

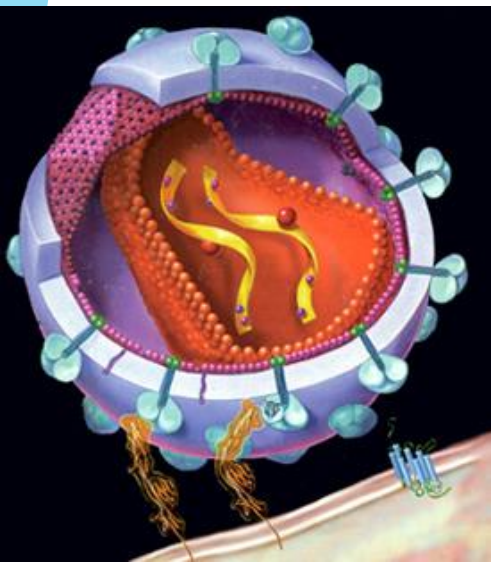


*In the
name of
GOD*

Challenges in Clinical- Laboratory Diagnosis of HIV/AIDS

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ID, Clinical HIV/AIDS fellowship



HIV and AIDS

- ▶ More than forty years has been passed since the first case of HIV was diagnosed, and with the spread of the disease worldwide, it has left millions dead during that time.
- ▶ Despite all the measures taken and available treatments, it is still a major health problem in the world
- ▶ A very important point in education is to explain the difference between HIV and AIDS

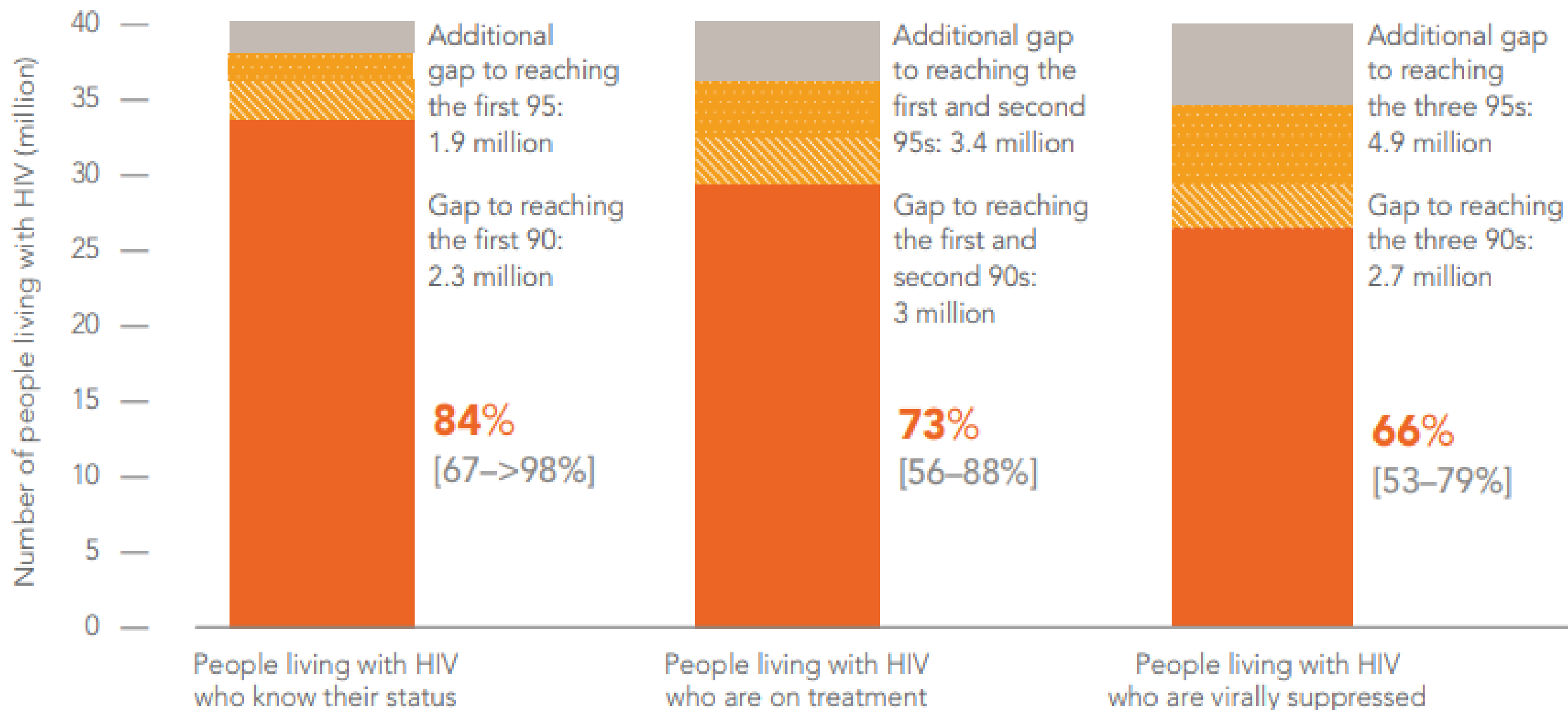
GLOBAL SUMMARY OF THE AIDS EPIDEMIC, 2020

Number of people living with HIV	Total	37.7 million	[30.2 million–45.1 million]
	Adults	36.0 million	[28.9 million–43.2 million]
	Women (15+ years)	19.3 million	[15.5 million–23.1 million]
	Children (<15 years)	1.7 million	[1.2 million–2.2 million]
People newly infected with HIV in 2020	Total	1.5 million	[1.0 million–2.0 million]
	Adults	1.3 million	[910 000–1.8 million]
	Women (15+ years)	660 000	[450 000–920 000]
	Children (<15 years)	150 000	[100 000–240 000]
AIDS-related deaths in 2020	Total	680 000	[480 000–1.0 million]
	Adults	580 000	[400 000–850 000]
	Women (15+ years)	240 000	[170 000–360 000]
	Children (<15 years)	99 000	[68 000–160 000]

Source: UNAIDS epidemiological estimates, 2021 (<https://aidsinfo.unaids.org/>).

- ▶ **It's estimated 37 million people in the world are currently living with HIV, of which about 10.2 million are untreated.**
- ▶ **Of those who are not treated, 4.1 million are unaware of their condition and 6.1 million do not have access to treatment despite knowing they have the disease.**

HIV TESTING AND TREATMENT CASCADE, GLOBAL, 2020



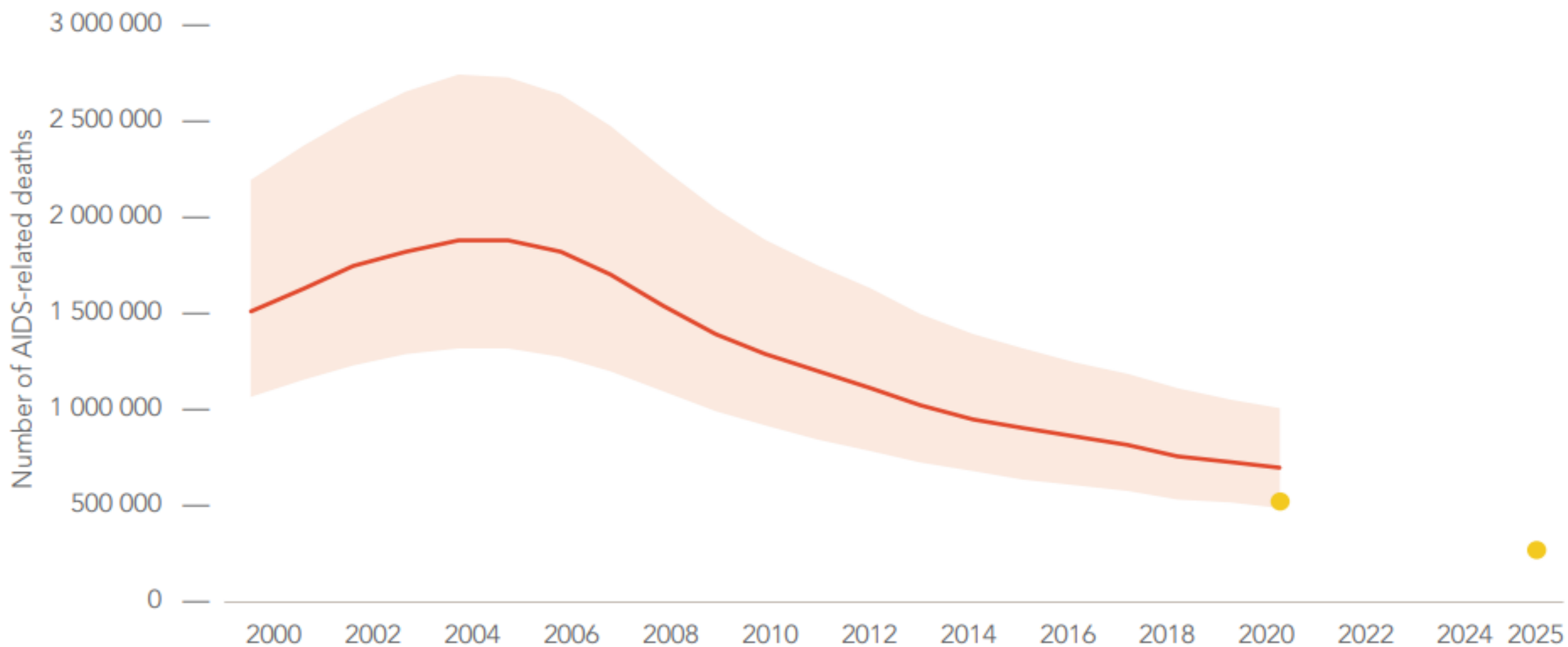
Source: UNAIDS special analysis, 2021.

