

DPP® HIV-SYPHILIS Assay

CAUTION- Investigational Device. Limited by Federal (or United States) Law to Investigational Use.
FOR IN VITRO DIAGNOSTIC USE
FOR PROFESSIONAL USE ONLY

A Qualitative Screening Test Kit for the Detection of Antibodies to HIV 1/2 and/or Syphilis *Treponema* in Fingerstick Whole Blood, Venous Whole Blood, Serum, or Plasma Specimens.

Read this Product Insert completely before using the product. Follow the instructions carefully when performing the test as not doing so may result in inaccurate Test Results.

STORAGE: Store at 2 to 30°C (36 to 86°F)

INTENDED USE

The Chembio DPP® HIV-SYPHILIS 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) and Syphilis *Treponema pallidum* in fingerstick whole blood, venous whole blood, serum, or plasma samples. The Chembio DPP® HIV-SYPHILIS Assay is intended for use as a point-of-care test to aid in the diagnosis of infection with HIV and/or Syphilis. This test cannot tell the difference between an acute or long-term infection. This test is suitable for use in multi-test algorithms designed for the statistical validation of rapid HIV and/or Syphilis test results. When multiple rapid HIV or Syphilis tests are available, this test should be used in appropriate multi-test algorithms.

SUMMARY AND EXPLANATION**HIV**

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].

By the end of 2007 there were approximately 33 million people living with HIV/AIDS. An estimated 2.7 million people were newly infected with HIV in 2007. In the same year more than 2 million died of AIDS-related illness; 270,000 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 DPP HIV-Syphilis Assay is a rapid immunochromatographic test, which is simple and easy to use. The Chembio DPP HIV-Syphilis Assay utilizes immobilized antigens for the detection of antibodies to HIV 1/2 in serum, plasma or whole blood.

Syphilis

Syphilis is sexually transmitted disease (STD) caused by the spirochete *Treponema pallidum*. It is a chronic bacterial infection that remains a public health concern worldwide especially in resource poor settings. Syphilis can be transmitted from infected women to their unborn child during pregnancy. Worldwide, 12 million individuals are diagnosed with syphilis each year, 90% of them in developing countries, and are at risk of becoming infected with and transmitting HIV.

Early and appropriate diagnosis and treatment prevent transmission and development of severe complications. The availability of rapid serological tests greatly enhances public health efforts to decrease the spread of this infection. The serologic detection of specific antibodies to *T. pallidum* is important in the diagnosis of syphilis. Various serologic tests are currently available such as Venereal Disease Research Laboratory (VDRL), rapid plasma reagin (RPR), fluorescent treponemal antibody absorption (FTA-ABS) test, *T. pallidum* hemagglutination (TPHA) test, immunoenzymatic assay (EIA), *Treponema pallidum* particle agglutination (TPPA) test and Western blot (WB) test

In the United States, CDC health officials reported over 36,000 cases of syphilis in 2006, including 9,756 cases of primary and secondary (P&S) syphilis. In 2006, half of all P&S syphilis cases were reported from 20 counties and 2 cities; and most P&S syphilis cases occurred in persons 20 to 39 years of age. The incidence of P&S syphilis was highest in women 20 to 24 years of age and in men 35 to 39 years of age. Reported cases of congenital syphilis in newborns increased from 2005 to 2006, with 339 new cases reported in 2005 compared to 349 cases in 2006 [11].

Between 2005 and 2006, the number of reported P&S syphilis cases increased 11.8 percent. P&S rates have increased in males each year between 2000 and 2006 from 2.6 to 5.7 and among females between 2004 and 2006. In 2006, 64% of the reported P&S syphilis cases were among men who have sex with men (MSM). Pregnant women with the disease can pass it to the babies they are carrying.

What is the link between syphilis and HIV?

Syphilis commonly co-exists in patients with HIV (prevalence is 14–36%). Genital sores (chancres) caused by syphilis make it easier to transmit and acquire HIV infection sexually. There is an estimated 2- to 5-fold increased risk of acquiring HIV if exposed to that infection when syphilis is present.

