# CURRENT HIV TESTING AND AVAILABLE IMMUNOASSAYS

## Background

Around 38 million people were living with human immunodeficiency virus (HIV) in 2019; of these, 36.2 million were adults and 1.8 million were children below 14 years old. About 1.7 million of newly infected people were also diagnosed in the same year[1].

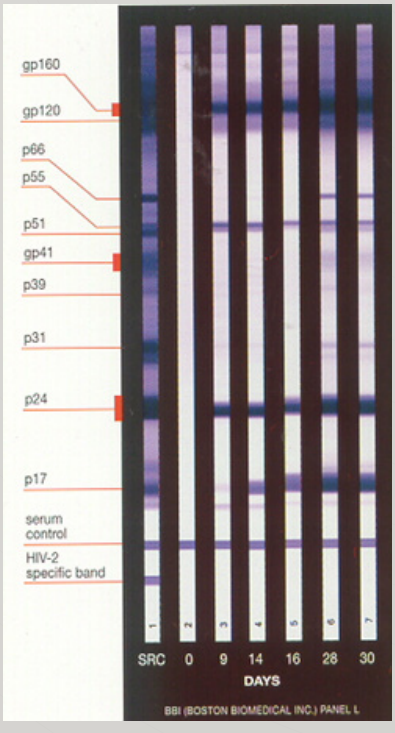
The HIV has two major types: HIV type-1 (HIV-1) and HIV type-2 (HIV-2). HIV-1 is more virulent and responsible for most of the infection cases worldwide. HIV-1 and HIV-2 differ in some of their antigenic components and geographic distribution[2]. The details of the differences in the gene and products are summarized in table 1. Each of the antigenic components can typically be identified in the Western Blot (Fig. 1).

### Differences in antigenic components between HIV-1 and HIV-2 *(Table 1)*

| | HIV-1 | HIV-2 |
|---|---|---|
| ***env*** | | |
| Env Precursor | gp160 | gp140 |
| External Glycoprotein | gp120 | gp105/125 |
| Transmembrane Protein | gp41 | gp36/41 |
| ***pol*** | | |
| Reverse Transcriptase | p66 | p68 |
| Reverse Transcriptase | p51 | p53 |
| Endonuclease | p31 | p31/34 |
| ***gag*** | | |
| Gag Precursor | p55 | p57 |
| Matrix | p17 | p17 |
| Capsid | p24 | p26 |