EVERY DAY THERE ARE 4000 NEW HIV INFECTIONS (ADULTS AND CHILDREN), 2020

- **60% are in sub-Saharan Africa**
- **10% are among children under 15 years of age**
- **90% are among adults aged 15 years and older, of whom:**
 - **51% are among women**
 - **31% are among young people (15–24)**
 - **20% are among young women (15–24)**

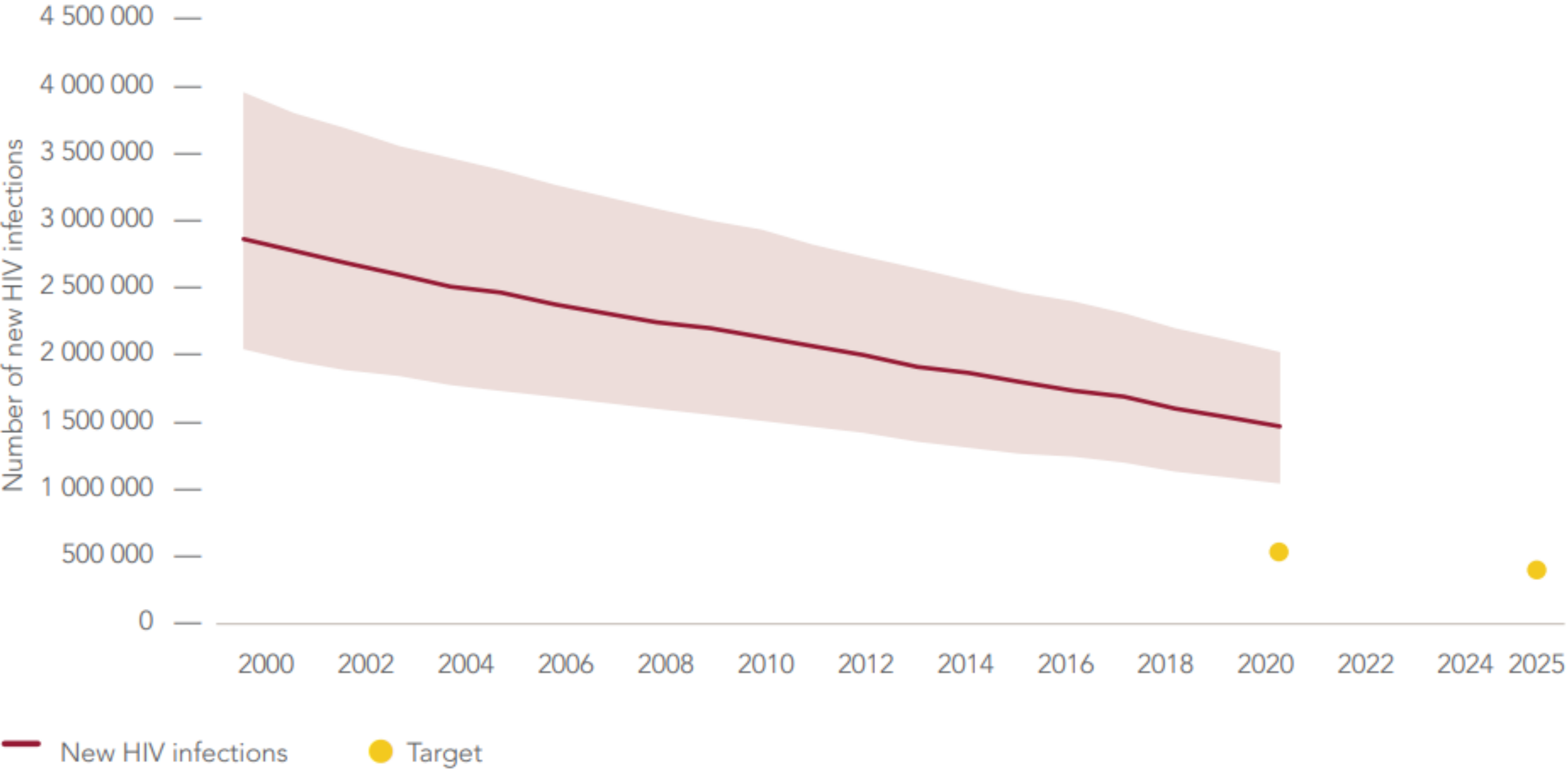
Source: UNAIDS epidemiological estimates, 2021 (<https://aidsinfo.unaids.org/>).

