

HEALTH-CHEM DIAGNOSTICS LLC

3341 S.W 15th STREET – POMPANO BEACH, FL 33069 - USA – (954) 979-3845 Fax: (954) 979-7997
Website: //www.healthchemdiagnostics.com

RAPIDTEST HIV® SCREEN – LATERAL FLOW

For use with SaniSal™ – Saliva Collection Device

NAME AND INTENDED USE

The **RAPIDTEST HIV® Lateral Flow** test is for the rapid detection of HIV-1 and HIV-2 antibodies in saliva, whole blood, plasma or serum. Both the testing and the results of the testing are intended to be used by medical and legal professionals only, unless otherwise authorized by regulation in the country of use. Not intended for sale in the USA or its Protectorates. The test should not be used without appropriate supervision. The **RAPIDTEST HIV® Lateral flow** test is intended only as an initial screening test and reactive samples should be confirmed by a supplemental assay such as the Western Blot test.

<i>Simple:</i>	Requires no expensive equipment - No incubations.
<i>Rapid:</i>	Takes only 3-5 minutes to perform (<i>results in 15 minutes or less</i>).
<i>Accurate:</i>	Correlates greater than 99% with Western Blot.
<i>Convenient:</i>	All reagents are ready to use.
<i>Safe:</i>	Contains <u>no</u> infectious materials.
<i>Easy to read:</i>	Two Lines indicate a POSITIVE - One Line indicates a NEGATIVE.
<i>Complete:</i>	Contains all materials needed to collect the specimen and run the test.

EXPLANATION OF THE TEST

The Human Immunodeficiency Virus (HIV) is the causative agent of Acquired Immune Deficiency Syndrome (AIDS). The normal method of detecting infection with HIV is by observing the presence of antibodies to the virus by the ELISA method, followed by confirmation with the Western Blot Test.

Most manufacturers have produced the ELISA test in a micro- titer plate format which requires sophisticated and expensive equipment to obtain a result. Also, it requires highly skilled personnel to perform the test. In many developing world countries the equipment and staff to run these sophisticated tests are limited, and as a result infected blood is still being used for transfusions and many people remain undiagnosed. Therefore, there is a great need for a simple, manual, visual and robust test that can be used to quickly test blood or saliva for HIV antibodies, The **RAPIDTEST HIV® Lateral flow** test is a membrane-based solid phase assay* which uses only one reagent and can provide a definitive result in as little as 3-5 minutes. The assay contains antigens which capture the antibodies from a plasma, serum or saliva sample on a membrane. The antigen/antibody complex is visualized by the reaction of a secondary antibody gold complex which produces a pink to red color. A positive sample produces two distinct red lines on the membrane while a negative sample produces only one line. The antigens used consist of the specific reactive components of both HIV-1 and HIV-2.

The capture antigens used in this new generation test are recombinant peptides which correspond to highly immunoreactive regions of HIV-1 and HIV-2. Five (5) surface and three (3) core binding sites have been

identified. The antigens are absorbed on a nitrocellulose membrane and can capture any HIV antibody present in the sample.

The assay starts with the application of a saliva sample followed by the accompanied diluent applied to the sample well. A HIV antibody-binding colloidal gold-antigen conjugate embedded in the sample pad reacts with the HIV antibody present in the saliva, serum or plasma sample forming conjugate/HIV antibody complex. As the mixture is allowed to migrate along the test strip, the conjugate/HIV antibody complex is captured by an antibody-binding protein A immobilized on a membrane forming a pink test band in the test region. A negative sample does not produce a test band due to the absence of colloidal gold conjugate/HIV antibody complex. The antigens used in the conjugate test are recombinant proteins which correspond to highly immunoreactive regions of HIV-1 and HIV-2. A pink control band in the control region appears at the end of test procedure regardless of test result. This control band is the result of colloidal gold conjugate binding to an anti-HIV antibody immobilized on the membrane. The control band indicates that the colloidal gold conjugate is functional.

MATERIALS PROVIDED

1. Test cassette
2. Sample diluent
3. Saliva collection device (SaniSal®)
4. Instruction for use.

MATERIALS SUGGESTED BUT NOT PROVIDED

- Positive and Negative Controls (*available as separate items*).

WARNINGS AND PRECAUTIONS

- For in vitro diagnostic and laboratory investigational use only.
- Keep out of reach of children.
- For professional medical and legal use only, unless otherwise authorized by regulation in the country of use.
- All positive results may be confirmed by an alternate method.
- Treat all specimens as though potentially infectious. Wear gloves and protective clothing when handling specimens.
- Devices used for testing should be autoclaved before disposal.
- Do not use kit materials beyond their expiration dates.
- Do not interchange reagents from one kit lot to another.

