



CD4 STATUS (WB)

One-Step Diagnostic Screening Test for the assessment of Immune Status in HIV/AIDS Patients through Qualitative Detection of Specific Proteins that Directly Correlate to CD4 Counts (CD4 Equivalents) using human Whole Blood Samples

For Professional In Vitro Diagnostic use only

Read Instructions before use

US AND FOREIGN PATENTS PENDING

PACKAGE INSERT

(New Test Cutoff: CD4 Counts 350)

Amgenix **OnSight™ – CD4 STATUS (WB)** rapid test is NOT DESIGNED NOR INTENDED to replace standard quantitative tests such as Flow Cytometry where they are routinely available and affordable.

OnSight™ – CD4 STATUS (WB) Rapid Test is designed for the qualitative determination of immune status in HIV/AIDS patients under conditions where such testing methods are not readily available or is cost prohibitive especially in resource poor and or rural settings.

Description

AIDS is characterized by changes in the amount of T-cell lymphocytes. The virus, in infected individuals, causes a depletion of the T-helper cells, which are a sub population of T-cells. This leaves patients susceptible to opportunistic infections and potential malignancies. The presence of the virus itself causes the immune system to deteriorate as AIDS progresses. In normal and immune suppressed individuals there are specific proteins that are detectable that correlate to CD4 counts and which can be detected by the **OnSight™ – CD4 STATUS (WB)** Rapid Test. Tests such as CD4 counts are among the most widely used method for determining the immune status of an HIV/AIDS of infected patients and to establish the efficacy of and or timing of the start of ART (anti-retroviral therapy) and or HAART (highly active anti-retroviral therapy).

Principle of the Test

OnSight™ – CD4 STATUS (WB) test is a rapid membrane based screening test to detect the presence of specific proteins that correlate to CD4 counts. This test is the newest generation lateral flow immunochromatographic type assay. These are among the simplest and easiest to use POC (point of care) assays requiring no instrumentation or highly skilled individuals to perform.

OnSight™ – CD4 STATUS (WB) test can be used with fresh EDTA or Heparin anti-coagulated whole blood. The test employs the use of an antigen binding monoclonal antibody conjugated to a colloidal gold particle and a unique combination of monoclonal antibodies immobilized on the membrane.

Once the sample is added to the test cassette, the whole blood is separated allowing the plasma to pass into and through the antigen binding/gold complex, which then binds the specific target protein in the sample. As this complex passes over the immobilized antibodies on the membrane, if any specific CD4 equivalent proteins are present the antibodies capture them in turn. This produces pink/purple bands in the T zone of the test card. The remaining complex continues to migrate to a control area in the test card and produces a pink/purple band in the C area. This control band indicates that the test has been performed properly.

Kit Components

Each test kit contains:

1. **OnSight™ – CD4 STATUS (WB)** test packs – 25
2. Package Insert

Materials Required but not Supplied

1. Whole blood: Vacutainer tube, or other appropriate tube, containing heparin or EDTA as an anticoagulant.
2. Micropipette (0-200 µL range) and pipette tips
3. Timer or clock.

Stability and Storage Conditions

The **OnSight™ – CD4 STATUS (WB)** tests are stable at any temperature between 4-30°C when in the unopened pouches. DO NOT FREEZE the kit or expose to temperature extremes. Stability of the kit is 18 months from the date of manufacture – dating is indicated on the pouch and kit label.

General Precautions

- The test is for *In Vitro* Diagnostic Use only.
- Appropriate infection control and handling procedures should be followed – do not smoke, eat or drink in the area where the test is to be performed. Use suitable clothing and gloves when handling samples and when performing the test.
- Do not pipet any samples or reagents by mouth.
- All materials should be considered as potentially infectious. To disinfect, either autoclave materials at 121.5°C for 1 hour or treat with Sodium hypochlorite (1 percent solution).
- Do not use test beyond the expiration date indicated.

Specimen Collection and preparation

1. **OnSight™ – CD4 STATUS (WB)** test is performed using **fresh EDTA or Heparin anti-coagulated whole blood**.
2. The blood specimen should be collected under standard laboratory conditions.
3. **Avoid hemolyzed and or lipemic samples** as large amounts of hemoglobin and or lipids can interfere with test results.
4. Heat inactivation of specimens, which may cause hemolysis and protein denaturation, should be avoided.
5. Patient samples performed best when tested immediately after collection.
6. Samples can be stored at 2-8°C after collection for up to 24 hours if testing cannot be done immediately. Allow sample to reach room temperature before proceeding. **Don't freeze the Blood Samples.**
7. Sodium azide can be added as a preservative up to 0.1% without affecting the test results.
8. Any shipment of samples should comply with all applicable regulations for transporting such materials.