Ulcerative STDs that cause sores, ulcers, or breaks in the skin or mucous membranes, such as syphilis, disrupt barriers that provide protection against infections. The genital ulcers caused by syphilis can bleed easily, and when they come into contact with oral and rectal mucosa during sex, increase the infectiousness of and susceptibility to HIV. Having other STDs is also an important predictor for becoming HIV infected because STDs are a marker for behaviors associated with HIV transmission.

Pregnancy: HIV and Syphilis

If a pregnant woman is infected with HIV, she can transmit the virus to her baby during pregnancy, labor and delivery, or breastfeeding. Without treatment, around 15-30 percent of babies born to HIV-infected women will become infected with HIV during pregnancy and delivery. A further 5-20 percent will become infected through breastfeeding [12] A woman who knows that she or her partner is HIV positive before she becomes pregnant can find out about interventions that may be able to protect herself, her partner or her baby from becoming infected with HIV. Doctors will be able to advise which interventions are best suited to her situation, and whether she should adjust any treatment she is already receiving if she is HIV positive.

The syphilis bacterium can infect the baby of a woman during her pregnancy. Depending on how long a pregnant woman has been infected, she may have a high risk of having a stillbirth (a baby born dead) or of giving birth to a baby who dies shortly after birth. An infected baby may be born without signs or symptoms of disease. However, if not treated immediately, the baby may develop serious problems within a few weeks. Untreated babies may become developmentally delayed, have seizures, or die.

Early and appropriate diagnosis and treatment prevents the transmission and development of severe complications. For example, the American Academy of Pediatrics and the American College of Obstetricians and Gynecologists recommend [13] that all pregnant women be screened for HIV and syphilis with serologic testing at the first prenatal visit, after exposure to an infected partner, and at the time of delivery. In some states, effective January 1, 2010, a second HIV test must be conducted during the third trimester, and upon her admission for delivery, if no record of the third trimester HIV test is available. They also recommend that pregnant women who are considered at high risk for acquiring syphilis should also be tested at the beginning of the third trimester. Forty-six of the 50 states (90%) and the District of Columbia have laws regarding antenatal syphilis screening [14]. Thirty-four of the 46 statutes (76%) mandate one prenatal test, usually at the first prenatal visit or early in pregnancy [14]. Twelve laws (26%) include third-trimester testing for all or high-risk women [14].

The Chembio DPP® HIV-Syphilis Test is a novel rapid lateral flow test that provides results at the point of care in 15 minutes. It is unique in that it is an aid in the simultaneous diagnosis of infection with HIV and/or Syphilis. The ability to screen for HIV and Syphilis on the same test on the same patient at the same time has a superior advantage to both the patient and the physician over other rapid tests. The Chembio DPP® HIV-Syphilis Test allows for a reduction in costs. Direct costs due to expenditure on medicines, transport, diagnostics, or other health services, and indirect costs, such as lost productivity or the opportunity cost due to time spent seeking care are both reduced for the care giver and the pregnant woman.

PRINCIPLE OF THE TEST

The Chembio DPP® HIV-SYPHILIS test employs a unique combination of an antibody binding protein, which is conjugated to colloidal gold particles, and a cocktail of HIV 1/2 antigens and Syphilis recombinant antigen which are separately bound to the membrane solid phase. The sample is applied to the round well #1 along with a buffer. The buffer facilitates the lateral flow of the released products and promotes the binding of antibodies to the antigens. If antibodies to HIV and/or Syphilis are present, they bind to the specific antigen (HIV and/or Syphilis) immobilized in the TEST (1)(2) area. After the sample and buffer have migrated onto the S2 test strip, additional buffer is added to the square well #2. The conjugated gold particles then migrate on the nitrocellulose membrane and are captured by the antibody-antigen complex in the test area producing pink/purple line(s). Test line 1 indicates the presence of Syphilis antibodies while test line 2 indicates the presence of HIV antibodies. In the absence of antibodies to HIV or Syphilis, there are no pink/purple lines in the TEST (1)(2) area. Unbound conjugated gold particles continue to migrate along the membrane and produce a pink/purple line in the CONTROL (C) area containing an antibody binding protein. This procedural control serves to demonstrate that the 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 DPP® HIV-SYPHILIS Individually Pouched Test Devices
- 20 Disposable Sample Loops (10µL)
- 20 DPP® SampleTainer™ (1mL, Black and White Cap)
- 1 DPP® HIV-Syphilis Running Buffer Bottle (6mL, Green Cap)
- 1 Product Insert