SAMPLE COLLECTION AND HANDLING

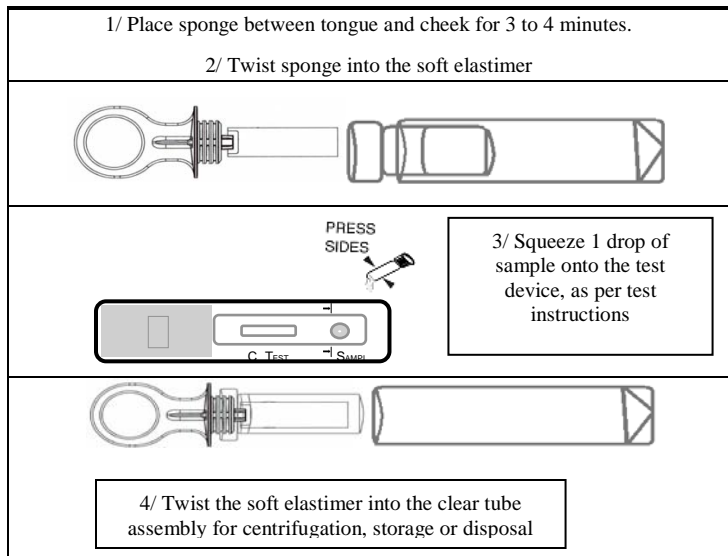
While saliva is recommended as the sample, whole blood, plasma, and serum will also work with the test cassette. Note: This procedure is written ONLY for Saliva. Use fresh saliva collected with a SaniSal®

Saliva Collection Device. If the test is not performed immediately, saliva samples should be kept refrigerated (5°C - 8°C). Do Not Freeze.

SaniSal® - Saliva Collection Device



SaniSal® - Saliva Collection Device Illustration of use



For saliva collection, use the following procedure:

1. Hold the SaniSal saliva collection device **by the cap only**. Twist and pull the device from the flexible test tube. Place the foam part of the device between the cheek and gum for about 4 minutes or until the foam sponge has expanded and thoroughly wetted.
2. Remove the saliva collection device from the mouth (again handling only by the plastic cap), and re-insert the wet device into its flexible test tube by twisting gently.
3. A saliva sample may be tested immediately by gently squeezing the polyethylene cover, to compress the foam, allowing sample drops to be applied to the sample well of a test device. **NOTE:** Avoid dispensing foam or drops containing air bubbles by discarding the first drop or two if necessary.
4. For later testing or submission to a laboratory, the polyethylene section should be inserted in the accompanying test tube with the cap sealed securely on the rim of the test tube.
5. The re-assembled device can now be transported or discarded. For further testing, the assembled SaniSal may

be centrifuged and the saliva sample may be transferred from the bottom of the test tube using a dropper pipette.

6. Disposal: use appropriate precautions and procedures for hazardous waste disposal. • Saliva specimens may be infectious, although the risk of infection from saliva is lower than the risk from plasma or serum. Properly handle and dispose of all used reaction devices in an approved biohazard container.

HOW TO USE THE RAPID TEST INDIVIDUAL HIV SAMPLE DILUENT

1. Tap the ampoule gently on a hard surface to force contents to the bottom of the tube, next to the applicator tip
2. Carefully cut (or break) the top of the applicator tip off, with a sharp knife or scissors.
3. After adding sample to the cassette (*step 4 below*) invert ampoule and gently squeeze the entire contents of the ampoule into the sample well.

INSTRUCTIONS - SAMPLE PREPARATION & ASSAY PROCEDURE

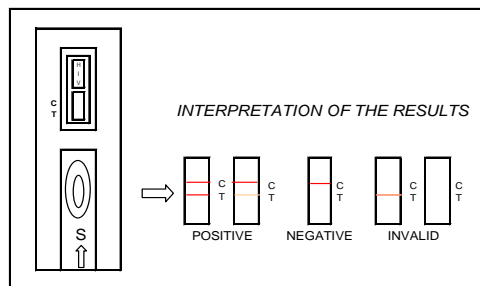
1. Bring diluent and specimens to room temperature.
2. Remove the HIV cassette and buffer from sealed pouch.
3. For saliva, add 1 drop of Saliva from the flexible cover of the sponge of the SaniSal Saliva Collection Device, by squeezing gently (avoid adding foam or bubbles). Allow to be absorbed into the sample hole.
4. Dispense the entire contents of the buffer into the sample well. This should be accomplished by following the “*RAPID TEST INDIVIDUAL HIV SAMPLE DILUENT*” instructions given above. When using a multi test bottle of buffer, add 2 drops of diluent from the dropper bottle.
5. **REACTION TIME:** The Health-Chem Diagnostics RAPID HIV® SCREEN – LATERAL FLOW is a membrane-based solid phase assay, which uses only one reagent and can provide a definitive result in as little as 3 minutes. Read the test results within 10 minutes for easiest interpretation. A very weak positive could react in as long as 15 minutes. Do Not interpret the results after twenty (20) minutes.

Note: Positive result could appear as soon as 1 minute for a sample with high levels of HIV antibodies.

PRECAUTIONS

- If at all possible, use fresh samples with the test.
- Saliva specimens may be infectious, although the risk of infection from saliva is lower than the risk from plasma or serum. Properly handle and dispose of all used reaction devices in an approved biohazard container.

READING THE TEST RESULTS



1. **Positive:** Both purplish red test band and purplish red control band appear on the membrane. The lower the antibody concentration, the weaker the test band.
2. **Negative:** Only the purplish red control band appears on the membrane. The absence of a test band indicates a negative result.
3. **Invalid:** There should always be a purplish red control band in the control region regardless of test result. If control band is not seen, the test is considered invalid. Repeat the test using a new test device.

