SYPHILIS HEALTH CHECK

Rev. O, 04/15

CLIA Complexity: WAIVED for Fingerstick Whole Blood Specimens ONLY

For *in vitro* diagnostic use only

Rx Only

A Certificate of Waiver is required to perform this test in a CLIA waived setting. To obtain a Certificate of Waiver, please contact your state health department. Additional CLIA waiver information is available at the Centers for Medicare and Medicaid website at www.cms.gov/CLIA or from your state health department.

Failure to follow the instructions or modification to the test instructions will result in the test no longer meeting the requirements for waived category.

INTENDED USE

Syphilis Health Check is a qualitative rapid membrane immunochromatographic assay for the detection of *Treponema pallidum* (syphilis) antibodies in human whole blood, serum or plasma. This product can be used as an initial screening test or in conjunction with a nontreponemal laboratory test and clinical findings to aid in the diagnosis of syphilis infection. This test is not intended for use in screening blood or plasma donors.

SUMMARY AND EXPLANATION

Syphilis is a sexually transmitted disease (STD) caused by the spirochete organism *Treponema pallidum* (TP). As this organism cannot be cultured on artificial media, the diagnosis of syphilis depends on the correlation of clinical data with antibodies demonstrated by serological tests. Two types of antibody responses normally result: non-specific (anti-cardiolipin) [1,2] and specific (anti-treponemal). While non-specific antibodies occur in the majority of infected individuals, many other conditions can give rise to false positive results, yielding an overall specificity of about 50% in the general population[3].

Treponemal specific tests are based on the use of treponemal antigens in the assay. For decades, treponemal tests, such as Microhemagglutination for *Treponema pallidum* antibodies (MHA-TP) and Fluorescent Treponemal Antibody Absorption (FTA-Abs) tests for *T. pallidum*, were largely used to confirm positive results obtained by non-specific nontreponemal tests, such as Rapid Plasma Reagin (RPR) and Venereal Disease Research Laboratory (VDRL) tests. More recently, many treponemal tests, such as the Enzyme Immunoassay (EIA) and Chemiluminescence Immunoassay (CIA) based tests, are used to confirm positive results of nontreponemal tests, or as a screening procedure for syphilis following a reverse sequence syphilis screening algorithm [4,5,6].Treponemal tests are considered reliable for detection of past treated or current untreated infections; treponemal antibody generally persists after successful treatment with the exception of treatment during early primary syphilis [7]. Syphilis is a chronic infection which progresses through distinct stages of infection: primary, secondary, latent, or late (tertiary) syphilis. The treponemal antibody results are not quantitative; hence no assumptions about staging of disease or efficacy of treatment can be made based on treponemal tests results.

PRINCIPLE OF THE TEST

Syphilis Health Check is a rapid qualitative screening test for detection of human antibodies to TP in serum, plasma or whole blood.

The method employs a unique combination of anti-human immunoglobulins gold conjugate and highly purified TP recombinant proteins to specifically detect anti-TP antibodies.

As the samples flow through the absorbent device, the anti-human immunoglobulins/protein A dye conjugate binds to the human immunoglobulins forming an antigen-antibody complex. This complex binds to the recombinant protein in the positive reaction zone and produces a pink-rose colored band. In the absence of anti TP antibodies, there is no line in the positive reaction zone. The reaction mixture continues flowing through the absorbent device past the reaction and control zones. Unbound conjugate binds to the reagents in the control zone producing a pink-rose color band, demonstrating that the reagents are functioning correctly.

MATERIALS SUPPLIED

Each kit contains everything needed to perform 20 tests.

- SYPHILIS HEALTH CHECK Test devices : 20

- Disposable plastic fixed volume pipettes : 20
- Diluent in a dropper bottle containing saline buffer, detergent and sodium azide (NaN3, 0.1%) : 5 mL
- Package insert 1

STORAGE

All SYPHILIS HEALTH CHECK kit components should be stored at (4º - 30°C). Test cassettes should be stored in their sealed pouch.

Do not freeze the test kit.

The SYPHILIS HEALTH CHECK- kit is stable until the expiry date stated on the package label.

