Human Immunodeficiency Virus (HIV)

Overview of the HIV infection and diagnostic testing guidelines as outlined by CDC and APHL (2018)

Human Immunodeficiency Virus (HIV) belongs to the genus *Lentivirus* within the *Retroviridae* family. It primarily targets CD4+ T lymphocytes. If it is left untreated, it can lead to acquired immunodeficiency syndrome (AIDS), which is 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, which enables the identification of both established HIV-1/HIV-2 infections and acute HIV-1 infection.

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 both types of HIV: HIV-1 and HIV-2. This step is critical for ensuring accurate diagnosis and appropriate clinical management. (1)

HIV virion structure and the diagnostic roles of gp120, gp41, gp36, and p24/p26

The structure of the HIV particle is similar in both 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 that is derived from the host cell.

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 referred to in scientific literature as the HIV-2 p26 antigen.
- The *env* gene encodes the envelope glycoproteins:
  - In HIV-1, the *env* gene encodes the precursor gp160, which is cleaved into 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, which makes them ideal targets for HIV tests.
  - In HIV-2, the *env* gene encodes the precursor gp160, which is cleaved into the surface glycoprotein gp120 and the transmembrane glycoprotein gp36, the latter being functionally analogous to gp41 in HIV-1.
- Finally, the *pol* gene encodes enzymes that are essential for viral replication, including reverse transcriptase, integrase, and protease.

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**

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). Meanwhile, Group O (Outlier) accounts for approximately 1-2% of HIV-1 infections. While the prevalence of Group O has remained low at 1-2% in Cameroon, certain European countries (France, Spain, and Belgium) have reported the highest prevalence outside of Africa. The sequence diversity between HIV-1 Group O and Group M strains is huge, reaching 50% and 30% in the *env* and *pol*, respectively. This diversity has hindered the diagnosis, monitoring, and treatment of Group O-infected patients. Due to the presence of the C181 mutation in Reverse transcriptase (RT) enzyme in Group O, more than 60% of individuals that live with this virus are faced with the challenge of drug resistance to some antiretroviral therapies (2).

Groups N and P are extremely rare. Meanwhile, HIV-2 comprises eight known groups that are designated from A through H, with groups A and B being the primary groups associated with widespread transmission. Conversely, groups C through H are considered rare (see Table 1).

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 (3). 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%. Therefore, the sensitivity and specificity of HIV p24 antigen detection are of critical importance.

P24 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 or HIV Ag/Ab combo assays.

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 (4). Details of the recommended antibody pairs and their corresponding LoD data are summarized in Table 2. Under our test conditions, the recommended pairs also demonstrated greater detection capacity for HIV-1 p24 and HIV-2 p26 reagents than the antibodies used in Abbott's and Roche's assays. In addition, external clinical sample testing shows the specificity exceeds 99.95%, which meets clinical requirements.

Moreover, for the sandwich-ELISA platform, we recommend the pairs for HIV p24 immunodetection shown in Table 3. All recommended pairs were validated in-house and demonstrated high specificity in sandwich-ELISA immunoassays.

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/BF rec, 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 1.

All pairs are capable of detecting WHO HIV-1 p24 international standard material (NIBSC code: 90/636), recombinant HIV-1 p24 antigen, and recombinant HIV-2 p26 antigen.

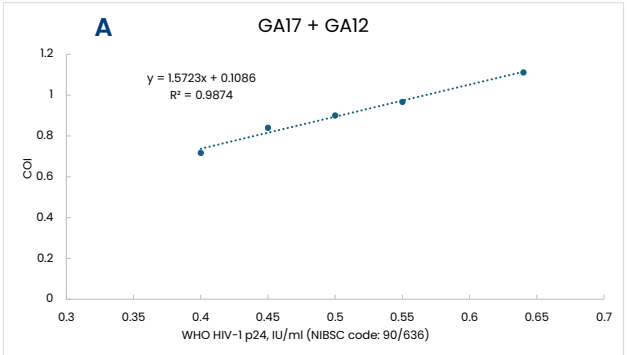

Figure 1.

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).

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

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	GA54	0.55	4	1.5	15
GA17	GA12	0.64	2	2	8
GA17	GA38	0.55	1.5	1.5	8
GA34	GA54	0.5	2	1.5	20
GA38	GA54	0.55	4	1.5	20
Abbott Alinity HIV Ag/Ab combo reagent kit		1.0	5	4	n/d when HIV-2 p26 is 1 ng/ml
Roche CombiPT HIV reagent kit		1.3	5	6	20

Table 3.

Recommended pairs for HIV p24 immunodetection in a sandwich-ELISA platform.

Capture	Detection
GA17	GA12
GA15	GA18
GA18	GA12
GA32	GA18
GA32	GA15

RECOMBINANT ANTIGENS FOR HIV-1/HIV-2 IGM/IGG DETECTION

Hytest also offers a panel of antigens for HIV-1/HIV-2 IgM/IgG detection. During infection, different antibodies appear at different times (5). Antibodies against gp41 are among the first to arise in HIV-1 infections. They are often detectable within 2–3 weeks post-infection, which means gp41 is a valuable target for early diagnostic assays. In contrast, antibodies against gp120 (V3 loop) generally appear at a later stage, although the V3 region is highly immunogenic once exposed. To provide broad and reliable diagnostic coverage, Hytest antigens contain immunodominant and conserved regions, such as HIV-1 gp41, gp120 V3 loop, and HIV-2 gp36. These antigens were selected based on their early antibody response, high immunogenicity, and their ability to represent the genetic diversity of HIV-1 (groups M and O, subtypes, CRFs) and HIV-2.