AIDS-RELATED DEATHS, GLOBAL, 2000–2020, AND 2020 AND 2025 TARGETS



— AIDS-related deaths ● Target

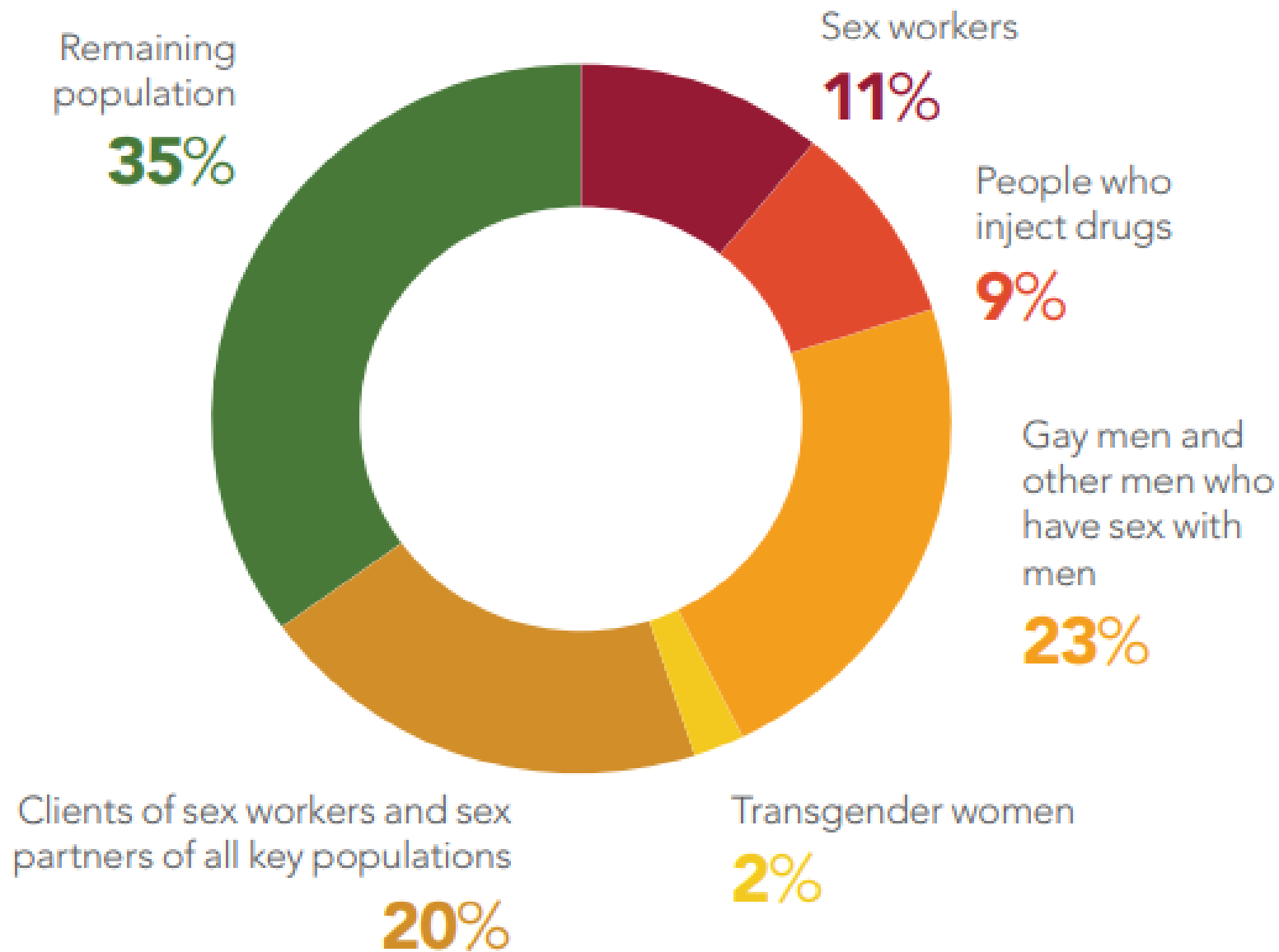
NEW HIV INFECTIONS, GLOBAL, 2000–2020, AND 2020 AND 2025 TARGETS



REGIONAL HIV AND AIDS STATISTICS AND FEATURES, 2020

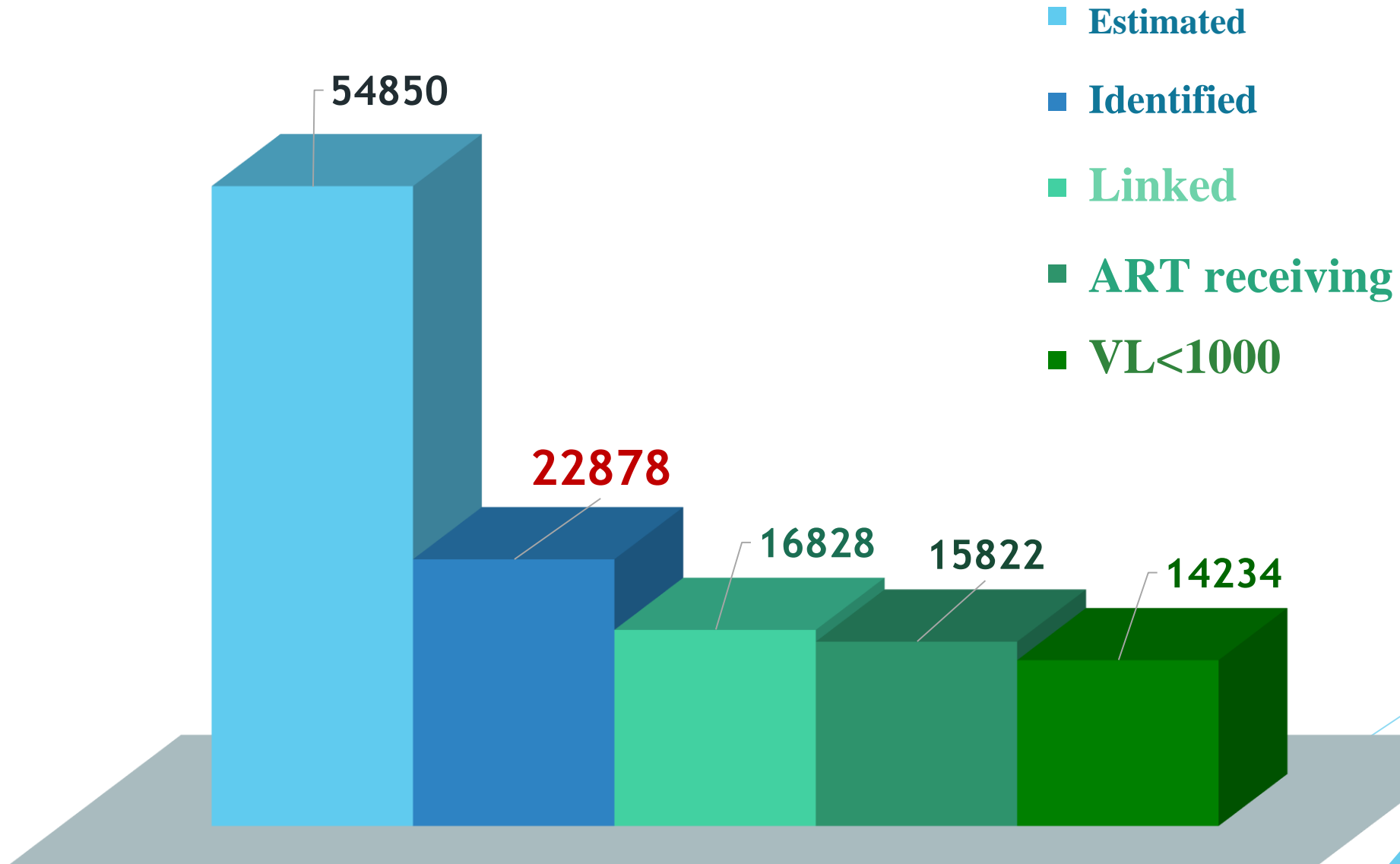
	Adults and children living with HIV	Adults and children newly infected with HIV	Adult and child deaths due to AIDS
Eastern and southern Africa	20.6 million [16.8 million–24.4 million]	670 000 [470 000–930 000]	310 000 [220 000–470 000]
Western and central Africa	4.7 million [3.9 million–5.8 million]	200 000 [130 000–330 000]	150 000 [100 000–210 000]
Middle East and North Africa	230 000 [190 000–310 000]	16 000 [12 000–28 000]	7900 [6000–13 000]
Asia and the Pacific	5.8 million [4.3 million–7.0 million]	240 000 [170 000–310 000]	130 000 [87 000–200 000]
Latin America	2.1 million [1.4 million–2.7 million]	100 000 [66 000–150 000]	31 000 [20 000–46 000]
Caribbean	330 000 [280 000–390 000]	13 000 [8700–18 000]	6000 [4300–8500]
Eastern Europe and central Asia	1.6 million [1.5 million–1.8 million]	140 000 [120 000–160 000]	35 000 [28 000–43 000]
Western and central Europe and North America	2.2 million [1.9 million–2.6 million]	67 000 [53 000–81 000]	13 000 [9200–17 000]
GLOBAL	37.7 million [30.2 million–45.1 million]	1.5 million [1.0 million–2.0 million]	680 000 [480 000–1.0 million]

