REF 65-9525-0
20 Test Kit

CHEMBIO
DIAGNOSTIC SYSTEMS, INC.

DPP® HIV-SYPHILIS Assay

FOR I*N VITRO* DIAGNOSTIC USE
FOR PROFESSIONAL USE ONLY

A Qualitative, Multiplex, Immunochromatographic Test for the Detection of Antibodies to HIV-1/2 and/or *Treponema Pallidum* in Serum, Plasma, Venous Whole Blood or Fingerstick Whole Blood 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. Users of this test should follow the CDC Universal Precautions for prevention of transmission of Human Immunodeficiency Virus, Hepatitis B Virus, and other bloodborne pathogens.[1]

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

10-6307-0 Rev 1
January 2014

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I. NAME AND INTENDED USE

The Chembio DPP® HIV-SYPHILIS Assay is a single-use rapid, visual, qualitative, multiplex, immunochromatographic test for the detection of antibodies to Human Immunodeficiency Virus Types 1 and 2 (HIV-1/2), and/or *Treponema pallidum* bacteria (the causative agent of syphilis) in human serum, plasma, venous whole blood or finger stick whole blood samples. The Chembio DPP® HIV-SYPHILIS Assay is intended for use in clinical and point-of-care (POC) settings 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 HIV tests are available, this test should be used in appropriate multi-test algorithms. This test is not intended for screening blood or plasma donors. The Chembio DPP® HIV-SYPHILIS Assay is intended for use in clinical and POC settings as an initial screening test or in conjunction with non-treponemal laboratory test and clinical findings, to aid in the diagnosis of syphilis infection. The test cannot distinguish between an acute or long-term infection.

II. RESTRICTIONS

1. **Sale of the Chembio DPP HIV-SYPHILIS Assay is limited by Federal (or United States) Law for Export Only.**
2. **The Chembio DPP HIV-SYPHILIS Assay is not approved for use to screen blood, plasma, cell or tissue donors.**

III. SUMMARY AND EXPLANATION

Syphilis is a global public health problem, resulting in an estimated 12 million new infections per year.[2] The CDC estimates that, annually, 55,400 people in the United States get new syphilis infections. [3] In 2011, 13,970 of these cases were primary and secondary (P&S) syphilis, the earliest and most infectious stages of syphilis. [3] Similar to the HIV epidemic, the current syphilis epidemic predominantly affects three groups: men who have sex with men (MSM), injection-drug users, and individuals who engage in sex for money or drugs. In 2011, 72% of P&S syphilis occurred among men who have sex with men. [3] Women account for a much smaller proportion of cases, but the incidence in this group has been increasing during the past 10 years[2] — a trend that could be related to the high proportion of MSM who engage in sex with women. There were also 360 reports of children with congenital syphilis in 2011. [3]

HIV and *Treponema pallidum* coinfection is relatively common, accounting for approximately 25% of cases of P&S syphilis reported in the U.S.[4] Syphilis facilitates both HIV transmission and HIV acquisition, reflecting the complex interplay between the two diseases. Chancres cause epithelial and mucosal breaches, facilitating the transmission of HIV virions.[5] In addition, *T. pallidum* and its pro-inflammatory components can induce expression of CCR5 (the major coreceptor for HIV entry) on human monocytes within chancres, thereby enhancing the susceptibility of these cells to HIV infection.[4] Furthermore, flow-cytometric studies have demonstrated that during the course of secondary syphilis, *T. pallidum* induces a potent innate and adaptive cellular immune response in both skin and peripheral blood.[6] This immune activation, measured by the increased percentage of activated CD4 cells, may enhance transmission of HIV; however, the immune response has no meaningful effect on the course of syphilis, nor does it prevent recurrent infection.

Pregnant women can transmit STDs to their unborn child, thus it is recommended 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.[7] HIV, if untreated, will result in *in-utero* transmission around 20% of the time[8], with additional transmission at the time of delivery or during breast-feeding [9,10]. 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. A global study showed that maternal syphilis was responsible for 460,000 abortions and stillbirths, 270,000 cases of congenital syphilis and low birth weight and premature babies annually and it is more serious in developing countries. [11,12] Without treatment, syphilis in pregnancy can directly cause adverse pregnancy outcomes in around 50% of cases. [13] Negative outcomes include late fetal loss, stillbirth, premature delivery, low birth weight, infant death, or an infant with reactive serology and clinical symptoms and signs (classically called “congenital syphilis”) [13]. The likelihood of transmission (and hence the risk of adverse outcome) will vary according to both the stage of syphilis infection in the mother (primary, secondary or latent)[15], and the timing of any intervention delivered during the pregnancy [16,17]and may drop to as low as to 1-2% with treatment.[18] If mothers are also infected with HIV, a syphilis infection will increase the risk of HIV transmission from mother-to-child.[19] Pregnant women living with both HIV and syphilis are twice as likely to pass HIV on to their babies compared to a woman infected with HIV alone. [19]

The Chembio DPP® HIV-SYPHILIS Test 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 the patient, the physician and health surveillance initiatives over other rapid tests. The Chembio DPP HIV-SYPHILIS Test has the potential, as a rapid test, to lead to simultaneous, independent diagnosis of two infectious diseases in short turnaround time for the test result, counseling and treatment. Screening allows clinicians to identify affected patients and begin treatment earlier in the course of disease, potentially improving outcomes and avoiding the health consequences that may occur in later stages of the disease. Treatment also reduces the likelihood of spread to others. Screening and early detection are also key to averting costs associated with disease progression and long-term complications. Thus, 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 patient.

IV. BIOLOGICAL PRINCIPLES OF THE TEST

The Chembio DPP HIV-SYPHILIS test employs Chembio’s patented DPP (Dual Path Platform) technology and consists of a sample path and a reagent path, which intersect in the antibody detection TEST (S) (H) and CONTROL (C) areas in the readout window of the test cassette. To initiate the test, a specimen is collected and applied to the SAMPLE+BUFFER Well of the DPP test cassette. The sample flows along the sample path membrane and is delivered to the TEST (S) (H) area of the reagent strip, where specific HIV antigens, a Syphilis recombinant antigen and Protein A are immobilized. HIV

antibodies and/or Syphilis antibodies, if present in the sample, bind instantly to the immobilized HIV and/or Syphilis antigens in the TEST (S) (H) area, while non-specific IgG binds to the Protein A in the CONTROL (C) area. Successful sample application is indicated by the dissolution of soluble dye lines in the TEST and CONTROL areas. Five minutes after adding the sample, buffer is added to the BUFFER Well. The buffer hydrates the dried antibody-binding colored conjugate, which migrates to the TEST area. If the sample contains Syphilis antibodies, the complex binds to the antigens immobilized in the TEST area producing a pink/purple line at TEST (S). If the sample contains HIV-1 and/or HIV-2 antibodies, the complex binds to the viral antigens immobilized in the TEST area producing a pink/purple line TEST (H). In the absence of Syphilis antibodies, there is no pink/purple line in the TEST (S) area. In the absence of HIV-1 and HIV-2 antibodies, there is no pink/purple line in the TEST (H) area. The liquid continues to migrate through the membrane, producing a pink/purple band in the CONTROL (C) area containing Protein A. This procedural control serves to demonstrate that specimen and reagents have been properly applied and have migrated through the device.

V. MATERIALS

i. MATERIALS PROVIDED

Each kit contains the items to perform 20 tests:

20 Individually Pouched DPP HIV-SYPHILIS Test Devices, each containing:

- 1 DPP HIV-SYPHILIS Test Device
- 1 Desiccant Pouch

20 Disposable 10µL Sample Loops
20 DPP HIV-SYPHILIS SampleTainer™– BLACK and WHITE Cap, 1 mL
1 DPP HIV-SYPHILIS Running Buffer – GREEN Cap, 6 mL
1 Product Insert for the DPP HIV-SYPHILIS Assay

ii. MATERIALS REQUIRED BUT NOT PROVIDED

- Clock, watch, or other timing device
- Pipettor capable of delivering 10µL of sample may be used in lieu of the disposable 10µL sample loop supplied with the Kit (for other than fingerstick whole blood specimens)
- Disposable gloves
- Sterile gauze (for fingerstick whole blood specimens)
- Antiseptic wipes
- Biohazard disposal container
- Sterile Safety Lancet (for fingerstick whole blood specimens)
- Collection devices (for venous whole blood or serum specimens)

VI. WARNINGS

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. Individuals infected with HIV-1 and/or HIV-2 who are receiving highly active antiretroviral therapy (HAART) may produce false negative results.

