The HIV 1+2 Ag/Ab ELISA is an enzyme-linked immunosorbent assay (ELISA) intended for qualitative detection of antibodies and p24 antigen in human serum or plasma. This kit can be used to detect antibodies to HIV-1 and HIV-2 in-vitro by the acquisition of the acquired immunodeficiency syndrome (AIDS).

**SUMMARY**

The human immunodeficiency viruses type 1 and type 2 are the etiological agents of the acquired immunodeficiency syndrome (AIDS). Since the first report, the HIV 1+2 Ag/Ab ELISA has been isolated from patients with AIDS. AIDS related complex (ARC) and from healthy individuals at high risk for AIDS. Infection with HIV is followed by an acute flu-like illness. This phase may be so mild that the patient is not aware of having contracted it. The acute phase is typically followed by an asymptomatic carrier state, which progresses to AIDS in about 50% of infected individuals within 10 years after seroconversion. Serological evidence of infection with HIV may be obtained by testing for presence of HIV antigens or antibodies in serum of individuals suspected for HIV infection. Antigens can generally be detected during both acute phase and the asymptomatic phases of AIDS only. The antibodies can be detected in the serum of HIV-1 and/or HIV-2 can be detected throughout the whole infection period, starting at or shortly after the acute phase appearance. The ELISA tests for detection of HIV infection are characterized with high sensitivity, specificity and simple operation procedure. They are most appropriate for testing of large numbers of specimens and currently, internationally available are hundreds of HIV tests used in routine blood screening or clinical diagnosis. Since the first HIV ELISA was sold in the market, it has been tested for over four million specimens, and the results have been based on viral isolates derived from viruses that are known to immunodeficiency virus type (HIV). The presence of HIV in human sera constitutes an important ease for the propagation of virology and virological studies. A virus persistence in the asymptomatic phase of infection can be shown by the detection of antibodies to HIV in human sera. Therefore, all donations of blood or plasma should be tested due to the risk of HIV transmission through contaminated blood.

**THE ELISA TESTS FOR DETECTION OF HIV INFECTION ARE CHARACTERIZED WITH HIGH SENSITIVITY, SPECIFICITY AND SIMPLE OPERATION PROCEDURE. THEY ARE MOST APPROPRIATE FOR TESTING OF LARGE NUMBERS OF SPECIMENS AND CURRENTLY, INTERNATIONALLY AVAILABLE ARE HUNDREDS OF HIV TESTS USED IN ROUTINE BLOOD SCREENING OR CLINICAL DIAGNOSIS. Since the first HIV ELISA was sold in the market, it has been tested for over four million specimens, and the results have been based on viral isolates derived from viruses that are known to immunodeficiency virus type (HIV). The presence of HIV in human sera constitutes an important ease for the propagation of virology and virological studies. A virus persistence in the asymptomatic phase of infection can be shown by the detection of antibodies to HIV in human sera. Therefore, all donations of blood or plasma should be tested due to the risk of HIV transmission through contaminated blood.

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**QUALITYCONTROL AND CALCULATION OF THE RESULTS**

Each microplate should be considered separately when calculating and interpreting the results of the assay, regardless of the number of plates concurrently processed. The results are calculated by relating each specimen absorbance (A) value to the Cut-off value (C.O.) of the plate. If the Cut-off reading is based on single filter reader, the results should be calculated by subtracting the Blank well A value from the print report values of specimens and controls. In this case the reading is based on dual filter plate reader, do not subtract the Blank well A value from the print report values of specimens and controls.

Calculation of the Cut-off value (C.O.) = A + ±±12 (±± mean absorbance value for three negative controls).

**Quality control (assay validation):**

The test results are valid if the Quality Control criteria are fulfilled. It is recommended that each laboratory must establish appropriate quality control system with quality control material similar to or identical with the patient sample being analyzed.

- The A value of the Blank well, which contains only Chromogen and Stop solution, is ≤ 0.080 at 450 nm.
- The A values of the Positive control must be ≥ 0.800 at 450 nm or 455 nm after blanking.
- The A values of the Negative control must be ≤ 0.100 at 450 nm or 455 nm after blanking.

If one of the Negative control A values does not meet the Negative control criteria, it should be discarded and the mean value calculated again using the remaining two values. If more than one Negative control A values do not meet the Quality Control Range specifications, the test is invalid and must be repeated.

**Example:**

1. **Quality Control:**
   - Blank well A value = 0.025 at 450nm (Note: blanking is required only when reading with single filter at 450nm)
2. **Well No.:**
   - B1 C1 D1
   - Negative control A values after blanking: 0.020 0.012 0.016
3. **Well No.:**
   - E1 F1 G1
   - Positive control A values after blanking: 2.421 2.439 2.593

INTERPRETATION OF THE RESULTS

**Negative Results** (A/C.O. < 1): Specimens giving absorbance less than the Cut-off value are negative for this assay, which indicates that no HIV-1 antibodies or p24 antigen have been detected with this HIV 1+2 Ag/Ab ELISA, therefore the patient is probably not infected with HIV-1 and the blood unit do not contain antibodies to HIV-1 or p24 antigen and could be transferred in case that other infectious diseases markers are also absent.

**Positive Results** (A/C.O. ≥ 1): Specimens giving an absorbance equal to or greater than the Cut-off value are considered positive for HIV-1 antibodies and/or p24 antigen which indicates that HIV-1 antibodies and/or p24 antigen have probably been detected using this HIV-1+2 Ag/Ab ELISA. All initially reactive specimens should be retested in duplicates using this HIV-1+2 Ag/Ab ELISA. Reactive positive speciment can be considered positive for antibodies to HIV 1/2 and/or p24 antigen which this HIV 1+2 Ag/Ab ELISA.

**Borderline (A/C.O. = 0.9-1.1) Specimens:**

Specimens with absorbance to Cut-off ratio between 0.9 and 1.1 are considered borderline and retesting of these specimens is duplicate is required to confirm the initial results.

**Follow-up, confirmation and supplementary testing of any positive specimen with other analytical system (e.g. WB, FCR) is required. Clinical diagnosis should not be established based on a single test result. It should integrate clinical and other laboratory data and findings.**

**LIMITATIONS**

- If, after retesting of the initially reactive samples, both wells are negative results (A/C.O.<0.9), these samples should be considered as non-repeatable positive (false positive) and recorded as negative. As with many sensitive ELISA assays, false positive results can occur due to the several reasons, most of which are connected with, but not limited to, inadequate washing step.
- If after retesting of duplicates, one or both wells are positive results, the result is correct. Reactive positive specimens: Reactively positive specimens can be considered positive for antibodies to HIV 1/2 and/or p24 antigen, therefore the patient is probably infected with HIV/12 and the blood unit must be discarded.
- If after retesting in duplicates, samples with values close to the Cut-off value are interpreted with caution and considered as "borderline" zone samples, or uninterpretable for the time being.

**REFERENCES**


**SUMMARY OF THE MAJOR COMPONENTS OF THE KIT:**

Use this summary only as a reference and always follow the comprehensive method sheet when performing assay. The note: the components of individual kits are not interchangeable.

**SUMMARY OF THE ASSAY PROCEDURE:**

Use this summary only as a reference and always follow the detailed method sheet when performing assay:

**EXAMPLE SCHEME OF CONTROLS / SAMPLES DISPENSING:**

**SYMBOLS:**

- **IND» non-interpretable**
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