Adopted from:
https://www.hiv.uw.edu/go/key-populations/hiv-2/core-concept/all

### Visualization of HIV antigenic components on MP Diagnostics HIV Blot 2.2 *(Fig 1)*



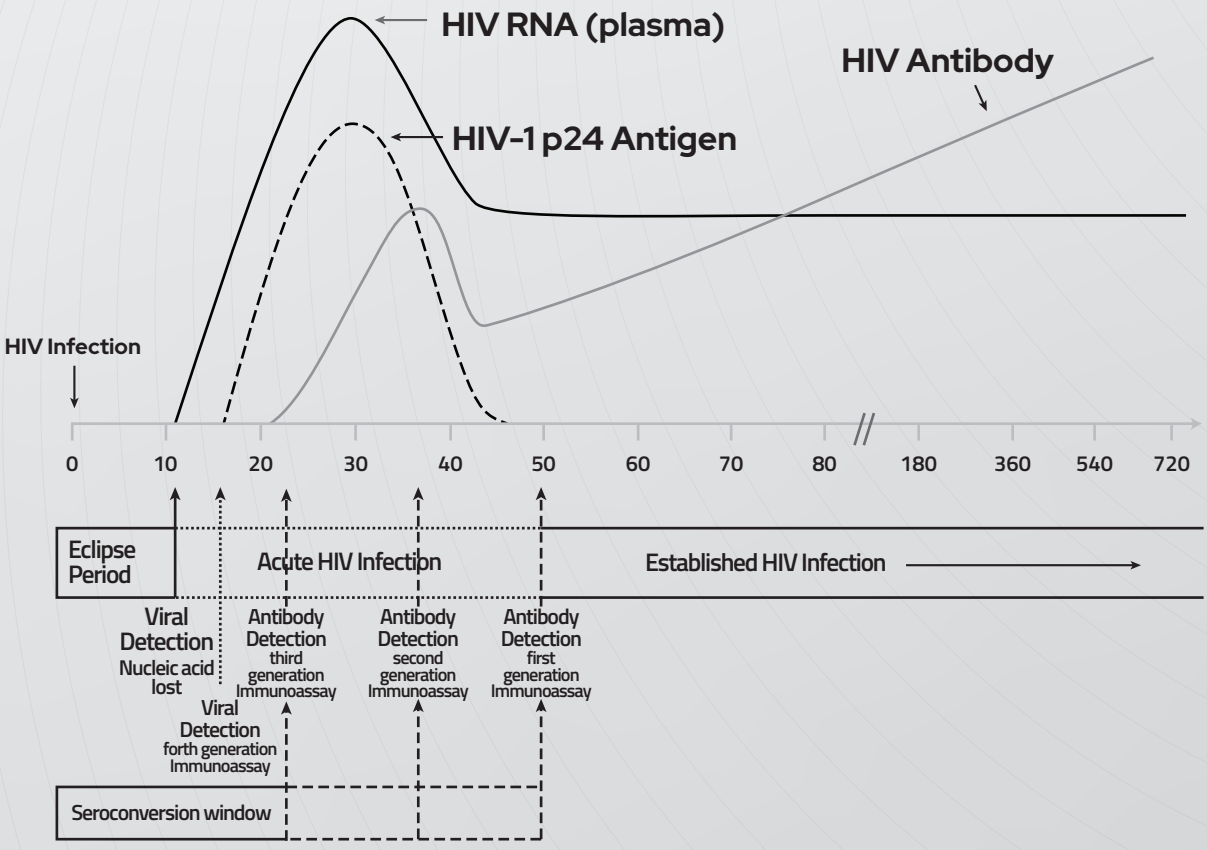
# Laboratory markers for HIV diagnosis

The use of the specimens from seroconversion panels has allowed the establishment of sequential appearance of different laboratory markers during HIV infection (Fig. 2). The first marker that appears in the plasma sample (approximately 10 days post-infection with HIV-1) is HIV-1 RNA. The subsequent detectable marker is p24 antigen (approximately week 2 to week 3 post-infection with HIV-1). Once the antibodies against p24 have developed and formed the immune complexes with p24 antigen, the test sensitivity for p24 antigen will decrease.

The first detectable antibodies are in the form of IgM (approximately week 3 to week 4 post-infection with HIV-1). The final detectable antibodies are in the form of IgG (approximately week 4 to week 5 post-infection with HIV-1). The IgG antibodies typically persist throughout the course of HIV infection.

The discovery of the above laboratory markers is facilitated by the various HIV immunoassays which have evolved over the years, from the 1st generation which uses HIV lysate and detects IgG antibodies until the 4th generation which uses synthetic peptide or recombinant protein (as the antigen) and detects IgM, IgG antibodies, as well as p24 antigen. The evolution of these various HIV immunoassays is partly driven by the update of the laboratory HIV testing algorithm by the health organizations, such as Centers for Disease Control and Prevention (CDC), World Health Organization (WHO). Some examples of the commercial test kits which can be used in various steps of the HIV testing algorithm is presented in Fig. 3 and Fig. 4.

