HIV 1/2 STAT-PAK™ Assay

FOR EXPORT ONLY
FOR IN VITRO DIAGNOSTIC USE
FOR PROFESSIONAL USE ONLY

A Qualitative Screening Test Kit for the Detection of Antibodies to HIV-1/2 in Human Fingerstick and Venous Whole Blood, Serum and Plasma

STORAGE: Store at 8 to 30°C (46 to 86°F)

INTENDED USE
The Chembio HIV 1/2 STAT-PAK™ assay is a single-use immunochromatographic, rapid screening test for the detection of antibodies to Human Immunodeficiency Virus Types 1 and 2 (HIV 1/2) in fingerstick whole blood, venous whole blood, serum or plasma specimens. The Chembio HIV 1/2 STAT-PAK™ assay is intended for use as a point-of-care test to aid in the diagnosis of infection with HIV 1 and HIV 2. This test is suitable for use in multi-test algorithms designed for the statistical validation of rapid HIV test results. When multiple rapid HIV tests are available, this test should be used in appropriate multi-test algorithms.

SUMMARY AND EXPLANATION
Discovered in 1983, the Human Immunodeficiency Virus (HIV) is a retrovirus identified as the etiologic agent for Acquired Immunodeficiency Syndrome (AIDS) [1]. AIDS is characterized by changes in the population of T-cell lymphocytes that play a key role in the immune defense system. In the infected individual the virus causes a depletion of a subpopulation of T-cells, called T-helper cells, which leaves these patients susceptible to opportunistic infections and certain malignancies. The major routes of transmission are sexual contact, contamination by blood or blood products and mother-to-newborn transmission [2-4].

Although there has been a decrease in the rate of infection in certain countries, the number of persons infected with HIV globally has continued to increase. By the end of 2005 there were approximately 40.3 million people living with HIV/AIDS, an increase from nearly 37.5 million in 2003. An estimated 5 million people were newly infected with HIV/AIDS in 2005. In the same year more than 3 million died of AIDS-related illness; more than 500,000 of these were children [5].

While the HIV virus consists of a genomic RNA molecule protected by a capsid and an envelope, the HIV envelope is the major target for humoral antibody response. The presence of the virus in patients causes the immune system to elicit the production of antibodies to HIV. The detection of these antibodies can be used as a diagnostic tool.

ELISAs, Western Blots, PCR-based assays and various other test systems are currently available for HIV 1/2 detection [6-10]. The Chembio HIV 1/2 STAT-PAK™ assay is a rapid immunochromatographic test, which is simple and easy to use. The Chembio HIV 1/2 STAT-PAK™ assay utilizes immobilized antigens for the detection of antibodies to HIV 1/2 in serum, plasma or whole blood.

PRINCIPLE OF THE TEST
The Chembio HIV 1/2 STAT-PAK™ assay employs a unique combination of a specific antibody binding protein, which is conjugated to colloidal gold dye particles, and HIV 1/2 antigens, which are bound to the membrane solid phase. The sample is applied to the SAMPLE (S) well followed by the addition of running buffer. The buffer facilitates the lateral flow of the released products and promotes the binding of antibodies to the antigens. If present, the antibodies bind to the gold conjugated antibody binding protein. In a reactive sample, the dye conjugated-immune complex migrates on the nitrocellulose membrane and is captured by the antigens immobilized in the TEST (T) area producing a pink/purple line. In the absence of HIV antibodies, there is no pink/purple line in the TEST (T) area. The sample continues to migrate along the membrane and produces a pink/purple line in the CONTROL (C) area containing immunoglobulin G antigens. This procedural control serves to demonstrate that specimen and reagents have been properly applied and have migrated through the device.

MATERIALS PROVIDED
Each kit contains the items to perform 20 tests:
- 20 STAT-PAK™ Individually Pouched Test Devices
- 1 HIV Running Buffer (3.5mL)
- 20 Disposable 5μL Sample Loops
- 1 Product Insert

MATERIALS AVAILABLE AS AN ACCESSORY TO THE KIT
Chembio Rapid HIV 1/2 Antibody Test Kit Controls (Catalog # HIV104)
Each package contains:
- HIV 1 Reactive Control
- HIV 2 Reactive Control
- Nonreactive Control
- Product Insert for the HIV104
MATERIALS REQUIRED BUT NOT PROVIDED

- Clock, watch or other timing device
- Automatic pipettor capable of delivering 5 µL of sample may be used in lieu of the disposable 5 µL sample loop supplied with the kit, for other than fingerstick specimens
- Sterile alcohol swab (for fingerstick samples only)
- Sterile lancet (for fingerstick samples only)
- Collection devices for samples other than fingerstick

PRECAUTIONS

Safety Precautions
1. Do not eat, drink or smoke in the area where specimens and kit reagents are handled. Avoid any contact between hands, eyes or mouth during specimen collection and testing.
2. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when handling patient specimens.
3. Dispose of all specimens and materials used in the test procedure in a biohazard waste container. Lancets should be placed in a puncture-resistant container prior to disposal. The recommended method of disposal of biohazard waste is autoclaving for a minimum of 1 hour at 121°C. Disposable materials may be incinerated. Liquid wastes may be mixed with appropriate chemical disinfectants. A freshly prepared solution of 10% bleach (0.5% solution of sodium hypochlorite) is recommended. Allow 60 minutes for effective decontamination.
NOTE: Do not autoclave solutions that contain bleach.

