CLIA Waiver by Application Approval Determination

Decision Summary

A. Document Number

CW210001

B. Parent Document Number

BP180191

C. CLIA Waiver Type:

CLIA Waiver by Application

D. Applicant

Chembio Diagnostic Systems, Inc.

E. Proprietary and Established Names

DPP HIV-Syphilis System

F. Measurand (analyte)

HIV-1 Antibody
HIV-2 Antibody
Treponemal antibodies to *Treponema pallidum*

G. Sample Type(s)

Capillary fingertip whole blood

H. Type of Test

Qualitative, Multiplex, Immunoassay

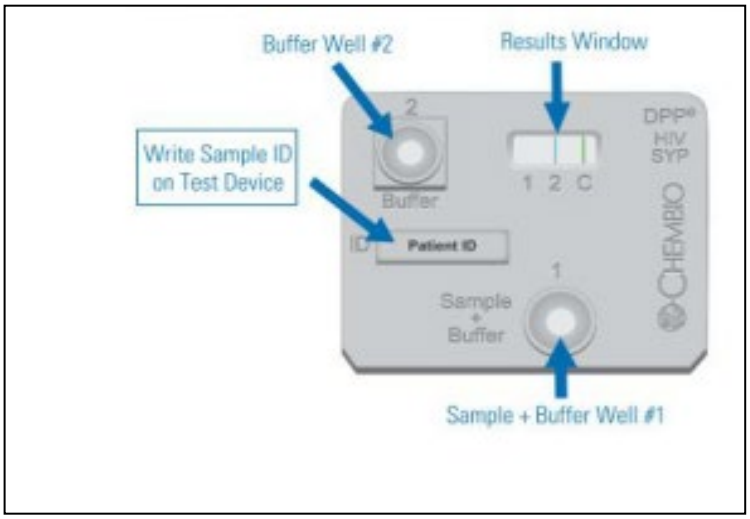
I. Test System Description

1. Overview

The DPP HIV-Syphilis test is a single-use rapid, qualitative, multiplex, immunoassay 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). The 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 and control) zones in the readout window of the test cassette. A specimen is collected and applied to the SAMPLE+BUFFER Well 1 of the test cassette to initiate the test. The sample flows along the sample path membrane and is delivered to the test zone of the reagent strip, where specific HIV antigens (Test Line 1), a *T. pallidum* recombinant antigen (Test Line 2), and Protein A (Control Line) are immobilized. Antibodies to HIV and/or *T. pallidum*, if present in the sample, bind instantly to the immobilized HIV and/or *T. pallidum* antigens in the TEST area, while nonspecific IgG binds to the Protein A in the CONTROL area. The dissolution of the soluble TEST and CONTROL lines indicates successful sample application. Five minutes after adding the sample, DPP Running Buffer is added to the BUFFER Well 2. The buffer releases the antibody-binding colored conjugate, which migrates to the TEST and CONTROL Line area.

Figure 1: The Chembio DPP HIV-Syphilis Test Cassette



The test results are interpreted using the DPP Micro Reader between 10 and 25 minutes after DPP Running Buffer is added to BUFFER Well 2. The DPP Micro Reader is a reflectance reader for use with DPP HIV-Syphilis. The DPP Micro Reader is a portable, battery-powered instrument that uses assay-specific algorithms to analyze the test and control line reflectance to determine the presence or absence of the antibodies to HIV and/or to *T. pallidum* in the specimen. The DPP Micro Reader verifies the presence of the control line and measures color intensity at each of the test line positions; it interprets the results using an algorithm including assay-specific cut-off values, and displays a Reactive (R), Nonreactive (NR) or invalid (INV) result on an LCD Panel for HIV and syphilis (*T. pallidum*) after approximately 3 seconds. The results are displayed through a 14-segment liquid crystal display (LCD) on the top of the instrument.

The DPP Micro Reader has been developed to minimize human interpretation errors. The DPP Micro Reader is maintenance-free, not configurable by the user and is operated by a

single, multi-function button. The DPP Micro Reader is a stand-alone instrument. It does not have interfaces with external devices except for the USB power cord, which is used to upload the test specific software at Chembio. The same USB power cord is used as an alternative power source by the end user and is not intended for or capable of data transfer.

2. Test Components

Each kit contains the following items to perform 20 tests.

- 20 individually pouched DPP HIV-Syphilis test devices, each containing:
  - One DPP HIV-Syphilis Test Device
  - One Desiccant Pouch
- 20 disposable 10 µL sample loops with break point – BLUE
- 20 SampleTainer Bottle - BLACK Cap
  1 mL; contains phosphate, sodium chloride, EDTA, Tween 20, Avidin, and chicken serum, antimicrobials and sodium azide as preservative.
- One DPP Running Buffer - GREEN Cap
  6 mL; contains phosphate, sodium chloride, EDTA, Tween 20, Avidin, and chicken serum, urea, antimicrobials and sodium azide as preservative.

The following are required but must be ordered separately:

DPP HIV-Syphilis Rapid Test Control Pack
Each package contains:

- HIV-1 Reactive Control
  One Vial containing 0.5 mL of heat inactivated human plasma positive for antibodies to HIV- 1, diluted in normal human plasma. Negative for Hepatitis B surface antigen, Hepatitis C antibody and HTLV I/II antibodies.
- HIV-2 Reactive Control
  One Vial containing 0.5 mL of heat inactivated human plasma positive for antibodies to HIV-2, diluted in normal human plasma. Negative for Hepatitis B surface antigen, Hepatitis C antibody, HTLV I/II antibodies and treponemal antibodies.
- *Treponema pallidum* Reactive Control
  One Vial containing 0.5 mL of human plasma positive for treponemal antibodies to *T. pallidum*, diluted in stabilizing matrix containing normal human plasma; negative for Hepatitis B surface antigen, Hepatitis C antibody and HTLV I/II antibodies.
- Non-reactive Control
  One Vial containing 0.5 mL of normal human plasma negative for antibodies to HIV-1, HIV-2 and *T. pallidum*. Negative for Hepatitis B surface antigen, Hepatitis C antibody and HTLV I/II antibodies.