MATERIALS REQUIRED BUT NOT PROVIDED

- Clock, watch or other timing device
- Pipettor capable of delivering 10µL to be used for serum, plasma or venous blood specimens
- Sterile gauze (for fingerstick samples only)
- Disposable gloves
- Antiseptic wipes
- Biohazard disposal containers
- Sterile Safety Lancet (for fingerstick whole blood samples)
- Collection devices for samples other than fingerstick

For IN VITRO diagnostic use

1. Read the Product Insert completely before using this assay. Follow the instructions carefully as not doing so may result in inaccurate test results.
2. Use of this test kit with sample types other than those specifically approved for use with this device may result in inaccurate test results.
3. This test should be performed at 18 to 30°C (64 to 86°F). If stored refrigerated, ensure that the pouch and buffers are brought to operating temperature before performing testing.
4. If the test kit is stored at temperatures outside the storage temperature 2 to 30°C (36 to 86°F), or used outside the operating temperature 18 to 30°C (64 to 86°F), use the Chembio DPP HIV-SYPHILIS Assay Controls to ensure proper performance of the test.
5. Individuals infected with HIV-1 and/or HIV-2 who are receiving highly active antiretroviral therapy (HAART) may produce false negative results.

PRECAUTIONS

Safety Precautions

1. Handle the samples and materials contacting samples as if capable of transmitting infection.
2. Do not eat, drink or smoke in the area where samples and kit reagents are handled. Avoid any contact between hands, eyes or mouth during sample collection and testing.
3. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when handling patient samples.
4. Dispose of all samples 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.

5. Use 10% bleach or other appropriate disinfectants to wipe all spills. The bleach solution should be made fresh each day.
6. For additional information refer to: Centers for Disease Control (CDC): Updated U.S. Public Health Service Guidelines for the Management of Occupational Exposures to HIV and Recommendations for Postexposure Prophylaxis [15].

Handling Precautions

1. If Desiccant Packet is missing, DO NOT USE. Discard test device and use a new test device.
2. Do not use any test device if the pouch has been perforated.
3. Each test device is for single use only.
4. Do not use the test beyond the expiration date printed on the pouch. Always check expiration date prior to testing.
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 DPP® HIV-SYPHILIS test devices should be stored in unopened pouches at 2 to 30°C (36 to 86°F). Do not freeze. Do not open pouch until you are ready to perform a test. When stored as indicated, test devices are stable until the expiration date marked on the pouch. Both Running Buffer and Sample Buffer should be stored at 2 to 30°C (36 to 86°F) in their original bottles. Do not use beyond the indicated expiration date.

SAMPLE COLLECTION

The Chembio DPP® HIV-SYPHILIS Assay can be performed on fingerstick whole blood, venous whole blood, serum or plasma samples.

Fingerstick Whole Blood

Before collecting the sample, write the sample ID on the SampleTainer with the BLACK CAP (Figure 1). Remove (unscrew) the WHITE CAP keeping the BLACK CAP screwed onto the white part of the cap. Prepare to perform the fingerstick collection procedure. Clean the finger of the person being tested with an antiseptic wipe. Allow the finger to dry thoroughly or wipe dry with a sterile gauze pad. Using a sterile lancet, puncture the skin just off the center of the finger and wipe away the first drop of blood with sterile gauze. Avoid squeezing the fingertip to accelerate bleeding as this may dilute the blood with excess tissue fluid.

Collect the sample from the second drop touching the disposable Sample Loop provided to the drop of blood until the Sample Loop is full as shown in Figure 1.

Insert the filled Sample Loop into the SampleTainer with the BLACK CAP, such that the loop is touching the bottom of the bottle. Snap and twist the shaft at the break notch to dislodge the loop into the bottle, as shown in Figure 2. Replace the BLACK/WHITE CAP assembly onto the bottle and gently shake the bottle for 10 seconds. Test immediately, following Test Procedure instructions.