Handling Precautions
1. Do not use any device if the pouch has been perforated.
2. Each device is for single use only.
3. Always check expiration date prior to testing.
4. Do not use the test beyond the expiration date printed on the pouch.
5. Do not mix reagents from different lot numbers of kits.
6. Adequate lighting is required to read the test results.

STORAGE AND STABILITY

The HIV 1/2 STAT-PAK™ test devices should be stored in unopened pouches at 8 to 30°C (46 to 86°F). Do not freeze. Do not use beyond the indicated expiration date. Test devices are stable until the expiration date marked on the pouch, when stored as indicated. Running Buffer should be stored at 8 to 30°C (46 to 86°F) in the original vial.

SPECIMEN COLLECTION

The Chembio HIV 1/2 STAT-PAK™ test can be performed on fingerstick whole blood, venous whole blood, serum or plasma specimens.

Fingerstick Whole Blood
Following laboratory procedure, prick the finger and wipe away the first drop. Collect the sample from the second drop with the included disposable sample loop. Follow test procedure instructions.

Venous Whole Blood
Draw blood following laboratory procedure for obtaining venous blood. Depending on use, collect sample in a tube containing citrate, heparin or EDTA. Be sure the tube of blood is well mixed before sampling. Follow test procedure instructions.

Serum or Plasma
Collect serum or plasma (EDTA) in a clean container, following standard laboratory procedures. Follow test procedure instructions.

Patient samples perform best when tested immediately after collection. If not tested immediately, specimens should be refrigerated at 2 to 8°C (36 to 46°F) and can be used up to 3 days after collection. If testing within 3 days is not possible, serum or plasma specimens should be frozen at -20°C (-4°F) or colder. DO NOT FREEZE WHOLE BLOOD!

NOTE: If specimens are to be shipped, they should be packed in compliance with regulations covering the transportation of etiologic agents.

CONFIRMATION OF TEST PERFORMANCE

When the test is complete, you will see a pink/purple line in the CONTROL (C) area of the test device on nonreactive as well as reactive samples. This control line serves as an internal control and gives confirmation of sample addition and proper test performance. Pink/purple lines in both the TEST (T) and CONTROL (C) areas indicate a reactive sample.
**TEST PROCEDURE**

If the specimen to be tested is refrigerated, remove it from the refrigerator and allow it to come to a temperature of 18 to 30° C (64 to 86°F) prior to testing.

1. Remove the Chembio HIV 1/2 STAT-PAK™ test device from its pouch and place it on a flat surface (it is not necessary to remove the desiccant from the pouch).
2. Label the test device with patient name or identification number. (See Figure 1 below)

![Figure 1](image)

3. Touch the 5 µL sample loop provided to the specimen, allowing the opening of the loop to fill with the liquid.
4. Holding the sample loop vertically, touch it to the sample pad in the center of the SAMPLE (S) well of the device to dispense ~5 µL of sample (serum, plasma or whole blood) onto the sample pad. (See Figure 2 below)

![Figure 2](image)

5. Invert the Running Buffer bottle and hold it vertically (not at an angle) over the sample well. Add 3 drops (~105 µL) of buffer slowly, dropwise, into the SAMPLE (S) well. (See Figure 3 below)

![Figure 3](image)

6. Read the test result 10 minutes after the addition of the Running Buffer. In some cases a test line may appear in less than 10 minutes however, 10 minutes are needed to report a nonreactive result. Read results in a well-lit area. **Do not read results after 20 minutes.**

**NOTE:** Discard the used sample loop, test device and any other test materials into a biohazard waste container.

**QUALITY CONTROL**

**Built-in Control Feature**

A pink/purple line will appear in the Control (C) area if the test has been performed correctly and the device is working properly. It serves as a built-in procedural control.

**External Quality Control**

Good Laboratory Practices necessitates testing external control material along with the test samples to ensure proper performance of the test kit. Chembio HIV Reactive and Nonreactive Controls (Catalog # HIV104) are available separately for use with the Chembio HIV 1/2 STAT-PAK™ test. The HIV Controls are used to verify the operator’s ability to properly perform the test and to interpret the results. The Reactive Control will produce a reactive test result and has been manufactured to produce a faint line in the TEST (T) area. The Nonreactive Control will produce a nonreactive test result. Run the controls as per the TEST PROCEDURE and INTERPRETATION OF THE RESULTS sections of this insert.