VII. PRECAUTIONS

i. 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 121ºC. 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.[1]
7. The running buffer contains Sodium Azide (0.2%). Avoid skin contact with this reagent. Sodium Azide may react with lead and copper in plumbing and form highly explosive metal oxides. Flush with large volumes of water to prevent azide build up in the plumbing.

ii. 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.

VIII. 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 SampleTainer should be stored at 2 to 30°C (36 to 86°F) in their original bottles.

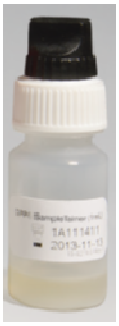
IX. SAMPLE COLLECTION

The Chembio DPP HIV-SYPHILIS Assay can be performed on fingerstick whole blood, venous whole blood, serum or plasma samples. All specimens should be collected, centrifuged (if applicable) and stored following local clinical or laboratory procedures. No special preparation of the patient is necessary prior to collection by approved techniques. Though fresh serum is preferable, specimens may be stored at 4-8°C for up to 24-48 hours in case of delay in testing.[20] Do not use turbid, lipemic and haemolyzed specimens.

i. 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

Figure 1



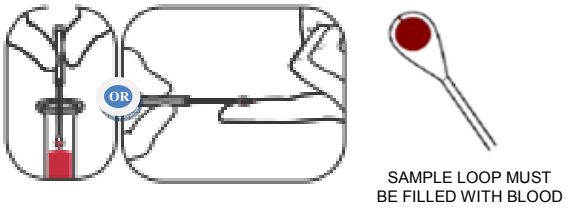
Prepare to perform the fingerstick collection procedure. Clean the finger of the person being tested with an antiseptic wipe. Allow the finger to dry thoroughly or wipe dry with a sterile gauze pad. Using a sterile lancet, puncture the skin just off the center of the finger and wipe away the first drop 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 2.

Insert the filled Sample Loop into the SampleTainer with the BLACK CAP, such that the loop is touching the bottom.

Snap and twist the shaft at the break notch to dislodge the loop into SampleTainer, as shown in Figure 3. Replace the BLACK/WHITE CAP assembly onto the SampleTainer and gently shake the bottle for 10 seconds. Test immediately, following Test Procedure instructions.

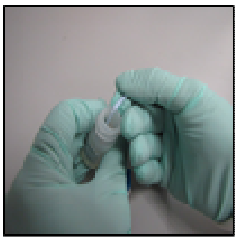
Figure 2



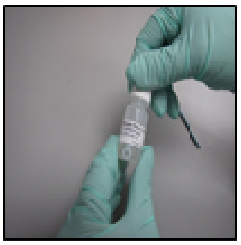
SAMPLE LOOP MUST BE FILLED WITH BLOOD

Figure 3

SAMPLE LOOP INSERTED INTO SAMPLETAINER



SNAP AND TWIST THE SHAFT AT THE BREAKPOINT TO DISLODGE LOOP INTO SAMPLETAINER



REPLACE CAP ON SAMPLETAINER AND SHAKE

ii. Venous Whole Blood

Draw blood following laboratory procedure for obtaining venous blood. Collect sample in a tube containing EDTA. Be sure the tube of blood is well mixed before sampling.

Dip the Sample Loop into the blood and allow it to fill as shown in Figure 2 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.

Snap and twist the shaft at the break notch to dislodge the loop into the SampleTainer, as shown in Figure 3. Replace the BLACK/WHITE CAP assembly onto SampleTainer and shake 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.

iii. 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 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 10µL 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. Snap and twist the shaft at the break notch to dislodge the loop into the SampleTainer, as shown in Figure 3. Replace the BLACK/WHITE CAP assembly onto SampleTainer and shake 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.

X. SPECIMEN SHIPPING

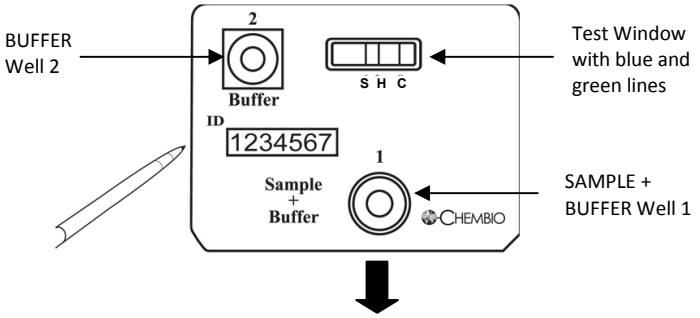
If specimens are to be shipped, they should be packed in compliance with regulations covering the transportation of etiologic agents. Venous whole blood, serum, and plasma specimens should be shipped refrigerated with cold packs or wet ice.

XI. TEST PROCEDURE

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.

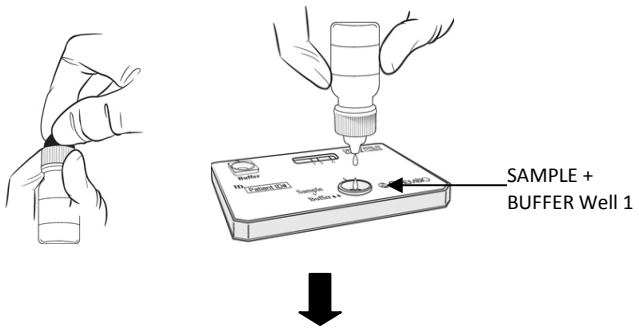
1. Remove the Chembio DPP HIV-SYPHILIS Test Device from its pouch and place it on a flat surface (it is not necessary to remove the Desiccant Packet from the pouch). Note: If Desiccant Packet is missing, DO NOT USE, discard Test Device and a new Test Device should be used.

Label the Test Device with patient ID or identification number. Note that the DPP Test Device has 3 colored lines in the Test Window; the 2 test lines are blue and the control line is green. If the 3 colored lines are absent, DO NOT USE, discard Test Device and a new Test Device should be used.



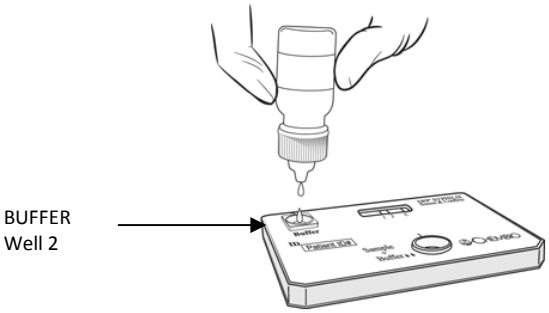
2. Remove (unscrew) the BLACK CAP keeping the WHITE CAP screwed onto the SampleTainer™ bottle.

Invert the SampleTainer™ bottle, containing the collected sample, and hold it vertically (not at an angle) over the SAMPLE + BUFFER Well 1. Add 2 drops slowly, dropwise, into the SAMPLE + BUFFER Well 1.



3. Wait 5 minutes. The blue and green colored lines should have disappeared from the rectangular TEST and CONTROL window. If not, DO NOT USE, discard Test Device and a new Test Device should be used.

Invert the Running Buffer bottle (GREEN CAP), and hold it vertically (not at an angle) over BUFFER Well 2. Add 4 drops of Buffer (GREEN cap) slowly, dropwise, into BUFFER Well 2.

