HIV Diagnosis

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Objectives

1. Understand the distinguishment between HIV and AIDS
2. Describe the pathophysiology of HIV infection and disease progression
3. Discuss screening and diagnostic tests for HIV

Case Study

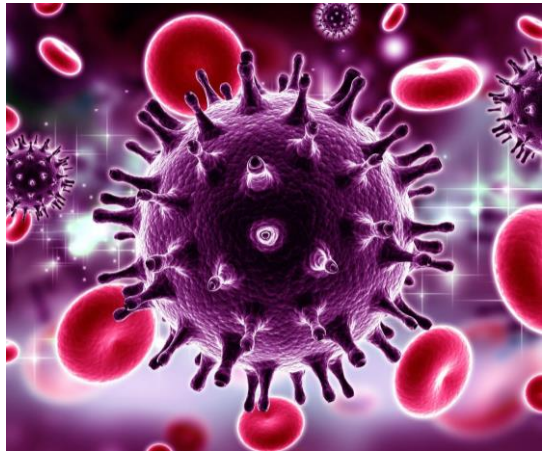
A 30-year-old female patient had been well until approximately 6 days earlier, when she began to have a vaginal discharge, followed by headache, fatigue, sore throat, and anorexia.

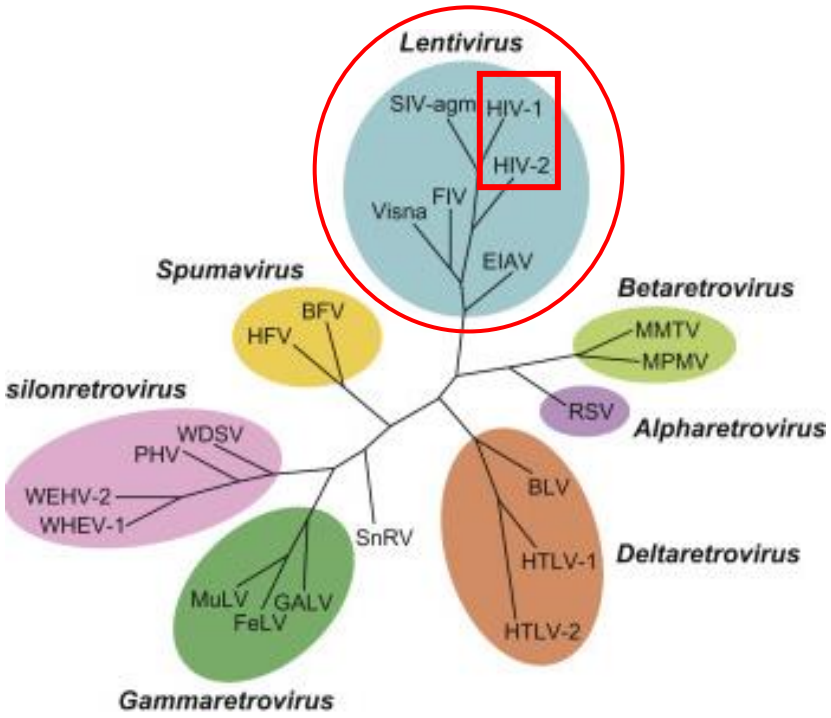
On the fifth day of symptoms, she went to an outpatient clinic in an urban area. On examination, she had a vaginal discharge; the temperature and the remainder of the examination were reportedly normal.

Patient has had multiple sexual partners and hasn't always used protection (condoms) for intercourse. Patient has a history of STDs (gonorrhea, syphilis) that has previously been treated. Lab screen for gonorrhea and syphilis *is negative*.

What is HIV?

- Human Immunodeficiency Virus
- Family: Retrovirus
- Subfamily: Orthoretrovirinae
  - reverse transcriptase dependent
- Genus: Lentiviruses (slow viruses)
  - distributed worldwide
  - hosted in several mammals
- HIV is transmitted via the exchange of a variety of body fluids from infected people
  - E.g., blood, breast milk, semen and vaginal secretions

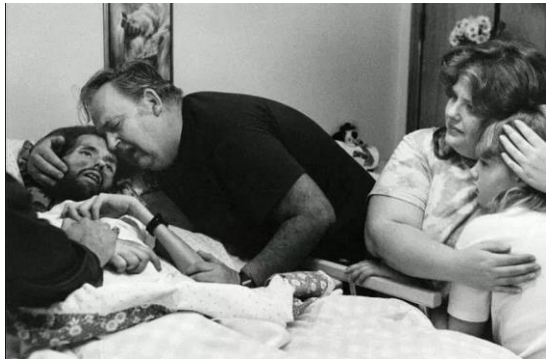


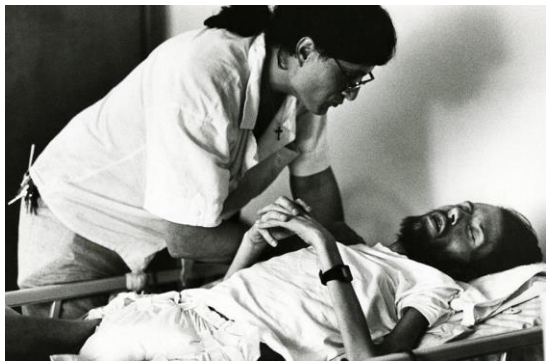


Fenner's Veterinary Virology, Editor(s): N. James MacLachlan, Edward J. Dubovi,Chapter 14 - Retroviridae,5th edition,Academic Press, 2017, p269-297.
https://www.aidsmap.com/about-hiv/hiv-lifecycle

What is HIV-AIDS?

- No verified cure for HIV
- Treatments available
- **AIDS** - Acquired Immunodeficiency Syndrome
- Marked by damage to the immune system
  - » Opportunistic infections:
    - e.g., TB, Candidiasis, Pneumocystis pneumonia, Cytomegalovirus
  - » Cancers: e.g., Kaposi's sarcoma, Lymphomas
  - » Wasting syndrome

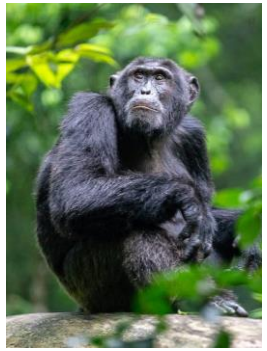




https://www.bio-rad.com/en-us/product/bioplex-2200-hiv-ag-ab?ID=NQD4K915
https://allthatsinteresting.com/david-kirby

HIV : Types

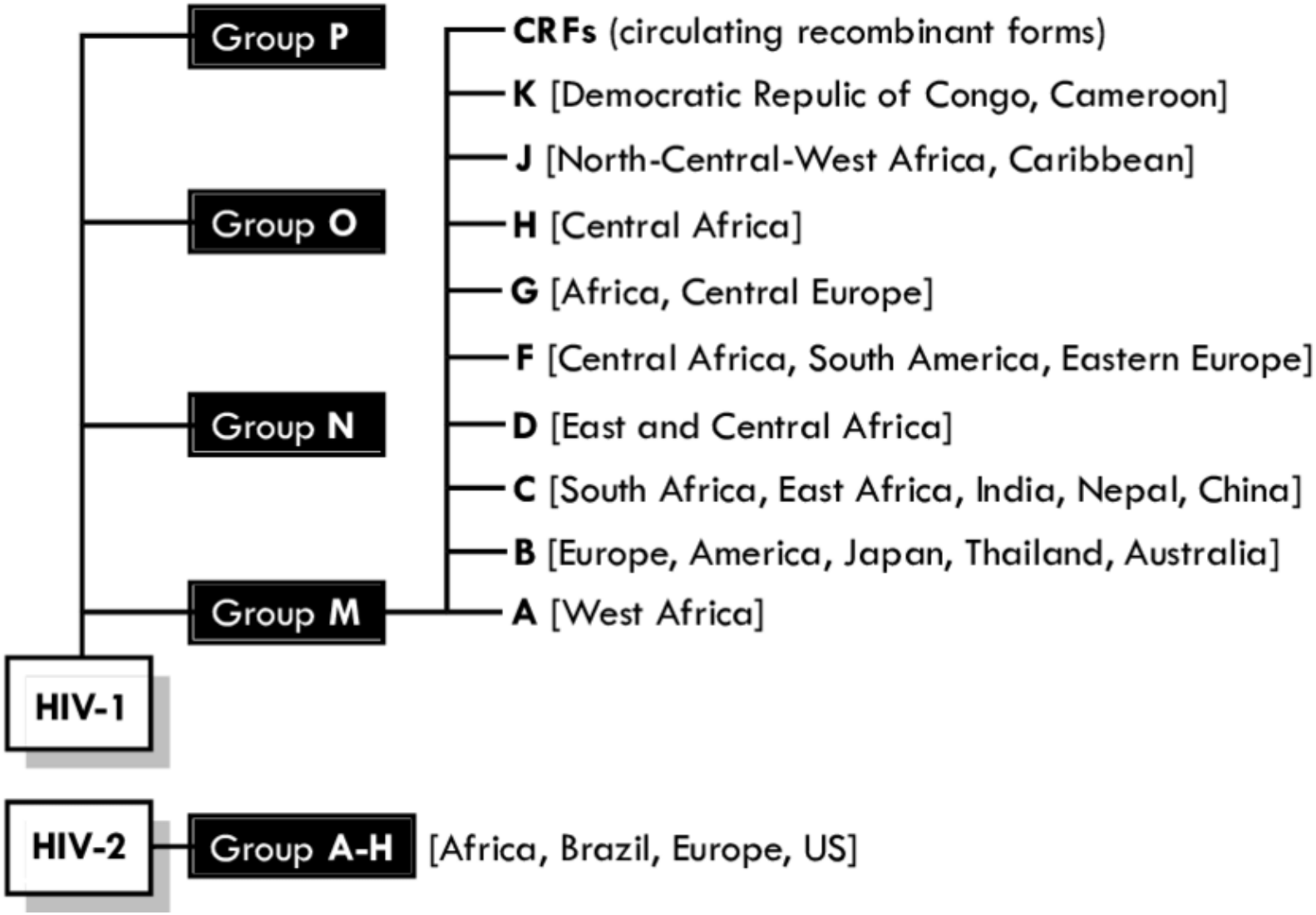
HIV Type-1	HIV Type-2
More common and with a global distribution	Predominant in West Africa
Faster progression to AIDS	Slower progression to AIDS
Higher plasma viral loads	Lower Plasma viral loads