MATERIALS REQUIRED BUT NOT PROVIDED

Timer - 20 min.
Syphilis Health Check Control Set, order from Diagnostics Direct LLC at (866) 358-9282.
25 µL Pipettes not included

WARNINGS AND PRECAUTIONS

1. Do not use test cassettes if foil pouches are opened or defective.
2. Make sure the materials in the kit are at room temperature before use.
3. Always wear gloves when performing Syphilis Health Check.
4. Place the device on a clean flat surface facing up.
5. Use the pipette included in the kit only.
6. This test is designed for "*in vitro diagnostic*" use
7. Read instructions carefully before using this test
8. A positive test must be followed by or reflexed to a laboratory nontreponemal syphilis assay with titer information.
9. Clinical judgment is necessary for interpreting the test results
10. A positive result may not be useful for establishing a diagnosis of syphilis infection. In some situations, such a result may reflect a prior treated infection; a negative result can exclude a diagnosis of syphilis except for cases of incubating or early primary disease where syphilis antibodies are not yet detectable.
11. Blood specimens may be potentially infectious. Avoid contact with skin by wearing gloves and proper laboratory attire. Properly handle and discard all used test devices in an approved biohazard container.
12. Avoid any contact between hands and eyes or nose during specimens collection and testing
13. Do not use the buffer or cassette after the expiration date printed on the outside of each foil pouch.
14. Test cassettes are single use only.
15. Adding sample and buffer in the wrong order will result in an incorrect result.
16. Test buffer and Controls contain sodium azide as preservative that is a poison and may be harmful if swallowed. Seek medical help if buffer is swallowed.
17. Persons performing the test must be tested for colorblindness before performing the test.

COLLECTION AND STORAGE OF SPECIMENS

For Fingerstick Whole Blood Collection:

1. Rub the chosen finger towards the tip and wipe the end of the finger with an alcohol wipe and a sterile pad.
2. Alcohol will affect the test**.** Let dry thoroughly.
3. **Two drops of** whole blood (50 µL) is required to perform the test.
4. Stick fingertip with a lancet.
5. The first drop of blood should be wiped clean with a sterile pad. **NOTE**: It is important that the first drop should NOT be used to avoid any potential interference from the alcohol.
6. Rub the finger towards the tip for two more drops of blood.
7. Using the fixed volume pipette provided in the kit, touch the end of the pipette to the drop of blood.

8. Holding the pipette horizontally, allow the blood to flow into the pipette on its own, making sure that there are no air bubbles or empty spaces or gaps in the specimen. If air bubbles or empty spaces or gaps are present, collect another sample.
9. It may be necessary to rub the finger for an additional drop of blood to get two drops.
10. Whole Blood samples collected by finger-stick should be used on the Syphilis Health Check test devices immediately after collection.

For Venous Whole Blood Collection:

The serum or plasma specimen should be collected aseptically under the standard laboratory conditions, avoiding hemolysis. Fresh samples should be used for testing.

If the test is to be run within 8 hours after collection, the specimen should be stored in the refrigerator (2° to 8°C). If testing is NOT performed within 8 hours, the sample must be converted to serum or plasma and can be stored refrigerated (2 - 8ºC) up to 5 days. If testing is delayed more than 5 days, serum and plasma specimens should be frozen. The frozen specimen must be completely thawed, thoroughly mixed and brought to room temperature prior to testing. Avoid repeated freezing and thawing.

1. Draw venous whole blood sample into a syringe or a vacuum collection tube containing EDTA as an anticoagulant for plasma or a red top tube for serum.
2. Remove tube cap and touch the end of the 50 µL pipette included in the kit to the blood in the tube by slightly tipping the tube and holding the pipette so the tip is in the blood.
3. Aspirate the blood into the end of the pipette (> 2 drops) making sure that there are no air bubbles or empty spaces or gaps in the specimen. If a whole blood (with red cells) sample is used, **TWO drops** of whole blood (50 µL) is needed for the assay. If the red blood cells are separated, then **ONE drop** of serum or plasma (25 µL) is required to perform this test. If air bubbles or empty spaces or gaps are present, collect another sample.
4. Replace cap on tube.

ASSAY PROCEDURE

1- Allow the Syphilis Health Check test device to come to room temperature prior to testing. Venous whole blood, serum, or plasma samples should be allowed to come to room temperature prior to testing. Finger-stick whole blood samples should be used immediately after collection.

2- Remove the reaction device from its protective wrapper by tearing along the notch.

3- Label device with the patient's name or control number.

4- Fill the pipette with specimen (whole blood, serum or plasma).

5- Hold the pipette vertically, dispense one drop (25 µl) of serum or plasma into the sample well (small circle). If whole blood is used, dispense two drops (50 µl) into the sample well.

6- Allow sample to be absorbed into the pad.