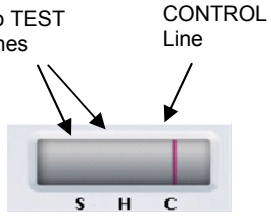
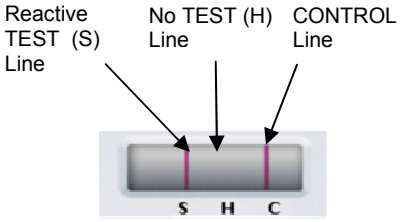
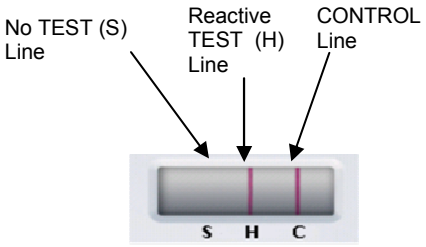
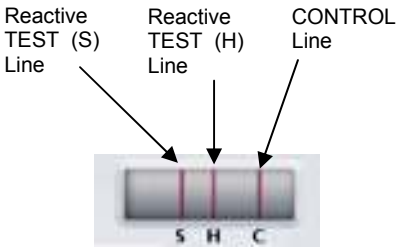
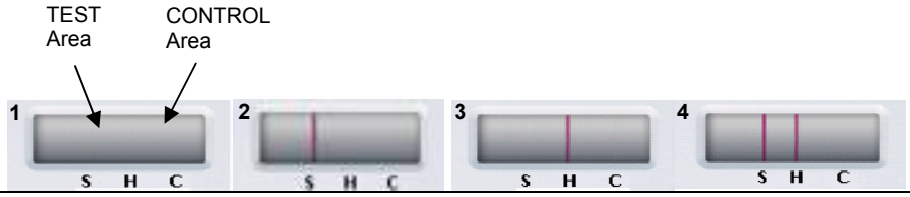


4. Fingerstick, Venous Whole Blood, Serum or Plasma
Read the Test Result between 10 and 25 minutes after the addition of the Running Buffer to BUFFER Well 2.

NOTE: Discard the used Sample Loop, Test Device, and any other test materials into a biohazard waste container

XII. INTERPRETATION OF TEST RESULTS

S. SYPHILIS H. HIV C. Control

NONREACTIVE:	One pink/purple line in the CONTROL (C) area, with no lines in the TEST (S) or TEST (H) areas indicates a nonreactive result. A nonreactive result indicates that there are no detectable TREPONEMA PALLIDUM or HIV antibodies in the sample. A nonreactive result does not exclude the possibility of SYPHILIS and/or HIV infection.	
SYPHILIS REACTIVE **HIV NON-REACTIVE:**	Two pink/purple lines, one in the TEST (S) area and one in the CONTROL (C) area indicates the presence of TREPONEMA PALLIDUM antibodies and a SYPHILIS reactive result. The line in the TEST (S) area may look different from the line in the CONTROL (C) area. Intensities of the TEST (S) and CONTROL (C) lines may vary. A test result with visible lines in both TEST (S) and CONTROL (C) areas with no line in the TEST (H) area, regardless of intensity, is considered REACTIVE for SYPHILIS and NON-REACTIVE for HIV.	
SYPHILIS NON-REACTIVE **HIV REACTIVE**	Two pink/purple lines, one in the TEST (H) area and one in the CONTROL (C) area indicate the presence of HIV antibodies and a HIV reactive result. The line in the TEST (H) area may look different from the line in the CONTROL (C) area. Intensities of the TEST (H) and CONTROL (C) lines may vary. A test result with visible lines in both TEST (H) and CONTROL (C) areas with no line in the TEST (S) area, regardless of intensity, is considered NON-REACTIVE for SYPHILIS and REACTIVE for HIV.	
SYPHILIS REACTIVE **HIV REACTIVE**	Three pink/purple lines, one in the TEST (S) area, one in the TEST (H) area and one in the CONTROL (C) area indicates the presence of HIV and TREPONEMA PALLIDUM antibodies and a HIV and SYPHILIS reactive result. The lines in the TEST (S) and TEST (H) areas may look different from the line in the CONTROL (C) area and different from each other. Intensities of the TEST (S), TEST (H) and CONTROL (C) lines may vary. A test result with visible lines in TEST (S), TEST (H) and CONTROL (C) areas, regardless of intensity, is considered REACTIVE for both SYPHILIS and HIV.	
INVALID:	A pink/purple line should always appear in the CONTROL (C) area, whether or not a line appears in the TEST (S) or TEST (H) 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.	
		

XIII. 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 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 25 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 HIV REACTIVE result using the Chembio DPP HIV-SYPHILIS Assay on the HIV line suggests the presence of antibodies to HIV-1 and/or HIV-2 in the sample and the REACTIVE test result is interpreted as Preliminary Positive for HIV-1 and/or HIV-2 antibodies. The Chembio DPP HIV-SYPHILIS Assay is intended as an aid in the diagnosis of infection with HIV-1/2. AIDS-related conditions are clinical syndromes, and their diagnosis can only be established clinically.
9. A person who has antibodies to HIV-1 or HIV-2 is presumed to be infected with the virus, except that a person who has participated in an HIV vaccine study may develop antibodies to the vaccine and may or may not be infected with HIV.
10. An individual infected with HIV-1 and/or HIV-2 who is receiving highly active antiretroviral therapy (HAART) may produce a false negative result.
11. A SYPHILIS REACTIVE result using the Chembio DPP HIV-SYPHILIS Assay on the TREP line suggests the presence of antibodies to *Treponema pallidum* in the specimen. The Chembio DPP HIV-SYPHILIS Assay is intended as an aid in the diagnosis of infection with Syphilis.
12. An individual infected with Syphilis who is receiving antibacterial therapy may produce false negative results.
13. REACTIVE test results are confirmed by additional testing.
14. For a REACTIVE result, the intensity of the test line does not necessarily correlate with the titer of antibody in the sample.
15. This assay has not been evaluated for newborn screening, cord blood specimens, or blood donor screening.
16. 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.

XIV. 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).

XV. PERFORMANCE CHARACTERISTICS

The DPP HIV-SYPHILIS is almost identical to its predicate the Chembio DPP® HIV 1/2 Assay, approved in December 2012 by the United States Food and Drug Administration (FDA). The only difference in these two products is that the DPP HIV-SYPHILIS Assay has an additional test line (S) for the detection of antibodies to *Treponema pallidum*, the causative agent of syphilis, in addition to the HIV test line (H) and Control Line (C) which is indistinguishable to that of the DPP HIV 1/2 Assay. A clinical comparability study was conducted to evaluate the performance of the DPP HIV-SYPHILIS assay compared to the FDA approved DPP HIV 1/2 Assay in efforts to demonstrate that the addition of the syphilis analyte did not impact the performance of the product in detecting HIV. A total of 1,500 well characterized, prospectively collected specimens obtained as part of a United States Clinical Evaluation to support PMA approval for the DPP HIV 1/2 Assay were tested. Specimens were from three cohorts, each composed of 500 samples: HIV-1-positive individuals, individuals suspect or at high risk for infection with STDs (intended use population), and apparently healthy subjects not suspected of having an STD infection. The final results of this study demonstrated overall agreement of 100% (1500/1500=100% with 95% CI 99.8 - 100%) between the HIV results of the DPP HIV-SYPHILIS test and DPP HIV 1/2 Assay using the HIV clinical samples.

As the clinical comparability study demonstrated equivalent performance between the DPP HIV-SYPHILIS Assay compared to the DPP HIV 1/2 Assay, it is acceptable to reference the approved supporting data for the HIV test line generated from the DPP HIV 1/2 studies to the DPP HIV-SYPHILIS Assay. The Chembio DPP HIV 1/2 Assay was evaluated in prospective clinical studies at five geographically distinct sites. The specimens were tested from three groups of individuals: Known infected with HIV-1, at high risk for infection with HIV-1, and at low risk for infection with HIV-1. The Chembio DPP HIV 1/2 Assay was tested in parallel on fingerstick whole blood, venous whole blood, serum and plasma specimen matrices. The serum/plasma specimens from study subjects were also tested using a licensed Enzyme Immunoassay (EIA). Specimens with discordant results were further tested using licensed Western Blot and/or FDA approved NAT assay.

The following data, although generated using the DPP HIV 1/2 platform, is applicable to the HIV test line of the DPP HIV-SYPHILIS Assay as the HIV test lines are identical and performance of the two products is equivalent. In the following HIV Performance Characteristic sections, for simplification, the Chembio product is referred to as “Chembio DPP Assay”.

HIV

A) HIV Sensitivity

i. HIV-1 Sensitivity- Diagnostic performance

CAPILLARY (FINGERSTICK) WHOLE BLOOD

The sensitivity of the Chembio DPP Assay to detect infection with HIV-1 in capillary (fingerstick) whole blood was evaluated using 868 specimens from individuals known to be infected with HIV-1. All 868 specimens tested repeatedly reactive using an FDA licensed EIA. Of these, 867 specimens tested positive using HIV-1 WB and one tested positive using HIV-1 NAT. Eight hundred sixty seven specimens (867) out of 868 tested Reactive using the Chembio DPP Assay.