Chimpanzees

Sooty mangabeys

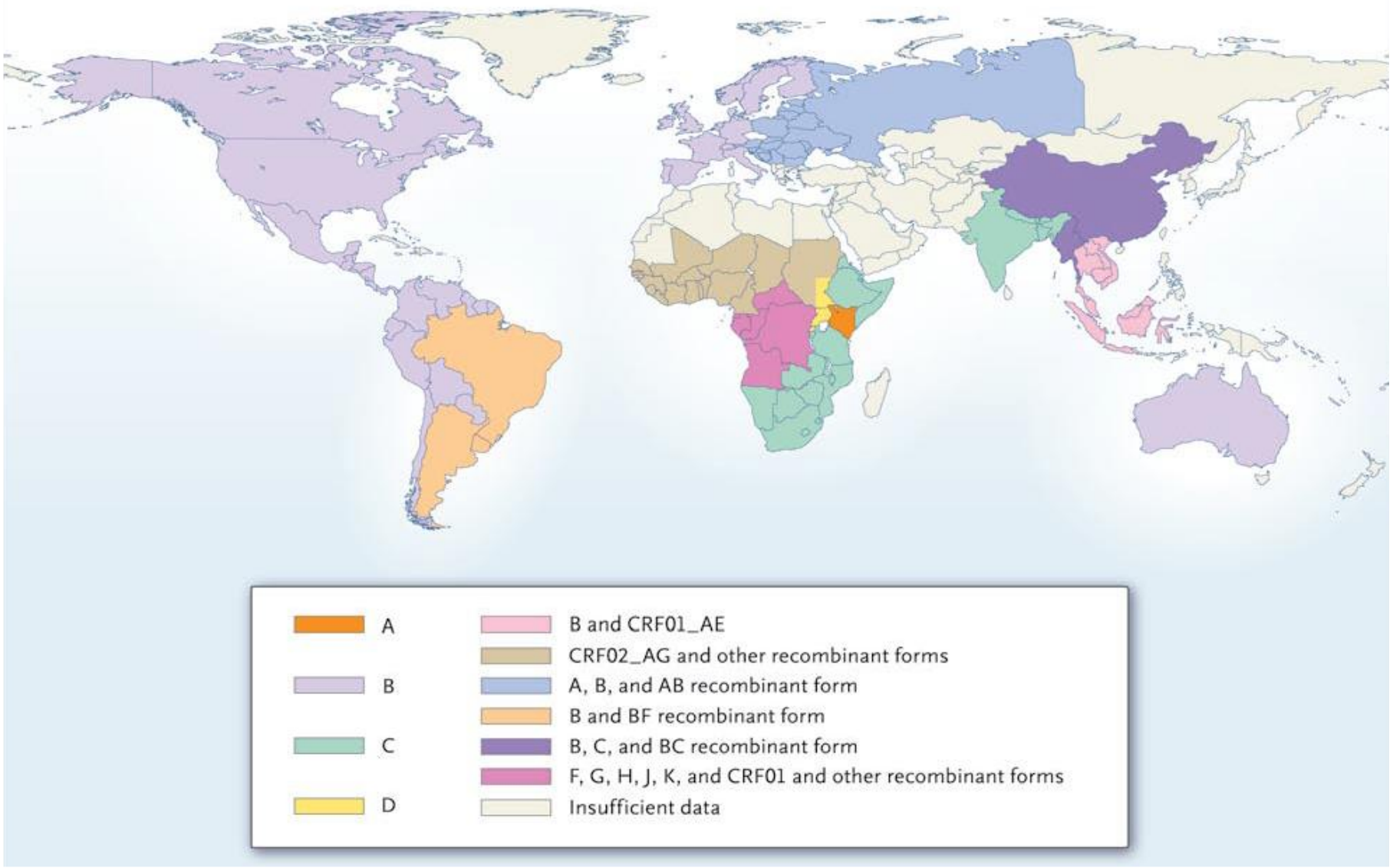
HIV : Global Epidemiology



Mai, Hung. (2015). Synthesis of N-Hydroxypyrazinones as Potential HIV Inhibitors. PhD Thesis - Katholieke Universiteit Leuven, DOI: 10.13140/RG.2.1.1098.7048

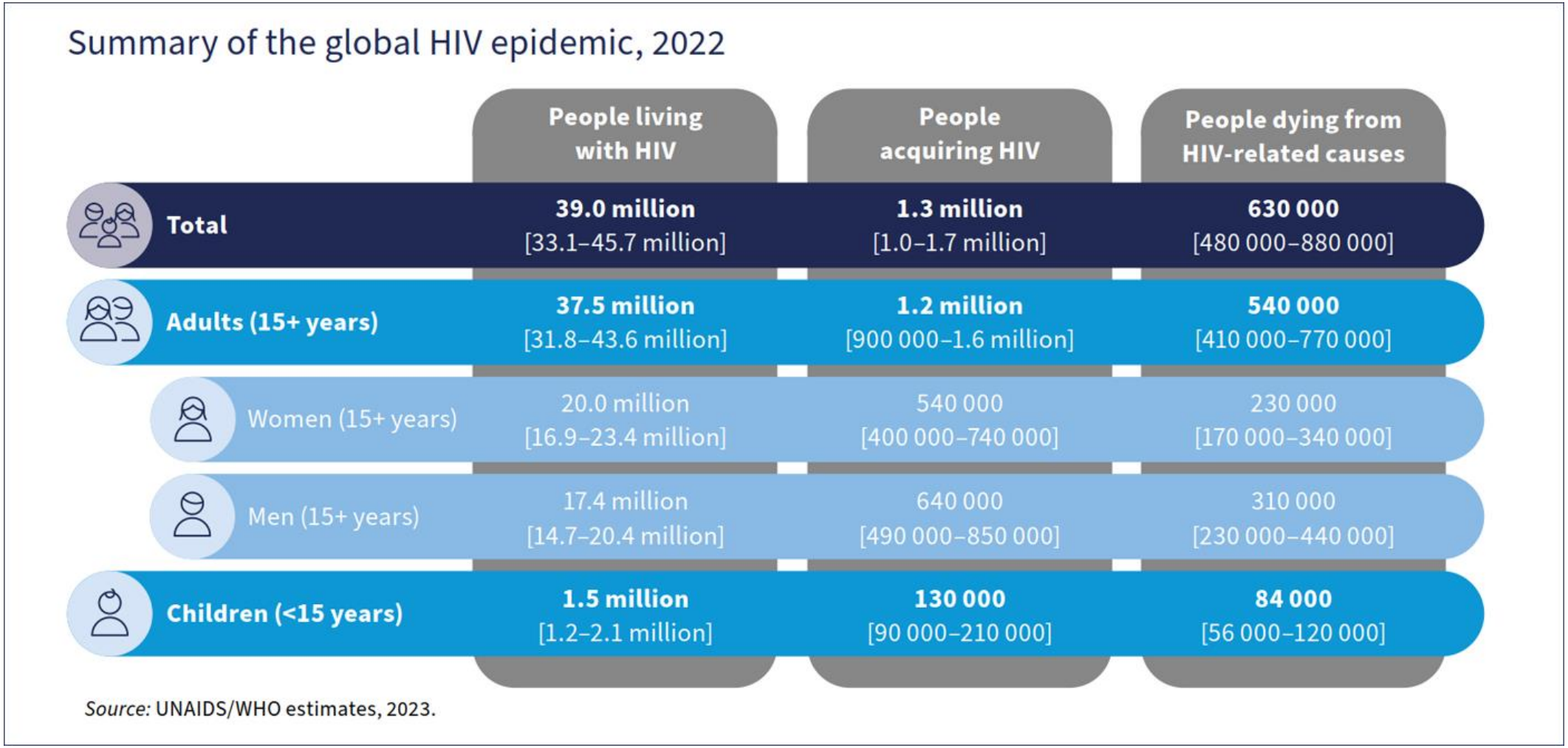
HIV : Global Epidemiology

Current Global Distribution of HIV-1 Subtypes and Recombinant Forms.



N Engl J Med 2008;358:1590-602.

HIV : Global Epidemiology



Summary of the global HIV epidemic, 2022

	People living with HIV	People acquiring HIV	People dying from HIV-related causes
Total	**39.0 million** [33.1–45.7 million]	**1.3 million** [1.0–1.7 million]	**630 000** [480 000–880 000]
Adults (15+ years)	**37.5 million** [31.8–43.6 million]	**1.2 million** [900 000–1.6 million]	**540 000** [410 000–770 000]
Women (15+ years)	20.0 million [16.9–23.4 million]	540 000 [400 000–740 000]	230 000 [170 000–340 000]
Men (15+ years)	17.4 million [14.7–20.4 million]	640 000 [490 000–850 000]	310 000 [230 000–440 000]
Children (<15 years)	**1.5 million** [1.2–2.1 million]	**130 000** [90 000–210 000]	**84 000** [56 000–120 000]

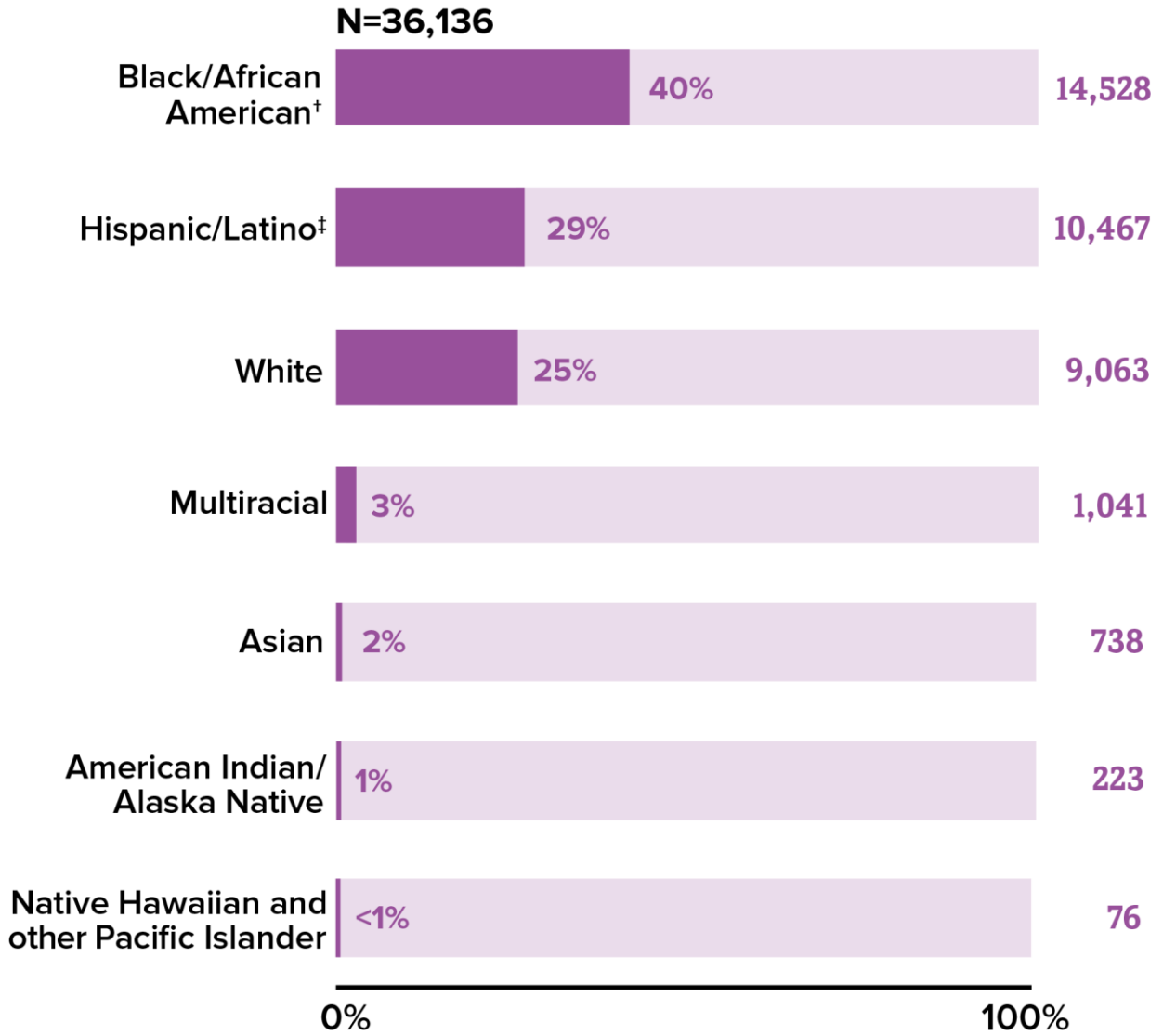
Source: UNAIDS/WHO estimates, 2023.