One DPP Micro Reader
Each kit contains:

- 1 DPP Micro Reader configured for use with DPP HIV-Syphilis System
- 3 Lithium-ion, type CR2032 (3 V/230 mAh), coin cell batteries (installed)
- 1 DPP Test Device Holder
- 1 USB Wall Power Adapter (5v/1000 mA) with cable

3. Workflow

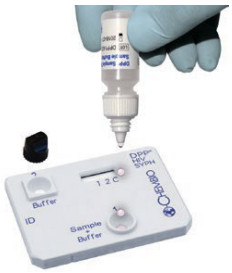
Collect and Prepare Fingerstick Sample:
Perform a fingerstick prick as per normal laboratory procedures. Wipe away the first drop of blood and collect the sample from the second drop with the provided loop. Ensure loop is properly filled with sample.

Insert Sample Loop into DPP SampleTainer bottle.

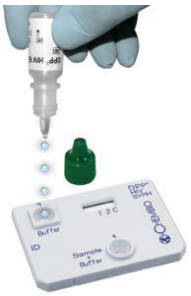
SNAP and TWIST the shaft at the BREAK-NOTCH to dislodge loop into the SampleTainer bottle.

Replace BLACK cap on DPP SampleTainer bottle and SHAKE for 10 seconds.

Run the Test:
Unscrew the DPP SampleTainer bottle BLACK CAP keeping the WHITE CAP screwed onto the bottle. Invert the DPP SampleTainer bottle, containing the collected sample, and hold it vertically over the SAMPLE + BUFFER Well 1. Add two drops (~65 µL) slowly, into the SAMPLE + BUFFER Well 1. Wait for five minutes.



Invert the DPP Running Buffer bottle (GREEN CAP) and hold it vertically over BUFFER Well 2. Add four drops (~135 µL) slowly, into BUFFER Well 2. Wait ten minutes for results.



Using the DPP Micro Reader (Reader):

Place the DPP Test Device Holder on a flat surface. Match the Reader with the DPP Test Device Holder by inserting the base of the Reader so that the 'slanted edge' meets the corresponding 'slanted corner' in the Test Device Holder socket. Connect the Reader to the supplied Test Device Holder as shown below. The Reader is secure in the DPP Test Device Holder once a 'clicking' sound is heard. At the time indicated for reading test results, place Reader and Holder over the Test Device; press button. The Reader will go through the start-up process and then display 'RDY'. Press the button again; the Reader will show 'RUN' and will then display the test results.

4. Result Interpretation

The DPP Micro Reader interprets the results using an algorithm including assay-specific cut-off values, and displays a Reactive (R), Nonreactive (NR) or invalid (INV) result on an LCD Panel for HIV and syphilis (*T. pallidum*) after approximately 3 seconds.

Interpretation of Test Results	Reader Display
NON-REACTIVE for HIV-1 or HIV-2 Antibodies	HV.NR
NON-REACTIVE for Treponemal Antibodies	TP.NR
REACTIVE for HIV-1 or HIV-2 Antibodies	HV. R
NON-REACTIVE for Treponemal Antibodies	TP.NR
NON-REACTIVE for HIV-1 or HIV-2 Antibodies	HV.NR
REACTIVE for Treponemal Antibodies	TP. R

REACTIVE for HIV-1 or HIV-2 Antibodies REACTIVE for Treponemal Antibodies	
INVALID RESULT An invalid result cannot be interpreted. It is recommended that the invalid test be repeated with a new device.	

5. External Controls

   The DPP HIV-Syphilis Rapid Test Control Pack containing Reactive/Nonreactive Controls are available separately for use with the DPP HIV-Syphilis test system. The controls are used to verify the operator's ability to properly perform the test and to interpret the results. The HIV-1/Syphilis Reactive Control will produce an HIV and Syphilis reactive test result when read using the DPP Micro Reader. The HIV-2 Reactive Control will produce an HIV reactive and Syphilis nonreactive test result when read using the DPP Micro Reader. The Nonreactive Control will produce a nonreactive test result for HIV and Syphilis when read using the DPP Micro Reader.

J. Demonstrating "Simple"

- The test uses direct unprocessed specimens, capillary blood (fingerstick) whole blood.
- The test needs only basic, non-technique-dependent specimen manipulation. An untrained operator can conduct the test by performing a few simple steps:
  1. Collect sample using the provided sample loop.
  2. Add sample into the SampleTainer bottle; mix by shaking.
  3. Dispense two drops from the SampleTainer bottle onto the test cartridge into Well 1; wait five minutes.
  4. Add four drops of the DPP Running Buffer into Well 2.
  5. Wait 10 minutes.
  6. Place the DPP Micro Reader on the test cartridge to read and display the result.
- The test requires no calibration.
- The buffer and sample wells are clearly labeled on the test cassette with both words 'Buffer' and 'Sample + Buffer' and are numbered in the order of the steps.
- The bottles of the SampleTainer and Buffer have different color caps, so they are not confused.
- The test cassette contains control lines which serves as a built-in internal procedural control and gives confirmation of sample addition and proper test execution.
- Sample collection utilizes a loop, and no pipetting is required.
- The SampleTainer bottle has a built-in dropper, so pipetting is not required at the time of sample addition to the test device.
- The test does not require any operator intervention during the analysis step.
- The test result lines are read by the DPP Micro Reader and displayed on the Reader screen.
- The results do not require interpretation, or calculation. The reader produces results that are easy to determine, such as 'positive' or 'negative.'

- Technical or specialized training is not required for troubleshooting or error code interpretation. If an error code is shown, the operator is instructed to repeat the test.
- There are no required electronic or mechanical maintenance tasks.
- The test cartridge is for single use.
- The DPP Micro Reader does not require calibration.
- Contains a Quick Reference Instruction sheet that is written in simple language at a 7th grade reading level and includes clear diagrams to guide the user.

