Human Immunodeficiency Virus (HIV)

Human Immunodeficiency Virus (HIV) belongs to the genus *Lentivirus* within the *Retroviridae* family. It primarily targets CD4+ T lymphocytes and, if left untreated, it can lead to Acquired Immunodeficiency Syndrome (AIDS), a condition characterized by severe immune suppression and increased susceptibility to opportunistic infections and certain cancers.

According to the 2018 Quick Reference Guide from the Centers for Disease Control and Prevention (CDC) and the Association of Public Health Laboratories (APHL), initial laboratory testing for HIV should be performed using an FDA-approved antigen/antibody immunoassay. This assay is designed to detect both HIV-1 and HIV-2 antibodies, as well as the HIV-1 p24 antigen, allowing for the identification of both established HIV-1/HIV-2 infections and acute HIV-1 infection.

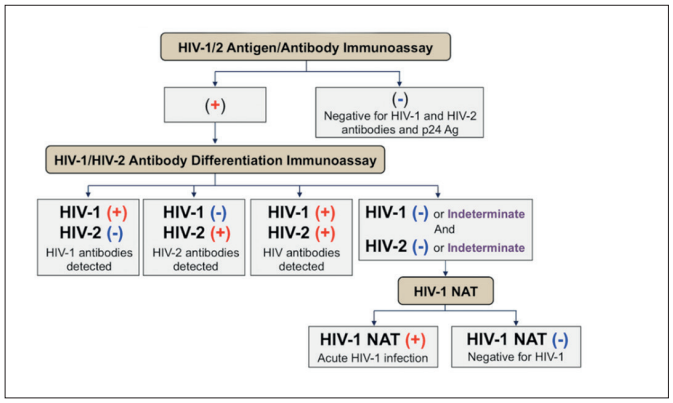

Figure 1.
CDC and APHL Recommended Laboratory Testing Algorithm for the Diagnosis of HIV infection. (1)

If the initial screening test is reactive, a supplemental antibody differentiation test should be conducted to confirm the presence of HIV infection and to differentiate between HIV-1 and HIV-2. This step is critical for accurate diagnosis and appropriate clinical management. The detailed HIV diagnosis algorithm is shown in Figure 1.

HIV virion structure and the diagnostic roles of gp120, gp41, and p24

The structure of the HIV particle is similar in both types of HIV: HIV-1 and HIV-2. It is roughly spherical, with a diameter of approximately 120 nm, and it is surrounded by a lipoprotein-rich membrane. Like other retroviruses, the HIV genome contains three major genes—*gag*, *env*, and *pol*—which encode the key structural and functional proteins of the virus. The *gag* gene encodes several internal structural proteins, most notably the capsid protein, p24, which forms the viral core and serves as an important early marker in HIV infection. HIV-1 expresses the p24 antigen, while HIV-2 expresses a homologous protein that is commonly referred to as the p24 antigen, although it is also known in scientific literature as the HIV-2 p26 antigen.

CLINICAL UTILITY

- **Screening and monitoring HIV infections in targeted populations to help curb the HIV epidemic**
- **Early detection and treatment to improve quality of life**

The *env* gene encodes the envelope glycoproteins gp120 and gp41, which are located on the surface of the virus. gp120 binds to CD4 receptors on host T cells, initiating viral entry, while gp41 anchors gp120 to the viral membrane and mediates fusion between the virus and host cell. Both gp120 and gp41 trigger strong antibody responses, making them ideal targets for HIV tests.

Finally, the *pol* gene encodes enzymes essential for viral replication, including reverse transcriptase, integrase, and protease.

HIV classification and geographic distribution

While HIV-1 is further divided into four groups—M, N, O, and P, Group M is the most prevalent and it includes multiple genetic subtypes (A, B, C, D, F, G, H, J, and K) as well as circulating recombinant forms (CRFs). Group O (Outlier) accounts for approximately 1% of HIV-1 infections and it is mainly distributed in Cameroon and neighboring countries. Groups N and P are extremely rare.

Meanwhile, HIV-2 comprises eight known groups, which are designated from A through H, with groups A and B being the primary groups that are associated with widespread transmission, while groups C through H are considered rare (See Table 1). Therefore, the sensitivity and specificity of HIV p24 antigen detection are of critical importance.

Early HIV detection markers and IVDR performance Criteria for p24 antigen assays

HIV RNA can be detected 7–10 days after infection, while the p24 antigen can be detected 10–15 days post-infection, and HIV antibodies are typically detectable within 2 to 8 weeks after infection (See Figure 2). The combined HIV p24 antigen and antibody testing can shorten the diagnostic window period to approximately 14 days. According to the EU IVDR Class D regulatory requirements, the lower limit of detection (LoD) for the WHO HIV-1 p24 international standard (NIBSC code: 90/636) must be ≤ 2 IU/mL, and the assay specificity must be ≥ 99.5%.