7- Add 4 full drops of Diluent (200 µl) to the sample well (small circle). One more drop can be added, if the sample does not flow down the membrane. DO NOT USE WATER OR OTHER LIQUIDS.

8- Set the cassette on a flat surface and incubate at room temperature (20 - 26ºC) for 10 minutes.

9- Read the results after 10 minutes. The result can be read up to 15 minutes.

PLEASE NOTE: Do not read after 15 minutes.

INTERPRETATION OF RESULTS

The assay is calibrated against commercially available serum "standardized" against the WHO Reference Material and the cut-off confirmed with results obtained with uninfected patient samples and borderline treponemal positive samples diluted to assess the imprecision around the cut-off of the assay.

A. Negative

One pink/red line of any intensity appears in the "C" control area. This indicates a Non-Reactive result that is interpreted as Negative for Syphilis antibodies. No visible line in the test area is considered a negative result.

B. Positive

A pink/red line of **any** intensity appears in the device window adjacent to "T" Test and a second line of any intensity appears adjacent to "C" Control. This indicates a Reactive result that is interpreted as Presumptive Positive for Syphilis antibodies. Any visible red/pink line is considered positive.

C. Invalid

If there is no color line visible in the "C" control area, whether or not there is a line in the "T" test area, the test is invalid and cannot be interpreted. In this case, repeat the test with a fresh specimen using a fresh device.

Contact Diagnostic Direct Technical Services at 866-358-9282 if you are unable to produce a valid result upon repeat testing.

IMPORTANT: In addition to the pink line by the Control mark ANY line that is seen near the Test mark of the cassette at the 10-minute time is considered a positive result. The intensity of the line does not matter.

The following Table provides an algorithm to aid in interpreting and reporting syphilis serology results for diagnosis of *T. Pallidum* infection status, using both a treponemal test and a nontreponemal test .

A positive SHC result is not diagnostic of syphilis without additional nontreponemal serologic testing and a full clinical evaluation.

A new venous whole blood specimen must be obtained for further testing.

Nontreponemal Result (NT)	Treponemal Result	Report/interpretation for all except neonates or infants*
Nonreactive	Negative (Nonreactive)	No serologic evidence of infection with *T. pallidum (*incubating or early primary syphilis cannot be excluded).
Reactive	Negative (Nonreactive)	Current infection unlikely; probability of Biological False Positive (BFP) secondary to other medical conditions (febrile diseases, immunizations, intravenous drug use, autoimmune diseases, etc.). Recommend repeat testing (nontreponemal, and treponemal by other test method).
Nonreactive	Positive (Reactive)	Probably past treated infection or untreated latent infection (e.g., if no history of previous treatment); rarely due to potential cross-reactivity with other spirochetes/related antigens. Recommend to treat (if untreated latent infection) or additional testing consistent with clinical findings/history.** Possibility of false negative NT due to incubating syphilis or prozone in secondary syphilis, late latent syphilis, or late neurosyphilis.
Reactive	Positive (Reactive)	Presumptive evidence of current infection or inadequately treated infection, persistent infection, reinfection, or serofast if prior history of treated syphilis). Recommend additional testing consistent with clinical assessment.**
Nonreactive	Not done	Current infection unlikely; effectively treated infection if previously treated; cannot exclude incubating or early primary syphilis; cannot exclude latent or neurosyphilis. Treponemal testing advised if clinical suspicion is present of latent or neurosyphilis. Recommend repeat testing if risks are present.
Not done	Negative (Nonreactive)	Current or past treated infection unlikely unless treated early in the incubating or early primary syphilis stage; cannot exclude incubating or early primary syphilis. Recommend repeat testing if risks are present.

*HIV-infected individuals may have delayed sero-reactivity or negative serology albeit very rarely.
**Nontreponemal testing with titer; clinical history; repeated (sequential) serological testing for changes in titer

QUALITY CONTROL

Built-in Controls:

Syphilis Health Check contains built-in quality control features. A pink line in the Control Zone should always be seen and shows: 1) that enough volume is added and 2) that proper flow is obtained. If this line is missing, the test was not run correctly or failed to function correctly. The test is invalid and the test should be repeated using a new cassette.