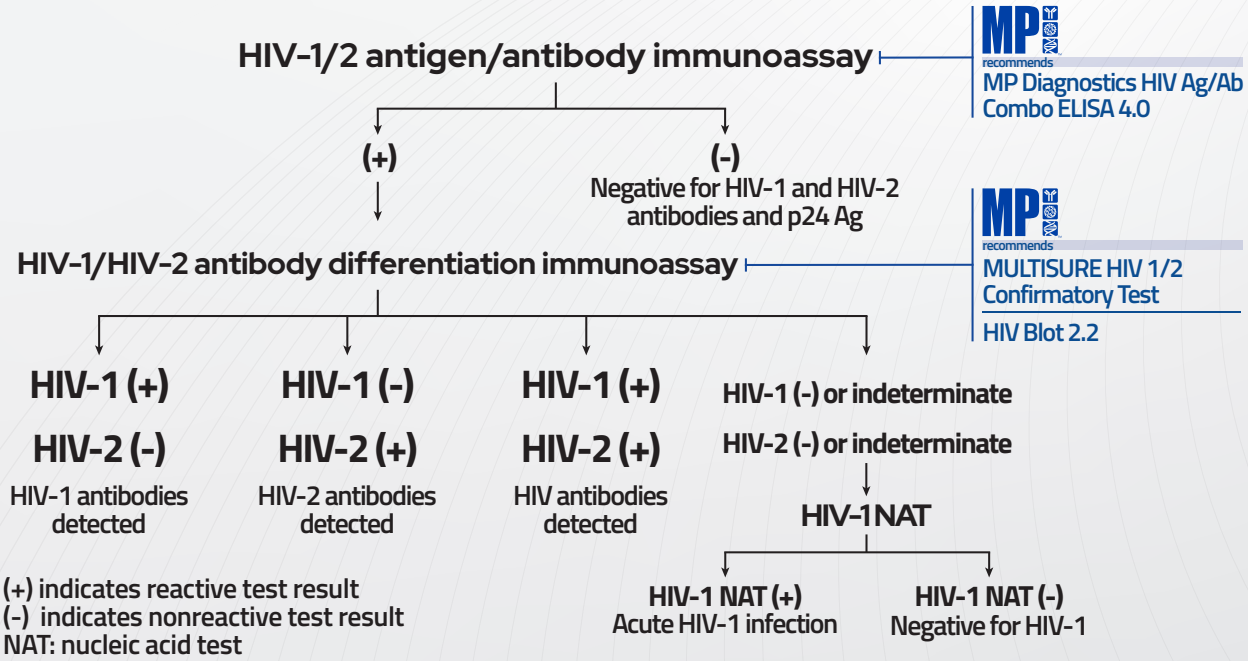
## Sequence of appearance of laboratory markers for HIV-1 infection *(Fig 2)*




Source: Centers for Disease Control and Prevention and Association of Public Health Laboratories. Laboratory Testing for the Diagnosis of HIV Infection: Updated Recommendations.

Available at http://dx.doi.org/10.15620/cdc.23447.
Published June 27, 2014.

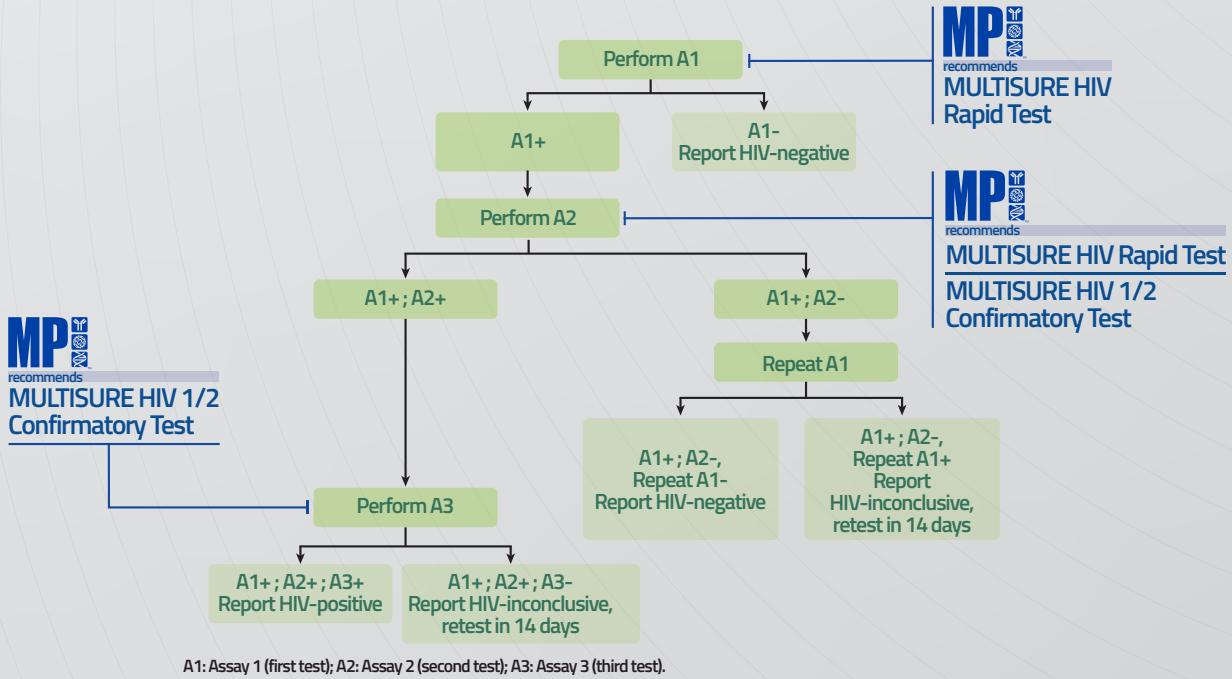
## Proposed uses of MP Diagnostics products in various steps of the CDC's recommended laboratory testing algorithm *(Fig 3)*