K. Demonstrating "Insignificant Risk of an Erroneous Result"- Failure Alerts and Fail-safe Mechanisms

1. Risk Analysis

   Risk analysis was performed by the sponsor, Chembio Diagnostics Systems, Inc. using the Failure Modes and Effects Analysis (FMEA) Method. The detailed analysis was included in the submission. A two-tiered approach, recommended in the FDA's *Guidance for Industry and FDA staff: Recommendations for Clinical Laboratory Improvement Amendments (CLIA) of 1988 Waiver Application for Manufacturers of In Vitro Diagnostics Devices*, was used where a comprehensive risk analysis was conducted to demonstrate that the device is robust and has appropriate and effective risk control measures. The risk analysis was conducted per *ISO 14971:2012 - Medical Devices - Application of Risk Management to Medical Devices*. All risks of harm to the patient or operator were mitigated to an acceptable level and were supported by flex studies and/or operator instructions.

2. Fail-Safe and Failure Alert Mechanisms:

   The DPP HIV-Syphilis test was designed with several failure alert mechanisms and procedural controls intended to reduce the risk of device malfunction and procedural errors when performing the assay.

   Dyed Test and Control Lines
   The DPP HIV-Syphilis Test and Control lines are dyed blue and green respectively, and dissolve upon contact with the DPP Sample Buffer contained in the SampleTainer bottle. This serves as a failure alert mechanism to verify that the DPP SampleTainer contents were successfully delivered to the Test and Control zone before proceeding to the next step, the application of running buffer to BUFFER Well 2. If the blue and green colored lines do not disappear after five minutes after DPP SampleTainer contents are applied to the SAMPLE + BUFFER Well 1, this indicates that sample application was unsuccessful. In this case, the user is instructed to discard the test device and repeat the test procedure with a new device.

   Protein A Procedural Controls
   The DPP HIV-Syphilis control line is composed of Protein A and captures human IgG in the sample. After the application of the DPP SampleTainer bottle contents to the test

device, the Protein A in the control line will capture IgG present in the sample. Following the addition of DPP Running Buffer, the colored antibody-binding conjugate is delivered to the test zone, which will bind to the captured IgG antibodies forming a visible pink/purple control line. If no sample is added to the DPP SampleTainer bottle, or if the contents of the DPP SampleTainer bottle are not delivered to the test device, the control line will not develop, and the device will produce an invalid result.

External Controls

The DPP HIV-Syphilis Rapid Test Control Pack, containing Reactive/Nonreactive quality control reagents, is provided separately, as an accessory to the DPP HIV-Syphilis test. These controls are described above. As per the DPP HIV-Syphilis test product insert, users are instructed to run kit 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 - 25°C (36 - 77°F)
- If the temperature of the testing area falls outside of 18 - 25°C (64 - 77°F)
- At periodic intervals as indicated by the user facility

If the DPP HIV-Syphilis controls produce unexpected results, the user is instructed to contact Chembio Customer Service.

DPP Micro Reader

The DPP Micro Reader is a reflectance reader for use with DPP HIV-Syphilis. The DPP Micro Reader is a portable, battery-powered instrument that verifies the presence of the control line and measures color intensity at each of the test and control line positions. It interprets the results using an assay-specific algorithm and reports a positive, negative, or invalid result after approximately 3 seconds and displays it through a 14-segment liquid crystal display (LCD) on the top of the instrument. The DPP Micro Reader has been developed to minimize human interpretation errors (i.e., interpretation of a multiple test lines or weak test lines), therefore the results cannot be visually interpreted by the operator. The DPP Micro Reader is maintenance-free, not configurable by the user and is operated by a single, multi-function button.

DPP SampleTainer Collection

The DPP SampleTainer bottle contains 1 mL of sample collection buffer which allows for one sample collection event to be used to run multiple DPP HIV-Syphilis test devices, as only 2 drops (~ 65 µL) are required to operate a single device. This allows the user to repeat the assay with a second test device if an invalid result due to procedural error or device failure, eliminating the need to repeat sample collection, provided that the initial sample collection was conducted properly.

3. Flex Studies

A comprehensive risk analysis, using the FEMA method described above, was performed and moderate user risks were identified and assessed in flex studies described below.

The flex studies focused on three areas of potential risks:
a. Specimen and reagent handling
b. Operator errors in performing the test
c. Environmental conditions

The test samples used in the flex studies were contrived in negative whole blood screened for negativity to both HIV1/2 and syphilis prior to use. Reactive plasma (HIV reactive and syphilis reactive) was purchased from a supplier and diluted into whole blood (10 µL unless other sample volumes are indicated) to prepare test samples with the target concentrations of antibodies relative to the assay cut-off as follows:

- Negative (un-spiked)
- Weakly Reactive: HIV and syphilis (2-3x cut-off)
- Reactive: HIV and syphilis (4-6x cut-off)

Each positive and negative sample was tested in six replicates for each condition being evaluated. Some studies only used a Weakly Reactive sample. All instructions were followed correctly except the identified condition in the flex study. Each flex study included testing the samples under control (normal) conditions. Minimum of two operators participated (three replicates per operator) in each study.

Specimen and Reagent Handling

- *Use of Improperly Stored Reagents:*

  This study evaluated the effect of using unopened pouches of DPP HIV-Syphilis tests stored outside the specified temperature of 2°C-25°C.
  1. Unopened pouched tests and buffer bottles were placed at -20°C for seven days prior to use. Six replicates of the Negative and Weakly Reactive (2-3x cut-off) samples were tested for each condition. All positive and negative samples produced expected results, demonstrating that inadvertent storing of unopened tests in a freezer had no effect on the performance of the DPP HIV-Syphilis test.
  2. Opened pouches of tests and buffer bottles were placed at -20°C for seven days prior to use. Six replicates of the Negative and Weakly Reactive (2-3x cut-off) samples were tested for each condition. All positive and negative samples produced expected results, demonstrating that inadvertent storing of opened tests in a freezer had no effect on the performance of the DPP HIV-Syphilis test.
  3. Unopened pouched tests and buffer bottles were placed at 45°C in a humidity chamber with minimum humidity of 80% for seven days prior to use. Six replicates of the Negative and Weakly Reactive (2-3x cut-off) samples were

tested for each condition. All positive and negative samples produced expected results, demonstrating that storing of unopened tests at an elevated temperature had no effect on the performance of the DPP HIV-Syphilis test.