External Controls:

The Positive and Negative Controls, which are provided separately from the manufacturer, should be run according to the laboratory requirements. These controls should be run like an unknown patient specimen, at a minimum in the following circumstances:

- Each new lot
- Each new shipment (even if from the same lot previously received)

- Each new operator (an individual who has not run the tests for at least two weeks)
- Monthly, as a continued check on storage conditions
- Whenever problems (storage, operator, or other) are identified
- Or other times as required by your laboratory's standard QC procedures. If the controls do not give expected results (Positive or Negative), patient results must not be reported, and the test should be re-run.

If your local or state regulations require more frequent testing of quality control material, quality control must be performed in compliance with those regulations.

If the test does not show any Control or Test line in the window or a smudged or partial line, the test cassette should be discarded. Do not report the results. Run the test again with a new cassette and follow the procedure exactly. If the second test does not show lines, please contact Technical Services at 866-358-9282. For any other concerns regarding Syphilis Health Check please call 866-358-9282 8am -6pm EST. Problems may also be reported using the MedWatch reporting system at http://www.fda.gov/Safety/MedWatch/HowToReport/ or calling 1-800-FDA-1088 (1-800-332-1088).

LIMITATIONS

1- The results obtained from this assay are intended to aid in diagnosis only. As with all serological treponemal tests for syphilis, interpretation of results obtained with the Syphilis Health Check Treponemal Antibody test must be used in conjunction with a nontreponemal syphilis serologic test with titer, the patient's clinical symptoms, medical history and other clinical and/or laboratory findings to produce an diagnosis of syphilis by stage.

2- A positive treponemal test requires a reflexive second test with a nontreponemal assay with titer, such as RPR, along with a clinical evaluation, for diagnosis of syphilis.

3- Very early stage of infection could lead to false negative results, due to the low concentration of anti-*Treponema pallidum* antibodies in the serum, plasma or whole blood samples.

4- A positive result does not exclude the presence of other pathogens. A positive result can also be obtained in cases of other treponemal diseases such as yaws, pinta and bejel.

5- The Syphilis Health Check test is specific for detecting *Treponema pallidum* antibodies in serum, plasma or whole blood samples. It does not detect *T. pallidum* directly.

6- All treponemal tests tend to remain reactive following treatment and cannot be quantified; therefore, they should not be used to evaluate responses to therapy. Because of the persistence of reactivity, probably for the life of the patient, the treponemal tests are of no value to the clinician in determining relapse or re-infection in a patient who has had a treated infection.

7- Treponemal antibodies after treatment are not indicative of immunity to future syphilis infections.

8- Performance characteristics of this device have not been established for matrices other than whole blood, serum or plasma.

9- Assay performance characteristics have not been established for immunocompromised or immunosuppressed patients, cord blood, neonatal specimens, or infants.

10- Performance characteristics of this device have not been established with specimens containing heterophile antibodies which are known to cause false positive results in various immunoassays.

11- Treponemal tests are not recommended in neonates to diagnose congenital syphilis as passive transfer of maternal antibodies can cause false positive results.

EXPECTED RESULTS

To assess the normal range (Expected Values) of the Syphilis Health Check test a series of ninety-eight (98) samples were obtained from various hospital laboratories in different U.S. geographical locations. The samples came from a general "presumed" healthy population (ages 20 – 66) whose serum or plasma were collected by hospital laboratories for routine serology testing, not related to STDs.

All of the samples were tested with RPR, TPPA and Syphilis Health Check tests. Two samples were found positive by the treponemal tests with both samples non-reactive with RPR. These 2 samples were confirmed with MHA-TP. Based on the results, among the 98 presumed healthy samples 2 were confirmed positive, resulting in a 2% positivity rate.

In a prospectively collected population of drug users visiting STD clinics, of 138 males and 13 females (ages 18 - 61 yrs) thirteen (8.6%) were found to be Syphilis Health Check test positive with eleven of those also reference method positive.

In a series of 69 pregnant women, ages 20 - 40 yrs, that were tested for routine screening, one sample (1.4%) was found to be Syphilis Health Check positive and confirmed by reference methods.

Eight hundred eighty (880) patients were prospectively collected in a population of individuals visiting STD and a hospital clinics and POC sites complaining and/or exhibiting signs and symptoms of STD infections, ages 16 - 81 yrs, and having 53% to 47% male to female ratio. It was found that a range of 3% - 6% of the patients identified as suspected syphilis

were found positive by Syphilis Health Check and yielded a Percent positive agreement to RPR of 98.3% and 95.7% to treponemal tests. Percent Negative Agreement to RPR and Treponemal test was 93.4% and 97.8% respectively. Overall Agreement to RPR and Treponemal tests was 94.1% and 97.4% respectively.