Global

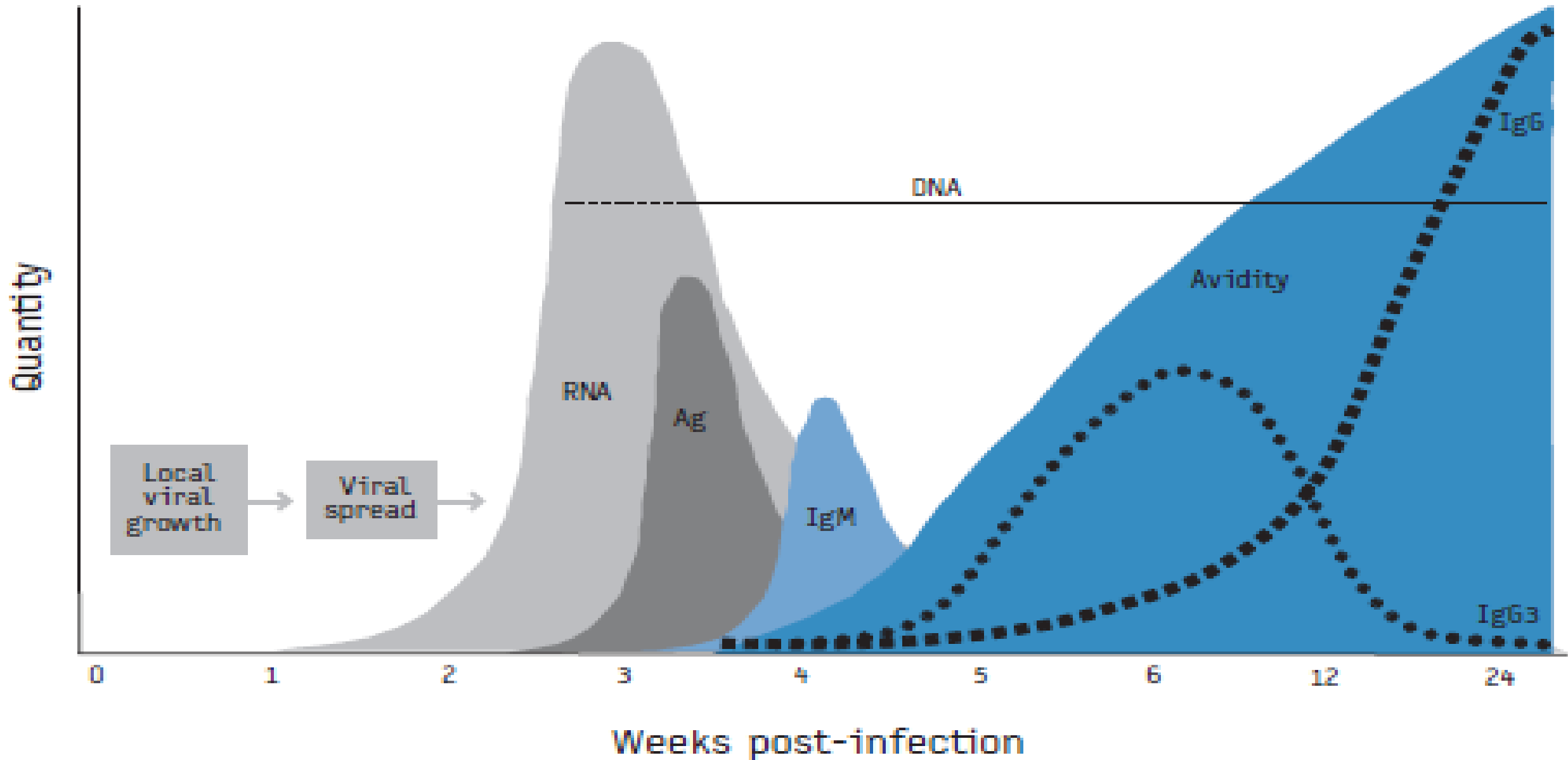


**PLWH
disaggregated
by population
2020**

Iran HIV treatment cascade

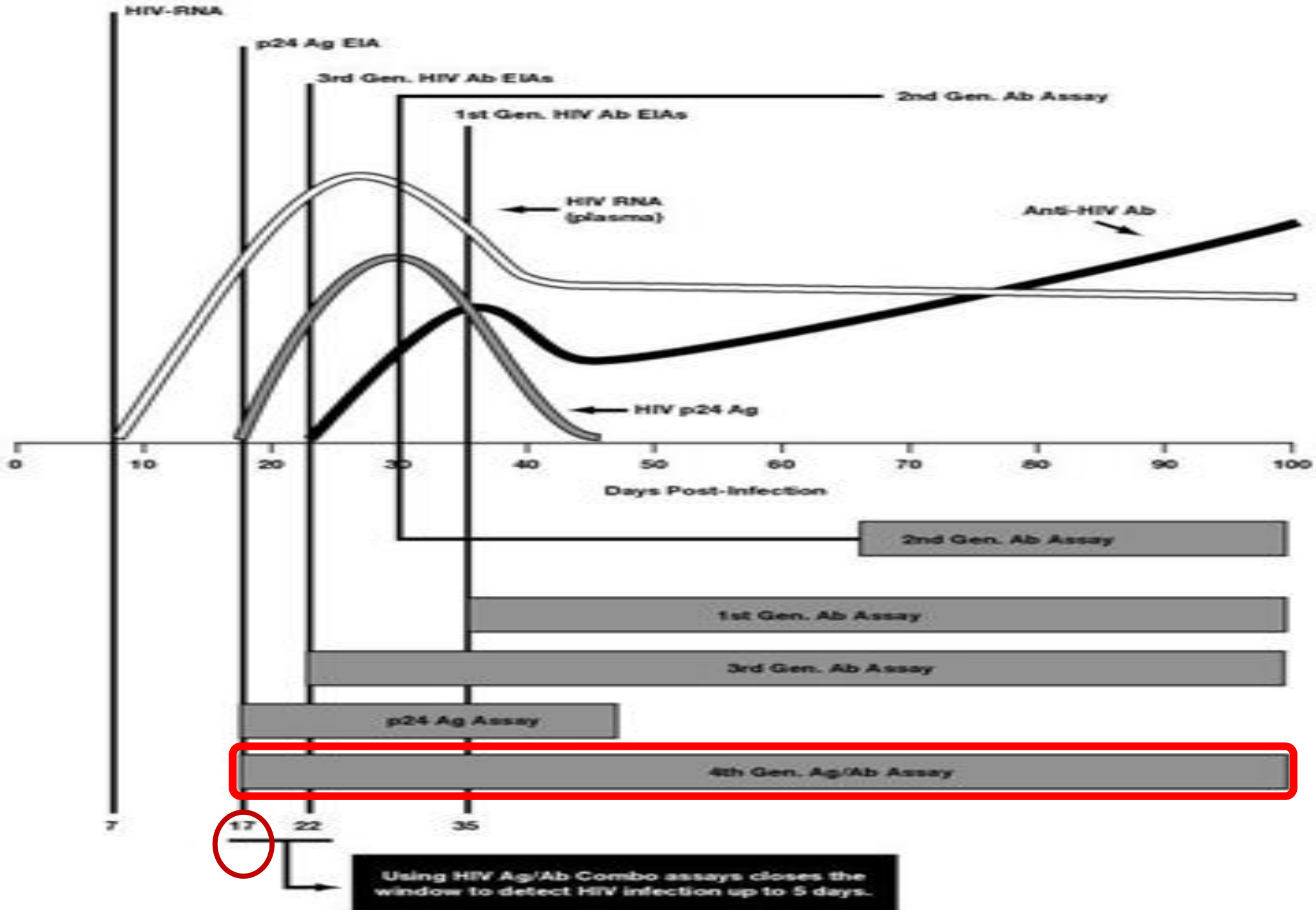


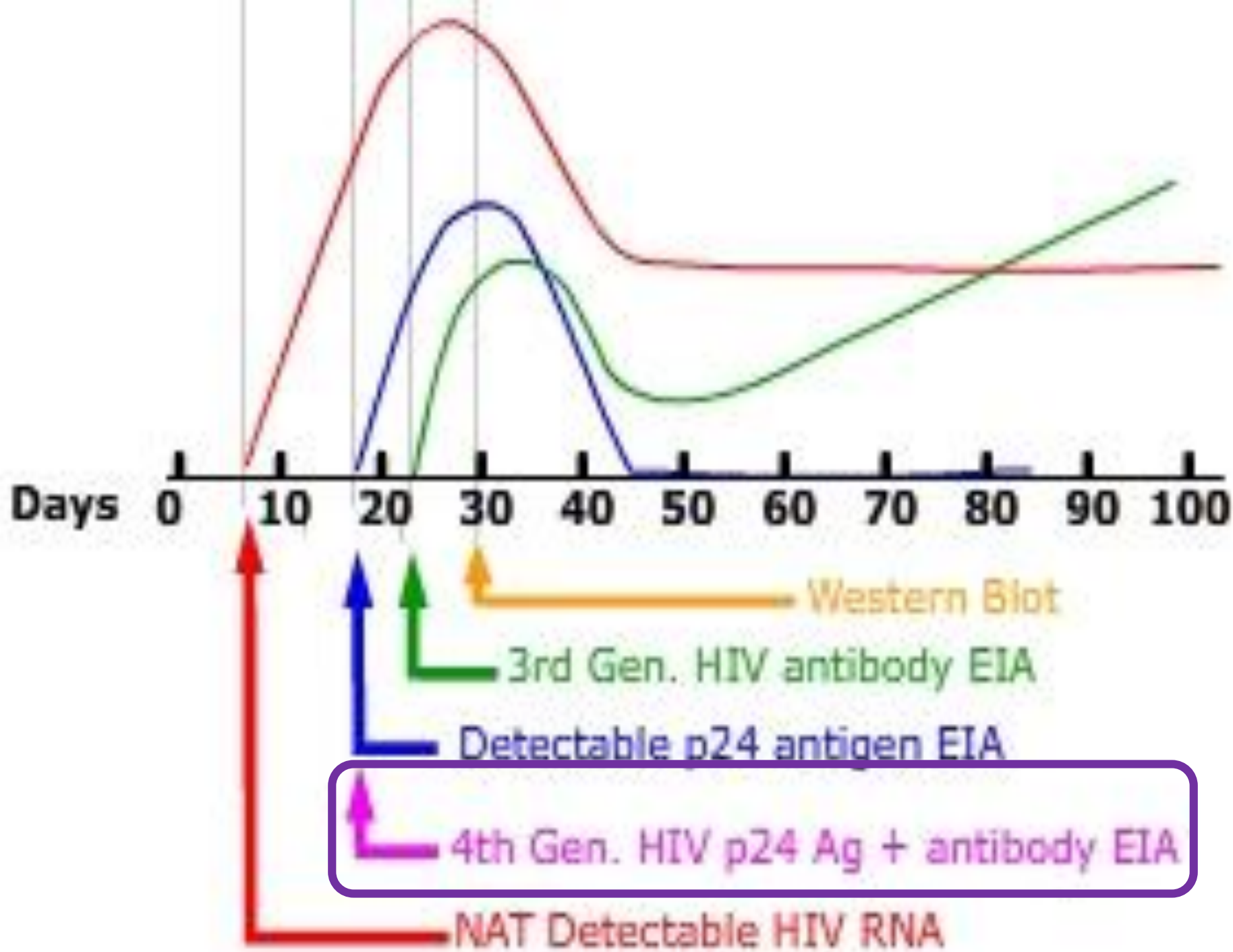
Laboratory changes in the course of HIV infection