Figure 1

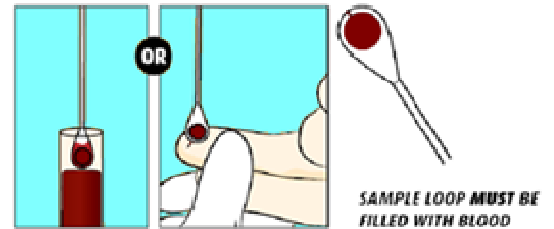
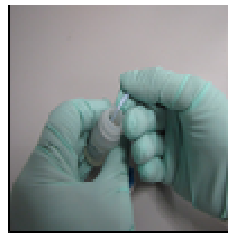


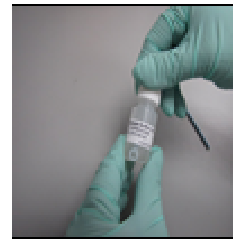
Figure 2



SAMPLE LOOP
INSERTED INTO BOTTLE



SNAP AND TWIST THE SHAFT
AT THE BREAKPOINT
TO DISLUDGE
LOOP INTO THE BOTTLE



REPLACE CAP ON BOTTLE
AND GENTLY SHAKE BOTTLE FOR
10 SECONDS

Venous Whole Blood

Draw blood following laboratory procedure for obtaining venous blood. Collect sample in a tube containing citrate, heparin or EDTA. Be sure the tube of blood is well mixed before sampling. Dip the Sample Loop into the blood and allow it to fill or use a laboratory pipet to withdraw 10µL of the blood. Pipette the sample or insert the filled Sample Loop into the SampleTainer with the BLACK CAP, such that the loop is touching the bottom of the bottle. Snap and twist the shaft at the break notch to dislodge the loop into the bottle, as shown in Figure 3. Replace the BLACK/WHITE CAP assembly onto the bottle and shake the bottle for 10 seconds. Test immediately, following Test Procedure instructions. If tested the same day, venous whole blood may be kept at room temperature. Venous whole blood may be stored for up to 3 days between 2 and 8°C (36 to 46°F) before testing.

DO NOT FREEZE WHOLE BLOOD! Allow refrigerated sample to reach room temperature and mix gently before testing.

Serum or Plasma

Draw blood following laboratory procedure for obtaining serum or plasma samples. Collect serum samples in tubes that do not contain any anticoagulant (serum). Collect plasma samples in tubes containing citrate, heparin, or EDTA. Collect sample in a clean container following standard laboratory procedures. Be sure that the tube of serum or plasma is well mixed before sampling. Use a laboratory pipet to withdraw 10 μ L of the sample. Pipette the sample or insert the filled Sample Loop into the SampleTainer with the BLACK CAP, such that the loop is touching the bottom of the bottle. Snap and twist the shaft at the break notch to dislodge the loop into the bottle, as shown in Figure 3. Replace the BLACK/WHITE CAP assembly onto the bottle and shake the bottle for 10 seconds. Test immediately, following Test Procedure instructions. Serum and plasma specimens may be tested immediately after collection. If specimens are not tested immediately, refrigerate them at 2 to 8°C (36 to 46°F) or freeze at 20°C (-4°F) or colder following collection.

Specimen Shipping

If specimens are to be shipped, they should be packed in compliance with regulations covering the transportation of etiologic agents. Venous whole blood, serum, and plasma specimens should be shipped refrigerated with cold packs or wet ice.

TEST PROCEDURE

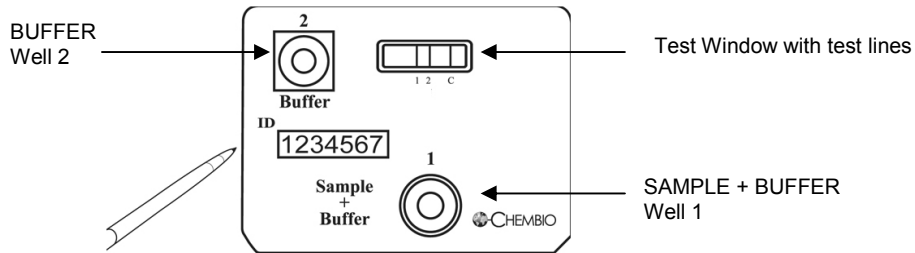
Kit component preparation

All components for the Chembio DPP® HIV-SYPHILIS Assay are ready to use as supplied. Follow directions as indicated. If the sample and / or kit components have been refrigerated, remove them from the refrigerator and allow them to come to a temperature of 18 to 30° C (64 to 86°F) prior to testing.