In addition, specimens from 976 individuals at high risk for infection with HIV-1 were tested. Of these, 96 specimens tested repeatedly reactive using an FDA licensed EIA, and positive using HIV-1 WB (true positive). On testing these 976 specimens using the Chembio DPP Assay, 95 specimens tested Reactive and 881 tested Nonreactive.

The sensitivity of the Chembio DPP Assay was evaluated using 964 specimens (868 known positives and 96 true positive identified from the high risk population). Of these, 962 specimens tested Reactive using the Chembio DPP Assay (867 known positive and 95 high risk), (see Table 1). In these studies, the Chembio DPP Assay gave false nonreactive results for one known positive specimen and for one confirmed positive specimen from a high risk individual when capillary whole blood specimens were tested. The calculated sensitivity of the Chembio DPP Assay for capillary (fingerstick) whole blood specimens in these studies was 962/964 = 99.8% (95% confidence interval 99.2 to 99.9%).

Table 1: Detection of antibody to HIV-1 in capillary whole blood (fingerstick) specimens from individuals known to be infected with HIV-1 and at high risk for infection with HIV-1

True Status	Chembio DPP Assay		Total
	Reactive	Nonreactive	
Positive[1]	962	2	964
Negative	0	880	880
Total	962	882	1844

1. Based on repeatedly Reactive test results using an EIA and positive using an FDA-licensed WB or NAT assay.

VENOUS WHOLE BLOOD

The sensitivity of the Chembio DPP Assay to detect infection with HIV-1 in venous whole blood was evaluated using 868 specimens from individuals known to be infected with HIV-1. All 868 specimens tested repeatedly reactive using an FDA licensed EIA. Of these, 867 specimens tested positive using HIV-1 WB and one tested positive using HIV-1 NAT. All these 868 specimens tested Reactive using the Chembio DPP Assay.

In addition, specimens from 975 individuals at high risk for infection with HIV-1 were tested. Of these, 96 specimens tested repeatedly reactive using an FDA licensed EIA, and positive using HIV-1 WB (true positive). On testing these 975 specimens using the Chembio DPP Assay, 95 specimens tested Reactive and 880 specimens tested Nonreactive.

The sensitivity of the Chembio DPP Assay was evaluated using 964 (868 known positives and 96 true positives identified from the high risk population). Of these, 963 specimens tested Reactive using the Chembio DPP Assay (868 known positive and 95 high risk) (see Table 2). In these studies, the Chembio DPP Assay gave false negative results for one confirmed positive specimen from a high risk individual when venous whole blood specimens were tested. The calculated sensitivity of the Chembio DPP Assay for venous whole blood specimens in these studies was 963/964 = 99.9% (95% confidence interval 99.4 to 99.9%).

Table 2: Detection of antibody to HIV-1 in venous whole blood specimens from individuals known to be infected with HIV-1 and at high risk for infection with HIV-1

True Status	Chembio DPP Assay		Total
	Reactive	Nonreactive	
Positive[1]	963	1	964
Negative	0	879	879
Total	963	880	1843

1. Based on repeatedly Reactive test results using an EIA and positive using an FDA-licensed WB or NAT assay

PLASMA

The sensitivity of the Chembio DPP Assay to detect infection with HIV-1 in plasma specimens was evaluated using 868 specimens from individuals known to be infected with HIV-1. All 868 specimens tested repeatedly reactive using an FDA licensed EIA. Of these, 867 specimens tested positive using HIV-1 WB and one tested positive using HIV-1 NAT. All 868 specimens tested Reactive using the Chembio DPP Assay.

In addition, specimens from 975 individuals at high risk for infection with HIV-1 were tested. Of these, 96 specimens tested repeatedly reactive using an FDA licensed EIA, and positive using HIV-1 WB (true positive). On testing these 975 specimens using the Chembio DPP Assay 95, specimens tested Reactive and 880 tested Nonreactive.

The sensitivity of the Chembio DPP Assay was evaluated using 964 specimens (868 known positives and 96 true positives identified from the high risk population). Of these, 963 specimens tested Reactive using the Chembio DPP Assay (868 known positive and 95 high risk) (see Table 3). In these studies, the Chembio DDP Assay gave false nonreactive results for one confirmed positive specimen from a high risk individual when plasma specimens were tested. The calculated sensitivity of the Chembio DPP Assay for plasma specimens in these studies was 963/964 = 99.9% (95% confidence interval 99.4 to 99.9%).

Table 3: Detection of antibody to HIV-1 in plasma specimens from individuals known to be infected with HIV-1 and at high risk for infection with HIV-1

True Status	Chembio DPP Assay		Total
	Reactive	Nonreactive	
Positive[1]	963	1	964
Negative	0	879	879
Total	963	880	1843

1. Based on repeatedly Reactive test results using an EIA and positive using an FDA-licensed WB or NAT assay

SERUM

The sensitivity of the Chembio DPP Assay to detect infection with HIV-1 in serum specimens was evaluated using 868 specimens from individuals known to be infected with HIV-1. All 868 specimens tested repeatedly reactive using an FDA licensed EIA. Of these, 867 tested positive using HIV-1 WB and one tested positive using HIV-1 NAT. All 868 specimens tested Reactive using the Chembio DPP Assay.

In addition, specimens from 976 individuals at high risk for infection with HIV-1 were tested. Of these, 96 specimens tested repeatedly reactive using FDA licensed EIA, and positive using HIV-1 WB (true positive). On testing these 976 specimens using the Chembio DPP Assay, 95 specimens tested Reactive and 881 specimens tested Nonreactive.

The sensitivity of the Chembio DPP Assay was evaluated using 964 specimens (868 known positives and 96 true positive identified from the high risk population). Of these, 963 specimens tested reactive using the Chembio DPP Assay (868 known positive and 95 high risk) (see Table 4). In these studies, the Chembio DPP Assay gave false nonreactive results for one confirmed positive specimen from a high risk individual when serum specimens were tested. The calculated sensitivity of the Chembio DPP Assay for serum specimens in these studies was 963/964 = 99.9% (95% confidence interval 99.4 to 99.9%).

Table 4: Detection of antibody to HIV-1 in serum specimens from individuals known to be infected with HIV-1 and at high risk for infection with HIV-1

True Status	Chembio DPP Assay		Total
	Reactive	Nonreactive	
Positive[1]	963	1	964
Negative	0	880	880
Total	963	881	1844

1. Based on repeatedly reactive test results using an EIA and positive using an FDA-licensed WB or NAT assay

B) HIV Sensitivity- Analytical performance

i. Reactivity with HIV-1 Specimens of Different Virus Subtypes

To assess the ability of the Chembio DPP Assay to detect the HIV-1 antibodies directed to different HIV-1 group M subtypes and HIV-1 Group "O", specimens (serum/plasma) from different world wide geographical regions such as Africa (Ghana, Cote d'Ivoire, Mozambique, Uganda, Zimbabwe), Asia (Thailand, China and India), Europe (England, France, Spain and Belgium) and Latin America (Brazil and Argentina) were tested. Of these 204 specimens, 203 specimens tested Reactive with the Chembio DPP Assay. One subtype D tested false nonreactive. The results are presented in Table 5.

Table 5: Testing HIV-1 Specimens from various Geographic Regions using Chembio DPP Assay

HIV Subtype	Number of Specimens	Chembio DPP Assay Reactive
A	7	7
AD	3	3
AE	13	13
AG	21	21
B	64	64
B/D	2	2
C	22	22
D	**16**	**15**
F	9	9
G	18	18
H	5	5
J	4	4
K	11	11
O	9	9
TOTAL	204	203

ii. Seroconversion Panels (Comparison to EIA)

Twenty-one commercial seroconversion panels (serum/plasma) were tested. Each panel consisted of sequential collections from a single individual who seroconverted. The table below presents the days elapsed from the date of the initial bleed to the last Nonreactive sample and first Reactive sample (Table 6). Data are presented for two FDA licensed EIA tests and the Chembio DPP Assay. In comparing test performance, positive numbers indicate earlier detection of HIV antibodies by the Chembio DPP Assay and negative numbers indicate earlier detection of HIV antibodies by EIA. The Chembio DPP Assay detected HIV antibodies in 15 out of 21 seroconversion panels as early as EIA 1 and later than EIA 1 in remaining six panels. The Chembio DPP Assay detected HIV antibodies earlier than EIA 2 in 11 out of 21 seroconversion panels, as early as EIA 2 in eight out of the remaining 10, and later than EIA 2 in two panels. At least 40 early seroconversion HIV samples were tested. The results conform to the state of the art.