- ✓ ~ 85.6 million infected since the start of the epidemic
- ✓ ~ 40.4 million AIDS-related deaths since the start of the epidemic

HIV : Epidemiology

USA Statistics

- ✓1,189,700 people had HIV at the end of 2019
- ✓87% knew they had HIV
- ✓30,635 new cases in 2020

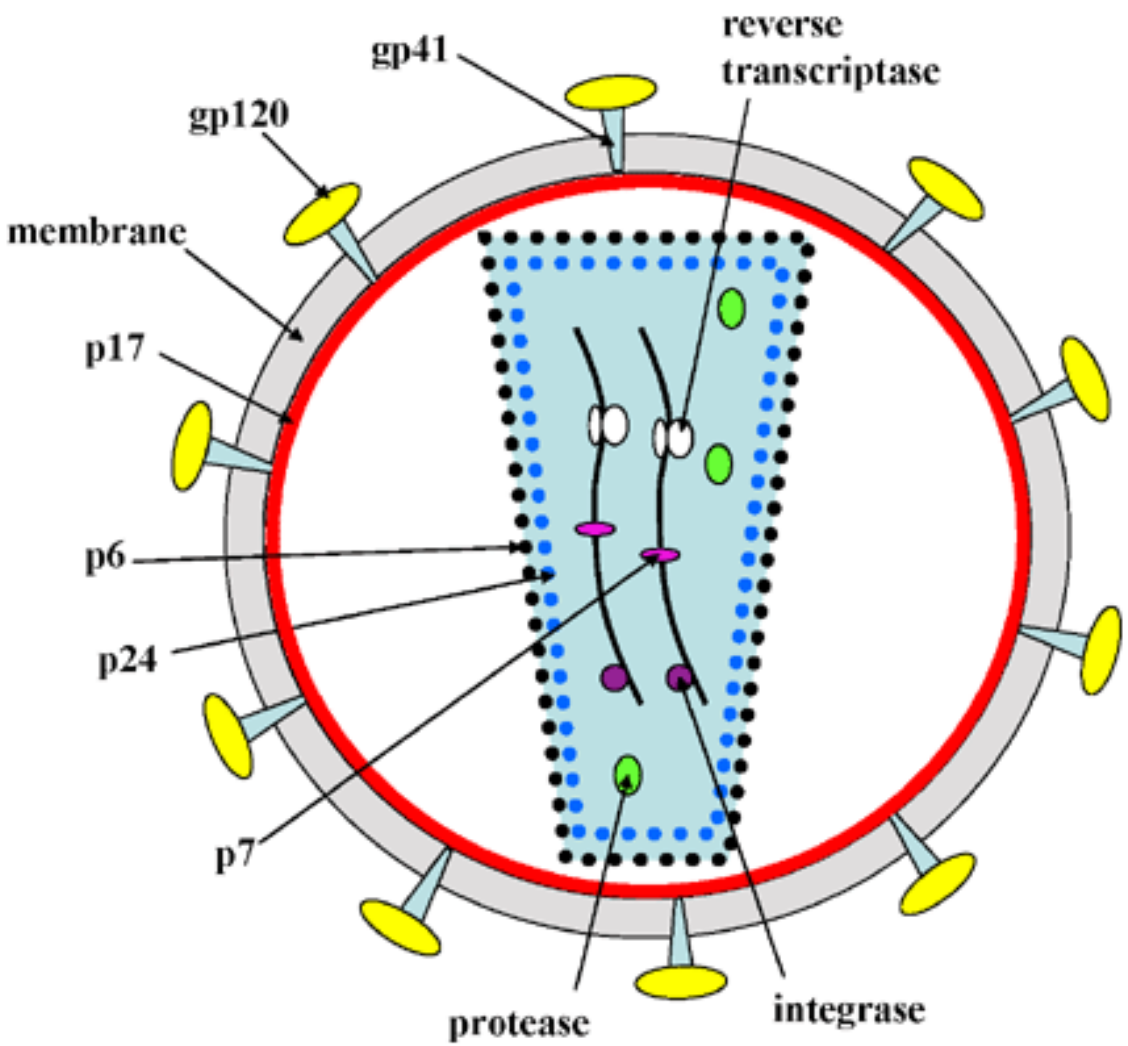


* Rates are per 100,000 people.

† Among adults, adolescents, and children under the age of 13. Hispanic/Latino people can be of any race

N Engl J Med 2008;358:1590-602.
https://www.cdc.gov/hiv/statistics/overview/diagnoses.html
https://www.cdc.gov/hiv/library/reports/hiv-surveillance/vol-33/index.html

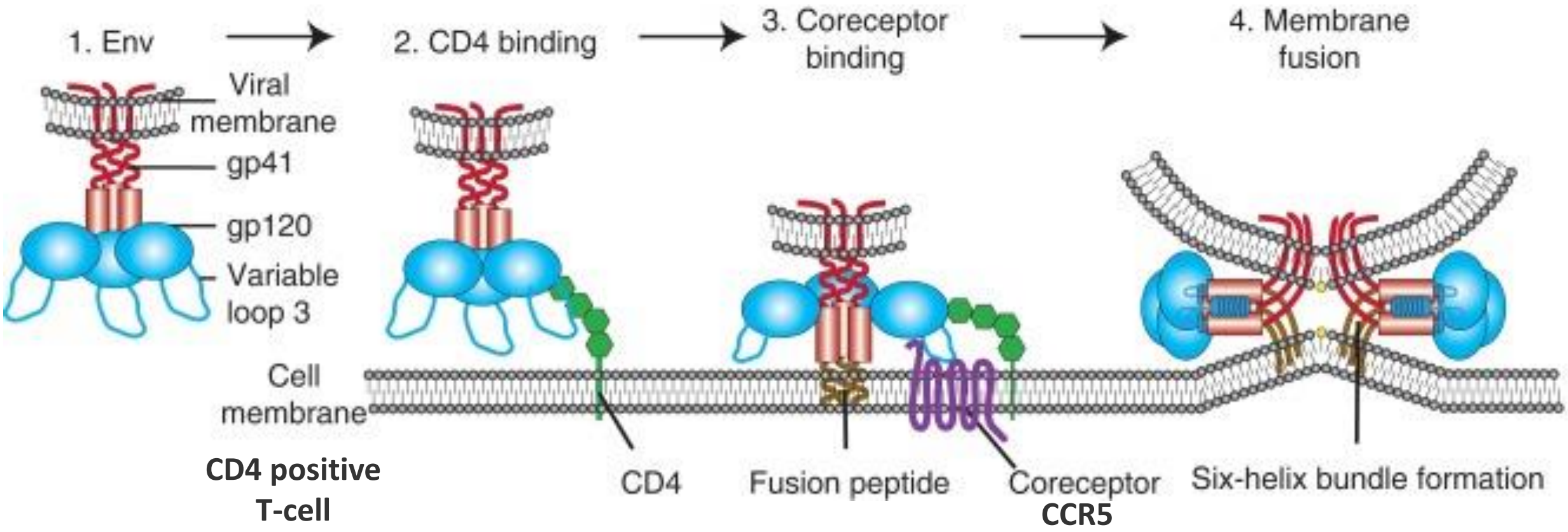
HIV : Structure



Key components

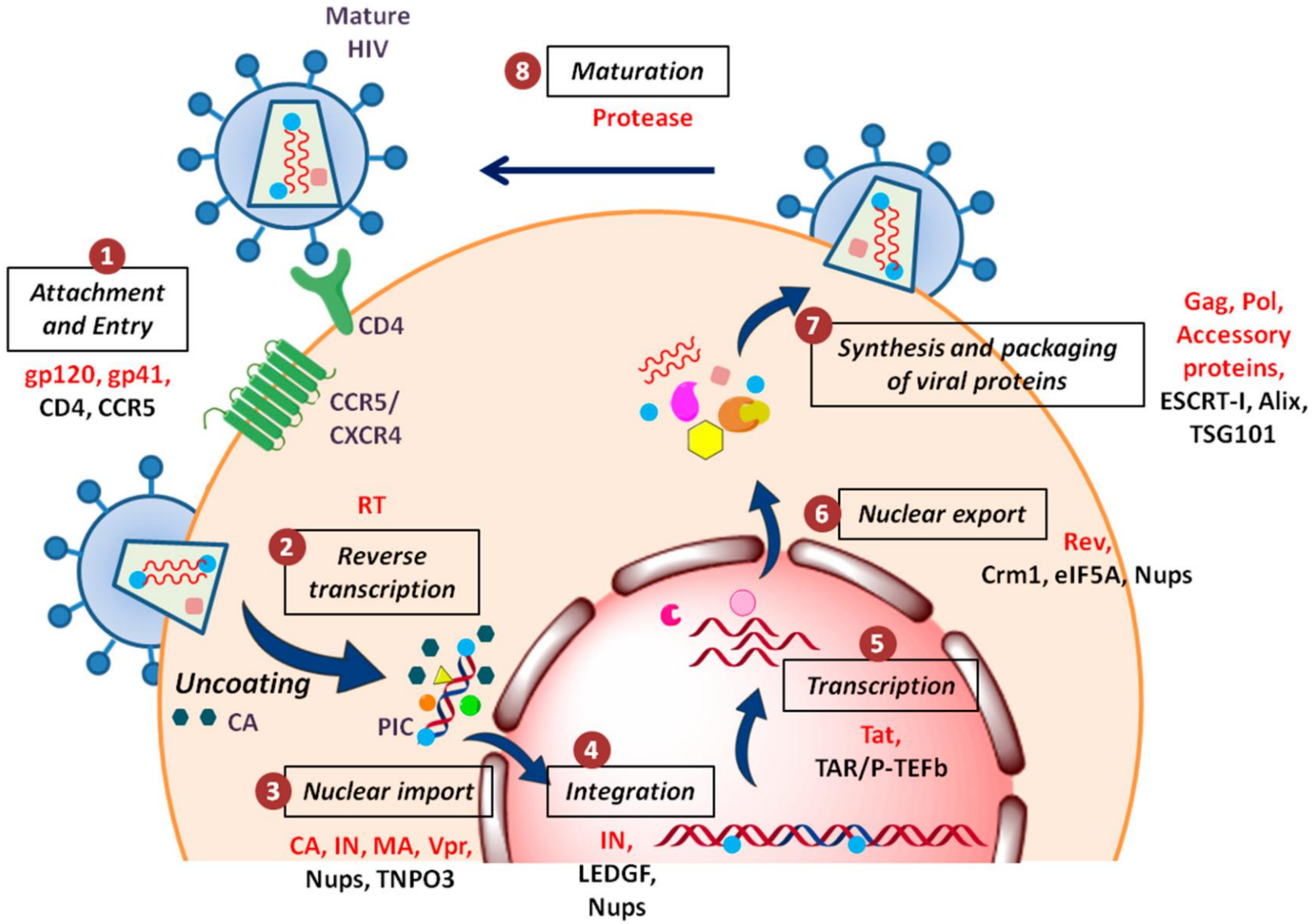
- gp120
- gp41
- p24
- Reverse transcriptase
- Integrase
- Protease