Cat. # 8H12, HIV-1, gp41-gp120 N-Fc, is a recombinant antigen expressed in a mammalian cell line with an approximate molecular weight of 66 kDa. Its theoretical pI is 6.55. The antigen is engineered by fusing HIV-1 gp120 and gp41 sequences derived from HIV-1 Groups M and O. This design presents epitopes from both *env* subunits, with the aim of identifying antibodies of different specificity across HIV-1 strains. Fc-tag was added to the N-terminus of the protein to facilitate solubility and further purification.

Cat. # 8H13, HIV-1, gp41 N-HSA, is a recombinant antigen expressed in a mammalian cell line with an approximate molecular weight of 87 kDa. Its theoretical pI is 6.39. The protein is based on a gp41 sequence from HIV-1 group M, engineered with a protein tag (HSA) and His-tag for protein solubility and protein purification, respectively.

Cat. # 8H16, HIV-1, gp120 C-Fc, is a recombinant antigen expressed in a mammalian cell line with an approximate molecular weight of 33.3 kDa. Its theoretical pI is 8.34. To address the high sequence diversity of gp120, the construct includes two fragments corresponding to the V3 region from Group M and Group O within a single fusion protein. The protein carries a C-terminal Fc fragment to facilitate protein solubility and further purification.

Cat. # 8H24, HIV-2, gp36 N-HSA, is a recombinant antigen expressed in a mammalian cell line, with an approximate molecular weight of 86.6 kDa. Its theoretical pI is 6.5. This construct contains several immunodominant regions and four confirmed N-glycosylation sites. The protein includes an N-terminal HSA tag and C-terminal His-tag to facilitate solubility and further purification. In addition, the protein is highly sequence-divergent from the HIV-1 gp41 protein.

Cat. # 8H25, HIV-2, gp36 C-TnC, is a recombinant antigen expressed in a mammalian cell line, with an approximate molecular weight of 37.6 kDa. Its theoretical pI is 4.7. This construct is comparable to Cat. # 8H24 but differs in tag type and placement: Cat. # 8H25 carries a C-terminal human cardiac troponin C (TnC) tag and C-terminal His-tag. The designs of Cat. # 8H24 and Cat. # 8H25 give assay developers flexibility across diagnostic formats and platforms, while supporting improved HIV-2 antibody detection.

Hytest HIV recombinant antigens evaluated on a CLIA platform demonstrate high sensitivity and specificity

Using Hytest prototype assays on the CLIA platform, 11 undiluted HIV-1–positive samples and 11 diluted HIV-2–positive samples were assessed. All Hytest HIV-1 and HIV-2 antigen pairs demonstrated 100% sensitivity (11/11 reactive) in their respective positive specimen panels.

Analytical specificity was assessed against various interference samples and clinical specimens. Hytest HIV-1 antigen pairs met the performance requirement defined in Commission Implementing Regulation (EU) 2022/1107 (specificity >99.5%). Using the same specificity assessment approach, Hytest HIV-2 antigen pairs also met the (EU) 2022/1107 performance requirement (specificity >99.5%).

HYTEST HIV AB/AG COMBO PROTOTYPE ASSAY EVALUATION

Hytest has further evaluated the HIV Ab/Ag combo assay prototypes on a CLIA platform. All combo prototype assays were able to detect all tested genotypes (A, B, C, C/CRF_BC, C/CRF08_BC, C/CRF31_BC, CRF_BG, CRF01_AE, CRF02_AG, CRF03_AB, CRF22_01A1, D, F2, G). The prototypes show high specificity (>99.9%) and high sensitivity, as demonstrated using seroconversion panels, which assess early-infection detection across sequential specimens collected during the transition from antibody-negative to antibody-positive, including low-titer bleeds where p24 antigen and/or antibodies are emerging.

REFERENCES

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ORDERING INFORMATION

MONOCLONAL ANTIBODIES

Product name	Cat. #	MAb	Isotype	Remarks
Monoclonal anti-HIV1/2 p24	3H24	GA12	IgG1	*In vitro*, CLIA, EIA
		GA15	IgG1	*In vitro*, CLIA, EIA
		GA17	IgG1	CLIA, EIA
		GA18	IgG1	*In vitro*, CLIA, EIA
		GA32	IgG	CLIA, EIA, recombinant rabbit antibody
		GA34	IgG	CLIA, EIA, recombinant rabbit antibody
		GA38	IgG	CLIA, EIA, recombinant rabbit antibody
		GA39	IgG	CLIA, EIA, recombinant rabbit antibody
		GA54	IgG1	CLIA, EIA, recombinant chimeric antibody

ANTIGENS

Product name	Cat. #	Purity	Source
Human Immunodeficiency Virus 1 Antigen (HIV-1, gp41-gp120 N-Fc), recombinant	8H12	>80%	Recombinant
Human Immunodeficiency Virus 1 Antigen (HIV-1, gp41 N-HSA), recombinant	8H13	>85%	Recombinant
Human Immunodeficiency Virus 1 Antigen (HIV-1, gp120 C-Fc), recombinant	8H16	≥90%	Recombinant
Human Immunodeficiency Virus 2 Antigen (HIV-2, gp36 N-HSA), recombinant	8H24	>90%	Recombinant
Human Immunodeficiency Virus 2 Antigen (HIV-2, gp36 C-TnC), recombinant	8H25	>80%	Recombinant

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Hytest

SCIENTIFIC EXCELLENCE FOR IVD

HYTEST LTD
Intelligate 1, 6th floor, Joukahaisenkatu 6 • FI-20520 Turku, FINLAND
Tel. +358 2 512 0900 • E-mail: hytest@hytest.fi • **HYTEST.FI**
HYTEST NORTH AMERICA INC.
800 MacArthur Blvd, STE 100, 07430 Mahwah, New Jersey
Tel. 833-368-3489 • E-mail: hytestna(at)hytest.fi • **HYTESTNA.COM**