Table 6: Testing seroconversion panels using the DPP Assay

Panel	EIA 1 and Chembio DPP Assay			EIA 2 and Chembio DPP Assay		
	EIA 1 Repeatedly Reactive Test Result on Day	DPP Assay Reactive Test Result on Day	Difference in Days to Anti-HIV Reactive Result: EIA minus DPP Assay	EIA 2 Repeatedly Reactive Test Result on Day	DPP Assay Reactive Test Result on Day	Difference in Days to Anti-HIV Reactive Result: EIA minus DPP Assay
PRB904	92	92	0	92	92	0
PRB910	26	26	0	26	26	0
PRB914	0	0	0	4	0	4
PRB916	30	30	0	30	30	0
PRB917	65	65	0	72	65	7
PRB919	9	9	0	11	9	2
PRB922	0	4	-4	>11	4	>7
PRB924	35	35	0	>40	35	>5
PRB926	27	27	0	27	27	0
PRB927	33	33	0	40	33	7
PRB928	111	111	0	120	111	9
PRB929	25	28	-3	28	28	0
PRB930	10	10	0	>10	10	>0
PRB933	21	27	-6	27	27	0
PRB934	7	7	0	11	7	4
PRB939	103	103	0	103	103	0
PRB944	14	14	0	16	14	2
PRB952	17	17	0	>21	17	>4
PRB953	10	>10	<0	10	>10	<0
PRB958	15	>17	<-2	15	>17	<-2
PRB959	9	14	-5	14	14	0

iii. Reactivity with HIV-1 Low Titer Panel

A 15-member HIV-1 commercially available low titer panel of serum and plasma specimens was used to evaluate the Chembio DPP Assay and the results were compared to FDA licensed HIV-1 EIAs and Western blot (WB). The Chembio DPP Assay detected the presence of antibodies to HIV-1 low-titer specimens similarly to licensed HIV EIAs and WB. In no case was the Chembio DPP Assay Nonreactive when both licensed EIAs were Repeatedly Reactive or WB was Positive (Table 7).

Table 7: Testing HIV-1 low titer panel using the Chembio DPP Assay

Panel Member ID	Chembio DPP Assay	EIA 1	EIA 2	WB
PRB108-1	R	R	R	P
PRB108-2	NR	NR	NR	N
PRB108-3	R	R	R	IND
PRB108-4	R	R	R	P
PRB108-5	R	R	R	P
PRB108-6	R	R	R	IND
PRB108-7	R	R	R	P
PRB108-8	R	R	R	P
PRB108-9	R	R	R	P
PRB108-10	R	R	NR	IND
PRB108-11	R	R	R	P
PRB108-12	NR	R	NR	N
PRB108-13	R	R	NR	IND
PRB108-14	NR	R	NR	N
PRB108-15	R	R	R	IND

R=Reactive, NR=Nonreactive, P=Positive, N=Negative, IND=Indeterminate

iv. HIV-2 Sensitivity

The sensitivity of the Chembio DPP Assay to detect HIV-2 antibody was determined by testing 210 serum/plasma specimens that were positive for HIV-2 antibodies only. These specimens were obtained from repository sources. A total of 554 specimens from an area endemic for HIV-2 infection were also tested. All specimens reactive by an FDA approved/licensed HIV-1/2 assay were also Reactive with the Chembio DPP Assay (see Table 8). The sensitivity of Chembio DPP Assay for detection of antibodies to HIV-2 in these studies was calculated to be 210/210 = 100% (95% confidence interval 98.3 to 100%).

Table 8: Detection of antibody to HIV-2 in known HIV-2 positive specimens and specimens from endemic populations

Study Population	Samples	Chembio DPP Assay Reactive	True HIV-2 Positive Only[1]
Known HIV-2 Positive	210	210	210
Endemic Samples	554	201[2]	0
Total	764	411	210

1. Confirmation based on results using a research use HIV-2 WB and not positive on an HIV-1 WB.
2. Of these 201 Reactive specimens, 93 were Positive on HIV-1 WB only, 108 were Positive on HIV-1 WB and HIV-2 WB.

C) HIV Specificity

i. Clinical Trial Data

CAPILLARY (FINGERSTICK) WHOLE BLOOD

The specificity of the Chembio DPP Assay was evaluated by testing capillary (fingerstick) whole blood specimens from 961 low risk and 976 individuals at high risk for infection with HIV-1 at five clinical study sites. Samples from 96 high risk and 26 low risk individuals were Repeatedly Reactive on a licensed EIA and Positive on Western Blot and were excluded from the study. All the remaining 1815 specimens tested Nonreactive using the Chembio DPP Assay (see Table 9). Based on these studies, the specificity of Chembio DPP Assay in capillary (fingerstick) whole blood specimens was calculated to be 1815/1815 = 100% (95% confidence interval 99.8 to 100%).

Table 9: Performance of the Chembio DPP Assay on capillary whole blood (fingerstick) specimens from individuals presumed to be negative for HIV-1

Study Population	Samples	True Negative	Chembio DPP Assay Nonreactive[1]
Low Risk	961	935	935
High Risk	976	880	880
Total	1937	1815	1815

1. One specimen tested Nonreactive using the Chembio DPP Assay and positive using HIV-1 WB

VENOUS WHOLE BLOOD

The specificity of the Chembio DPP Assay was evaluated by testing venous whole blood specimens from 961 low risk and 975 individuals at high risk for infection with HIV-1 at five clinical study sites. Samples from 96 high risk and 26 low risk individuals were Repeatedly Reactive on a licensed EIA and Positive on Western Blot and were excluded from the study. Of the remaining 1814 specimens, one specimen from individual at low risk for infection with HIV-1 tested Reactive using the Chembio DPP Assay (false positive) that tested negative using HIV-1 WB. One specimen from an individual at high risk for infection with HIV-1 tested Nonreactive (false negative) using Chembio DPP Assay that tested positive using WB, (see Table 10). Based on these studies, the specificity of Chembio DPP Assay in venous whole blood specimens was calculated to be 1813/1814 = 99.9% (95% confidence interval 99.7 to 99.9%).

Table 10: Performance of the Chembio DPP Assay on venous whole blood specimens from individuals presumed to be negative for HIV-1 infection

Study Population	Samples	True Negative	Chembio DPP Assay Nonreactive[1]
Low Risk	961[1]	935	934
High Risk	975[2]	879	879
Total	1936	1814	1813

1. One specimen tested Reactive using the Chembio DPP Assay that tested negative using HIV-1 WB.
2. One specimen tested Nonreactive using the Chembio DPP Assay that tested positive using HIV-1 WB.

PLASMA

The specificity of the Chembio DPP Assay was evaluated by testing plasma specimens from 961 low risk and 975 individuals at high risk for infection with HIV-1 at five clinical study sites. Samples from 96 high risk and 26 low risk individuals were Repeatedly Reactive on a licensed EIA and Positive on Western Blot and were excluded from the study. Of the remaining 1814 specimens, one specimen from individuals at low risk for infection with HIV-1 tested Reactive using the Chembio DPP Assay (false positive) that tested negative using HIV-1 WB (see Table 11). One specimen from an individual at high risk for infection with HIV-1 tested Nonreactive using Chembio DPP Assay (false negative) that tested positive using WB. Based on these studies, the specificity of Chembio DPP Assay in plasma specimens was calculated to be 1813/1814 = 99.9% (95% confidence interval 99.7 to 99.9%).