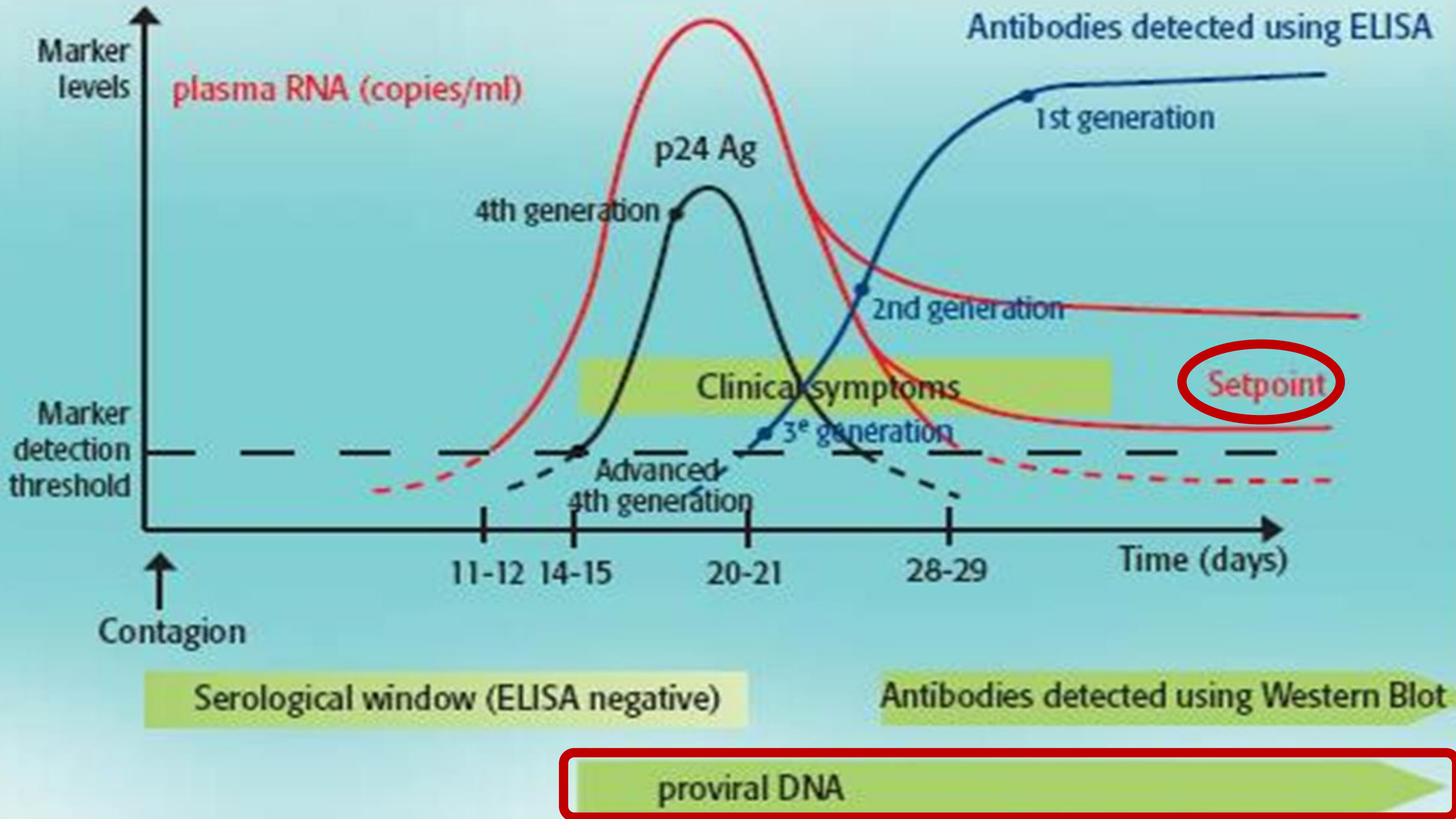


Type of laboratory tests

- ▶ **Detect antibodies**
- ▶ **Detect antigen**
- ▶ **Detect both antigen and antibodies**
- ▶ **Detect or monitor viral load**
- ▶ **Drug resistance**







ELISA

■ First generation:

- Purified HIV lysates
- Very low sensitivity and specificity

■ Second generation:

- Recombinant or synthetic peptides

■ Third generation:

- Sandwich ELISA, Ag or Ab conjugated

■ Fourth generation:

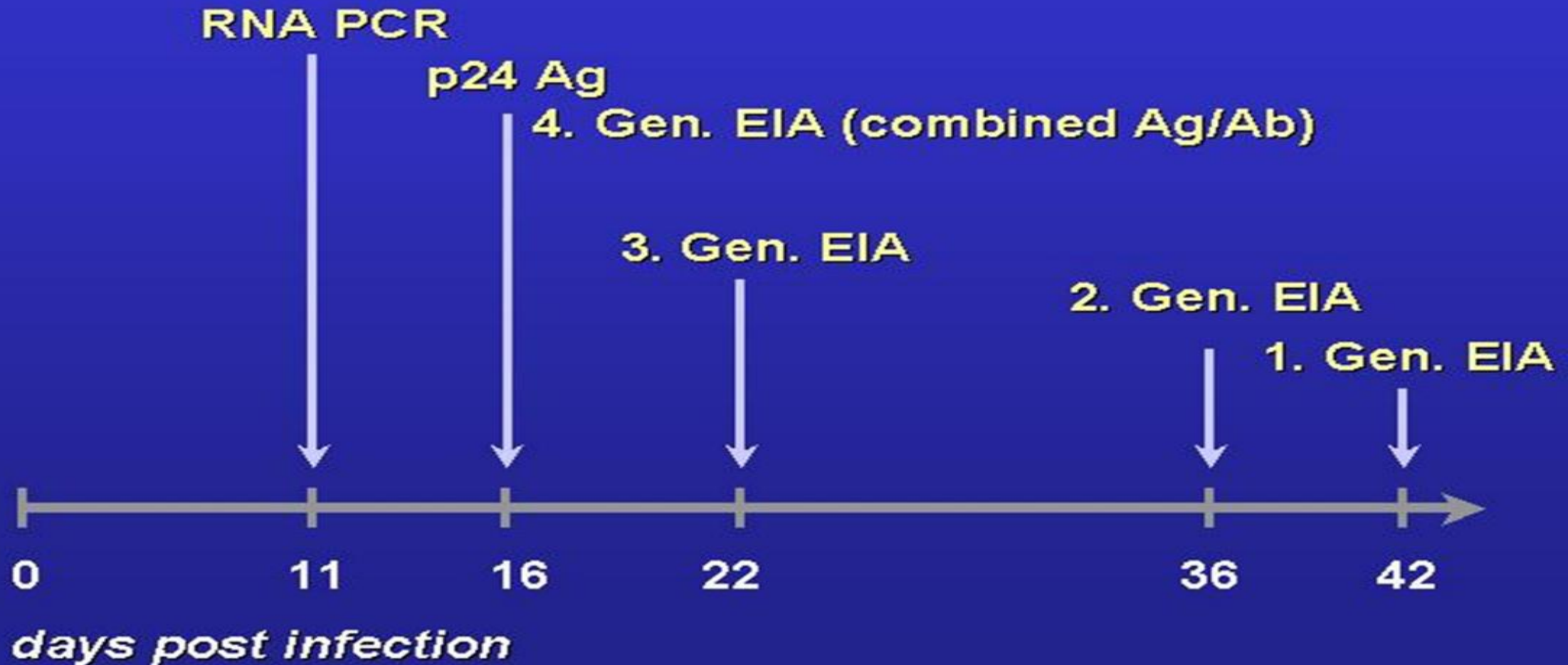
- Ag and Ab detection

ELISA

- ▶ **It is based on HIV antibody assay and is 98% to 100% sensitive, The specificity of this method is almost 99%.**
- ▶ **With the advancement of Elisa technique and in order to shorten the window period, 3rd and 4th generations of Elisa were developed.**
- ▶ **Third generation kits are capable of reacting to IgM. In fourth generation, the window period is shortened by adding P24 antigen (6 weeks)**

Time of HIV tests positivity based on days post infection

HIV infection: diagnostic window



False-Positive HIV Test Results

High HIV prevalence of 2%

10,000 tested; test specificity is 99.8%

True Positives
n=200

False Positives
n~20

91% True Positive (200/220)

Low HIV prevalence of 0.1%

10,000 tested; test specificity is 99.8%

True Positives
n=10

False Positives
n~20

33% True Positive (10/30)

NAAT (nucleic acid amplifications tests)