4. Opened pouched tests and buffer bottles were placed at 45°C in a humidity chamber with minimum humidity of 80% for seven days prior to use. Six replicates of the Negative and Weakly Reactive (2-3x cut-off) samples were tested for each condition. All positive and negative samples produced invalid results, demonstrating that the assay performance was safeguarded from generating false results after storing of opened tests at an elevated temperature. The labeling clearly warns: *Do not open pouch until you are ready to perform a test.*

Labelling for DPP HIV-Syphilis test states that DPP HIV-Syphilis Test Devices should be stored in unopened pouches at 2°C to 25°C and they should not be stored frozen. Labelling also states that the Running Buffer and DPP SampleTainer Bottles should be stored at 2°C to 25°C in their original containers. If the kit components have been refrigerated, IFU instructs the users to remove them from the refrigerator and allow them to come to a temperature of 18°C to 25°C prior to testing.

- *Use of Different Lots of DPP SampleTainer and Running Buffer:*

  This study evaluated testing using mis-matched lots of DPP Sample Buffer in SampleTainer bottles and Running Buffer. Six replicates of each of the Negative, Weakly Reactive (2-3x cut-off) and Reactive (4-6x cut-off) samples were tested with mis-matched lots of the DPP buffers. All positive and negative samples produced expected results for all testing conditions.

<u>Operator Errors in Performing the Test</u>

- *Incorrect Volume of Sample Added to SampleTainer:*

  During the test procedure approximately 10 µL of sample is delivered to the SampleTainer bottle using the provided Sample Loop. This study evaluated the risk of adding insufficient or excess volume of sample to the SampleTainer bottle. Reactive (4-6x cut-off), Weakly Reactive (2-3x cut-off) and Negative samples were tested at various volumes to determine the minimum and maximum volume of the sample that is required to obtain accurate results. Samples were evaluated at volumes ranging from one-fourth to three times the nominal volume (i.e., 0, 2, 5, 10, 30 µL sample). The results showed that adding insufficient volume (2 µL), rather than the recommended 10 µL of the HIV/Treponema Weakly Reactive whole blood sample, resulted in false negative results on both HIV and Treponema test lines of the device. The assay is insensitive to excess input volume up to three times (30 µL) of the nominal assay input, i.e., 10 µL.

  The risk of the user adding less than optimal volume of sample is mitigated through the use of the provided Sample Loop for collection of whole blood sample. The clear

instructions and figures on how to use the Sample Loop in device labelling (Instructions For Use (IFU) and QRI) further mitigate errors in sample transfer.

- *Incorrect Mixing of Sample in SampleTainer Bottle (with and without bubbles):*

  The device labelling states that the user must shake the DPP SampleTainer for 10 seconds after sample addition before applying it to Well 1 on the test cassette. This study evaluated the risk of incorrectly mixing the sample with the buffer and foaming during mixing in the SampleTainer bottle. Testing was conducted by shaking the Negative and Weakly Positive (2-3x cut-off) sample with the buffer (after sample addition) in the DPP SampleTainer for 0, 5, 10, and 15 seconds with and without creating bubbles. All samples yielded expected results, indicating that under or over mixing of the sample with the buffer in the SampleTainer bottle and foaming during mixing does not affect the test outcome.

- *Incorrect Time Between Sample Addition to SampleTainer and Addition of Sample into Well 1:*

  The test procedure specifies that after the sample is added to the SampleTainer and mixed for ten seconds, two drops of the mix is added to Well 1. This study evaluated the risk of incorrect timing between adding samples to the SampleTainer and adding the mix from the SampleTainer bottle to Well 1. During this study, after addition of samples to the SampleTainer, effect of a wait period of 1, 5, 10, 30, 60 minutes and 8 hours to add the diluted sample into Well #1 for Negative and Weakly Reactive (2-3x cut-off) samples was evaluated. All samples yielded expected results, indicating that waiting time between sample addition to the SampleTainer and addition to Well 1 does not affect the test outcome.

- *Incorrect Sample Volume from SampleTainer Added to Well 1:*

  Device labelling states that during the test procedure, add two drops (~65 µL) of the Sample+Buffer mix from the SampleTainer slowly, into the Well 1. This study evaluated the risk of adding insufficient or excess number of drops from the SampleTainer to the Well 1. Weakly Reactive (2-3x cut-off) and Negative samples were tested to determine the minimum and maximum drops from the SampleTainer that is required to obtain accurate results by evaluating number of drops from one-half to 2.5x the nominal volume (i.e., 1, 2, 3, 4, and 5 drops of sample). The results showed that adding insufficient number of drops (one drop), rather than the recommended two drops of the sample, resulted in failure of disappearance of blue/green lines in the Result window of the device (Wait Time Failure; WTF). In an event of WTF, user is instructed to discontinue running the test. Addition of excess number of drops (four and five drops) resulted in a device overflow with no results. All tests returned expected results on addition of two or three drops of the sample.

The risk of erroneous results due to the user adding less or more than optimal number of drops (two drops) from SampleTainer was considered insignificant. Furthermore, clear instructions on number of drops to be added to Well 1 in device labelling (IFU and QRI) further mitigate errors in sample and buffer mix addition from the SampleTainer. Also, the user is instructed to wait for five minutes after addition and check to make sure the blue and green colored lines have disappeared from the Result window ensuring that the sample has been added correctly. In case they do not disappear (WTF), user is instructed to discard the test and repeat testing using a new DPP test device.

- *Incorrect Time Between SampleTainer Mix Addition into Well 1 and Buffer Addition to Well 2:*

  The test procedure specifies that after the mix is added to Well 1 using the SampleTainer, wait for five minutes and then add four drops of DPP Running Buffer to Well 2. This study evaluated the risk of incorrect timing between addition of mix from the SampleTainer to Well 1 and Running Buffer addition to Well 2. Negative and Weakly Reactive (2-3x cut-off) samples were added to Well 1 followed by a wait time of 1, 3, 5, 10, 20, 30, 40, 50, 60 and 120 minutes before addition of the Running Buffer into Well 2. Wait time of one minute resulted a Wait Time Failure (WTF) with no results for Negative sample and the expected results for both HIV and syphilis for Weakly Reactive samples. Testing with excess wait times of 10, 20, 30 and 40 minutes, yielded expected results on all Negative and Weakly Reactive whole blood samples. Waiting for 50 and 60 minutes for the Negative sample resulted in false positive results on the *Treponema* test line of the device. This error is mitigated by clear instructions: "*Wait 5 minutes. The blue and green colored lines should have disappeared from the rectangular Results window. If not, DO NOT USE, discard test device and repeat the procedure with a new DPP test device.*" Further, as wait times of 50 and 60 minutes are ten-fold longer than the recommended wait time of 5 minutes in the labelling, the likelihood of occurrence appears low.