Table 11: Performance of the Chembio DPP Assay on plasma specimens from individuals presumed to be negative for HIV-1 infection

Study Population	Samples	True Negative	Chembio DPP Assay Nonreactive
Low Risk	961[1]	935	934
High Risk	975[2]	879	879
Total	1936	1814	1813

1. One specimen tested Reactive using the Chembio DPP Assay that tested negative using HIV-1 WB.
2. One specimen tested Nonreactive using the Chembio DPP Assay that tested positive using HIV-1 WB.

SERUM

The specificity of the Chembio DPP Assay was evaluated by testing serum specimens from 961 low risk and 976 individuals at high risk for infection with HIV-1 at five clinical study sites. Samples from 96 high risk and 26 low risk individuals were Repeatedly Reactive on a licensed EIA and Positive on Western Blot and were excluded from the study. Of the remaining 1815 specimens, one specimen from an individual at low risk for infection with HIV-1 tested Reactive (false positive) using the Chembio DPP Assay that tested negative using HIV-1 WB. One specimen from an individual at high risk for infection with HIV-1 tested Nonreactive (false negative) using Chembio DPP Assay that tested positive using WB (see Table 12). Based on these studies, the specificity of Chembio DPP Assay in serum specimens was calculated to be 1814/1815 = 99.9% (95% confidence interval 99.7 to 99.9%).

Table 12: Performance of the Chembio DPP Assay on serum specimens from individuals presumed to be negative for HIV-1 infection

Study Population	Samples	True Negative	Chembio DPP Assay Nonreactive
Low Risk	961[1]	935	934
High Risk	976[2]	880	880
Total	1937	1815	1814

1. One specimen tested Reactive using the Chembio DPP Assay that tested negative using HIV-1 WB.
2. One specimen tested Nonreactive using the Chembio DPP Assay that tested positive using HIV-1 WB.

ii. Blood Donor Samples

The specificity of the Chembio DPP Assay was further evaluated by testing 1115 blood donations (not excluding first time donors) from two blood donation centers. Fifteen (15) of those donations were reactive for antibodies to HIV only on the Chembio DPP Assay. Of those 15, five (5) samples were reactive with p24/25 antigen and one (1) was reactive for p17/18 antigen when tested with an FDA-approved confirmatory HIV-1 Western Blot. Thus, all six samples can be categorized as HIV-1 indeterminates as per FDA/ CDC/ Consortium for Retrovirus Serology Standardization (CRSS) criteria and were excluded from final calculations. Therefore, the final specificity for the Chembio DPP Assay for the HIV band was 99.2% (1100/1109=99.2% with 95% CI 98.5- 0.99.6).

Syphilis

A) Syphilis Sensitivity

i. Clinical performance

One-hundred and seventy-seven (177) of the 1500 serum specimens collected during the US pivotal clinical trial previously described were reactive for *Treponema pallidum* antibodies on the Chembio DPP HIV-SYPHILIS Assay. These samples were further characterized via syphilis serology. Results obtained on the Chembio DPP HIV-SYPHILIS Assay were compared to results obtained on *Treponemal* specific EIAs, a *Treponemal* specific TP-PA, and a non- treponemal test, i.e. RPR. When results obtained on the DPP test were compared to RPR results, there was 100% agreement on all samples tested (177/177= 100% with 95% CI 97.9 – 100%). The first EIA found 172 of 176 samples reactive for *Treponema pallidum* (overall agreement: 172/176= 97.7% with 95% CI 94.3 – 99.4%) with one sample excluded due to inconclusive results. The second EIA found 176 of the 177 samples reactive for *Treponema pallidum* (overall agreement: 176/177= 99.4% with 95% CI 96.6 – 100%). Due to insufficient sample, only 176 specimens could be tested via TP-PA and one sample was later removed due to inconclusive results. Two (2) of the remaining 175 samples tested were found negative by the TP-PA (overall agreement: 173/175 = 98.9% with 95% CI 95.7 – 100%). Results are summarized below in Table 13.

Table 13: Characterization of the specimens reactive on the DPP HIV-SYPHILIS Assay for the syphilis analyte

Study Populations	DPP HIV-SYPHILIS Assay # reactive/total		EIA 1 # reactive/ total	EIA 2 # reactive/ total	TP-PA # reactive/total	BD RPR # reactive/total
	HIV	Syphilis	*IgG*	*Treponemal specific*	*Treponemal specific*	*non- treponemal test*
HIV Positive	500/500	110/500	105/109	109[1]/110	106[2]/108	110/110
High Risk/ Suspect	52/500	40/500	40/40	40/40	40/40	40/40
Healthy/ Not Suspected	7/500	27/500	27/27	27/27	27/27	27/27
Total	559/1500	177/1500	172/176	176/177	173/175	177/177
Overall agreement			97.7%	99.4%	98.9%	100%

[1] This sample was also found *Treponemal* negative by TP-PA.
[2] One sample was found reactive by EIA and RPR. The other sample was found negative by EIA and reactive by RPR.

ii. Well characterized Syphilis Specimens

A total of 139 specimens well characterized, clinically diagnosed, serum samples from patients with varying stages of syphilis procured from a commercial vendor were evaluated on the Chembio DPP HIV-SYPHILIS Assay. Of these 139 specimens, 18 were from a population at high risk for infection with syphilis (all untreated). Seventeen (17) of the 136 specimens were clinically diagnosed as having the first (primary) stage of syphilis. Of those 17, eleven (11) had received treatment for syphilis while 6 were untreated for syphilis. Thirty-nine (39) were clinically diagnosed as having the secondary stage of syphilis with 9 having received treatment and 30 being untreated for syphilis. The remaining 65 of the 139 specimens were clinically diagnosed as having a latent (hidden) syphilis infection. Of those 65, forty-two (42) had received treatment for syphilis while 23 were untreated for syphilis. The overall positive percent agreement (PPA) of the treponemal line of the DPP HIV-SYPHILIS Assay with reference results (i.e. EIA and TP-PA) for these specimens was calculated to be 137/139 = 98.6% (95% CI 94.9 – 99.8%). See Table 14.

Table 14: Performance of the DPP HIV-SYPHILIS Assay in Specimens Known to be Positive for Syphilis Infection

Stage Of Disease		EIA 1	EIA 2	TP-PA	RPR	DPP HIV-SYPHILIS Assay	
		IgG	*Treponemal*	*Treponemal*	*Non Treponemal*	SYP	PPA
High-Risk for Syphilis		18	18	18	18	17	94.4%
Primary Syphilis	Treated	11	11	11	11	11	100%
	Untreated	6	6	6	6	6	100%
Secondary Syphilis	Treated	9	9	9	9	9	100%
	Untreated	30	30	30	30	29	96.7%
Latent Syphilis	Treated	42	42	42	42	42	100%
	Untreated	23	23	23	23	23	100%
Number Reactive		139	139	139	139	137	98.6%
TOTAL tested		139	139	139	139	139	N/A

iii. Seracare syphilis-positive samples

To assess sensitivity of the Chembio DPP HIV-SYPHILIS Assay for Syphilis-positive specimens, one well characterized panel was used to verify the performance of the Chembio DPP HIV-SYPHILIS Assay. The results are presented in the Table 15 below and indicated that the Chembio DPP HIV-SYPHILIS Assay performed similarly to currently licensed immunoassays in detecting reactivity with Syphilis-positive specimens.

Table 15: Testing syphilis-positive specimens using the DPP HIV-Syphilis Assay

	DPP HIV-SYPHILIS				
Sample ID	Syphilis Trep	HIV	Treponemal specific EIA	RPR	HIV-1/2 EIA
9253813	R	NR	R	R	NR
9253814	R	NR	R	R	NR
9253815	R	NR	R	R	NR
9253816	NR	NR	NR	NR	NR
9253817	R	NR	R	R	NR
9253818	R	NR	R	R	NR
9253819	R	NR	R	R	NR
9253820	R	NR	R	R	NR
9253821	R	NR	R	R	NR
9253822	R	NR	R	R	NR
9253823	R	NR	R	R	NR
9253824	R	NR	R	R	NR
9253825	NR	NR	NR	NR	NR
9253826	R	NR	R	R	NR
9253827	R	NR	R	R	NR
9253828	R	NR	R	R	NR
9253829	R	NR	R	R	NR
9253830	R	NR	R	R	NR
9253831	R	NR	R	R	NR
9253832	R	NR	R	R	NR

R=Reactive, NR=Nonreactive

iv. HIV-2 samples

The performance of Chembio DPP HIV-SYPHILIS Assay was evaluated using 100 available HIV-2 samples obtained from a commercial vendor. All 100 were found to be positive on the Chembio DPP HIV-SYPHILIS Assay for HIV as indicated by a reactive HIV test line result. In addition, 12 of the HIV-2 confirmed specimens evaluated yielded reactive results for antibodies to *Treponema pallidum* on the DPP HIV-SYPHILIS Assay. These 12 specimens were further characterized via syphilis serology using the following FDA-licensed tests: two EIAs, a TPPA test and a RPR. All 12 specimens were reactive for both syphilis serology tests with EIA, TP-PA and concordant reactive on the DPP HIV-Syphilis Assay. 11 of the 12 specimens were reactive via RPR. See Table 16.

