

# RED-DOT HIV 1&2™

## GOLD COLLOIDAL CONJUGATE MEMBRANE FOR HIV-1 & 2 ANTIBODIES

ALL POSITIVE TEST  
MUST BE CONFIRMED WITH  
WESTERN BLOT  
OR  
IMMUNOFLUORESCENT TEST

### PRINCIPLE

AIDS (ACQUIRED IMMUNODEFICIENCY SYNDROME) is characterized by changes in the population of T cell lymphocytes. 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 some malignancies. The presence of the virus in the patient causes the immune system to elicit the production of anti-HIV-1 or HIV-2 antibodies. Tests to detect these anti-HIV-1 or HIV-2 antibodies are now widely used in order to identify infected patients and for the screening of blood derived products.

The synthetic peptides gp-41 derived from HIV-1 and transmembrane glycoprotein and gp-36 derived from HIV-2 represents a major antigenic site for anti-HIV antibodies. A fragment of this protein has been produced in the laboratory with the help of advanced genetic engineering technologies.

RED-DOT HIV-1 & 2™ is a rapid membrane-based immunodiagnostic assay for detection of HIV-1 & 2 antibodies. The assay utilizes a HIV peptide antigen (HIV-1/HIV-2) on the membrane surface which "captures" antibodies in the serum that are against HIV-1/HIV-2. Captured antibodies are visualized with a Protein A colloidal gold conjugate resulting in a red dot. Absence of antibodies in the serum is indicated by a clean white membrane.

The specimen is allowed to pass through the membrane immobilized with HIV peptide antigens. HIV-1 & 2 antibodies in the positive specimen will be bound to the antigens immobilized on the membrane. Protein-A gold will then be added to bind the conjugate portion of the HIV antibodies so as to give a distinct red dot on the membrane.

The test kit represents the second generation of modern HIV antibodies testing. First generation assays use HIV antigen that is purified from cells which have been infected with the virus. The present assay uses a viral protein as antigen which has been produced in the laboratory with the help of advanced genetic engineering technologies. The material used in this test is not isolated from the virus for the envelop protein. THIS MATERIAL IS THEREFORE 100% NONINFECTIOUS and highly specific.

This product is a SCREEN TEST for HIV antibodies. All positive tests must be confirmed with a second test (western Blot or Immunofluorescent test)

### REAGENTS

1. Serum Buffer.
2. Gold Conjugate.
3. Wash Buffer.
4. Reaction Cell (Test Device).

### STORAGE INSTRUCTIONS

1. Do not smoke or eat or drink or pipet by mouth in the laboratory,
2. Wear disposable gloves whenever handling patient's specimens.
3. Use a 5% Sodium Hypochlorite solution to wipe up spills.
4. Treat all materials used in the test as if they were infectious. Autoclave all materials for 1 hour at 121.5 degree Celsius. Add Sodium Hypochlorite to liquid waste in order to reach a final concentration of 1%.
5. Use a separate pipette for each sample and then discard it as a bio-hazardous waste.
6. Once the assay has been started, all subsequent steps should be completed without interruption and within the recommended time limits.

### SPECIMENS COLLECTION

1. Specimen can be drawn by venipuncture or convenient fingertip method. After complete clot reaction, the serum is separated for testing.
2. The serum specimens should be stored refrigerated. If testing is to be prolonged in excess of 24 hours, serum should be frozen. Bacterial contamination may cause protein to denature.
3. Serum specimens should be tested within six hours after collection, if kept at room temperature. If the testing is delayed, the specimens should be refrigerated (2° to 8°C) or kept frozen (for longer storage). Serum may be refrigerated for up to 24 hours.
4. When frozen specimens are thawed, they must be mixed thoroughly and excessive sediment removed by centrifugation before use.
5. If specimens are to be mailed, add 0.01% thimerosal (m/v) to the serum specimens and dispatch by the fastest means available.
6. If fresh blood specimens are to be used, make certain that anti-coagulant is added.

## TEST PROCEDURE

### A. PREPARATION FOR HIV TESTING

1. Prepare a data sheet to identify the individual pack for each specimen.
2. Bring all reagents to room temperature before use.
3. Open a pouch to obtain the test device when ready to test a specimen.