HIV : Entry into host cells



https://en.wikipedia.org/wiki/HIV

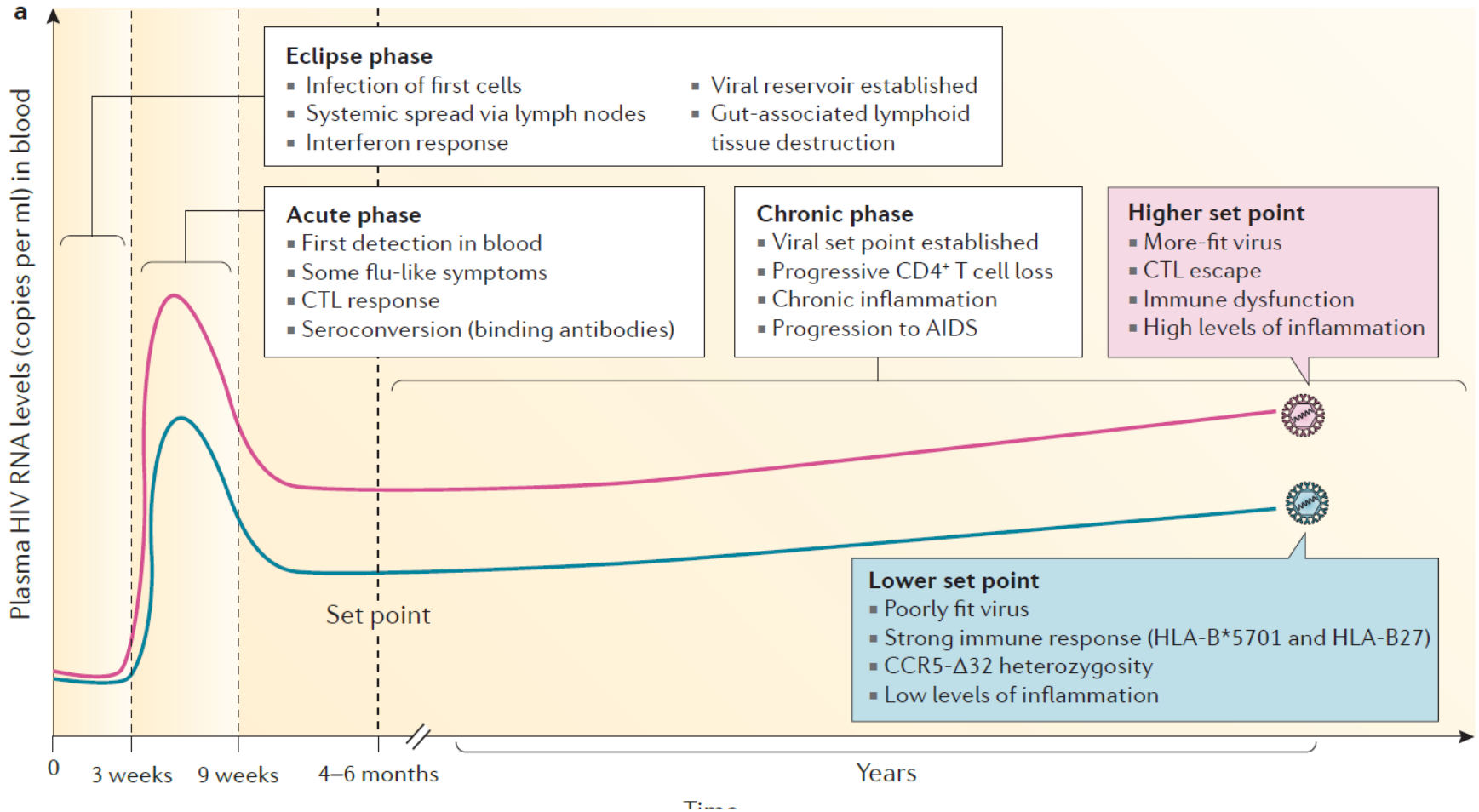
HIV : Replication



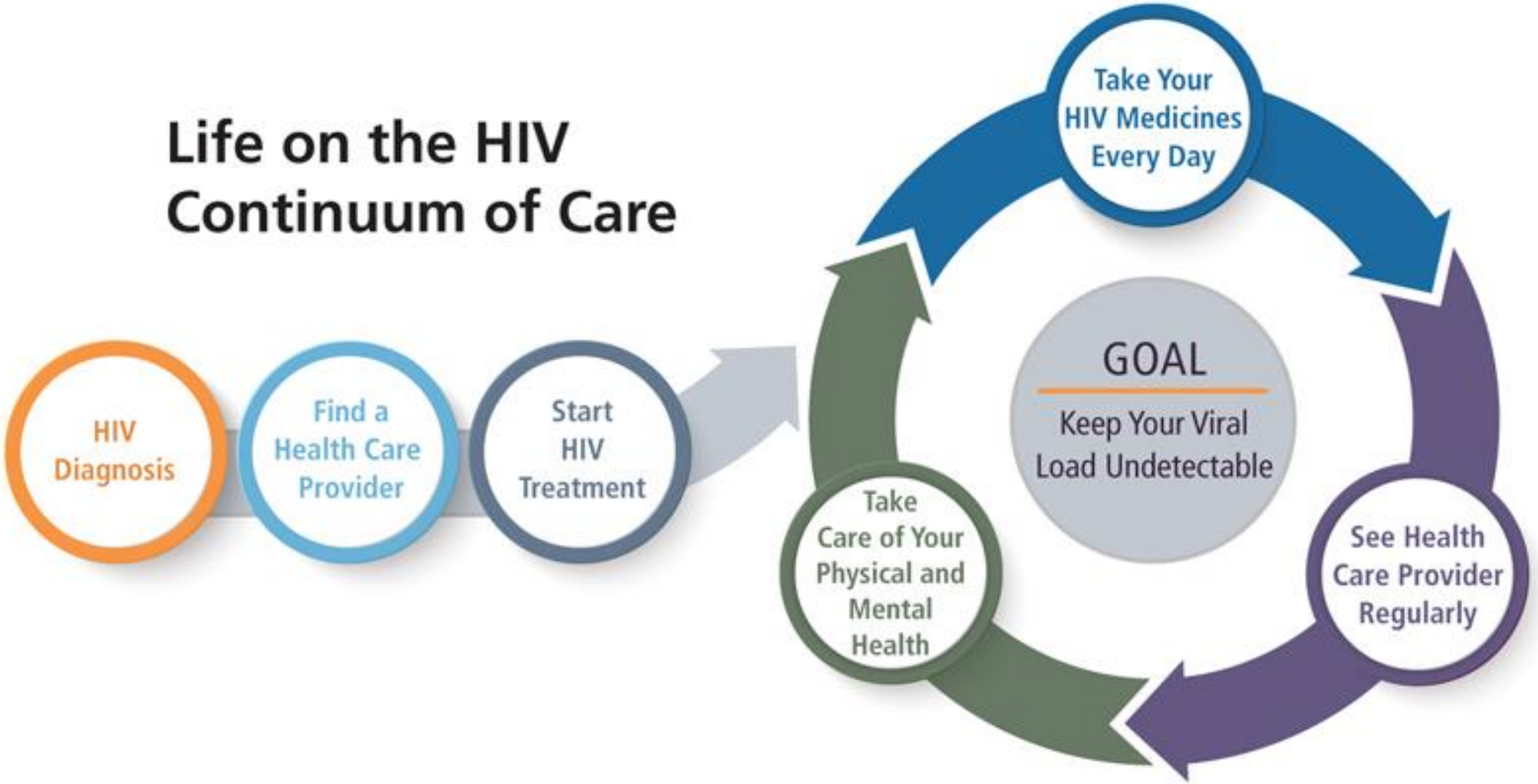
HIV : Infection timeline

Acute Phase Symptoms

- Fever
- Lymphadenopathy
- Rash
- Myalgias
- Malaise
- Rarely: meningitis



HIV: Care Cascade



https://clinicalinfo.hiv.gov/en/glossary/hiv-continuum-care

HIV : Screening Guidelines

Guideline Recommendations for HIV Screening	
CDC[a] (endorsed by AAFP, ACOG, ACP, IDSA)	All individuals 13-64 yrs of age (testing recommended at least once) Patients at high risk for HIV (annual screening recommended) Pregnant individuals (routine prenatal screening recommended)
USPSTF[b]	All individuals 15-65 yrs of age Individuals <15 yrs or >65 yrs at increased risk of infection Pregnant individual, including untested individuals who present in labor with unknown HIV status