Table 16: Characterization of Specimens Reactive on the DPP HIV-SYPHILIS Assay for the Syphilis Analyte

Chembio DPP HIV-SYPHILIS Assay		EIA 1	EIA 2	TP-PA	RPR
Syphilis Reactive	**HIV Reactive**	***IgG Reactive***	***Treponemal Reactive***	***Treponemal Reactive***	***Non Treponemal Reactive***
12	--	12	12	12	11

v. Mixed-titer panel

The Chembio DPP HIV-SYPHILIS Assay was tested against 20 members of Boston Biomedica, Inc. (BBI) mixed titer panels (PSS202). See Table 17 below.

Table 17: Performance of the Chembio DPP HIV-SYPHILIS Assay with mixed titer panel

	DPP HIV-SYPHILIS		RPR 1	RPR 2 [1]	ATA 1[2]	ATA 2[1]	ATA13	ATA 4[2]
	SYPHILIS	HIV						
PSS202-01	R	NR	128	128	>5.5	REACTIVE	REACTIVE	3.3
PSS202-02	R	NR	8	4	>5.5	REACTIVE	REACTIVE	3.7
PSS202-03	R	NR	4	4	>5.5	REACTIVE	REACTIVE	2.2
PSS202-04	NR	NR	NEGATIVE	NEGATIVE	0.3	NEGATIVE	NEGATIVE	0.2
PSS202-05	R	NR	1	NEGATIVE	>5.5	REACTIVE	REACTIVE	2.25
PSS202-06	R	NR	64	128	>5.5	REACTIVE	REACTIVE	4.6
PSS202-07	R	NR	8	8	>5.5	REACTIVE	REACTIVE	3.5
PSS202-08	R	NR	1	1	>5.5	REACTIVE	REACTIVE	0.2
PSS202-09	R	NR	32	32	4.5	REACTIVE	REACTIVE	2.1
PSS202-10	R	NR	16	64	>5.5	REACTIVE	REACTIVE	3.8
PSS202-11	R	NR	4	4	5.1	REACTIVE	REACTIVE	3.1
PSS202-12	R	NR	32	64	>5.5	REACTIVE	REACTIVE	2.0
PSS202-13	R	NR	1	2	>5.5	REACTIVE	REACTIVE	3.1
PSS202-14	R	NR	2	2	>5.5	REACTIVE	REACTIVE	3.7
PSS202-15	R	NR	32	64	>5.5	REACTIVE	REACTIVE	2.2
PSS202-16	NR	NR	NEGATIVE	NEGATIVE	0.3	NEGATIVE	NEGATIVE	0.2
PSS202-17	R	NR	2	2	>5.5	REACTIVE	REACTIVE	2.3
PSS202-18	R	NR	1	NEGATIVE	>5.5	REACTIVE	REACTIVE	2.3
PSS202-19	R	NR	2	2	>5.5	REACTIVE	REACTIVE	3.5
PSS202-20	R	NR	NEGATIVE	NEGATIVE	>5.5	REACTIVE	REACTIVE	2.1

[1] RPR results are endpoint dilutions.
[2]Immunoassay results are means of duplicates expressed as signal to cutoff ratios (s/co). Ratios ≥1.0 are considered reactive.
R=Reactive, NR=Nonreactive

B) Syphilis Specificity

i. Blood Donor Samples

The specificity of the Chembio DPP HIV-SYPHILIS Assay was evaluated by testing 1115 blood donations (not excluding first time donors) from two blood donation centers. All of the 1115 blood donations resulted in a non-reactive Trep band on the DPP HIV-SYPHILIS Assay indicating a non-reactive syphilis result. Therefore, the specificity for the DPP HIV-SYPHILIS Assay for the Trep band when using blood donor samples was 100% (1115/1115=100% with 95% CI 99.7 – 100%).

ii. HIV/Syphilis Negative Pregnant Women

The specificity of the Chembio DPP HIV-SYPHILIS Assay was further evaluated by testing 207 sera samples using pregnant women samples from a vendor that had known trimester, age and risk for infection with an STD. Samples were tested in parallel on the Chembio DPP HIV-SYPHILIS Assay, two FDA-Approved HIV 1/2 rapid tests and a CE-Marked HIV 1/2 Rapid Test. For reactivity to HIV, all 207 specimens yielded concordant non reactive results on the Chembio DPP HIV-SYPHILIS Assay, HIV1/2 FDA-licensed assays and the CE Marked HIV 1/2 Assay for a total of 207 non reactive specimens to HIV. Therefore, the specificity of the DPP HIV-SYPHILIS Assay when using specimens from pregnant women who are negative for HIV was 100% (207/207=100% with 95% CI 98.2-100%) when compared to these tests.

Of the 207 specimens evaluated, one specimen representative of the high risk population due to current STD infection was found to be reactive for antibodies to *Treponema pallidum* only on the DPP HIV-SYPHILIS assay. The sera sample that caused a reactive Trep band to develop on the DPP HIV-Syphilis Assay was further evaluated with a *Treponemal*-specific EIA and a non-treponemal specific RPR test. The DPP Trep-positive sera sample was also positive on the EIA and RPR tests. This specimen was excluded from final calculations for Syphilis. The specificity of the DPP HIV-Syphilis Assay when using specimens from pregnant women who are negative for Syphilis infection was 100% (206/206=100% with 95% CI 98.2-100%) when compared to the EIA and RPR.

Table 18 below summarizes these results.

Table 18: Performance of the DPP HIV-SYPHILIS Assay in detecting HIV and Syphilis in Pregnant Women

Study Populations	DPP HIV-SYPHILIS Assay # nonreactive/total		FDA HIV 1/2 rapid test 1 # nonreactive/total	FDA HIV 1/2 rapid test 2 # nonreactive/total	CE Marked HIV 1/2 Rapid Test # nonreactive/total	Agreement DPP HIV-SYPHILIS vs other rapid tests (HIV band only)
	HIV	Syphilis				
High Risk for STDs	59/59	58/59[1]	59/59	59/59	59/59	100%
Low Risk for STDs	148/148	148/148	148/148	148/148	148/148	100%
Total	207/207	206/207[1]	207/207	207/207	207/207	100%

[1]The Trep-band positive sample was also found positive by Treponemal-specific EIA and a non-treponemal specific RPR test.

C) HIV and Syphilis Co-infection

HIV and syphilis are often seen as co-infections since they share a common mode of transmission. A total of one-hundred and twenty-three (123) of the specimens collected during the US pivotal clinical trial previously described above were reactive for both HIV and *Treponema pallidum* antibodies on the Chembio DPP HIV-SYPHILIS Assay. One-hundred and six (106) of these samples were from HIV-1-positive individuals, 14 samples were from the Suspect/High Risk population and 3 were from apparently healthy subjects not suspected of having an STD. The results are summarized in Table 19 below.