- ▶ **PCR**

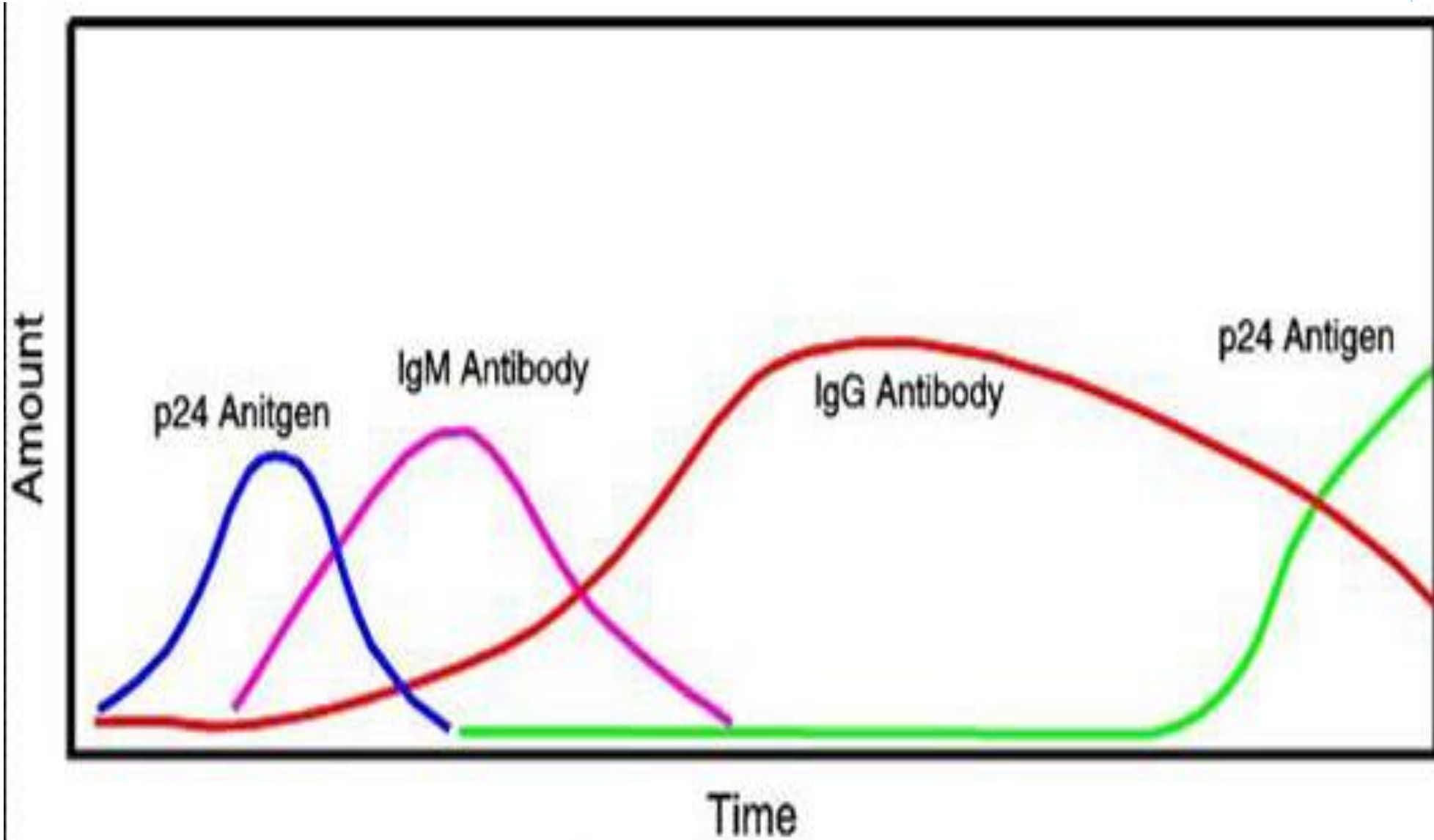
- ▶ **For early diagnosis and infant diagnosis**

- ▶ **Serodiscordant**

P24 Ag

- ▶ **The p24 Ag assay measures the viral capsid p24 protein in blood that is detectable earlier than HIV Ab during acute infection.**
- ▶ **P24 Ag characteristically appears early and late during infection.**
- ▶ **When Abs to HIV become detectable, P24 Ag is often no longer demonstrable, most likely due to Ag-Ab complexing in the blood.**

HIV Antigen Assay



Testing for P24 can be of value in:

- ▶ **Detecting early HIV infection**
- ▶ **Screening blood**

- ▶ **P24 Ag test must be preformed in addition to testing for HIV Ab.**

- ▶ **Combined assays for Ag and Ab detection can not substitute single Ag tests for blood donor screening.**
- ▶ **The detection limit of fourth-generation assays (20 to > 100 pg of p24 Ag/ml) is higher than that of Ag assays (3.5 to 10 pg of p24 Ag/ml) .**
- ▶ **Highly sensitive Ag assays detect primary infection on average 1 to 2 days earlier than fourth-generation enzyme immunoassays.**

HIV assays urine and saliva specimens

Advantages:

- ▶ Do not require a trained technician for specimen collection and processing.
- ▶ Do not require contact with possibly contaminated laboratory materials, e.g., used Needles or lancets that need biohazard waste facilities.
- ▶ A greater percentage of the target population may agree to be tested.

- ▶ **urine contains very low levels of IgG (1mg/l), and so very sensitive techniques are required to detect specific Ab.**
- ▶ **In most urine assay, HIV-1 gp160 coated.**
- ▶ **Oral fluid specimens consist mostly of saliva, which predominantly contains IgA class and oral mucosal transudates which mostly contain IgG, and so also have much lower level of IgG than serum (15mg/l)**

Rapid /simple assay

- ▶ **Rapid assays for detecting specific HIV antibody were developed in the late 1980s, and are defined as tests that can be performed in less than 30 minutes.**
- ▶ **Very useful tool for screening**

Nucleic Acid-Based HIV-1 Viral Load Assays

- ▶ Viral load refers to a quantitative measurement of the number of HIV-1 RNA copies in a patient's samples. The result is expressed as "HIV RNA copies per milliliter" of plasma, serum, or body fluids.
- ▶ Measuring levels of HIV-1 RNA provides a monitoring changes in viral load during therapy. It is usually performed at baseline and at regular intervals during treatment.

Nucleic Acid-Based HIV-1 Viral Load Assays

- ▶ High levels of HIV RNA are strongly predictive of clinical disease progression in HIV-infected patients.
- ▶ Reduction of HIV-1 RNA levels in response to initiation of antiretroviral therapy is associated with a delay in progression to AIDS and death and reduce transmission
- ▶ Currently ART could lead to undetectable VL, the result is:

Undetectable = Untransmissible

AIDSinfo, January 2022

- ▶ In the previous version of the guidelines, the Panel on Antiretroviral Guidelines for Adults and Adolescents suggested that an HIV RNA level of <10,000 copies/mL in a person suspected to have acute HIV may represent a false-positive test result.
- ▶ The section was updated to revise this threshold. The Panel noted that given the improved sensitivity and specificity of current HIV RNA tests in the presence of compatible symptoms or exposure history, even a low HIV RNA concentration (e.g., <3,000 copies/mL) in the setting of a negative or indeterminate HIV antibody test result may represent acute HIV.
- ▶ The Panel noted that, in rare cases, an HIV RNA <3,000 copies/mL may represent a false-positive quantitative test result. In that case, repeat testing should be done to confirm the diagnosis

Infant diagnosis

- ▶ Infants diagnosis is done by PCR (DNA or RNA)
- ▶ Serologic tests are not effective in diagnosis because of maternal Ab transmission
- ▶ The standard test for HIV infection in infants and children who are younger than 18 months of age is (PCR) testing.
- ▶ Repeat testing is necessary because HIV infection can occur perinatal and very recent infection will not be detectable.