Adopted from 2018 Quick reference guide: recommended laboratory HIV testing algorithm for serum or plasma specimens.

Available at: https://stacks.cdc.gov/view/cdc/50872.

## Possible uses of MP Diagnostics RDT products (MULTISURE HIV Rapid Test and MULTISURE HIV 1/2 Confirmatory Test) in various steps of the WHO standard HIV testing strategy *(Fig 4)*




A1: Assay 1 (first test); A2: Assay 2 (second test); A3: Assay 3 (third test).

Adopted from Consolidated guidelines on HIV testing services, 2019. Geneva: World Health Organization; 2020.

# Available Immunoassays


## MP Diagnostics HIV Ag/Ab Combo ELISA 4.0

The MP Diagnostics HIV Ag/Ab Combo ELISA 4.0 is an enzyme-linked immunosorbent assay (ELISA) intended for qualitative detection of antigens and/or antibodies to Human Immunodeficiency Viruses (HIV) type 1 (group M, O) and/or type 2 in human serum or plasma specimens.

**Performance Characteristics**

The analytical and clinical performance characteristics of MP Diagnostics HIV Ag/Ab Combo ELISA 4.0 were evaluated by two external evaluation centres: the Institute of Tropical Medicine (ITM) in Belgium and the German Red Cross – Baden-Württemberg-Hesseng GmbH (DRK).

**Diagnostic sensitivity:** 100% (500/500 positive samples) tested on 310 anti-HIV-1, 100 anti-HIV-2 and 40 anti-HIV-1 non B subtypes (A, C, D, F, G, H, J, K, O, CRF01_AE and other circulating recombinant forms) serum/plasma samples and 50 anti-HIV-Ab / HIV-1 Ag positive samples.

**Diagnostic specificity:** 99.96% tested on 5004 negative plasma samples of blood donors – based on the results after repeat testing of initially reactive samples.

**Analytical specificity:** 200/200 hospitalized patients were negative on the MP Diagnostics HIV Ag/Ab ELISA 4.0. 95/101 samples containing potentially cross-reactive substances, including samples from pregnant women, were negative on the MP Diagnostics HIV Ag/Ab ELISA 4.0. Serum to plasma equivalence is demonstrated on 25 positive and 25 negative serum / EDTA plasma / heparin plasma / sodium citrate plasma couples.

*p24 antigen analytical sensitivity: 1.25U/ml*


# MP Diagnostics MULTISURE HIV Rapid Test

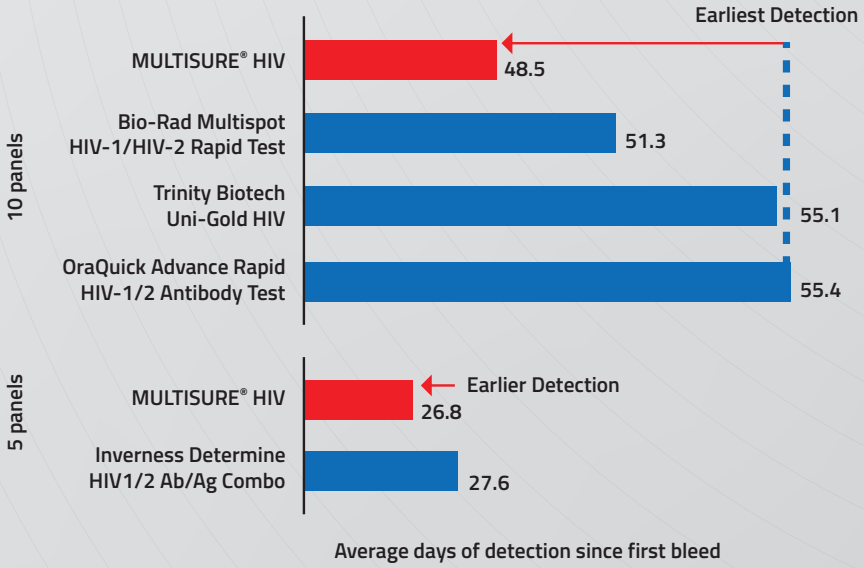
The MP Diagnostics MULTISURE HIV Rapid Test is a qualitative immunochromatographic assay for the rapid in vitro detection and differentiation of antibodies to HIV-1 and HIV-2 in human serum, plasma, finger pricked whole blood or whole blood with anti-coagulants.