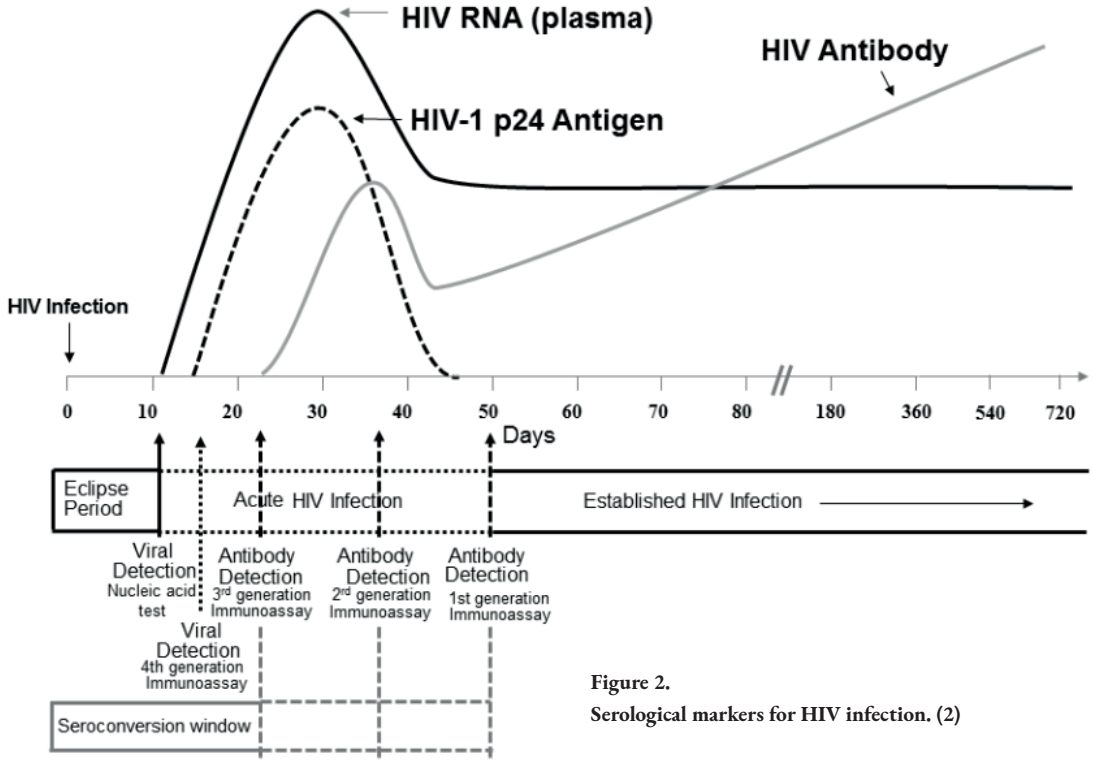

Figure 2.
Serological markers for HIV infection. (2)

Table 1.
HIV classification overview

Virus Type	Group	Prevalence	Key Features	Geographic Distribution
HIV-1	M	Most prevalent	Contains multiple subtypes (A, B, C, D, F, G, H, J, K) and many CRFs (Circulating Recombinant Forms)	Global
	O	~1% of cases	Genetically distinct ("Outlier")	Primarily in Cameroon and neighboring countries
	N	Extremely Rare	Limited circulation	Mainly in Cameroon
	P	Extremely Rare	Least characterized group	Very limited data
HIV-2	A&B	Most prevalent in HIV-2	Associated with widespread transmission	Mainly West Africa
	C-H	Rare	Limited transmission	Isolated cases, primarily in West Africa

Monoclonal antibodies for HIV immunoassay development

Hytest offers several monoclonal antibodies (MAbs) against HIV p24, which can be used for the development of next-generation HIV p24 antigen immunoassays. Sensitivity analysis on the CLIA platform using the WHO HIV-1 p24 international standard (NIBSC code: 90/636) indicates that the recommended pairs are able to achieve an analytical sensitivity of 0.5–0.64 IU/mL, which exceeds the requirements set by the EU IVDR Class D regulations (3). Details of the recommended antibody pairs and their corresponding LoD data are summarized in Table 2. It's worth mentioning that the recommended pairs exhibited a greater detection capacity for HIV-1 p24 and HIV-2 p26 reagents than antibodies used in Abbott and Roche's assays. In addition, external clinical sample testing shows that the specificity of the recommended pairs exceeds 99.95%, which meets clinical standards.

All of the recommended HIV p24 antibody pairs are capable of recognizing the WHO HIV-1 p24 international standard material (NIBSC code: 90/636), as well as the following HIV-1 subtypes: A1, B, C, D, F1/CRF12_BF/BFrec, G, CRF20_BG, CRF01_AE, CRF02_AG, H, and Group O (NIBSC code: 16/210). They also recognize the WHO International Reference Reagent for HIV-2 p26 Antigen (NIBSC code: 16/236). These data indicate that the presented antibodies recognize shared epitopes (or conservative fragments) of non-identical p24 proteins (HIV-1 p24 group M, group O, p26 from HIV-2). Representative calibration curves for the pairs GA17 (capture) -GA12 (detection) and GA17 (capture) -GA54 (detection) are shown in Figure 3.

