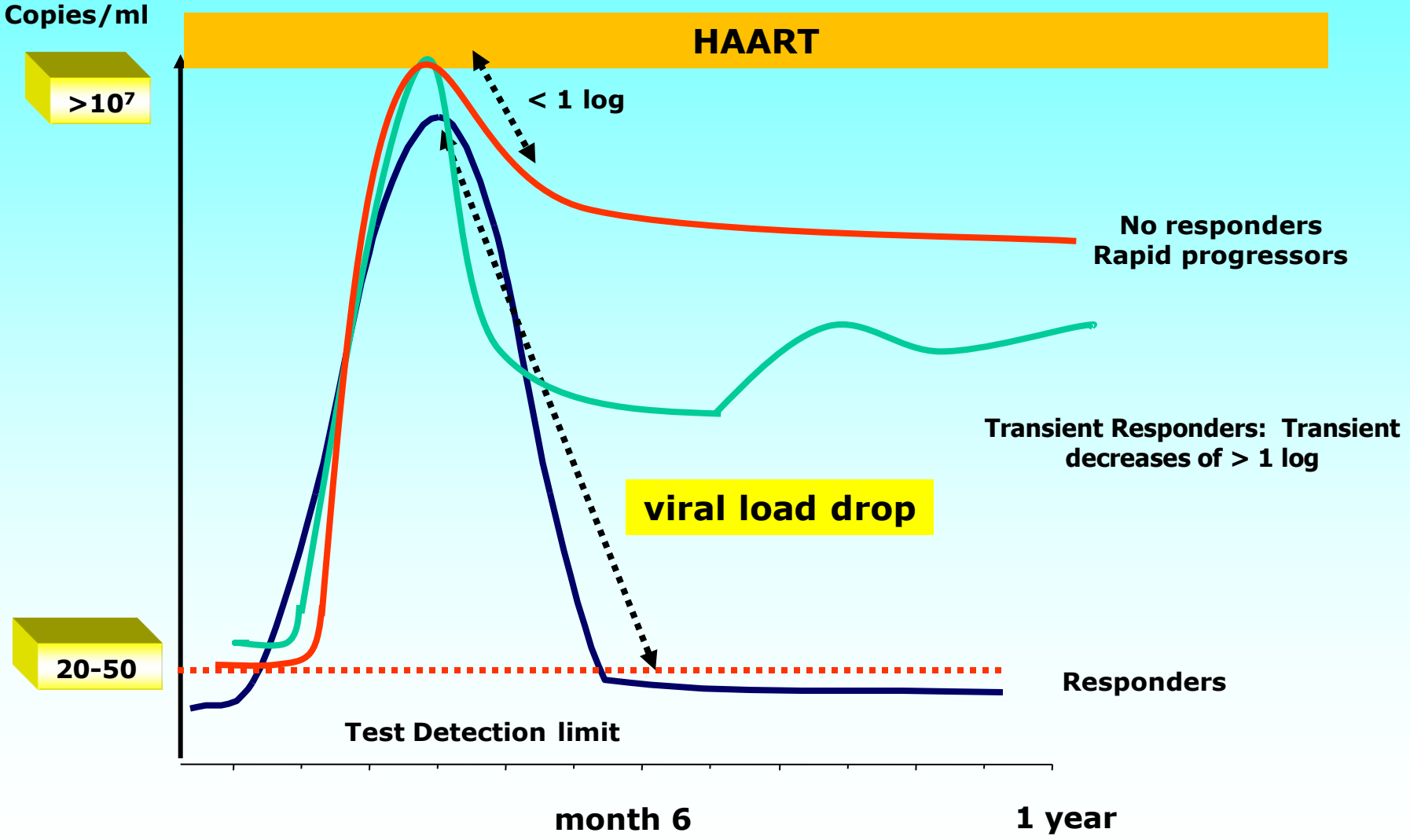
- ➔ **Identifica la presenza del virus e quanto il virus si replica**
- ➔ **Conferma la diagnosi sierologica**
  In tutti i casi **ed** ***è risolutivo*** **per:**
  1. **Fase finestra**
  2. **Test di screening+/western blot negativi o indeterminati**
- ➔ **Identifica il genotipo di HIV**
- ➔ **Consente il Monitoraggio e valutazione di efficacia della terapia**
- ➔ **Identifica la resistenza alla terapia**