When adding solutions to the device, be sure to allow solutions to soak in before proceeding to the next step. Solutions and sample (serum) should be added to the inner circle of the device. Refer to the diagram.

### B. QUALITATIVE TESTING FOR SERUM SAMPLES.

1. Add 2 drops of Buffer into the device.
2. Add 1 drop of Serum specimen using a disposable pipette provided in each pouch.
3. Add 2 drops of Buffer.
4. Add 2 drops of Wash Solution.
5. Add 2 drops of Gold Conjugate.
6. Add 2 drops of Wash Solution.
7. Read the result on the membrane within 2 minutes.

## INTERPRETATION OF RESULTS

**POSITIVE:** If two red spots are visible (figure 1), the specimen contains HIV-1 & 2 antibodies. The test dot may have any shape or pink/red and may be different color intensity.

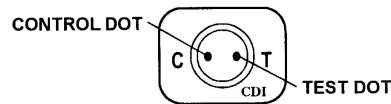
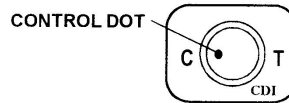


Figure 1

The positive procedural control is a spot of rabbit IgG that has been immobilized onto the membrane surface. This control verifies that the test reagents (colloidal gold conjugate, wash, buffer, and HIV-1& 2 devices) are functioning and that the reagents were added in the correct order.

**NEGATIVE:** If only one red spot (control dot) is visible (Figure 2), the specimen does not contain detectable HIV-1 & 2 antibodies. A slight pinkish color may develop on the overall surface of the membrane that can be considered as negative. Centrifugation of the serum specimen may help flow-through of the specimen on the membrane and reduce the pinkish color. A slight gray



test dot shall be considered as negative.

Figure 2

No positive or negative control serum is needed and therefore not included in the two-dot test.

## FROZEN SAMPLES

The HIV test is best used with fresh specimens that have not been frozen and thawed. However, most frozen specimens will perform well if the suggested procedure is followed.

1. Allow the specimen to thaw in a vertical position and do not shake the specimen. This allows particulates to settle at the bottom. If a centrifuge is available, the specimen can be centrifuged.
2. Insert the disposable pipette just below the surface of the specimen and withdraw on drop of specimen.

## PERFORMANCE CHARACTERISTICS

Specimens which are negative in the HIV assay are considered negative and no further testing is required. Positive specimens should be retested in order to confirm the initial result. Specimens repeatedly reactive in the two assays are considered to be positive for screening test.

This product is a SCREENING TEST for HIV-1 & 2 antibodies. All positive tests must be confirmed with a second test (Western Blot or Immunofluorescent test).

RED-DOT HM 1 & 2 shows 100% agreement with results obtained by the use of other qualified immunological HIV tests.

120 serum specimens of known HIV serology were blindly evaluated using the RED-DOT HIV 1 & 2™. Of these, 37 were ELISA and RED-DOT HIV positive, and 83 were ELISA and RED-DOT HIV negative.

Sensitivity: 100%  
Specificity: 100%

## REFERENCES

1. Gallo, R.C., et al., 1984 Frequent detection an isolation of cytopathic retrovirus (HTLV-III) from patients with AIDS and at risk for AIDS. *Since 224 (4648): 500-503*
2. Schupbach, J. M., et al., 1984 Serologic analysis of a subgroup of human T-lymphotrophic retroviruses (HTLV-III) associated with AIDS. *Since 224:503-505*
3. 1985 Provision Public Health Service Interagency recommendations for screening donated blood and plasma for antibody to the virus causing Acquired Immunodeficiency Syndrome. *MNWR 34: 1-5*
4. Spielberg, F., Fyder, R. W., Harris, J., et al., 1989 Field testing and comparative evaluation of rapid, visually read screening assays for antibody to human immunodeficiency virus. *Lancet 1: 580-584*
5. Spielberg, F., Kabeya, C. M., Quinn, T. C., et al., 1990 Performance and cost-effectiveness of a dual rapid assay system for screening and confirmation of Human Immunodeficiency Virus type 1 seropositivity, *J. of Clin. Micro, 28: 303-306*
6. Van der Groen, G., Van Kerchoven, T., Vercanten, G., et al., 1991 Simplified and less expensive confirmatory HIV testing. *Bull of the WHO, 69: 747-752.*

Jan. 2007