- *Incorrect Running Buffer Volume Added to Well 2:*

  The labelling states that during the test procedure after adding two drops of the sample mix from the SampleTainer slowly into the Well 1, wait for 5 minutes, verify that the blue and green lines in the Result window have disappeared, and then add four drops (~135 µL) of DPP Running Buffer to Well 2. This study evaluated the risk of adding insufficient or excess volume of DPP Running Buffer to Well 2. During the execution of the study, testing was conducted by applying Running Buffer volumes ranging from one-fourth to approximately four times the nominal volume (i.e., 1, 2, 3, 4, 5, 6, 8, 9, 12 and 15 drops). Tests were conducted with Negative and Weakly Reactive (2-3x cut-off) samples. The results showed that adding insufficient volume (one to two drops), rather than the recommended four drops of the Running Buffer, resulted in invalid results on the device whereas excess volumes of 12 to 15 drops resulted in device overflow. All tests returned expected results on addition of three to nine drops of the Running Buffer indicating

that the device is tolerant to incorrect Running Buffer addition to Well 2 and risks of erroneous results is minimal. Furthermore, clear instructions for the number of drops to be added to Well 2 in device labelling (IFU and QRI) further mitigate errors in Running Buffer addition.

- *Drop Test Cassette During Run:*

  The study examined the effect of dropping test cassettes from bench top to the floor during testing at two different steps of the workflow - After addition of sample from the SampleTainer to Well 1 and again after addition of Running Buffer to Well 2. Six (6) separate replicates of each sample type (Negative samples and Weakly Reactive for HIV and *Treponema* at 2-3x cut-off) were tested for each condition according to the Package Insert of the DPP HIV-Syphilis. All cassettes tested yielded expected results with the Negative sample (producing negative results) and the HIV and *Treponema* Weakly Positive sample (producing positive results).

- *Incorrect Timing of Reading Results:*

  The test procedure specifies that after addition of four drops of DPP Running Buffer to Well 2, test results can be read using the DPP Micro Reader between 10 and 25 minutes. This study evaluated varied timepoints for reading results and result stability of the DPP HIV-Syphilis using the DPP Micro Reader. Six replicates of Negative, Weakly Reactive (2-3x cut-off) and Reactive (4-6x cut-off) samples were tested per timepoint in this study. After addition of Running Buffer to Well 2, test results were read using the DPP Micro Reader at the following timepoints: 5, 10, 15, 25, 40, 50, 60, 90 and 120 minutes. All valid replicates produced expected results for all testing conditions. All samples (Negative, Weakly Reactive and Reactive) produced valid results for 5 to 60 minutes timepoints. DPP HIV-Syphilis results were stable for up to 35 minutes after the designated development time of 10 to 25 minutes. False negative results were reported for Weakly Reactive samples at both 90 and 120 minutes and false positive results were reported for Negative samples at 120 minutes. These errors are mitigated by clear labelling which instructs the user to read the test results within 10 to 25 minutes of addition of Running Buffer to Well 2.

- *Incorrect Order of Reagent Application and/or Incorrect Placement (Wrong Well) of SampleTainer Liquid or Running Buffer:*

  This study evaluated the risk of incorrect order of various procedural steps for DPP HIV-Syphilis test or adding sample from SampleTainer or Running Buffer into the wrong well. Six replicates of each type of sample, Negative and Weakly Reactive (HIV and *Treponema* at 2-3x cut-off) were tested per procedural variation. Each variation in the procedure that was tested in the study is described below:
  1. Add sample from SampleTainer to Well 1, wait five minutes and then add Running Buffer to Result window.
  2. Add sample from SampleTainer to Well 2 only.

3. Add sample from SampleTainer to Well 2, wait for five minutes and then add Running Buffer to Well 1 or Result window.
4. Add sample from SampleTainer to Result window, wait for five minutes and then add Running Buffer to either Well 1 or 2.
5. Add Running Buffer to Well 1, wait for five minutes and then add sample from SampleTainer to either Well 2 or the Result window.
6. Add Running Buffer to Well 2, wait for five minutes and then add sample from SampleTainer to either Well 1 or the Result window.
7. Add Running Buffer to Result window, wait for five minutes and then add sample from SampleTainer to either Well 1 or 2.
8. Add sample from SampleTainer to Well 1, wait for five minutes and then add sample from SampleTainer to Well 2.

All devices tested with each of the Negative and Weakly Reactive samples produced either invalid or no result indicating that the risk of getting an erroneous result when the test is not performed correctly is minimal.

- *DPP Micro Reader (Reader) Errors:*

For these studies a set of six replicates of dried test devices were prepared for each of the following samples: Negative, Weakly Reactive (HIV and Syphilis at 2-3x cut-off) and Reactive (HIV and Syphilis at 4-6x cut-off). DPP Micro Reader (Reader) was configured to display numerical results during these studies. All testing was performed by a minimum of two operators (three replicates per operator). The performance of the Reader was evaluated under the following stressed conditions:

1. Misalignment of the DPP Micro Reader in the Holder: The purpose of this study was to evaluate the effect of incorrect orientation of the Reader in the holder on reading the results. Three incorrect orientations of the Reader at 90°, 180° and 270° rotation with respect to the correct orientation in the holder were tested. Please refer to figures above for images of what the Reader and DPP HIV-Syphilis test device look like and how they fit together in the correct orientation. Results from these flex studies showed that when the Reader is misaligned with the holder while reading results, the assay may yield erroneous results. To mitigate the potential risk arising from the misalignment of the Reader with the holder, the holder was modified to include latches to fasten the Reader into place once it is engaged with the holder. Device labelling instructs the user to firmly seat the Reader into the holder receptacle by firmly inserting the base of the Reader so the ‘slanted edge’ meets the corresponding ‘slanted corner’ in the holder socket. When the user hears a “click” sound, it confirms that the Reader is secured into the holder in the correct orientation. The Reader will not be permanently locked into the holder and can be disengaged by releasing the latch mechanism. This will ensure correct placement of the Reader to prevent erroneous results. The risk control of using the modified holder demonstrated that the risks are reduced and acceptable.