Read Below Section Carefully Before Starting Test

1. When reading the test, any visible Pink/Purple line in the T (Test) area of the card within the prescribed time limit of the test indicates a valid result regardless of how light or dark the line may be.
2. **As the amount of CD4 Equivalent proteins decrease and approach the test cutoff (CD4 Count 350) the resulting test line will become much lighter until the test line disappears as the amount of CD4 Equivalent protein falls below the cutoff. The intensity of the line does not always reflect the amount of CD4 EQ protein present.**
3. The proteins detected by the test are disease “activated” and as such testing normal whole blood may, in some instances, not yield the results expected.

Procedure:

1. Bring all materials and specimens to room temperature.
2. Remove as many test cards from the pouches as needed. Lay on a clean flat surface.
3. Use micropipette to transfer 150 µL of whole blood sample, or place the transfer pipette supplied with the test device in the whole blood specimen and depress the bulb to withdraw a sample.
4. Hold the pipette in a vertical position over the sample well (S) of the test card and deliver 3-4 drops (120-160 µL) of sample into the sample well.
5. Read the result at 20 minutes.

Interpretation of Results:

POSITIVE:

Results are in relative CD4 cell count equivalents (CD4 EQ's)

If two colored bands are visible in both test (T) and control (C) areas within 20 minutes, the test result is positive and valid. The test result can be read as soon as a distinct colored band appears in the test area.

Test line visible which indicates CD4 EQ's of **greater than 350** (+/- 25) - **Satisfactory Immune Status.**

Note: Specimens containing very low levels of CD4 equivalent protein may develop two color bands over 20 minutes.

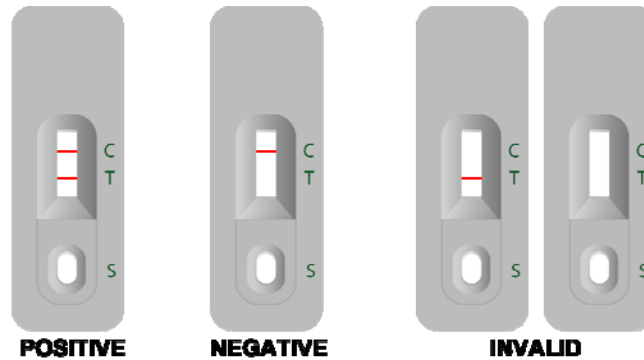
NEGATIVE:

If test area (T) has no color band and the control area (C) displays a colored band, the result is negative and valid.

No test line visible which indicates CD4 EQ's of **less than 350** (+/- 25). **Decreasing Immune Status.**

INVALID RESULT:

If only test bands appear in the Test area (T) or no band appears at all in the Control area (C). It is then recommended that a fresh device be used and the test repeated carefully following the directions in this insert. **Indeterminate.**



Quality Control

1. The control band is an internal reagent and procedural control. It will appear if the test has been performed correctly and the reagents are reactive.
2. Good Laboratory Practice recommends the daily use of control materials to validate the reliability of the device. Control materials which are not provided with this test kit are commercially available.
3. A known set of human based HIV/AIDS control samples with quantitative CD4 counts in the ranges specified above should be run to insure proper performance. All controls should be handled in the same manner as patient samples.

Limitations of the Test

1. The instructions for use and reading of the test instructions must be followed carefully for the test to perform properly.
2. The **OnSight™ – CD4 STATUS (WB)** test is designed to detect specific proteins that correlate to CD4 counts in whole blood.
3. For the accuracy of the test result, only fresh blood samples (up to 24 hours after collection) should be used for the testing. *Serum or plasma samples have higher CD4 Equivalent protein concentration and may give wrong information of CD4 level in patient samples.*
4. The test result should be used in conjunction with other clinical information such as clinical signs and symptoms and other test results to diagnose CD4 level.
5. **OnSight™ – CD4 STATUS (WB)** test only provides qualitative result at cut-off of CD Count 350. A quantitative assay method must be used to determine CD4 concentration.
6. For samples that test in the Decreasing Immune Status range by the **OnSight™ – CD4 STATUS (WB)** test, more specific confirmatory testing should be done. A clinical evaluation of the patient's situation and history should also be made before a final diagnosis is established. The use of a rapid test alone is not sufficient to determine immune status even if corresponding CD4 equivalent ranges of proteins are present. Also, a result in the Satisfactory Immune Status range does not preclude the possibility of infection with HIV 1 or 2 or AIDS or the need for further testing to determine the exact immune status of an individual.
7. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the result of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
8. Testing of any other body fluids has not been validated and may not yield appropriate results.

Performance Characteristics

As there are no true standards established for determining the absence or presence of HIV/AIDS CD4 equivalent proteins in whole blood samples it is recommended that the performance of the test be compared to established CD4 panels or reference materials. The **OnSight™ – CD4 STATUS (WB)** test was tested against internationally recognized CD4 panels as well as fully characterized commercially available HIV/AIDS patient samples and has shown to correlate approximately 89 percent when compared to flow cytometry.