## Performance Characteristics

| Diagnostic Parameter | Performance of MP Diagnostics MULTISURE HIV Rapid Test | 95% Confidence Interval |
|---|---|---|
| Sensitivity (n=801) | 100.00% | 99.54% to 100.00% |
| Specificity (n=2057) | 99.12% | 98.62% to 99.48% |
| Positive Predictive Value | 97.80% | 96.55% to 98.69% |
| Negative Predictive Value | 100.00% | 99.82% to 100.00% |

### Total Diagnostic Performance

**MULTISURE® HIV** detects the earliest seroconverion panels when compared against commercially available rapid test kits†.




† Commercial kits data extracted from COA of seroconversion panels from ZeptoMetrix Corporation and SeraCare Life Sciences


# MP Diagnostics MULTISURE HIV 1/2 Confirmatory Test

The MP Diagnostics MULTISURE HIV 1/2 Confirmatory Test is a qualitative immunochromatographic assay for the rapid in vitro detection and differentiation of antibodies to Human Immunodeficiency Virus Type 1 and 2 (HIV-1 and HIV-2) in human serum or plasma. It is intended for professional use as a supplemental test on human serum or plasma samples found to be repeatedly reactive by screening procedures.

## Performance Characteristics

### Total Diagnostic Performance

| Diagnostic Parameter | Performance of MP Diagnostics MULTISURE HIV 1/2 Confirmatory Test | 95% Confidence Interval |
|---|---|---|
| Diagnostic Sensitivity | 100.00% (396/396) | 99.1% to 100.0% |
| Diagnostic Specificity | 98.3% (510/519)<br>(9 Indeterminate for HIV Antibody) | 96.7% to 99.2% |
| Positive Predictive Value | 97.80% | 95.8% to 98.8% |
| Negative Predictive Value | 100.00% | N.A. |

### Differentiation of HIV-1 and HIV-2

| Serotype | Total Sample Size | Accuracy of differentiation by MP Diagnostics MULTISURE HIV 1/2 Confirmatory Test |
|---|---|---|
| HIV-1 | 275 | 100.0% (275/275) |
| HIV-2 | 120 | 100.0% (120/120) |

### Detection of HIV-1 subtypes

| Serotype^ | Total Sample Size | Performance of MP Diagnostics MULTISURE HIV 1/2 Confirmatory Test |
|---|---|---|
| HIV-1 (Group M)* | 80 | 100.0% (80/80) |
| HIV-1 (Group O) | 5 | 100.0% (5/5) |

* Including Group M (Subtype A, B, C, D, E, F, G, H, J, K, C/E, E/F, CRF01_AE, CRF02_AG, CRF03_AB, G/CRF02, H/A1, K/CRF09, CRF01/CRF15)

^ Source of panels:
1. HIV worldwide Performance Panel WWRB301, Sera Care Life Sciences
2. HIV worldwide Performance Panel WWRB304, Sera Care Life Sciences
3. HIV worldwide Performance Panel WWRB305, Sera Care Life Sciences
4. WHO International Standard-NIBSC Code 02/210, NIBSC
5. HIV-1 Group O samples, Biomex GmbH


# MP Diagnostics HIV BLOT 2.2

The MP Diagnostics HIV BLOT 2.2 is a qualitative enzyme immunoassay for the in vitro detection of antibodies to human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2) in human serum or plasma. It is intended for use as a more specific supplemental test on human serum or plasma specimens found repeatedly reactive by screening procedures.