2. Incorrect Orientation of DPP HIV-Syphilis Test Device in Holder: The purpose of this study was to evaluate the effect of incorrect orientation of the Test Device in the holder on reading the results by the Reader. Three incorrect orientations were tested with respect to the correct orientation of the Test Device in the holder: Test Device upside down from the correct orientation, 180° rotation from correct orientation and 180° rotation and upside down from correct orientation. Please refer to figure above for images of what the Reader and Test Device look like and how they fit together in the correct orientation. All samples yielded the expected invalid results for each condition tested showing that the potential for getting erroneous results when the DPP test device is incorrectly placed in the holder is minimal.

3. Reader Assembly Held at an Angle (approximately 30-45 degrees) While Reading Results: The purpose of this study was to evaluate the effect of placing the assembled DPP HIV-Syphilis Test Device in Holder with the Reader (assembled as per the Package Insert) at an angle of approximately 30-45 degrees facing the operator reading of the results. All samples (Negative, Weakly Reactive and Reactive) yielded the expected results for each condition tested. Device labelling instructs the users to place the DPP Test Device Holder on a flat surface.

4. DPP Micro Reader Button Pressed Repeatedly Throughout the Run: The labelling for the device states that after the assembly of the Test Device into the Holder and placement of the Reader on the top, press the Reader button once to begin the start-up process. Once the display reads 'RDY' indicating that the Reader is ready to read, press the button again to start the run. The Reader will show 'RUN'. The purpose of this study was to evaluate the effect of repeatedly pressing the Reader button throughout the run (from start of the run to result display). All samples yielded the expected results for each condition tested indicating that repeated pressing of the Reader button during the run does not affect the final test results.

5. Read Results Using the DPP Micro Reader Under the Illumination Between Full Daylight and Sunlight: The purpose of this study was to document the effect of environmental light on reading of test results by DPP Micro Reader. For this study, illumination between Full Daylight and Sunlight (between 10,752 and 107,527 Lux according to the National Optical Astronomy Observatory website), was used during reading of the results by the DPP Micro Reader. All samples yielded the expected results indicating the associated risks to be minimal.

Environmental conditions

- *DPP HIV-Syphilis Test Run in Excessive Temperature and Humidity*

The labeling specifies optimum operational conditions for performing the test to be

18°C to 25°C. The purpose of this study was to evaluate the effect of excessive temperatures (40°C±3°C) and humidity (≥80%) on the test performance of the DPP HIV-Syphilis test. Environmental chambers were used to simulate the test conditions. Weakly Reactive (2-3x cut-off) and Negative samples were used as test inputs for the condition described above. All pouched tests/Readers/buffer bottles were placed in the environmental chamber and allowed to equilibrate with the chamber temperature prior to testing. All samples generated expected results demonstrating that the risks of erroneous results due to extremes of temperature and humidity outside the specified conditions for testing are minimal.

- *Incorrect Placement of Device: Test Run on Uneven Surfaces and Severe Angles:*

  The purpose of this study was to evaluate the effect of non-leveled benchtop or work surface on the test performance of the DPP HIV-Syphilis test. Six replicates of each sample type (Negative and Weakly Reactive for HIV and *Treponema*) per condition were used in the study according to the package insert of DPP HIV-Syphilis test. Five different angles and conditions were tested: device vertical with Well 1 in down position, device vertical with Well 1 in up position, device vertical with Well 2 in up position, device vertical with Well 2 in down position and device in upside position. Each of the five angles and conditions tested produced expected results for HIV and *Treponema* testing except for one device which yielded incorrect false negative *Treponema* results when device was placed vertically with Well 2 in up position. To further evaluate this condition, the study was repeated by four different operators, with 20 replicates each for a total of 80 results for Weakly Reactive (2-3x cut-off) whole blood sample. All 80 tests produced the expected results for *Treponema* testing and risk was assessed to be minimal. The risks of erroneous results are mitigated by clear directions in the labelling, which directs users to place the device on a flat surface at the start of testing.

<u>Conclusion from Flex Studies</u>

The flex studies carried out in support of this CLIA waiver submission have effectively and comprehensively demonstrated the robustness of the system to generate correct results even when operated under conditions of stress. The combination of the device design and the built-in fail-safe features, along with the clear test instructions which include relevant cautions, render the risks of erroneous results to be minimal. As all identified sources of user errors have been addressed either by fail-safe features, failure alert mechanisms or labeling mitigations, the DPP HIV- Syphilis was shown to have an insignificant level of risk in generating erroneous results when used as directed in the test procedure instructions.

L. Demonstrating "Insignificant Risk of an Erroneous Result" –Accuracy

Clinical Performance

The clinical performance of the DPP HIV-Syphilis test when performed by untrained operators was evaluated in two multi-site prospective studies conducted in the U.S. Five geographically diverse sites representing CLIA waived testing locations across the U.S. participated in these studies. The sites consisted of community outreach centers, outpatient physicians' office, a blood donation center and a health and wellness center. A total of 50 operators performed testing with the DPP HIV-Syphilis test with DPP Micro Reader. The operators had no formal training or experience in medical laboratory testing methods. Information on the operators' current job title, education, laboratory experience and the number of years of relevant work experience was provided. The education of the operators ranged from high school graduates to postgraduate degrees. Operators were provided only with the instructions for use and the Quick Reference Instructions.

Subjects were recruited by the study sites. Demographic and clinical data were collected from subjects by interview and medical record review. Subjects who met inclusion criteria were enrolled in the studies. The first clinical study was conducted in May 2016 to April 2017, using two sites representative of high prevalence for HIV and syphilis infection, where the prevalence of HIV and syphilis was determined to be greater than 1%, and a third site which was representative of low prevalence for HIV and syphilis, where less than 1% of new HIV and syphilis infections were identified in the population presenting to the clinic. This study included individuals with known HIV infections (with unknown or negative syphilis status), known syphilis positive patients, individuals at 'High Risk' for HIV and syphilis infection based on certain preset criteria and 'Low Risk' individuals for HIV and syphilis infection, i.e., subjects who did not meet any of the criteria for "High Risk" and were of unknown HIV and syphilis status. The second clinical study was conducted in March 2022 to October 2022 using three sites representative of high prevalence for HIV and syphilis infection and included individuals with known HIV infections (with unknown or negative syphilis status) and known syphilis positive patients. One of the sites was used in both studies.