Whole Blood, Serum, Plasma Test Procedure

1. Remove the Chembio DPP® HIV-SYPHILIS test device from its pouch and place it on a flat surface (it is not necessary to remove the desiccant from the pouch). **NOTE:** If desiccant packet is missing, DO NOT USE. Discard test device and use a new test device. Label the test device with patient ID or identification number (see Figure 3 below). Note that the DPP® test device has 3 colored lines in the Test Window; two are blue and the other is green. If the 3 colored lines are absent, DO NOT USE. Discard the test device and use a new test device.

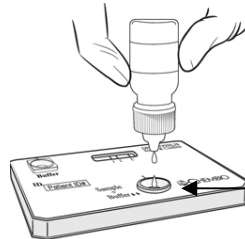
Figure 3



2. For all sample types (Blood, serum and plasma) invert the SampleTainer (black cap), containing the collected sample, and hold it vertically (not at an angle) over the SAMPLE + BUFFER Well 1. Slowly add 2 drops into the SAMPLE + BUFFER Well 1 (see Figure 4 below).

Figure 4

Holding the white portion of the cap, unscrew the **BLACK** portion only.



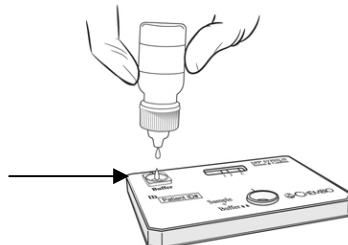
Well 1. Add Sample + Buffer mix here

3. **Wait 5 minutes.** The blue and green colored lines should have disappeared from the rectangular TEST and CONTROL window. If not, discard the test device and repeat the procedure with a new DPP® test device.

Slowly add 4 drops of Running Buffer (green cap) to BUFFER Well 2 (see Figure 5 below).

Figure 5

Well 2. After 5 minutes, add Running Buffer here.



4. Read the test result 10 to 15 minutes after the addition of the Running Buffer to BUFFER Well 2. In some cases test lines 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 15 minutes from the addition of the Running Buffer to BUFFER Well 2.**

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

LIMITATIONS OF THE PROCEDURE

1. The Chembio DPP® HIV-SYPHILIS Assay must ONLY be used with capillary (fingerstick) or venous whole blood, serum or plasma. Using other types of samples or testing of venipuncture whole blood samples 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 DPP® HIV-SYPHILIS Assay must be used in accordance with the instructions in this product insert to obtain accurate results.
3. Reading test results earlier than 10 minutes or later than 15 minutes after the addition of Running Buffer to BUFFER Well 2 may yield erroneous results.
4. Do not open the sealed foil pouch until just prior to use.
5. Do not use kit contents beyond labeled expiration date.
6. Ensure finger is completely dry before performing fingerstick.
7. Read results in a well-lit area.
8. A reactive result using the Chembio DPP® HIV-SYPHILIS Assay suggests the presence of antibodies to HIV and/or SYPHILIS in the sample. The Chembio DPP® HIV-SYPHILIS Assay is intended as an aid in the diagnosis of infection with HIV and/or Syphilis. Syphilis, HIV and AIDS-related conditions are clinical syndromes and their diagnosis can only be established clinically.
9. For a reactive result, the intensity of the test line does not necessarily correlate with the titer of antibody in the sample.
10. A nonreactive result does not preclude the possibility of exposure to HIV or Syphilis or infection with HIV or Syphilis. An antibody response to a recent exposure may take several months to reach detectable levels.
11. A person who has antibodies to HIV-1 or HIV-2 and/or Syphilis is presumed to be infected with the respective virus, except that a person who has participated in an HIV or Syphilis vaccine study may develop antibodies to the vaccine and may or may not be infected with that virus.

QUALITY CONTROL

Built-in Control Feature

The control line serves as a built-in internal control and gives confirmation of sample addition and proper test performance. A pink/purple line will appear in the CONTROL (C) area if the test has been performed correctly and the device is working properly (Please see: Interpretation of Test Results).