Table 19: Detection of Syphilis in HIV-infected specimens using the DPP HIV-Syphilis Assay

Study Populations	DPP HIV-SYPHILIS Assay # reactive/total		DPP HIV-SYPHILIS Assay Co-infected
	HIV	Syphilis	
HIV Positive	500/500	110/500	106
High Risk/Suspect	52/500	40/500	14
Healthy/ Not Suspected	7/500	27/500	3
Total	559/1500	177/1500	123

Of the 139 well characterized, clinically diagnosed, serum samples from patients with varying stages of syphilis procured from a commercial vendor that were evaluated on the Chembio DPP HIV-SYPHILIS Assay (Trep band results previous shown in Table 14), 86 specimens were reactive for both HIV and *Treponema pallidum* antibodies on the Chembio DPP HIV-Syphilis Assay. Ten (10) of these samples were from those at high risk for syphilis infection (all untreated). Thirteen (13) of the 86 samples were clinically diagnosed as having the first (primary) stage of syphilis. Of those diagnosed with the 1st stage of syphilis, 8 had received treatment for syphilis while 5 were untreated for syphilis. Nineteen (19) were clinically diagnosed as having the secondary stage of syphilis with 4 having received treatment and 15 being untreated for syphilis. The remaining 43 of the 86 specimens were clinically diagnosed as having a latent (hidden) syphilis infection. Of those 43, twenty-four (24) had received treatment for syphilis while 19 were untreated for syphilis. Samples were tested in parallel on the Chembio DPP HIV-SYPHILIS Assay, an FDA-Approved HIV 1/2 rapid test and a CE-Marked HIV 1/2 Rapid Test. The results are summarized in Table 20 below.

Table 20: Detection of HIV in Syphilis infected specimens using the DPP HIV-Syphilis Assay

Stage Of Disease		FDA HIV 1/2 Rapid test 1	CE Marked HIV 1/2 Rapid Test	DPP HIV-SYPHILIS Assay		DPP HIV-SYPHILIS Assay Co-infected
				SYP	HIV	
High-Risk for Syphilis		10	10	17	10	10
Primary Syphilis	Treated	8	8	11	8	8
	Untreated	5	5	6	5	5
Secondary Syphilis	Treated	4	4	9	4	4
	Untreated	15	15	29	15	15
Latent Syphilis	Treated	24	24	42	24	24
	Untreated	19	19	23	19	19
Number Reactive		86	86	137	86	86
TOTAL tested		139	139	139	139	N/A

XVI. EFFECT OF UNRELATED MEDICAL CONDITIONS ON ANALYTICAL SENSITIVITY AND SPECIFICITY

To evaluate the extent to which various medical conditions might cross-react with the Chembio DPP HIV-SYPHILIS Assay to yield false results, 163 naturally occurring serum and plasma specimens representing various medical conditions were tested. The DPP HIV-SYPHILIS test did not show cross reactivity with HIV1/2, HCV, HBsAg, HTLV, Syphilis, Lyme disease and Dengue. It only showed potential cross reactivity with one of ten Brazilian Leptospirosis Samples. The overall cross reactivity rate out of the 163 specimens tested was 0.6%. See Table 21 below.

Table 21: Effect of unrelated medical conditions on analytical sensitivity and specificity of the Chembio DPP HIV-SYPHILIS Assay

Unrelated Medical Conditions	Number of Positive Samples Tested for condition stated to the left	Unrelated Medical Conditions	Number of Positive Samples Tested for condition stated to the left
Chagas	14	14	0
Lyme	15	15	0
Brazilian Lyme Samples	3	3	0
Viral Co-infection	25	25	0
HCV	15	15	0
HCV	25	25	0
HIV1/2	15	15	0
Syphilis	20	20	0
Dengue	21	21	0
Brazilian Lepto Samples	10	9	1
TOTAL SAMPLES TESTED	163	162	1
OVERALL CROSS REACTIVITY RATE			1/163×100= 0.6%

XVII. EFFECT OF POTENTIALLY INTERFERING SUBSTANCES ON ANALYTICAL SENSITIVITY AND SPECIFICITY

To evaluate the influence of interfering substances on the performance of HIV/syphilis negative and positive samples on the specificity and sensitivity of Chembio DPP HIV-SYPHILIS Assay, nine potentially interfering substances , with each interfering substance being assessed at 3 levels (i.e. dilutions), were tested. Serum samples that were non-reactive for both HIV 1/2 and Syphilis Treponema were spiked with the interfering substances at a low, medium and high dilution. Each level of spiked sample was then tested with the DPP HIV-SYPHILIS test following the procedure described in the Product Insert. Likewise, serum samples that were reactive for both HIV 1/2 and Syphilis Treponema were spiked with the interfering substances at a low, medium and high dilution. Each level of spiked sample was then tested with the DPP HIV-SYPHILIS test following the procedure described in the Product Insert. The DPP HIV-SYPHILIS test was demonstrated to be robust to the nine (9) interfering substances at all levels tested when spiked into both negative and positive sera. Results are summarized below in Table 22.

Table 22: Effect of potentially interfering substances on analytical sensitivity and specificity of the Chembio DPP HIV-SYPHILIS Assay

	DPP HIV-SYPHILIS Test Result Key= N=Non-reactive, R=Reactive, NT=Not Tested							
Interfering Substances* spiked into HIV 1/2 and Syphilis Treponema NEGATIVE Serum Samples *(Ranges Tested)	**Saline Only (No interfering substance present)**		**LOW dilution level of interfering substance**		**MEDIUM dilution level of interfering substance**		**HIGH dilution level of interfering substance**	
	S	**H**	**S**	**H**	**S**	**H**	**S**	**H**
Hemoglobin Samples (0.98 – 500 mg/dL)	N	N	N	N	N	N	N	N
Glycerol Triolin (5.0 – 300 mg/dL)	N	N	N	N	N	N	N	N
Bilirubin (0.04 – 20 mg/dL)	N	N	N	N	N	N	N	N
Human Serum Albumin (5.6 – 11.0 g/dL)	N	N	N	N	N	N	N	N
EDTA (1.6 – 800 mg/dL)	N	N	N	N	N	N	N	N
Sodium Citrate(2.0 – 1,000 mg/dL)	N	N	N	N	N	N	N	N
Glucose (2.0 - 1,000 mg/dL)	N	N	N	N	N	N	N	N
IgG (0.1 g/dL)	N	N	NT	NT	NT	NT	N	N
Cholesterol (2.25– 5.5 g/dL)	N	N	N	N	N	N	N	N
Interfering Substances* spiked into HIV 1/2 and Syphilis Treponema POSITIVE Samples *(Ranges Tested)	**Saline Only (No interfering substance present)**		**LOW dilution level of interfering substance**		**MEDIUM dilution level of interfering substance**		**HIGH dilution level of interfering substance**	
	S	**H**	**S**	**H**	**S**	**H**	**S**	**H**
Hemoglobin Samples (0.98 – 500 mg/dL)	N	N	R	R	R	R	R	R
Glycerol Triolin (5.0 – 300 mg/dL)	N	N	R	R	R	R	R	R
Bilirubin (0.04 – 20 mg/dL)	N	N	R	R	R	R	R	R
Human Serum Albumin (5.6 – 11.0 g/dL)	N	N	R	R	R	R	R	R
EDTA (1.6 – 800 mg/dL)	N	N	R	R	R	R	R	R
Sodium Citrate(2.0 – 1,000 mg/dL)	N	N	R	R	R	R	R	R
Glucose (2.0 - 1,000 mg/dL)	N	N	R	R	R	R	R	R
IgG (0.1 g/dL)	N	N	NT	NT	NT	NT	R	R
Cholesterol (2.25– 5.5 g/dL)	N	N	R	R	R	R	R	R

XVIII. REPRODUCIBILITY

Reproducibility and repeatability was tested at three different laboratories on-site at Chembio using three separate lots of the Chembio DPP HIV-SYPHILIS Assay. A panel of seven blinded samples representing, HIV-1 weak positive, HIV-2 weak positive, *Treponema pallidum* weak positive, HIV-1 strong positive, HIV-2 strong positive, *Treponema pallidum* strong positive and nonreactive were run by a total of nine separate technicians. Results were read at both the lower and upper limits for the assay (10 and 25 minutes after the addition of Running Buffer to well 2) and were read qualitatively according to the DPP Evaluation Scale. A total of 189 data points were generated for each test line, at each assay time point, for a total

of 756 data points. The results as presented in indicate that the there was 100% reproducibility and repeatability (189/189) for both the Syphilis (S) and HIV (H) test lines, and also at both assay reading times.

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CHEMBIO DIAGNOSTIC SYSTEMS, INC.
3661 HORSEBLOCK ROAD
MEDFORD, NY 11763 USA

Tel: 1-800-327-3635
Tel: (631) 924-1135
Fax: (631) 924-2065

Email: **info@chembio.com**
Web Site: **www.chembio.com**

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