HIV confirmation in infant

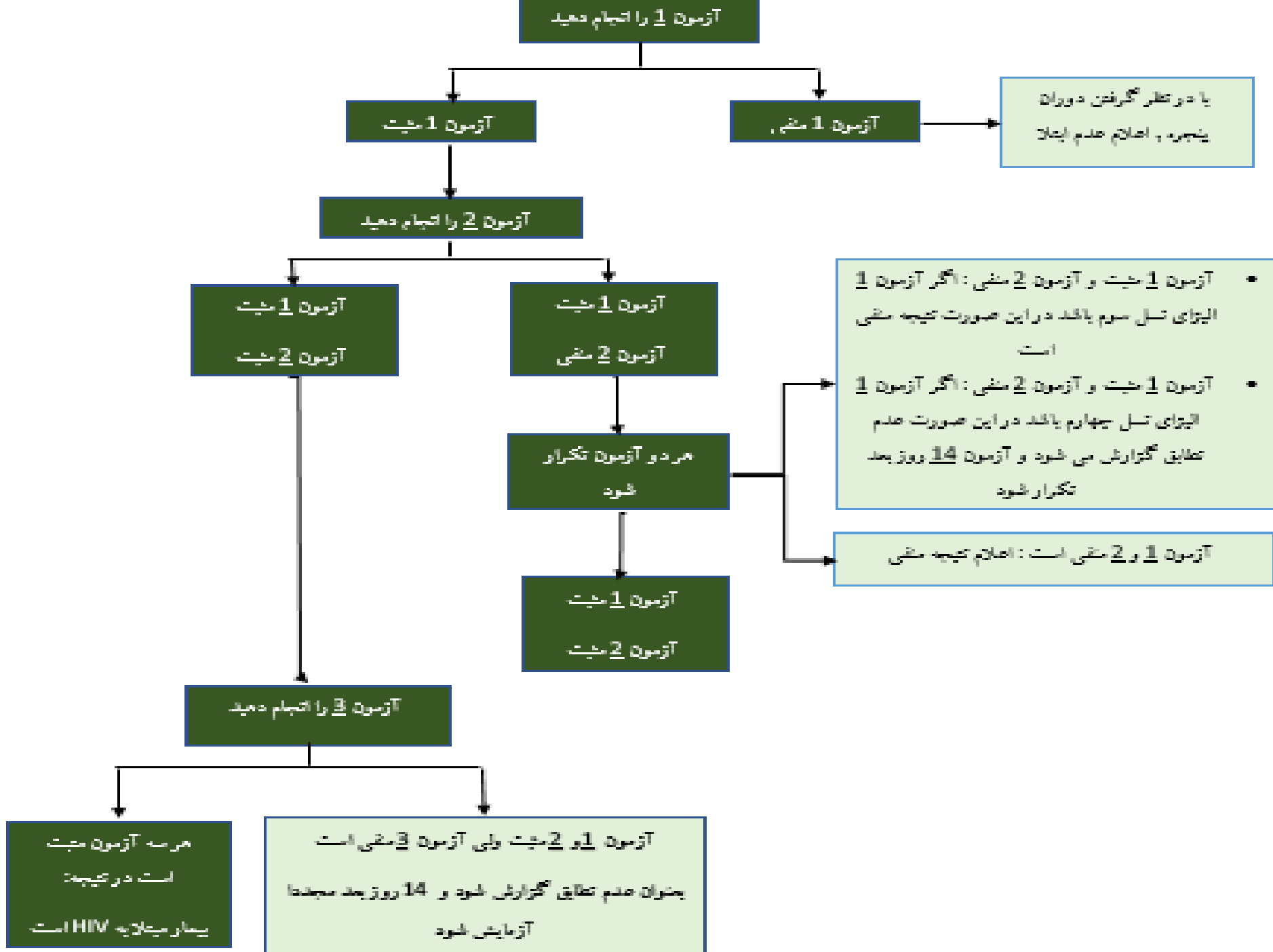
- ▶ **First test: during 48h after birth**
- ▶ **Second test: 8 weeks after birth**
- ▶ **Third test: 4-6 moths after birth**
- ▶ **2 consecutive positive test after 48h is necessary for confirming the diagnosis**
- ▶ **Two consecutive negative ELISA test after 6 moths indicates HIV negative**


False positive HIV ELISA

- ▶ Instrument error
- ▶ Personnel errors
- ▶ Autoimmune diseases
- ▶ Hyperimmunoglobulinemia
- ▶ Influenza vaccination
- ▶ Liver disease
- ▶ Frequent blood transfusions
- ▶ Heating to blood samples
- ▶ Participation of individuals in HIV-1 vaccine clinical trial trials
- ▶ *Covid-19 (disease, vaccination) ???*

False positive HIV ELISA

- ▶ Perform the test in the window period
- ▶ Instrument errors
- ▶ Personnel errors
- ▶ HIV-2 infection
- ▶ Severe immunosuppressive therapies
- ▶ Congenital Immunoglobulin deficiency
- ▶ Advanced stages of HIV infection
- ▶ People who have received PEP treatments show delayed antibody responses
- ▶ Certain types of HIV Subtype such as O if that test is not detectable.



- 
- ▶ Because there is a need for several testing steps in the HIV diagnosis process, each laboratory is now required to include the name of the kit and the test by which it assessed HIV status on the test results
 - ▶ This helps to standardize the diagnosis in the country and also to ensure that the kit is used in the correct stage.

Test 1

- ▶ The immunoassay tests listed in this algorithm for test 1 must have at least the following specifications:
 - ▶ Sensitivity is approximately equal to 100%
 - ▶ More than 99% Specificity
 - ▶ Ability to simultaneously identify HIV-1 and HIV-2

Test 2 & 3

- ▶ Test 2: One of the following immunoassays can be:
 - ▶ Fourth generation ELISA test
 - ▶ Third generation ELISA test

- ▶ Test 3: One of the following tests can be:
 - ▶ Fourth generation ELISA test
 - ▶ Third generation ELISA test

Window period

- ▶ The window period is 6 weeks if the first test is the fourth generation ELISA and 3 months if the first test is the third generation ELISA or rapid test.
- ▶ At present, according to the national instructions, it is necessary to perform tests 2 and 3 with the fourth generation ELISA.
 - ▶ In case of inconclusive result, repeat both test after 14
 - ▶ If repeated test results be inconclusive, NAAT test should be used for confirmation.
 - ▶ Western blot is practically removed from the tests and has no place.

- ▶ Blood sampling is different in the first and second samples, but the second and third tests should be performed on one sample and in one center.
- ▶ If the first test of the clients is positive, it is recommended to immediately connect to the Behavioral Diseases Counseling Centers to perform confirmatory tests and connect to the services, which will guide the patient to perform confirmatory tests while maintaining confidentiality and providing free services.

- ▶ Due to the importance of accuracy in performing tests, all other tests that have been performed in other laboratories or tests that have been used as a self-test and a positive result has been reported, are considered as **"zero test"** and from First, the diagnosis must be made based on the national diagnostic algorithm

آزمون صفر را انجام دهید

آزمون صفر منفی است

در صورتی که بیش از سه ماه از رفتار پرخطر گذشته باشد، نتیجه منفی قابل قبول است. توصیه می شود برای آشنایی بیشتر و دریافت خدمات پیشگیرانه به مراکز مشاوره مراجعه نمایید

آزمون صفر مثبت است

رد پای احتمالی از وجود بیماری HIV است و قطعیت ندارد. اما توصیه می شود هر چه سریعتر فرد برای انجام تست های تشخیصی تأییدی به مراکز مشاوره مراجعه نموده و در صورت تأیید تشخیص، درمان شروع شود

آزمون یک

ادامه مشابه الگو ریتم
تشخیص اچ آی وی

• **یک تست مثبت خود آزمون فقط یک رد پای اولیه از HIV است و باید با تست های معتبر تأیید شود**

Self Test

Self tests approaches

- ▶ "HIV self-test" can be presented to different audiences with two approaches. The difference between the two methods is in the amount and type of support that is provided to the audience during the test.
 - ▶ The first approach: "HIV self-examination" is supported
 - ▶ The second approach: "HIV self-examination" without support

- ▶ Viral RNA levels can transiently rise during acute illness, an outbreak of herpes simplex infection or vaccination against a variety of pathogens including influenza, pneumococcus, and tetanus.
- ▶ The increases may be quite dramatic, 1 log (tenfold) or greater; however, values usually return to baseline within one month.
- ▶ Thus, plasma HIV RNA levels should not be measured within one month of any of these events.

CD4 count

- ▶ The CD4 count is used in combination with the VL, to determine the staging and outlook of the disease.
- ▶ CD4+ T cell counts be measured at the time of diagnosis and generally every 3-6 months thereafter until the CD4 reach to >500 cell/ml in stable PLWH

CD4:CD8

- ▶ CD4:CD8 ratio is usually greater than 1 in immunocompetent individuals.
- ▶ However, in HIV infection, the CD4:CD8 ratio is less than 1.
 - ▶ This reflects increasing numbers of CD8+ T cells and depletion of CD4+T cells in chronic infection.
 - ▶ This ratio usually increases with the initiation of antiretroviral therapy (ART)

CD4 count is not a good prognostic factor (if alone)

- ▶ **Currently CD4 count is not a good tool**
- ▶ **Several situations can affect the CD4 count:**
 - ▶ **Different hours of the day**
 - ▶ **Fatigue**
 - ▶ **Viral infection**
 - ▶ **Medication**
 - ▶ **...**

- ▶ Modest decreases in the CD4 cell count have been noted in various acute infections (eg, CMV, EBV, HBV, TB, some bacterial infections, and histoplasmosis).
- ▶ Some data suggest that HCV coinfection is associated with a less robust immune recovery after initiation of ART compared with patients who are HCV seronegative

- ▶ Declines in absolute CD4 cell counts have been observed in HIV/HCV co-infected patients who are undergoing interferon therapy; however, CD4 T cell percentages usually remain the same or increase throughout HCV therapy.
- ▶ One dose of corticosteroids leads to a rapid and transient decline in the numbers of peripheral blood lymphocytes through a redistribution of circulating lymphocytes. This may lead to a profound drop in the absolute CD4 cell count

- ▶ With chronic administration, corticosteroids may lead to a leukocytosis; this may in turn lead to an elevated absolute CD4 cell count
- ▶ Alcohol use is associated with depression of CD4 cell counts, which improve after alcohol abstinence
- ▶ The mean absolute lymphocyte cell count, lymphocyte percentage, and absolute CD4+ cell count were significantly lower during pregnancy than during the 12 week post delivery period. In contrast, the mean absolute CD8 count was not significantly different

CD4 response

- ▶ With antiretroviral therapy and effective viral suppression, the expected CD4 cell response is an increase of 100 to 150 cells/mm at one year and an additional 20 to 50 cells/mm annually for the next three to five years .
- ▶ When ART is discontinued there is generally a rapid viral load rebound and sharp decline in CD4 count with a decrease of up to 100 to 150 cells/mL in three to four months

TLC

- ▶ The total lymphocyte count (TLC) is a crude surrogate for the CD4 cell count, but is advocated by the WHO in areas where flow cytometry may not be available.