External Quality Control

Chembio DPP HIV-Syphilis Reactive and Nonreactive Controls are available separately for use with the Chembio DPP HIV-Syphilis test. The HIV Controls are used to verify the operator's ability to properly perform the test and to interpret the results. Each Reactive Control will produce a reactive test result and has been manufactured to produce a faint line in the TEST (1)(2) area. The Nonreactive Control will produce a nonreactive test result. Run the controls as described in the Test Procedure section for a serum / plasma sample and follow the directions in the Interpretation of Results section of this product insert. It is the responsibility of each facility using the Chembio DPP HIV-Syphilis Assay to establish an adequate quality assurance program to ensure the performance of the device under specific locations and conditions of use.

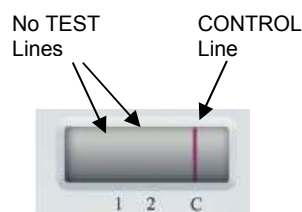
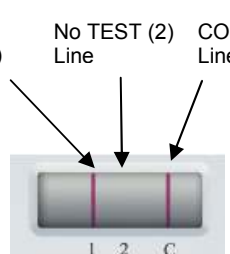
Run the DPP HIV-Syphilis Assay Controls under the following circumstances:

- Each new operator prior to performing tests on patient samples
- When opening a new test kit lot
- Whenever a new shipment of test kits is received
- If the temperature of the test storage area falls outside of 2 to 30°C (36 to 86°F)
- If the temperature of the testing area falls outside of 18 to 30°C (64 to 86°F)
- At periodic intervals as indicated by the user facility

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

INTERPRETATION OF TEST RESULTS

1. SYPHILIS 2. HIV C. Control

<p>NONREACTIVE:</p>	<p>One pink/purple line in the CONTROL (C) area, with no lines in the TEST (1) or TEST (2) areas indicates a nonreactive result. A nonreactive result at 10 to 15 minutes from the addition of buffer to Well 2 indicates that there are no detectable SYPHILIS or HIV antibodies in the sample. A nonreactive result does not exclude the possibility of SYPHILIS and/or HIV infection.</p>	
<p>SYPHILIS REACTIVE HIV NON-REACTIVE:</p>	<p>Two pink/purple lines, one in the TEST (1) area and one in the CONTROL (C) area indicate a SYPHILIS reactive result. The line in the TEST (1) area may look different from the line in the CONTROL (C) area. Intensities of the TEST (1) and CONTROL (C) lines may vary. A test result with visible lines in both TEST (1) and CONTROL (C) areas with no line in the TEST (2) area, regardless of intensity, is considered REACTIVE for SYPHILIS and NON-REACTIVE for HIV.</p>	

<p>SYPHILIS NON-REACTIVE</p> <p>HIV REACTIVE</p>	<p>Two pink/purple lines, one in the TEST (2) area and one in the CONTROL (C) area indicate a HIV reactive result. The line in the TEST (2) area may look different from the line in the CONTROL (C) area. Intensities of the TEST (2) and CONTROL (C) lines may vary. A test result with visible lines in both TEST (2) and CONTROL (C) areas with no line in the TEST (1) area, regardless of intensity, is considered NON-REACTIVE for SYPHILIS and REACTIVE for HIV.</p>	
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<p>SYPHILIS REACTIVE</p> <p>HIV REACTIVE</p>	<p>Three pink/purple lines, one in the TEST (1) area, one in the TEST (2) area and one in the CONTROL (C) area indicate an HIV and SYPHILIS reactive result. The lines in the TEST (1) and TEST (2) areas may look different from the line in the CONTROL (C) area and different from each other. Intensities of the TEST (1), TEST (2) and CONTROL (C) lines may vary. A test result with visible lines in TEST (1), TEST (2) and CONTROL (C) areas, regardless of intensity, is considered REACTIVE for both SYPHILIS and HIV.</p>	
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<p>INVALID:</p>	<p>A pink/purple line should always appear in the CONTROL (C) area, whether or not a line appears in the TEST (1) or TEST (2) areas. If there is no distinct pink/purple line visible in the CONTROL (C) area (see diagrams 1, 2, 3 and 4), then the test is INVALID. An INVALID test cannot be interpreted. It is recommended that the INVALID test be repeated with a new device.</p>	