Table 2.

HIV p24 antibody pairs LoD

Sample preparation: WHO HIV-1 p24 international standard material (NIBSC code: 90/636), HIV-1 p24 (group M) antigen , HIV-1 p24 (group O) antigen and HIV-2 p26 antigen.

Detection method: all of the samples were detected using sandwich-CLIA (alkaline phosphatase labelling).

The recommended pairs exhibited a greater detection capacity for HIV-1 p24 and HIV-2 p26 reagents than antibodies used in Abbott and Roche's assays.

Capture MAb	Detection MAb	LoD of WHO international standard p24, IU/ml	LoD of HIV1 p24_M, pg/ml	LoD of HIV1 p24_O, pg/ml	LoD of HIV-2 p26, pg/ml
GA17	GA12	0.64	4	1.5	15
GA17	GA38	0.55	1.5	1.5	8
GA17	GA54	0.55	4	1.5	15
GA34	GA32	0.55	2	4	8
GA34	GA39	0.55	2	4	5
Abbott Alinity HIV Ag/Ab combo reagent kit		1	5	4	n/d when HIV-2 p26 is 1ng/ml
Roche CombiPT HIV reagent kit		1.3	5	10	20

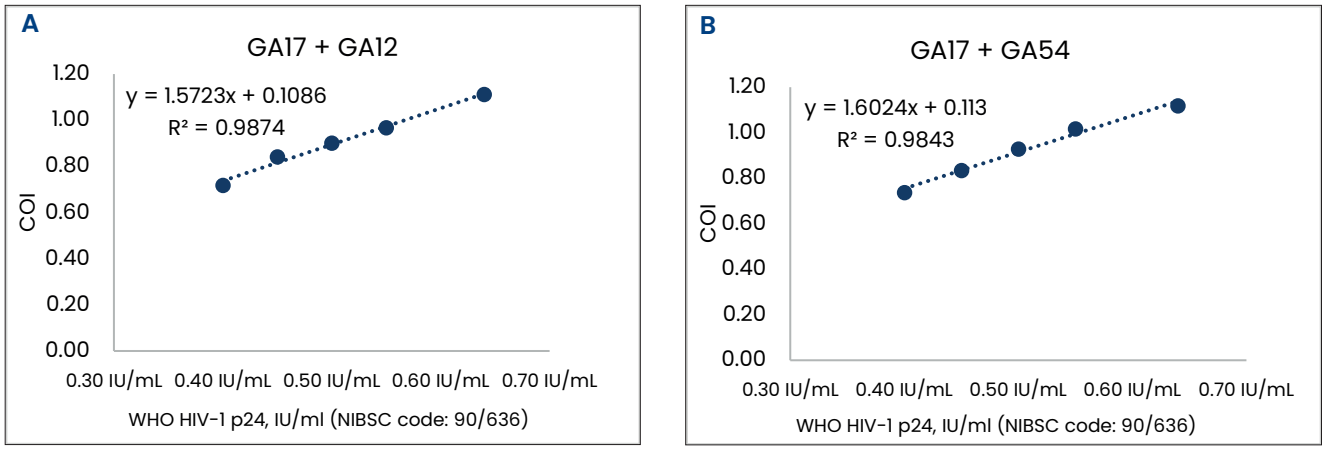

Figure 3.

Representative results for two antibody pairs: GA17 (capture)-GA12 (detection) (A), GA17 (capture)-GA54 (detection) (B) for detecting the WHO HIV-1 p24 International Standard Material (unit: COI). Detection method: sandwich - chemiluminescent assay (alkaline phosphatase labelling).

REFERENCES

1. Centers for Disease Control and Prevention and Association of Public Health Laboratories. 2018 Quick reference guide: Recommended laboratory HIV testing algorithm for serum or plasma specimens. Published January 27, 2018.
2. Branson, Bernard M. et al. (2014). Laboratory testing for the diagnosis of HIV infection : updated recommendations.
3. European Commission. Commission Implementing Regulation (EU) 2022/1107 of 4 July 2022 laying down common specifications for certain class D in vitro diagnostic medical devices. Official Journal of the European Union, L 174, 4 July 2022, pp. 3–42. At <https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX%3A32022R1107 >

ORDERING INFORMATION

MONOCLONAL ANTIBODIES

Product name	Cat. #	MAb	Isotype	Remarks
Monoclonal anti-HIV1/2 p24	3H24	GA12	IgG1	*CLIA, FIA, in vitro monoclonal antibody*
		GA17	IgG1	CLIA, FIA, in vivo monoclonal antibody
		GA32	IgG	CLIA, FIA, recombinant monoclonal antibody
		GA34	IgG	CLIA, FIA, recombinant monoclonal antibody
		GA38	IgG	CLIA, FIA, recombinant monoclonal antibody
		GA39	IgG	CLIA, FIA, recombinant monoclonal antibody
		GA54	IgG1	CLIA, FIA, recombinant chimeric antibody

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