If the HIV Control reagents do not produce the expected results, contact Chembio Diagnostic Systems’ Customer Service (+1-631-924-1135).
INTERPRETATION OF RESULTS

Nonreactive Results

**Nonreactive Result:**
One pink/purple line in the CONTROL (C) area, with no line in the TEST (T) area indicates a nonreactive result. A nonreactive result at 10 minutes indicates that there are no detectable HIV antibodies in the sample. A nonreactive result does not exclude the possibility of HIV infection.

Reactive Results

**Reactive Result:**
Two pink/purple lines, one in the TEST (T) area and one in the CONTROL (C) area indicate a reactive result. The line in TEST (T) area may look different from the line in the CONTROL (C) area.

**NOTE:** Intensities of the TEST (T) and CONTROL (C) lines may vary. If any visible line appears in the TEST (T) area and in the CONTROL (C) area, the result is reactive.

Invalid Results

**Invalid Result:**
An invalid result indicates a problem with running the test, either related to the specimen or to the device. Repeat the test with a new device and/or a new sample. Contact Chembio Diagnostic Systems at +1-631-924-1135 ext 114 or info@chembio.com if you are unable to obtain a valid result upon repeat testing.
LIMITATIONS OF THE PROCEDURE
1. The Chembio HIV 1/2 STAT-PAK™ assay must be used with capillary (fingerstick) or venous whole blood, serum or plasma only. Using other types of specimens or testing of venipuncture whole blood specimens collected using a tube containing an anticoagulant other than citrate, heparin or EDTA may not yield accurate results. For serum samples, collect blood without anticoagulant.
2. The Chembio HIV 1/2 STAT-PAK™ assay must be used in accordance with the instructions in this product insert to obtain accurate results.
3. Be careful not to add more than 3 drops of Running Buffer with blood sample as it may lead to the appearance of a red line at the base of the window.
4. Reading test results earlier than 10 minutes or later than 20 minutes may yield erroneous results.
5. Do not open the sealed foil pouch until just prior to use.
6. Do not use kit contents beyond labeled expiration date.
7. Ensure finger is completely dry before performing fingerstick.
8. Read results in a well-lit area.
9. A reactive result using the Chembio HIV 1/2 STAT-PAK™ assay suggests the presence of antibodies to HIV-1 or HIV-2 in the specimen. The Chembio HIV 1/2 STAT-PAK™ assay is intended as an aid in the diagnosis of infection with HIV-1/2. HIV and AIDS-related conditions are clinical syndromes and their diagnosis can only be established clinically.
10. For a reactive result, the intensity of the test line does not necessarily correlate with the titer of antibody in the specimen.
11. A nonreactive result does not preclude the possibility of exposure to HIV or infection with HIV. An antibody response to recent exposure may take several months to reach detectable levels.
12. An individual infected with HIV-1 and/or HIV-2 who is receiving highly active antiretroviral therapy (HAART) may produce false negative results.

EXPECTED RESULTS
This is a qualitative test for the detection of antibodies to HIV 1/2 in whole blood, serum or plasma. As described in the PERFORMANCE CHARACTERISTICS section below, the sensitivity of the Chembio HIV 1/2 STAT-PAK™ assay was found to be substantially equivalent to EIA and Western Blot tests when tested on selected performance panels.

PERFORMANCE CHARACTERISTICS
In-house studies demonstrate that the sensitivity of the Chembio HIV 1/2 STAT-PAK™ assay is substantially equivalent to the EIA and Western Blot test when tested on BBI performance panels PRZ204 Anti-HIV 1 / 2 Combo Performance Panel, PRF202 Anti-HIV 2 Performance Panel and PRB203 Anti-HIV 1 Mixed Titer Performance Panel as well as BBI Seroconversion panels PRB904 and PRB909.

In an external evaluation of the performance of the HIV 1/2 STAT-PAK™ assay using 336 confirmed nonreactive and reactive sera, plasma and whole blood samples, sensitivity was 100% (129/129) and specificity, 100% (207/207). This included 34 fingerstick blood samples – 29 reactive and 5 nonreactive. In this same study, excellent analytical sensitivity relative to EIA was demonstrated using BBI seroconverter panels, PRB940 and PRB931.

PRECISION
Intraassay
Within run precision was determined by using 10 replicates of two specimens containing different levels of HIV 1/2 antibodies. The nonreactive and reactive results were correctly identified 100% of the time.

Interassay
Between run precision was determined by using the same two specimens in 10 different replicates from three different lots of test devices. Again nonreactive and reactive results were observed 100% of the time.

Cross Reactivity and Interference
No cross reactivity was observed from Hepatitis B, Rheumatoid Factor (~80 IU/ml) and hCG (500 mIU/ml) with Chembio HIV 1/2 STAT-PAK™ assay. In addition, no interference from bilirubin, hemoglobin and triglycerides was observed.
REFERENCES

Available Accessories:
Catalog # HIV104 HIV Reactive and Nonreactive Controls

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