PERFORMANCE CHARACTERISTICS

SENSITIVITY

The sensitivity of the Chembio DPP® HIV-Syphilis Assay was evaluated using clinically collected samples. Samples were collected in the United-States during a pivotal clinical study. Results were compared to an FDA approved HIV enzyme immunoassay, Western blot for HIV-1 and HIV-2 antibodies, and a Treponemal specific EIA . In cases where there was a discordant result between the DPP rapid test result for syphilis and the Treponemal specific EIA, then a second, different-branded, Treponemal specific EIA was performed. However the results from the second EIA did not always agree with the results from the first EIA. Therefore as part of further resolution testing, a non- treponemal test, i.e. RPR, was performed on all EIA reactives (as per CDC recommendations).

398 samples were found to be positive for HIV via EIA and WB. 398 of the 398 HIV positive samples caused a reactive HIV band to develop on the Chembio DPP® HIV-Syphilis Assay making the sensitivity of the HIV band $398/398 = 100\%$ with the 95% confidence interval extending from 99.1% to 100%. Of the 398 specimens known-positive for HIV, 176 tested positive on the first EIA. The DPP test agreed with 83 of these test results, i.e. a reactive Trep band developed on the Chembio DPP® HIV-Syphilis Assay. Of the remaining 93 samples that were negative on DPP but positive on the first EIA, 63 were found to be negative on the second EIA. EIAs typically remain positive for years despite treatment, are unable to distinguish between past and current infections and therefore can give rise to false positive results. Follow-up testing with the nontreponemal test (RPR) revealed that all 30 of the samples found to be positive on the first Treponemal specific EIA but negative on the second EIA and DPP® HIV-Syphilis Assay were also RPR negative.

According to currently proposed algorithms, since the treponemal tests were reactive, the specimens were reflexed for an RPR. A non-reactive RPR after a positive EIA, may mean that the EIA is a false positive as EIAs cannot distinguish between new/active disease & old disease - treated or untreated, false positive results may occur in otherwise normal or healthy persons (biological false positives), and reports of false-positive results on serologic tests for syphilis in HIV-infected persons raise questions regarding the specificity and sensitivity of serologic diagnoses in such patients.

In the end, the sera gave discrepant results between EIAs and the significance of this could not be further characterized. Most likely, RPR-nonreactive, EIA-reactive cases were either old or treated cases. However, we cannot say this for certain because of the lack of clinical data and history regarding

syphilis on these HIV-positive patient samples. Furthermore, we cannot comment on the differences in levels of sensitivity and specificity between the two EIAs. The reason why certain sera are positive by one EIA and negative by another is not clear, therefore these 63 discrepant samples were excluded from final calculations.

In comparison to EIA only, the sensitivity of the Chembio DPP® HIV-Syphilis Assay for the Trep band is 83/113 = 73.5% with the 95% confidence interval extending from 64.3% to 81.3%.

However, syphilis sensitivity compared with EIA followed by RPR confirmation is 83/83= 100% with the 95% confidence interval extending from 95.7% to 100%.

Therefore the sensitivity of the Chembio DPP® HIV-Syphilis Assay in this set of samples is as follows:

			Sensitivity	95% Confidence Interval
DPP® HIV-Syphilis	HIV Band	398/398	100%	99.1 to 100 %
DPP® HIV-Syphilis	Syphilis Band	83/83	100%*	95.7 to 100 %

*Compared with ELISA followed by RPR Confirmation

In a separate CDC Foundation managed study, the sensitivity of the Chembio DPP® HIV-Syphilis Assay was evaluated using 723 serum samples positive for Trep via TPPA. 682 of the 723 Trep positive samples caused a reactive band to develop on the Chembio DPP® HIV-Syphilis Assay. Therefore the sensitivity of the Chembio DPP® HIV-Syphilis Assay was calculated to be as follows:

			Sensitivity	95% Confidence Interval
DPP® HIV-Syphilis	Trep Band	682/723	94.3%	92.4 to 95.9%