A total of 1385 subjects were enrolled in these studies evaluating performance of DPP HIV-Syphilis test at the CLIA waived sites. There were 192 fingerstick specimens excluded due to patient ineligibility or sample collection and handling issues with one sample generating an invalid result by the DPP HIV-Syphilis assay, leaving a total of 1193 fingerstick specimens to be included in the evaluation of the assay performance. The overall invalid rate observed during the clinical studies was 0.1% (one out of 1194 specimens) with 95% Confidence Interval (CI) of 0.0% to 0.5%. As per the device labelling, all invalid results were repeated immediately. All repeat testing generated valid results. The performance calculations of DPP HIV-Syphilis test for HIV and syphilis are discussed separately below.

- *HIV Clinical Performance of DPP HIV-Syphilis with Fingerstick Specimens*

The clinical performance for the DPP HIV-Syphilis test in detecting HIV antibodies was estimated by calculating the sensitivity and specificity of the DPP HIV-Syphilis test results with comparator testing. For subjects of unknown HIV status at enrollment, comparator testing was conducted according to the current recognized algorithm, the "4th Generation" algorithm, for the detection of HIV (CDC Laboratory Testing for the Diagnosis of HIV Infection: Updated Recommendations, 2014). Subjects, Known Positive for HIV, at or prior to enrollment, were tested for HIV by only an HIV 1/2 Ag/Ab EIA and only if unreactive by the test, were tested using an HIV-1/HIV-2 Differentiation Assay and HIV-1 Nucleic Acid Test (NAT). The following table shows how the final Patient Infected Status (PIS) for HIV was determined.

Patient Infected Status Determination Based on 4th Generation HIV Testing Algorithm

HIV 1/2 Ag/Ab Assay	HIV-1/HIV-2 Differentiation Assay	HIV-1 NAT	PIS Interpretation
Nonreactive	N/A	N/A	Negative
Reactive	HIV-1 Reactive HIV-2 Nonreactive	N/A	Positive for HIV-1
	HIV-1 Nonreactive HIV-2 Reactive	N/A	Positive for HIV-2
	HIV-1 & HIV-2 Reactive	N/A	Positive for HIV-1/2
	HIV-1 Nonreactive or Indeterminate and HIV-2 Negative	HIV-1 NAT Reactive	Positive for HIV-1, Acute Infection
		HIV-1 NAT Negative	Negative for HIV-1

Results obtained from 1160 fingerstick specimens were used in the data analysis. The clinical performance of the DPP HIV-Syphilis test for HIV for fingerstick whole blood specimen type, when in the hands of untrained operators, is presented below.

HIV Clinical Performance of DPP HIV-Syphilis Test with Fingerstick Specimens (Tested by Untrained Operators)

Population	N	TP	FN	TN	FP	PPA (95% CI)	NPA (95% CI)
Unknown HIV status at Enrollment	484	10	0	472	2	100% (72.3 - 100%)	99.6% (98.5 - 99.9%)
Known HIV positive at Enrollment	676	670	6[1]	0	0	99.1% (98.1 - 99.6%)	N/A
Combined	1160	680	6[1]	472	2	99.1% (98.1 - 99.6%)	99.6% (98.5 - 99.9%)

[1]Two of the DPP HIV negative samples were from HIV Known Positive subjects that were HIV-1 Indeterminate or Negative via HIV-1/HIV-2 Differentiation Assay and HIV-1 RNA was not detectable via Nucleic Acid Test (NAT).

PPA=Positive Percent Agreement, NPA=Negative Percent Agreement
N=Included Subjects, TP=True Positive, TN=True Negative, FN=False Negative, FP= False Positive

The 33 fingerstick specimens excluded from the calculations of Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) of the DPP HIV-Syphilis test for HIV included:

a. Fifteen specimens excluded due to incomplete or missing reference results
b. Nine specimens that were from subjects who could not be recalled to the clinical site as the original fingerstick whole blood specimen collection was inadequate
c. Nine specimens from subjects enrolled as HIV Known Positive which could not be confirmed HIV positive via reference testing.

- *Syphilis Clinical Performance of DPP HIV-Syphilis with Fingerstick Specimens*

The clinical performance of the treponemal test line of DPP HIV-Syphilis was evaluated by calculating the Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) of the assay with the final comparator result based on an algorithm of results from three commercially available FDA-cleared syphilis assays: a treponemal EIA, a non-treponemal Rapid Plasma Reagin (RPR) assay and a second *Treponema pallidum* Particle Agglutination (TPPA) assay. The final comparator result was determined using a two out of three rule. The following table shows how the final comparator result was determined.

Serologic Comparator Algorithm for Treponemal Antibodies

1st Treponemal Test (EIA)	Non-Treponemal (RPR)	2nd Treponemal Test (TPPA)	Final Comparator Result
Nonreactive	Nonreactive	Reactive	Negative
		Nonreactive	Negative
		Inconclusive	Negative
Nonreactive	Reactive	Reactive	Positive
		Nonreactive	Negative
		Inconclusive	Negative
Reactive	Reactive	Reactive	Positive
		Nonreactive	Positive
		Inconclusive	Positive
Reactive	Nonreactive	Reactive	Positive
		Nonreactive	Negative
		Inconclusive	Positive
Equivocal	Nonreactive	Reactive	Positive
		Nonreactive	Negative
		Inconclusive	Indeterminate
Equivocal	Reactive	Reactive	Positive
		Nonreactive	Negative
		Inconclusive	Indeterminate

Results obtained from 844 fingerstick specimens were used in the data analysis. The clinical performance of the DPP HIV-Syphilis test for syphilis for fingerstick specimen type, when in the hands of untrained operators, is presented below.