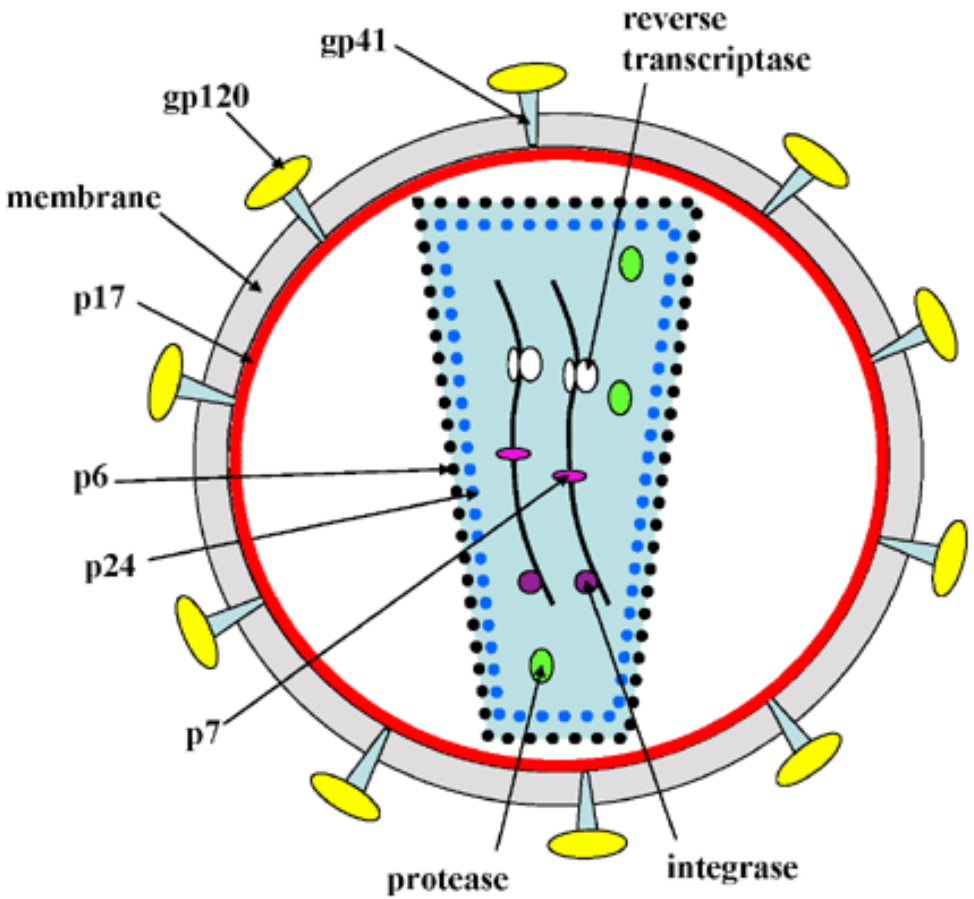
[a]Recommendations issued initially in 2006 and current in 2018.

[b]Recommendations issued in 2019.

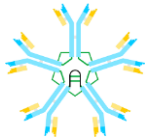
AAFP, American Academy of Family Physicians; ACOG, American College of Obstetricians and Gynecologists; ACP, American College of Physicians; IDSA, Infectious Diseases Society of America; USPSTF, U.S. Preventive Services Task Force

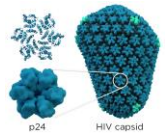
Sources: CDC, 2018[2]; USPSTF, 2019[10]; ACOG, 2014[11]; ACOG, 2018[12]

HIV : Diagnosis



Targets

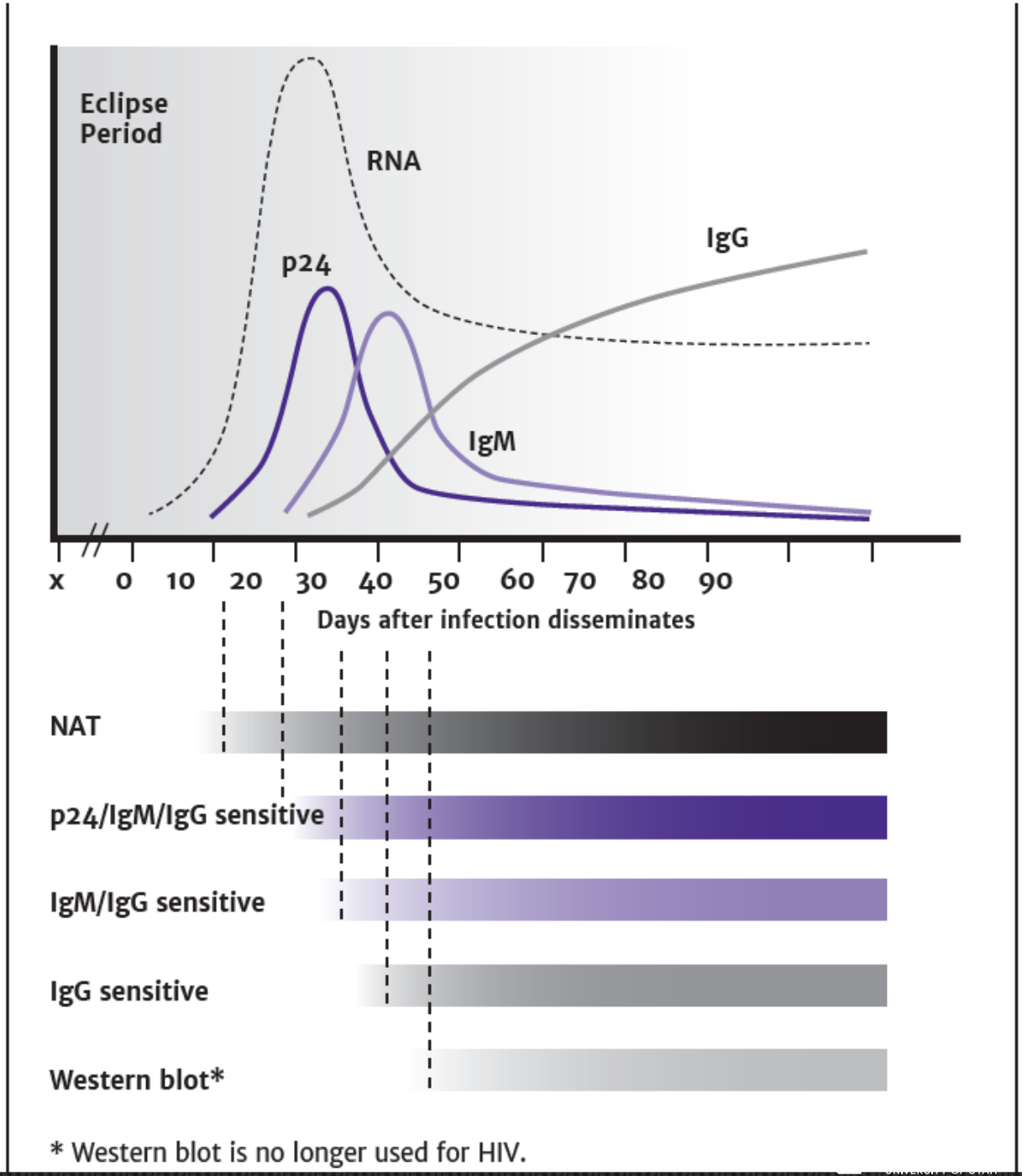
- Anti-HIV IgM 
- Anti-HIV IgG 

- Viral proteins e.g., p24 

- Viral RNA 

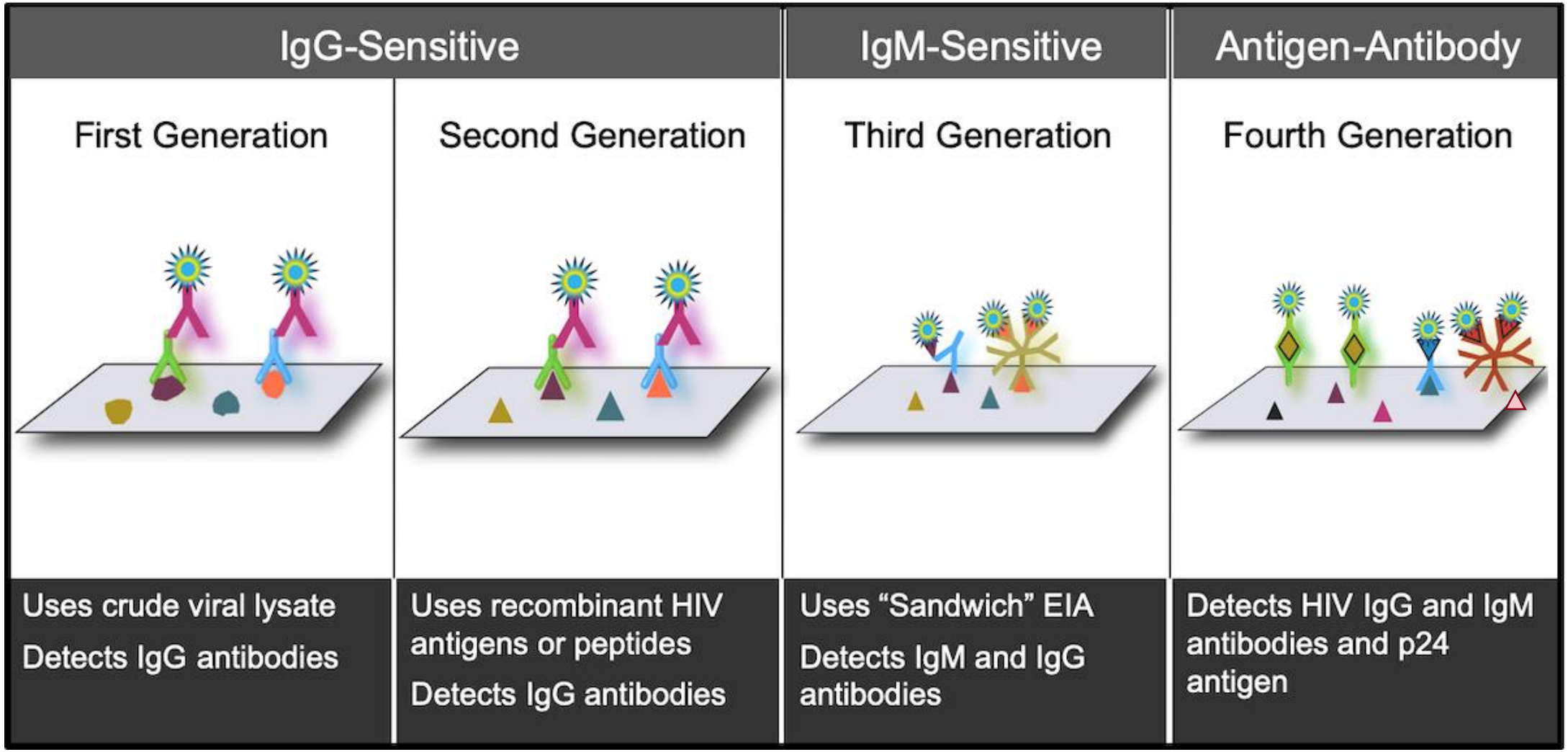
HIV : Diagnosis

- Antibody and protein marker concentrations vary over the course of infection
- Biomarker distribution over time must be considered during test selection