The overall sensitivity of the Trep band of the Chembio DPP® HIV-Syphilis Assay was calculated to be 682/723 = 94.3% with the 95% confidence interval extending from 92.4 - 95.9%

As with any diagnostic test, the positive predictive value (PPV) is dependent on the prevalence of the infection in the population being tested. Given these variables in interpretation, the importance of a careful clinical examination of HIV-infected patients at risk for syphilis cannot be overstated. Clinicians ordering syphilis serology should consider the clinical indication for performing the test and interpret the results based on confirmatory evidence for the diagnosis from any available source, including the patient's history, clinical findings, direct examination of lesion material for spirochetes, and serologic tests for syphilis.

SPECIFICITY

The specificity of the Chembio DPP® HIV-Syphilis Assay was evaluated by testing 330 blood samples, 202 sera samples and 407 plasma samples prepared from blood. These samples were either purchased from a commercial source or prepared from whole blood at Chembio. All the samples were found to be HIV1/2 EIA negative and Syphilis RPR Negative.

All 939 samples were nonreactive for HIV on the Chembio DPP® HIV-Syphilis Assay, making the overall specificity of the DPP HIV test line 939/939 = 100% with the 95% confidence interval extending from 99.6 to 100%.

Sample Matrix	Total Samples tested	DPP® HIV-Syphilis Assay		EIA HIV1/2		Specificity of DPP® HIV-Syphilis Assay	
		HIV1/2 (-)	HIV1/2 (+)	(-)	(+)	HIV1/2	95% CI
Blood	330	330	0	330	0	100%	98.9 to 100%
Sera	202	202	0	202	0	100%	98.2 to 100%
Plasma	407	407	0	407	0	100%	99.1 to 100%
TOTAL	939	939	0	939	0	100%	99.6 to 100%

320 of the 330 blood samples were nonreactive for Syphilis on the Chembio DPP® HIV-Syphilis Assay. All of these samples were Syphilis RPR Negative. No further Treponemal specific reactivity was evaluated.

The 202 sera samples and 407 plasma samples were also tested using a commercially available EIA screening test for the detection of antibodies to Syphilis Treponema pallidum. A subset of the samples that gave discordant results between DPP and the first Treponemal specific EIA were subsequently tested in a second Treponemal specific EIA. Follow up testing was conducted with a Syphilis RPR.

11 of the 407 plasma samples and 7 of the 202 sera samples gave a reactive result on the Trep line of the Chembio DPP® HIV-Syphilis Assay. Twenty-one of the 407 plasma samples and 8 of the sera samples were positive on the first run Treponemal specific EIA. However, there were two samples that were negative on the Treponemal specific EIA that gave a reactive result on the Trep line of the Chembio DPP® HIV-Syphilis Assay. When these two samples were tested on the second Treponemal specific EIA, the result was reactive matching the DPP result.

Sample Matrix	Total Samples tested	DPP® HIV-Syphilis Assay		Treponemal specific EIA 1		Syphilis RPR		Trep Band Specificity %	CI %
		Syphilis Trep (-)	Syphilis Trep (+)	(-)	(+)	(-)	(+)		
Blood	330	320	10	ND	ND	330	0		
Sera	202	195	7	194	8	202	0		
Plasma	407	396	11	386	21	407	0		
TOTAL	939	911	28	N/A	N/A	939	0	97.0%	95.7 - 98.0%

There were 13 samples that gave nonreactive results on the Trep line of the Chembio DPP® HIV-Syphilis Assay but were reactive on the first run Treponemal specific EIA. These samples were subsequently run on a second Treponemal specific EIA. Due to insufficient sample, one of the samples could not be tested further. Three of the samples were positive on the second Treponemal specific EIA. Nine of the 13 samples were nonreactive on the second EIA. All nine of these samples were also found to be Syphilis RPR negative.


DPP® HIV-Syphilis Assay	Treponemal specific EIA 1		
	Positive	Negative	Total
Positive	16	2 ^b	18
Negative	13 ^a	598	611
Total	29	600	629

^aNine out of these 13 samples were positive by a second Trep EIA. All 9 were subsequently found to be RPR negative.

^bThese two samples became positive by EIA upon repeat testing.

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





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