## Performance Characteristics

**Sensitivity study of HIV-1 viral antigen reactivity with HIV-1 seropositive samples. (Number of samples = 201)**

| Serological Profile | HIV Blot 2.2 |
|---|---|
| GAG, POL and ENV | 97.5% |
| p24, p31, gp41 and/or gp120/gp160 | 94.9% |
| ENV and GAG or POL | 100.0% |

**Specificity study of HIV-1 viral antigen reactivity with normal donor samples and sera with other viral infections**

| Sample Type | Number Tested | Positive | HIV-1 Reactivity | |
|---|---|---|---|---|
| | | | Indeterminate* | Negative |
| Normal Donors | 208 | 0 | 11 | 197 |
| HTLV-1 | 5 | 0 | 0 | 5 |
| CMV | 5 | 0 | 1 | 4 |
| EBV (IgM) | 5 | 0 | 1 | 4 |
| V. zoster (IgG) | 5 | 0 | 1 | 4 |
| Measles | 6 | 0 | 2 | 4 |
| Rubella | 5 | 0 | 1 | 4 |
| Mumps | 4 | 0 | 1 | 3 |
| Adenovirus | 5 | 0 | 2 | 3 |
| HSV | 5 | 0 | 0 | 5 |
| Dengue | 5 | 0 | 1 | 4 |
| **Total** | **258** | **0** | **21** | **237** |

*All showed as a p24 or p17 band only.

## Sensitivity study of HIV-2 peptide band with HIV-2 seropositive samples

| HIV-2 Western Blot Serological Profile[#] | HIV-2 Peptide Reactivity | |
|---|---|---|
| | Positive | Negative |
| GAG, POL and 2 ENV | 160 | 0 |
| GAG, POL and 1 ENV | 18 | 0 |

[#]Sera defined as positive by results of Pasteur New LAV Blot 2. Data provided by Dr. Oliviero E. Varnier and Dr. Flavia Lillo. Laboratory of Human Retroviruses. University of Genoa.v

## Specificity study of HIV-2 peptide band with HIV-1 seropositive sera, normal donor samples and sera with other viral infections

| Sample Type | Number Tested | HIV-2 Peptide Reactivity | |
|---|---|---|---|
| | | Positive | Negative |
| HIV-1 seropositive | 197 | 16[#] | 181 |
| Normal Donors | 208 | 0 | 208 |
| HTLV-1 seropositive | 5 | 0 | 5 |
| CMV | 5 | 0 | 5 |
| EBV (IgM) | 5 | 0 | 5 |
| V. zoster (IgG) | 5 | 0 | 5 |
| Measles | 6 | 0 | 6 |
| Rubella | 5 | 0 | 5 |
| Mumps | 4 | 0 | 4 |
| Adenovirus | 5 | 0 | 5 |
| HSV | 5 | 0 | 5 |
| Dengue | 5 | 0 | 5 |
| **Total** | **455** | **16** | **439** |

[#]When tested on the MP Diagnostics HIV-2 Western Blot, 6 of these samples had reactivity with ENV and GAG or POL, and 9 of these samples had reactivity to only GAG and/or POL while 1 sample was negative.

# Conclusion

The various immunoassays for HIV diagnosis are usually intended for either screening or confirmation purpose. For screening, the test device needs to be able to detect the positive specimen as early as possible (especially during seroconversion).

While, for supplemental use, the test device needs to be able to confirm the result of the repeatedly reactive specimen. MP Biomedicals has a full suite of screening and supplemental/confirmatory test devices which can be used in any step of the HIV testing algorithm.

### Order Information

| Catalog Number | Product Name | Pack Size |
|---|---|---|
| 0723040096 | HIV Ag/Ab Combo ELISA 4.0 | 96 tests |
| 0723040480 | | 480 tests |
| 0743030020 | MULTISURE HIV Rapid Test | 20 tests |
| 0793030020 | MULTISURE HIV 1/2 Confirmatory Test | 20 tests |
| 0711030018 | HIV Blot 2.2 | 18 tests |
| 0711030036 | | 36 tests |

## References


1. FACT SHEET– WORLD AIDS DAY 2020 (https://www.unaids.org/en/resources/fact-sheet).
2. Clavel F, Gue´tard D, Brun-Ve´zinet F, et al. Isolation of a new human retrovirus from West African patients with AIDS. Science 1986;233:343–6.