https://www.hivguidelines.org/hiv-testing-acute-infection/acute-hiv/#tab_1

Generations of HIV tests



Target

HIV-1 IgG

Targets

HIV-1 IgG
HIV-2 IgG

Targets

HIV-1 IgM and IgG
HIV-2 IgM and IgG
HIV-1 O IgM and IgG

Targets

HIV-1 IgM and IgG
HIV-2 IgM and IgG
HIV-1 O IgM and IgG

HIV assays over the last 30 years

Generation	1st	2nd	3rd	4th	5th
Antigen (Ag) Source	Virus Infected Cell Lysate	Lysate & Recombinant	Recombinant & Synthetic peptides	Recombinant & Synthetic peptides	Recombinant & Synthetic peptides
Specificity	95-98%	>99%	>99.5%	99.5%	99.5%
Sensitivity	99%	>99.5%	>99.5%	>99.8%	100%
Negative Window	8-10 weeks	4-6 weeks	2-3 weeks	2 weeks	2 weeks
Detects Antibody (Ab) and Ag	IgG Anti HIV-1	IgG anti HIV-1 and IgG anti HIV-2	IgG and IgM anti HIV-1, HIV-2 and Group O	IgG and IgM anti HIV-1, HIV-2 and Group O. Also detects HIV-1 p24 Ag	IgG and IgM anti HIV-1, HIV-2 and Group O. Also detects HIV-1 p24 Ag
Results	Single result	Single result	Single result	Single result; does not differentiate Ab from Ag positivity	Separate HIV-1 and HIV 2 Ab and Ag results
Confirming Tests	HIV-1 western blot (WB) or immunofluorescence (IFA)	HIV-1 WB or IFA, HIV-2 ELISA and WB if HIV-1 confirm is negative	HIV-1 WB or IFA, HIV-2 ELISA and WB if HIV-1 confirm is negative	HIV-1.2 differentiation Assay followed by qualitative HIV-1 RNA PCR if differentiation assay is negative	HIV-1.2 differentiation assay followed by HIV-1 NAAT

Alexander TS, *Clinical and Vaccine Immunology* **2016**, 23(4), 249-253

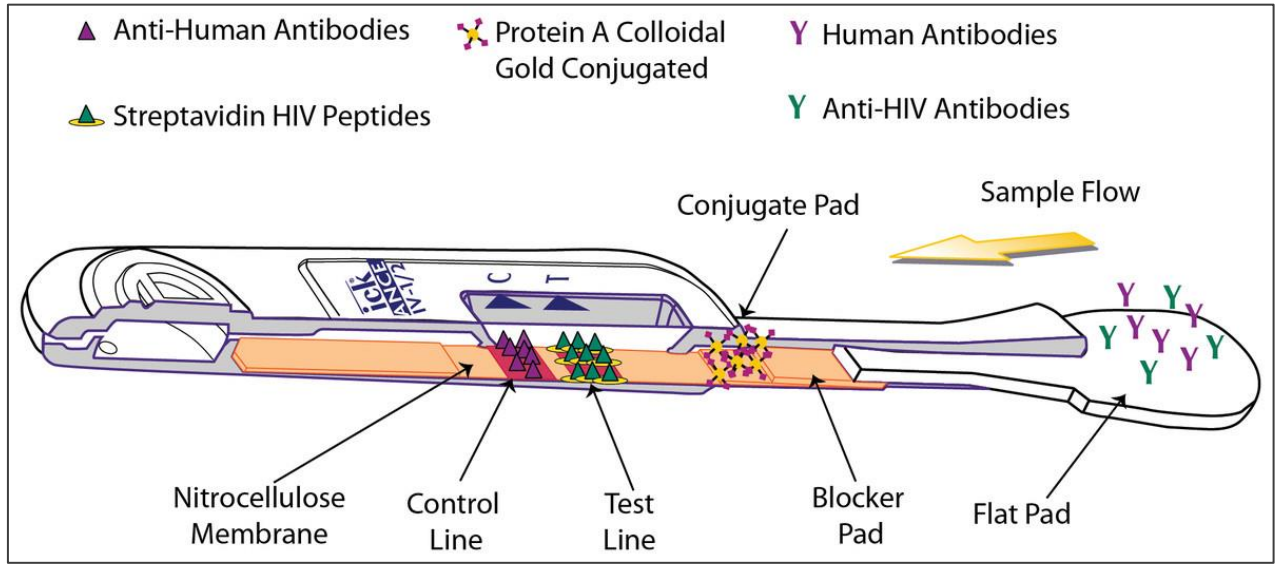
HIV Diagnosis: Rapid tests

IgG IgM

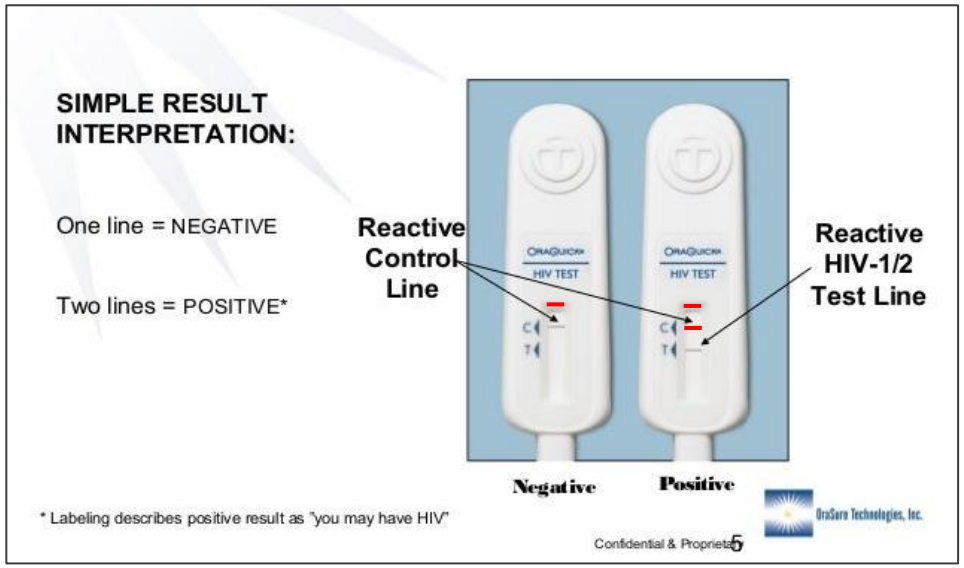
ORAQUICK® HIV SELF-TEST

3rd Generation

Assay Principle



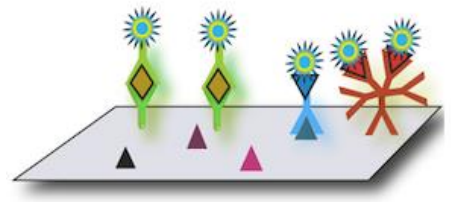
Results interpretation



https://www.slideshare.net/HIVScotland/self-testing-meeting-glasgow-reid3

Guillon et al. (2018), Human anti-HIV IgM detection by the OraQuick ADVANCE Rapid HIV 1/2 Antibody Test. PeerJ 6:e4430; DOI 10.7717/peerj.4430