Syphilis Clinical Performance of DPP HIV-Syphilis Test with Fingerstick Specimens (Tested by Untrained Operators)

Population	N	TP	FN	TN	FP	PPA (95% CI)	NPA (95% CI)
Subjects tested for Syphilis	844	295	13	509	27	95.8% (92.9 - 97.5%)	95.0% (92.8 - 96.5%)

PPA=Positive Percent Agreement, NPA=Negative Percent Agreement
N=Included Subjects, TP=True Positive, TN=True Negative, FN=False Negative, FP= False Positive

The 349 fingerstick specimens excluded from the calculations of PPA and NPA of the DPP HIV-Syphilis test for syphilis included:

a. Six specimens that could not be tested within the appropriate stability requirements for the comparator assay after receipt at the reference laboratory
b. Four specimens due to insufficient volume to complete the Serological Comparator Algorithm testing
c. Nine specimens from subjects which could not be recalled to the clinical site and were excluded for inadequate fingerstick blood collection
d. 330 specimens did not have all the comparator data as per the Serological Comparator Algorithm discussed above (these were subjects enrolled to supplement the HIV data for the estimation of clinical performance of the DPP HIV-Syphilis assay for the detection of HIV).

Device Performance with Analyte Concentrations Near the Cutoff

The performance of the DPP HIV-Syphilis test with samples at analyte concentrations near the assay cutoff was evaluated in a study conducted at three external sites representative of CLIA waived sites, with two untrained operators at each site. This study was a subset of the reproducibility study reviewed and documented under BP180191. The test samples were contrived in whole blood matrix and consisted of a six-member panel containing (a) low reactive HIV-1, (b) a near cutoff HIV-1, (c) a low reactive HIV-2, (d) a low reactive *T. pallidum,* (e) a near cutoff *T. Pallidum* sample, and (f) a sample nonreactive for both HIV and *T. pallidum*. The antibody concentrations were targeted based on the reflectance signal value from the DPP Micro Reader with low reactive samples at concentrations 3-5x assay cutoff and near cutoff samples at 1-2x assay cutoff for the analyte. Each operator tested each sample a total of 20 times over the course of the study. Testing was performed according to the test instructions in the QRI.

The data presented below show the performance of the DPP HIV-Syphilis test with samples at low reactive, near the cutoff concentrations, and negative samples, in the hands of

untrained operators. However, the focus of this evaluation was on the three samples near the assay cutoff, highlighted below.

Summary of Results Testing Samples with the DPP HIV-Syphilis Test by Untrained Operators

Sample	HIV Test Line					Treponemal Test Line				
	Reactive/Total (% Agreement with Expected Result)					Reactive/Total (% Agreement with Expected Result)				
	Site 1 (b) (4)	Site 2	Site 3	Total	95% CI	Site 1 (b) (4)	Site 2	Site 3	Total	95% CI
Low Reactive HIV-1 (3-5x LoD)	40/40 (100%)	40/40 (100%)	40/40 (100%)	120/120 (100%)	96.9-100%	3/40 (92.5%)	0/40 (100%)	0/40 (100%)	3/120 (97.5%)	92.9-99.2%
Near Cutoff HIV-1 (1-2x LoD)	35/40 (87.5%)	37/40 (92.5%)	40/40 (100%)	112/120 (93.3%)	87.4-96.6%	0/40 (100%)	1/40 (97.5%)	0/40 (100%)	1/120 (99.2%)	95.4-99.9%
Low Reactive HIV-2 (3-5x LoD)	40/40 (100%)	40/40 (100%)	40/40 (100%)	120/120 (100%)	96.9-100%	0/40 (100%)	0/40 (100%)	0/40 (100%)	0/120 (100%)	96.9-100%
Low Reactive *T. pallidium* (3-5x LoD)	0/40 (100%)	0/40 (100%)	0/40 (100%)	0/120 (100%)	96.9-100%	40/40 (100%)	40/40 (100%)	40/40 (100%)	120/120 (100%)	96.9-100%
Near Cutoff *T. pallidum* (1-2x LoD)	0/40 (100%)	0/40 (100%)	0/40 (100%)	0/120 (100%)	96.9-100%	37/40 (92.5%)	38/40 (95%)	40/40 (100%)	115/120 (95.8%)	90.6-98.2%
Non Reactive	0/40 (100%)	0/40 (100%)	0/40 (100%)	0/120 (100%)	96.9-100%	0/40 (100%)	1/40 (97.5%)	0/40 (100%)	1/120 (99.2%)	95.4-99.9%

The study results demonstrated that users untrained in the test procedure of the DPP HIV-Syphilis were able to perform the test correctly and the test provided the expected results for samples with organism concentration near the assay cutoff.

Operator Questionnaire

Following completion of the study, operators at each site were asked to complete a questionnaire to help assess whether participants understood how to use the DPP HIV-Syphilis test with the DPP Micro Reader correctly. The questionnaire consisted of a series of questions pertaining to the ease of use of the test with answers rated on a scale from one to five where one indicated the greatest ease of use and five indicated the least ease of use. Participants graded the overall ease of use at ~1.5. They found the test to be easy to use and the instructions easy to understand. Based on the operators' feedback, invalid results were easy to understand and the result screen on the Reader was clear and easy to interpret.

M. Labeling for Waived Devices

The labeling consists of:

1. Product Insert
2. Quick Reference Instructions (QRI)

The following elements are appropriately present:

- The Quick Reference Instructions are written in simple language and, where appropriate, contain graphic representation of system components and procedure steps.
- The Quick Reference Instructions identify the test for professional use only.
- The Product Insert contains a statement that a Certificate of Waiver is required to perform the test in a waived setting.
- The Product Insert identifies the sample types that are allowed for use in Waived Settings and for Moderate Complexity Laboratories.
- The Product Insert contains a statement that laboratories with a Certificate of Waiver must follow the manufacturer's instructions for performing the test. 42 CFR 493.15(e)(1).
- The User's Manual and Quick Reference Guide provide instructions for conducting quality control procedures.
- The labeling is sufficient and satisfies the requirements of 21 CFR Part 809.10.

N. Conclusion:

The submitted information in this CLIA waiver application supports a CLIA waiver approval decision.