Indication for plasma HIV RNA testing

U.S. Department of Health and Human Services *DHHS,*

Clinical Indication	Information	Use
Syndrome consistent with acute HIV infection (See Table 25.)	Establishes diagnosis when HIV antibody test is negative or indeterminate	**Diagnosis**
Initial evaluation of newly diagnosed HIV infection	**BASELINE**	Use in conjunction with CD4 count for decision to start or defer therapy
Every 3–4 months in patients not on therapy	Changes in viral load	Use in conjunction with CD4 count for decision to start therapy
2–8 weeks after initiation of change in antiretroviral the	**Therapy assessment After 4- 8 weeks from start Every 3-6 months**	to continue or change
3–4 months after start of thera		to continue or change apy
Every 3–4 months in patients on therapy	Durability of antiretroviral effect	Decision to continue or change therapy
Clinical event or significant decline in CD4 T-cells	Association with changing or stable viral load	Decision to continue, initiate, or change therapy

HIV RNA monitoring as a measure of the virologic response



HIV : Test di resistenza

Scopo della terapia anti-HIV

Azzerare la replicazione di HIV: HIV RNA non rilevabile

No replication = No Mutation = No Drug Resistance

Test genotipici

Basati sul sequenziamento del virus HIV

Profilo genetico virale
Profilo di suscettibilità a farmaci

Antivirogramma

HIV : Test di resistenza

Laboratorio di Microbiologia e Virologia
Ospedale Amedeo di Savoia

Test genotipici

Sequenziamento geni di HIV bersaglio di farmaci

Geni virali	Farmaci
POL/PRO per Proteasi	Inibitori della proteasi
POL/RT per Reverse transcriptasi	Inibitori transcriptasi
POL/IN per Integrasi	Inibitori integrasi
Envelope	Inibitori CCR5

HIV DRUG RESISTANCE DATABASE – Stanford University

STANFORD UNIVERSITY
HIV DRUG RESISTANCE DATABASE
A curated public database designed to represent, store, and analyze the divergent forms of data underlying HIV drug resistance.

HOME GENOTYPE-RX GENOTYPE-PHENO GENOTYPE-CLINICAL HIVdb PROGRAM

HIVdb: Genotypic Resistance Interpretation Algorithm

SeqID: 25366-08_1 25366-08 Fri Dec 12 13:20:09 CET 2008. 1275 bases. Date: 15-Dec-2008

Subtype and % similarity to closest reference isolate:

1. PR: CRF0
2. RT: CRF0

Sequence Quality Assessment

Gene	QA Problem
PR	Stop Codons, Frame Shifts:
PR	R,D,H,V,N:

NRTI Resistance Mutations: M184V

NNRTI Resistance Mutations: K103N, K238T

Other Mutations: V35T, E40D, V60IV, D123E, S162A, P170PS, K173T, Q174K, D177E, V179IV, T200A, Q207E, R211KR, V245K, P247AP, E248D, A272P, T286A, E291D, V292I, I293V, P294PT, P313PS

Nucleoside RTI		Non-Nucleoside RTI	
lamivudine (3TC)	High-level resistance	delavirdine (DLV)	High-level resistance
abacavir (ABC)	Potential low-level resistance	efavirenz (EFV)	High-level resistance
zidovudine (AZT)	Susceptible	etravirine (ETR)	Low-level resistance
stavudine (D4T)	Susceptible	nevirapine (NVP)	High-level resistance
didanosine (DDI)	Susceptible		
emtricitabine (FTC)	High-level resistance		
tenofovir (TDF)	Susceptible		

NRTI and NNRTI mutations

PI Major Resistan
PI Minor Resistan
Other Mutations:

atazanavir/r (ATV	
darunavir/r (DRV/	
fosamprenavir/r (	
indinavir/r (IDV/r)	
lopinavir/r (LPV/r)	Susceptible
nelfinavir (NFV)	Susceptible
saquinavir/r (SQV/r)	Susceptible
tipranavir/r (TPV/r)	Susceptible

PI mutations

several subtypes, it is the consensus residue.
• K20R/M/I/T/V are associated with resistance to multiple PIs when present with other mutations. K20R/M/I

3N in which case it
n. K103N is a rarer

ations (TAMs). AZT

Grazie a tutti per l'attenzione!