HIV Diagnosis: Automated systems

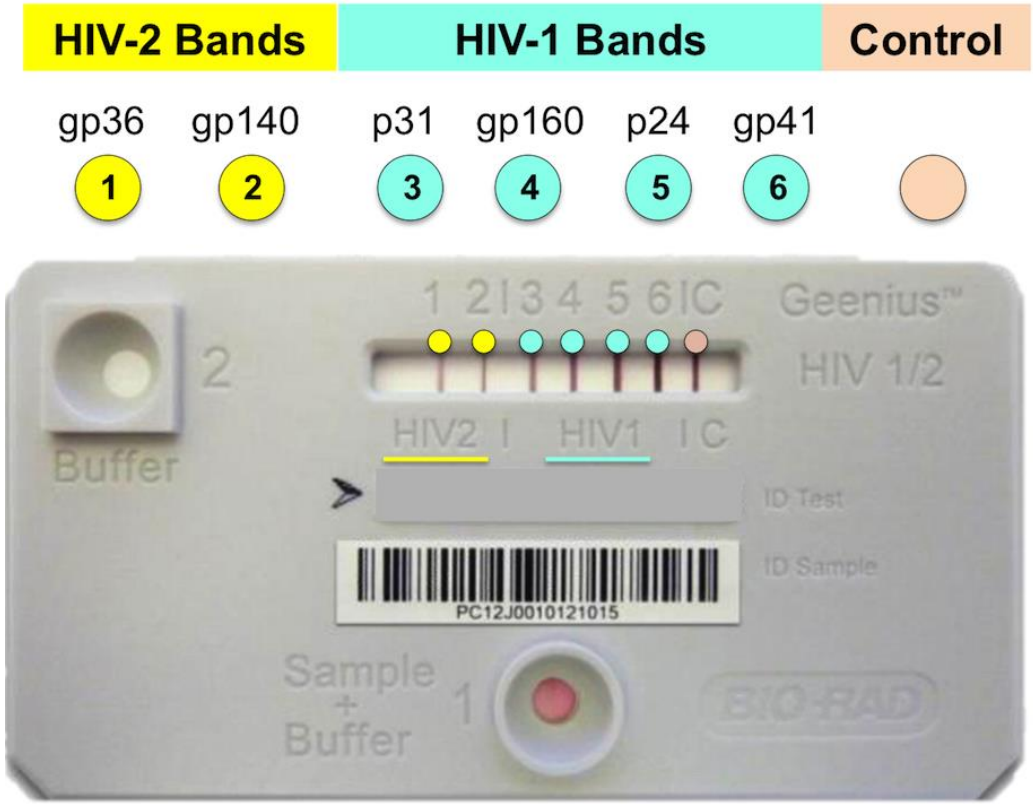


Examples of automated 4th generation platforms

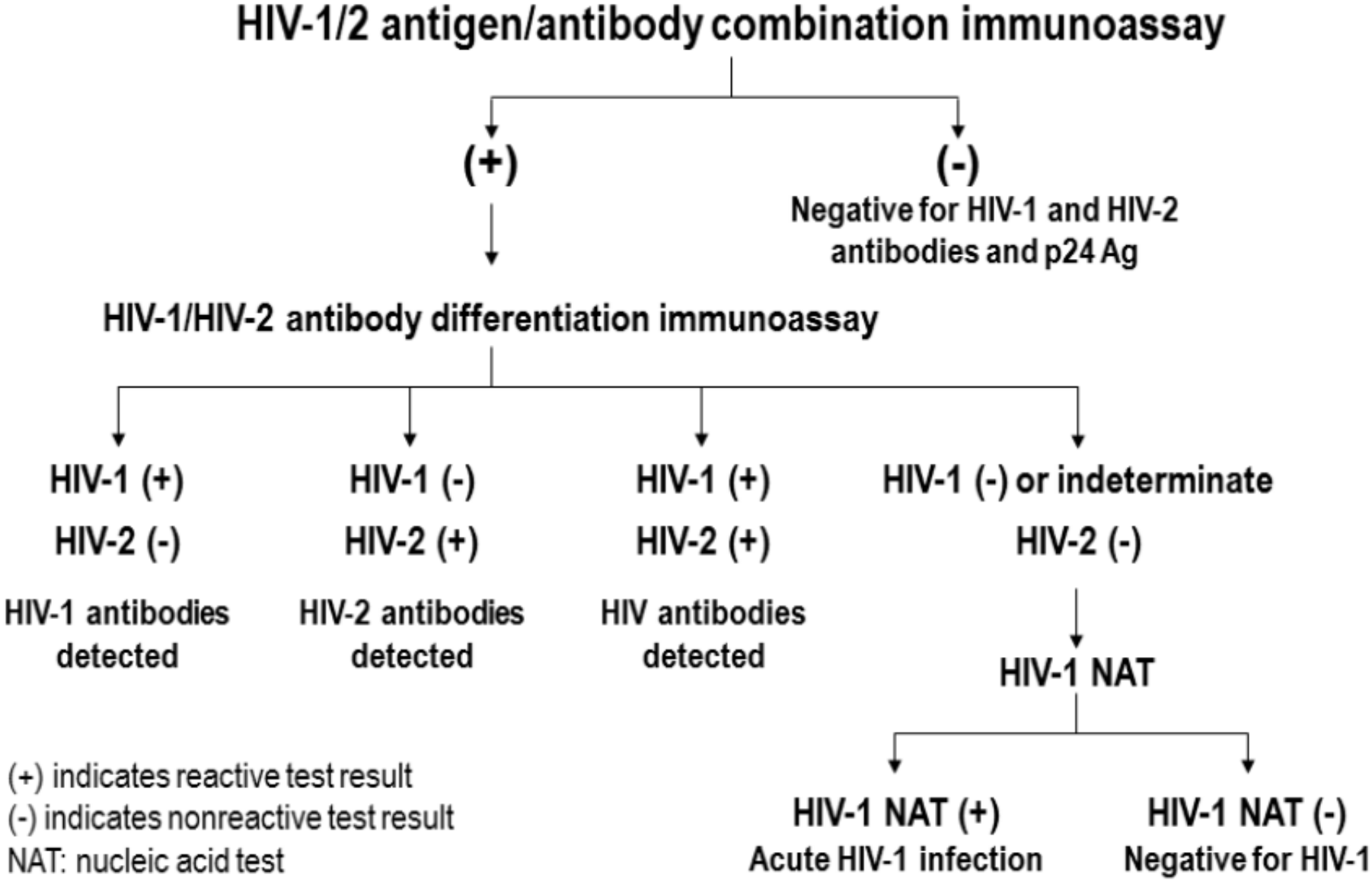
Test Name	Time to 1st test result	Target analyte	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	Approved specimen types and volume[c]	Assay format[d]
 **Abbott Architect HIV Ag/Ab Combo Assay (fully automated CLIA moderate assay)**	<30 min	HIV-1 p24 antigen and antibodies to HIV-1/2	Plasma/serum HIV-1: 100 (99.63-100) HIV-2: 100 (98.2-100) HIV-1 p24: 100 (94.3-100) at 18.1 pg/ml (range 17.8-19.7).	Plasma/serum 99.8 (99.6-99.9)	Plasma/serum 150 µl	Chemiluminescent microparticle immunoassay (CMIA)
 **Siemens ADVIA Centaur HIV Ag/Ab Combo (CHIV) (fully automated CLIA moderate assay)**	<1 hour	Antibodies to HIV p24 Ag, HIV-1 including group O, and/or HIV-2	Serum HIV-1 100.00 (99.61-100.00) HIV-2 100.00 (98.18-100.00)	Serum HIV-1 99.72 (99.56-99.84)	Serum/Plasma **175 µL**	Chemiluminescent microparticle immunoassay (CMIA)

HIV Diagnosis: Differentiation test

The Geenius HIV 1/2 Supplemental Assay by Bio-Rad®



HIV Testing Algorithm



HIV Diagnosis: 5th Generation Tests

BioPlex 2200 HIV Ag-Ab assay

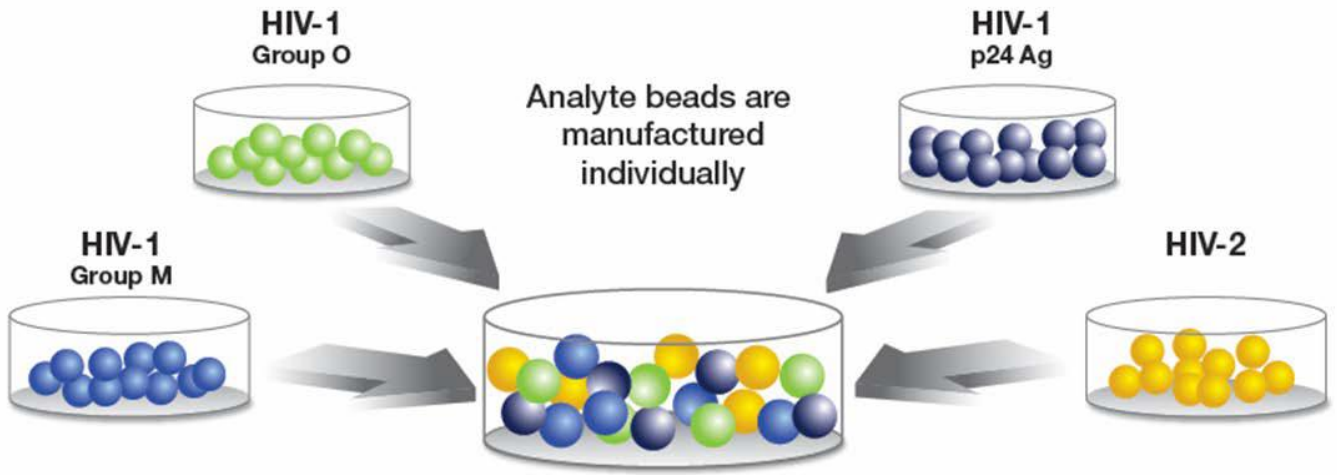
Simultaneously detects and reports a screen and three individual HIV results:

- » HIV Ag-Ab overall result

with

- » HIV-1 p24 Ag
- » HIV-1 Ab (Groups M & O)
- » HIV-2 Ab

- **Includes HIV-1 and HIV-2 Ab *Differentiation***
- ***Enhanced sensitivity* for p24 antigen detection**



Beads are combined into single "Bead Reagent" for multiplex analysis

HIV Diagnosis in infants

- Ab based serological testing is not recommended for the first 18 months of life

- Testing by NAAT recommended for infants
- Test must be performed
  - » At birth
  - » At age 6 weeks
  - » At age 4 to 6 months

- If all tests are negative, then prophylaxis is discontinued

HIV Diagnosis: NAAT

HIV-1 detection by Quantitative NAAT

- Can be used at 3rd step of HIV algorithm
  - ✓ For diagnosis of acute HIV infection
- Monitors viral load

APTIMA HIV-1 QNT Dx Kit

Detects (HIV-1) RNA from Group M, N and O subtypes

LOD: 17 copies/mL

AMR: 30-10,000,000 copies/mL

Panther Fusion System

Use in individuals living with HIV-1

- Baseline viral load establishment before initiating anti-HIV-1 drug therapy
- Viral load check every 4-8 weeks after treatment initiation, until viral load is undetectable
- In patients on a stable, suppressive ART, viral load measurement should be repeated every 3 to 4 months

After HIV Diagnosis, what next?

Prior to treatment initiation:

- Gather complete medical history

- Perform laboratory examination
  - » **Plasma HIV RNA (viral load)**
  - » **CD4 T lymphocyte (CD4) cell count**
  - » Complete blood count and chemistry profile
  - » Serologies for hepatitis A, B, and C viruses

- Genotypic drug-resistance testing

HIV Therapy

- The treatment for HIV is called antiretroviral therapy (ART)
- ART is a cocktail of drugs (called an HIV treatment regimen) taken daily on a life-long bases e.g., tenofovir disoproxil fumarate-emtricitabine (TDF-FTC)
- HIV drugs divided into seven classes which include:
  - nucleoside reverse transcriptase inhibitors (NRTIs)
  - non-nucleoside reverse transcriptase inhibitors (NNRTIs)
  - protease inhibitors (PIs)
  - fusion inhibitors
  - CCR5 antagonists
  - post-attachment inhibitors
  - Integrase strand transfer inhibitors (INSTIs)

FDA approved therapeutic injection

- Cabenuva treatment
  - — cabotegravir and rilpivirine
  - 2021 (monthly)
  - 2022 (once every 2-months)

- HIV medicines prevent HIV replication to reduce the viral load
  - Gives the immune system a chance to recover and produce more CD4 cells.
  - Reduces the chances of transmission

Post HIV Diagnosis: NGS Testing

- HIV-1 genotyping tests antiretroviral susceptibility for
  - » protease inhibitors (PI)
  - » reverse transcriptase inhibitors (NNRTI, NRTI)
  - » integrase inhibitors (INI)

- NGS can detect low frequency virus population

- NGS data may be compared to the gold standard Stanford database, ensuring up-to-date interpretation based on the most recent HIV clinical trials

Prevention of HIV Infection

Pre-Exposure Prophylaxis (PrEP)

Population	Recommendation
Adolescents and adults at increased risk of HIV	Clinicians should prescribe pre-exposure prophylaxis with effective antiretroviral therapy (**oral tenofovir disoproxil fumarate-emtricitabine (TDF-FTC)** and **injectable cabotegravir**)to persons who are at increased risk of HIV acquisition to decrease the risk of acquiring HIV infection.