Note: *It is normal to have a slightly lightened control band with very strong positive samples, as long as it is distinctly visible.*

STORAGE

It is recommended that all components provided should be stored REFRIGERATED at 5°C to 8°C. or in the coolest and driest area available. All components are stable, if stored and transported properly, for 12 months. DO NOT FREEZE. Allow solutions to reach room temperature before performing the test,

RECENT STUDY RESULTS

Specificity- 100%

In an in-house laboratory study, Saliva from 119 confirmed negative with Western Blot whole blood samples were evaluated with RAPIDTEST HIV® SCREEN - Lateral Flow test using both, EIA and Western Blot as reference tests. The study gave 100% specificity for the test.

Sensitivity- 100%

In the above mentioned study, RAPIDTEST HIV® SCREEN – LATERAL FLOW Test was evaluated with 64 confirmed whole blood positive samples. The sensitivity of RAPIDTEST HIV® SCREEN Lateral Flow test was found to be 100% relative to Western Blot assay.

A recent European study, 875 specimens. Sensitivity 99.1% and Specificity 99.2%. 100% correlation relevant to Western Blot and EIA. (Flavia Lillo, et. al. – San Raffaele Hospital, Milan, Italy. Published in New Microbiologica, 32, 391-396, 2009).

PATCH*: The Program for Appropriate Technology in Health (WHO Collaborating Center on AIDS). These studies were conducted in the following geographic regions, over a two year period:

Region: USA=230 / INDIA=84 / SUB-SAHARA-AFRICA=78 / SOUTHEAST ASIA=16 / MEXICO=49.

Sensitivity and Specificity were also confirmed by the following outside studies:

	PATCH*	Ghana	South Africa	Chan, et al Canada Ref#11	R.J. Sherman Ref# 12 (Saliva vs Western Blot)	San Raffaele Hospital, Milan, Italy
Sensitivity	99.5%	100%	98.0%	100%	99.9%	99.1%
Specificity	99.3%	100%	100%	98.1%	99.7%	99.2%
N =	457	100	107	296	1938	875

REFERENCES

1. Schable, C., Zekeng, L., Pau, C.P., Hu, D., Kaptue, L. & al. Lancet 1994; 344:1333-4. 2. Malone, J.D., Smith, E.S., Sheffield, J., Bigelow, D., Hyams, K.C., Breardsley, S.G., et al. J. Acquir. Immune. Deficiency Syndr. 1993; 6:115-9. 3. Irwin, K., Olivo, N., Schable, C. A., Weber, J.T, janssen, R., Ernst, J., et al. Ann. Int. Med. 1996: 111:44-53 - 4. Sato, P. A., Maskill, W.J., Tamashiro, H, Heymann, D.L., J. Clin. Microbiol, 1990: 28:819-22. - 5. Guyader, M., Emerman, M., Sonigo, P., et al. Genome organization and trans-activation of the human immunodeficiency virus type 2. Nature, 326:662-669. 1987. - 6. Blattner W., Gallo, R.C. and Temin. H.M. HIV causes AIDS. Science. 241:515, 1988. - 7. Curran, J,W, Morgan. W.M., Hardy, A.M., et al. The epidemiology of AIDS: Current status and future prospects. Science 229:1352-1357. 1985. - 8. Sarngadharan. M.J., Popovic M., Bruch, L, Schupback. J., and Gallo, R.C. Antibodies reactive with human T-lymphotropic retroviruses (HTLV- 111) in the serum of patients with AIDS. Science. 224:506-508. 1984. - 9. Weber, JN., Weiss, R.A., Roberts, C., et al. Human immunodeficiency virus infection in two cohorts of homosexual men: Neutralizing sera and association of anti-gag antibody with prognosis, Lancet 1:119-124. 1987. - 10. Clavel, F., Gultard. D., Brun-Vizinet, F., et al. Isolation of a new human retrovirus from West African patient with AIDS. Science 233:343-346. 1986. – 11. CHAN, ET AL: Published in the Proceedings of American Society for Microbiology, 95th General Meeting, Washington, DC, 24 May 1995. “Comparison of Rapid Test HIV, a Rapid HIV-1 and HIV-2 Antibody Test, versus Enzyme Immunoassay and Western Blot (Immunoblot)”. E. L. Chan, F. Sidaway, and G Horsman. Laboratory and Disease Control Services Branch, Regina, Saskatchewan, Canada. 12. R.J. Sherman: A new rapid test for HIV1 and 2® / AIDS for the rapid detection and screening of HIV1 & HIV2 antibodies in saliva, serum, and whole blood – (Clinical Study available upon request). - A membrane based test covered under US Patent 4,774,192. Other patents pending.

Manufactured by:
HEALTH-CHEM DIAGNOSTICS LLC
3341 S.W 15th Street - Pompano Beach, FL 33069 – USA
Tel: (954) 979-3845 – Fax: (954) 979-7997
website: www.healthchemdiagnostics.com