- ▶ CD4 counts tend to be lower in the morning and higher in the evening
- ▶ Fatigue and stress can also affect test results
- ▶ Try to use the same lab each time, have your tests done at the same time of day each time
- ▶ Wait for at least a couple of weeks after an infection or vaccination before getting a CD count test

CD4 Count and percent

- ▶ A CD4 percentage that is greater than 29% usually means that your immune system is functioning normally (i.e., your CD4 count is roughly >500 cells/mm³).
- ▶ A CD4 percentage of 14%-28% typically means your CD4 count is in the range of 200-500 cells/mm³.
- ▶ When your CD4 count is below 200 cells/mm³, your CD4 percentage is likely to be below 14%.

HIV Drug resistance Tests

- ▶ The ability of HIV to mutate and reproduce itself in the presence of antiretroviral drugs is called HIV drug resistance.
- ▶ The consequences of drug resistance include treatment failure, increased direct and indirect health costs associated with the need to start more costly second-line treatment for patients, the spread of resistant strains of HIV and the need to develop new anti-HIV drugs.

Categories of HIV resistance

- ▶ HIV drug resistance may be **transmitted or acquired**. Drug resistance could develop if patients interrupt their treatment or do not take it according to prescription. This phenomenon is called acquired drug resistance.
- ▶ If people are infected by others who had HIV drug resistance, it is called transmitted drug resistance.













Accumulation of mutations

- ▶ These results suggest that if virological monitoring were to be made routinely available, treatment failure could be identified at an early stage and patients offered effective second-line treatment.
- ▶ Earlier start of a second-line regimen after failure of first-line options can help patients avoid accumulation of drug resistance mutations and preserve efficacy of their second-line treatment.

- ▶ Two concepts are important to an understanding of the development of drug resistance:
 - ▶ First, HIV infection is characterized by high levels of virus production and turnover (10^8 - 10^{10} per day)
 - ▶ Second, the viral population in an infected person is highly heterogeneous
- ▶ Under these circumstances, it is easy to understand why if any of these mutations can confer some selective advantage to the virus, such as a decrease in its susceptibility to an antiretroviral agent

Detected Mutations

NRTI	V75M, M184V, T215Y
NNRTI	K101E, K103N, P225H
Other	V179I









Drug name	Class	Assessment		
		<i>Stanford</i>		
abacavir (ABC)	NRTI	 Low-Level Resistance		
zidovudine (AZT)	NRTI	 Intermediate Resistance		
stavudine (D4T)	NRTI	 High-Level Resistance		
didanosine (DDI)	NRTI	 Intermediate Resistance		
emtricitabine (FTC)	NRTI	 High-Level Resistance		
lamivudine (3TC)	NRTI	 High-Level Resistance		
tenofovir (TDF)	NRTI	 Susceptible		
doravirine (DOR)	NNRTI	 Intermediate Resistance		
efavirenz (EFV)	NNRTI	 High-Level Resistance		
etravirine (ETR)	NNRTI	 Low-Level Resistance		
nevirapine (NVP)	NNRTI	 High-Level Resistance		
rilpivirine (RPV)	NNRTI	 Intermediate Resistance		

Protease

This region was sequenced successfully and covers codons 1 - 99

Detected Mutations

Major	None
Accessory	None
Other	K20R, M36I, H69K, L89M





Drug name	Class	Assessment		
		<i>Stanford</i>		
atazanavir/r (ATV/r)	PI	 Susceptible		
darunavir/r (DRV/r)	PI	 Susceptible		
fosamprenavir/r (FPV/r)	PI	 Susceptible		
indinavir/r (IDV/r)	PI	 Susceptible		
lopinavir/r (LPV/r)	PI	 Susceptible		
nelfinavir (NFV)	PI	 Susceptible		
saquinavir/r (SQV/r)	PI	 Susceptible		
tipranavir/r (TPV/r)	PI	 Susceptible		

Integrase

This region was sequenced successfully and covers codons 1 - 288

Detected Mutations

Major	None
Accessory	None
Other	None

Drug name	Class	Assessment		
		<i>Stanford</i>		
bictegravir (BIC)	INSTI	 Susceptible		
dolutegravir (DTG)	INSTI	 Susceptible		
elvitegravir (EVG)	INSTI	 Susceptible		
raltegravir (RAL)	INSTI	 Susceptible		

Comments

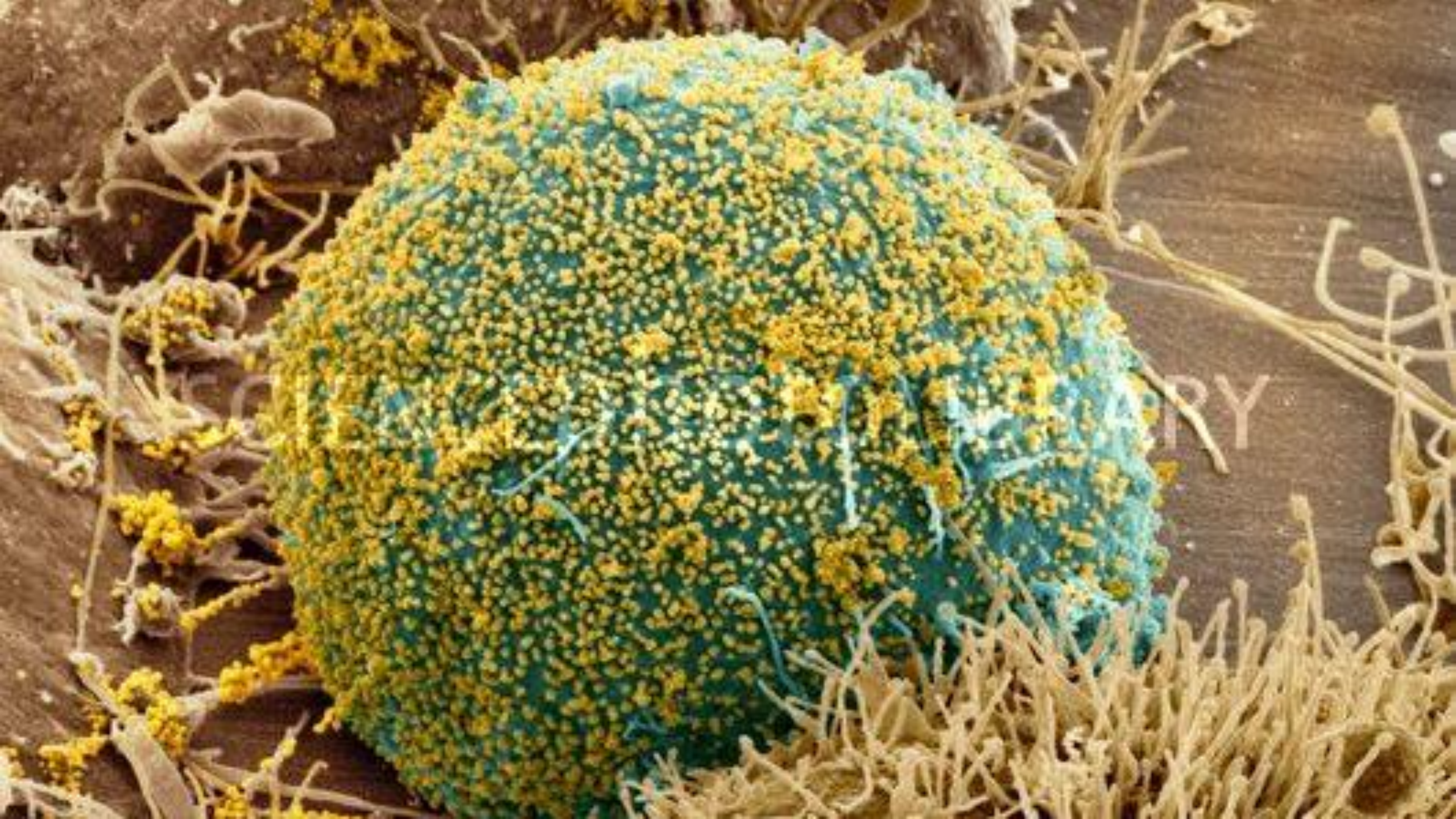
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