The USPSTF recommends that the following persons be considered for PrEP:

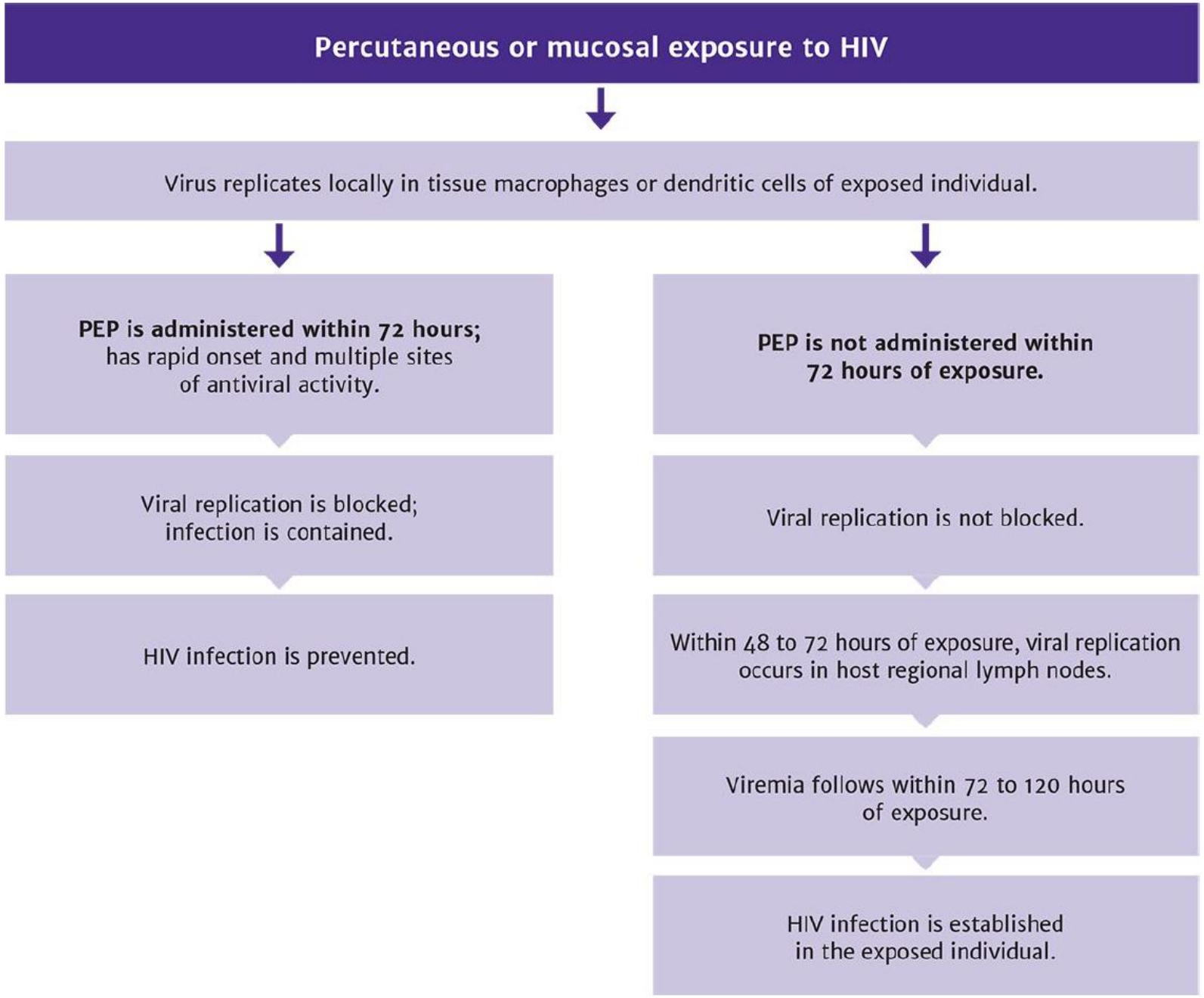
1. Sexually active adults and adolescents weighing at least 35 kg (77 lb) who have engaged in anal or vaginal sex in the past 6 months and have any of the following:
   - A sexual partner who has HIV
   - A bacterial sexually transmitted infection (STI) in the past 6 months.
   - A history of inconsistent or no condom use with sex partner(s) whose HIV status is not known; whether their sex partner or partners are in a group with a higher prevalence of HIV (e.g., men who have sex with men or with men and women, transgender women, persons who inject drugs, and persons who engage in transactional sex).
2. Persons who inject drugs and have a drug injecting partner who has HIV or who shares injection equipment.

What if an HIV exposure happens at work?

- **PEP (post-exposure prophylaxis)** means taking viral replication-blocking medication to prevent HIV after a possible exposure
  - » In a study, PEP reduced the risk of HIV acquisition by 81% for percutaneous exposures
- **PEP is best initiated within 2 hours of exposure**
  - » Initiate no later than 72 hours after an exposure
- PEP anti-retroviral regimens
  - » are safe in individuals who do not have HIV
  - » have minimal adverse effects
- PEP regimen is recommended for 28 days

HIV Exposure, With and Without Administration of PEP

- PEP should not be delayed while awaiting
  - » the source patient's HIV status/test result, or
  - » results of the exposed worker's baseline HIV test
- Discontinued PEP if test results subsequently show the source patient does not have HIV



HIV Testing Post-Exposure

- Baseline HIV test the exposed individual with Ag-Ab combination test
  - » preferably at the time of PEP initiation, but no later than 72 hours after exposure

- Subsequent HIV screen testing frequency
  - » Baseline
  - » Week 4
  - » Week 12

Baseline Laboratory Studies
☐ HIV testing
☐ Complete blood counts
☐ Renal and hepatic function tests
☐ Serologic testing for HBV and/or HCV (if indicated)
☐ Pregnancy test (if indicated)

- Clinicians should continue PEP in any individual who is suspected to be
  - » Seroconverting, or
  - » for whom HIV has not been ruled out at week 4

- If an exposed individual's HIV screening test result is reactive at any time, clinicians should perform an FDA-approved confirmatory HIV-1/HIV-2 Ab differentiation immunoassay

https://www.hiv.uw.edu/go/prevention/occupational-postexposure-prophylaxis/core-concept/all
https://www.ncbi.nlm.nih.gov/books/NBK562734/table/nycgpep.tab22/

Case Study

A 30-year-old black female patient had been well until approximately 7 days earlier, when she began to have a vaginal discharge, followed by headache, fatigue, sore throat, and anorexia. On the fifth day of symptoms, she went to an outpatient clinic in an urban area. On examination, she had a vaginal discharge; the temperature and the remainder of the examination were reportedly normal. Patient has had multiple sexual partners and hasn't always used protection (condoms) for intercourse. Patient has a history of STDs (gonorrhea, syphilis) that has previously been treated. Lab screen for gonorrhea and syphilis are negative.

What would be the next course of action if HIV is suspected?

4th generation lab serological test is run in the laboratory which came back as positive

What would be the next course of action?

Geenius test was performed to type the infection

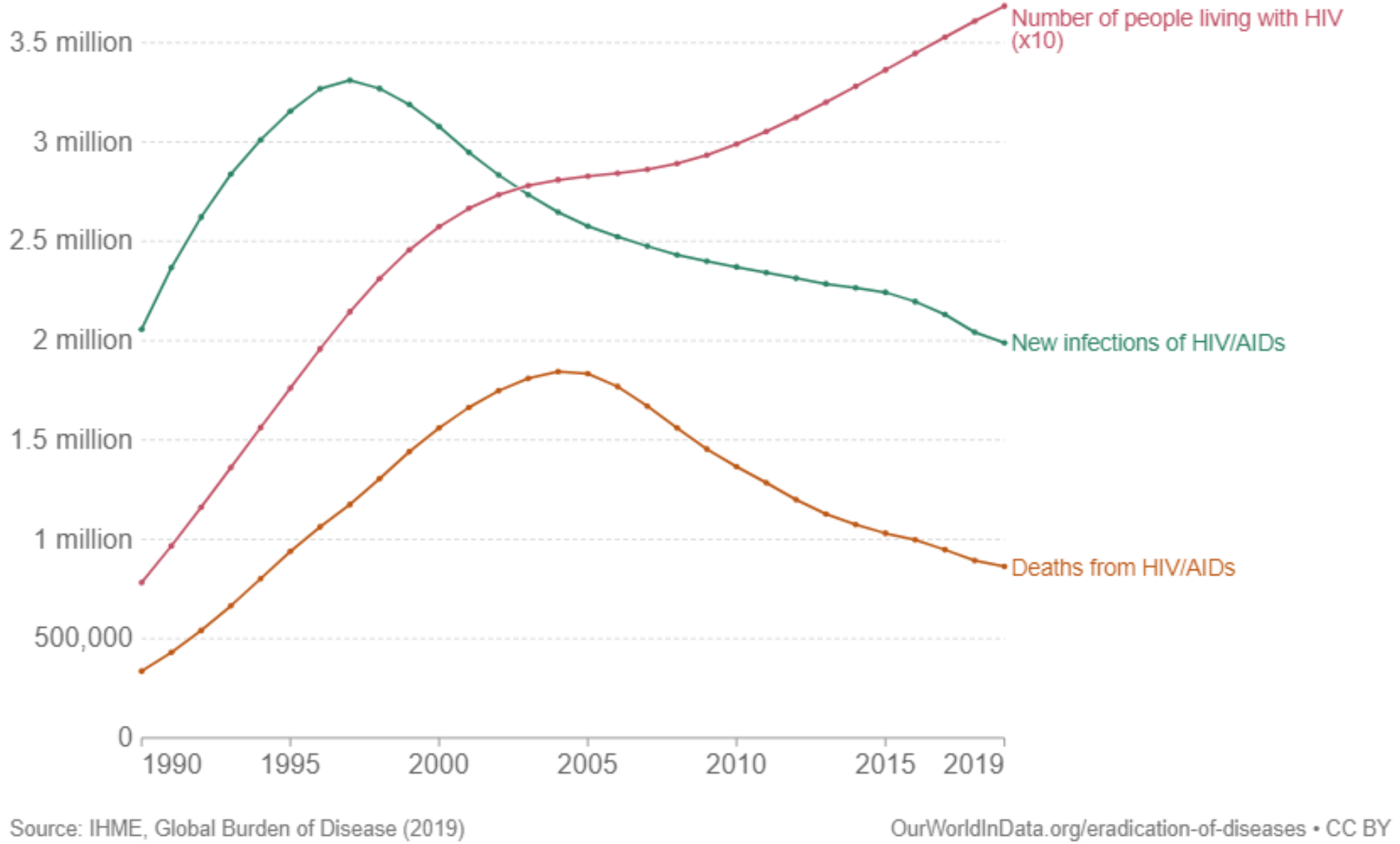
HIV-1 (positive)

HIV-2 (negative)

HIV-1 infection

What would be the next course of action?

HIV: Progress has been made!



HIV Outlook

The initiative's goal is to reduce the number of new HIV infections in the U.S. by:

75% BY 2025 & **90% BY 2030**

Diagnose

Diagnose all people with HIV as early as possible after infection.

Treat

Treat the infection rapidly and effectively to achieve sustained viral suppression.

Prevent

Prevent new HIV transmissions by using proven interventions, including pre- exposure prophylaxis (PrEP) and syringe services programs (SSPs).

Respond

Respond quickly to potential HIV outbreaks to get needed prevention and treatment services to people who need them.

https://www.hiv.gov/federal-response/ending-the-hiv-epidemic/overview/

Thank you!