These results are consistent with published rates for prevalence of antibody in the adult population. Prevalence may vary depending on a variety of community, population and social factors. [8,9,10]

PERFORMANCE CHARACTERISTICS

Comparison

In order to evaluate the performance of the Syphilis Health Check test a series of 783 patient samples were obtained that were collected and tested from five prospective study sites. In addition, a 412 frozen retrospective samples purchased from outside commercial vendors and blood centers. A total of 1195 patient samples, including known retrospective samples (412) and prospectively collected patient samples (783), were used to demonstrate the performance of the Syphilis Health Check test to nontreponemal RPR and other treponemal tests.

Prospective Studies were conducted at five clinical study sites which compared the Syphilis Health Check to RPR, a nontreponemal test, and treponemal tests such as TPPA, TPHA, or ELISA, using specimens from patients coming into four STD clinics and one hospital clinic. The patients enrolled in the study were identified by medical associates as suspected positive for syphilis and exhibiting symptoms.

Initial evaluations were performed at a university clinic and a hospital clinic to assess the performance of the Syphilis Health Check test versus RPR and their reference treponemal tests - FTA and TPHA. Only gender and age were collected from these patients. A more comprehensive study was performed at three study sites to collect further patient history information in order to identify a broader range of STD related patients. The information and data collected from these sites is presented below.

The Syphilis Health Check assay demonstrated 94.9% and 98.2% Percent Positive Agreement versus the nontreponemal test and treponemal tests respectively, and 93.3% and 97.3% Percent Negative Agreement respectively.

CUMULATIVE COMPARISON RESULTS

The results obtained for the prospective and retrospective samples yield the following results compared to nontreponemal RPR and treponemal tests.

TOTAL Nontreponemal Comparison - total combined sample results from the 5 prospective sites and the frozen known and suspected positive samples are presented.

		RPR - Nontreponemal		
		Positive	Negative	Total
Syphilis Health Check	Positive	411	51	462
	Negative	22	711	733
	Total	433	762	1195

Percent Positive Agreement: 94.9% (95% C.I. = 92.4 - 96.8%)
Percent Negative Agreement: 93.3% (95% C.I. = 91.3 - 95.0%)

TOTAL Treponemal Comparison - total combined sample results are presented from the 5 sites and the frozen known and suspected positive samples.

		Reference Treponemal tests		
		Positive	Negative	Total
Syphilis Health Check	Positive	444	20	464
	Negative	8	723	731
	Total	452	743	1195

Percent Positive Agreement: 98.2% (95% C.I. = 96.5 - 99.2%)
Percent Negative Agreement: 97.3% (95% C.I. = 95.9 - 98.3%)

PROSPECTIVE STUDIES - 5 SITES

TOTAL Nontreponemal Comparison - total combined sample results are presented from the 5 prospective sites.

		RPR - Nontreponemal		
		Positive	Negative	Total
Syphilis Health Check	Positive	56	25	81
	Negative	2	700	722
	Total	58	725	783

Percent Positive Agreement: 96.6% (95% C.I. = 88.1 - 99.6%)
Percent Negative Agreement: 96.6% (95% C.I. = 95.0 - 97.8%)

TOTAL Treponemal Comparison - total combined sample results are presented from the 5 sites.

		Reference Treponemal tests		
		Positive	Negative	Total
Syphilis Health Check	Positive	67	16	83
	Negative	7	693	700
	Total	74	709	783

Percent Positive Agreement: 90.5% (95% C.I. = 81.5 - 96.1%)
Percent Negative Agreement: 97.7% (95% C.I. = 96.4 - 98.7%)

COMPARISON BY SITE
University Clinic site

		RPR		
		pos	Neg	Total
Syphilis Health Check	Pos	32	1	33
	Neg	0	6	6
	Total	32	7	39

FTA		
pos	neg	Total
27	6	33
0	6	6
27	12	39

Percent Positive Agreement = 100.0% (95% C.I. = 89.1 - 100%) 100% (95% C.I. = 87.2 - 100%)
Percent Negative Agreement = 85.7% (95% C.I. = 42.1 - 99.6%) 50.0% (95% C.I. = 21.1 - 78.9%)

Hospital Clinic site

		RPR		
		pos	Neg	Total
Syphilis Health Check	Pos	3	3	6
	Neg	0	44	44
	Total	3	47	50

TPHA		
pos	neg	Total
6	0	
0	44	
6	44	50

Percent Positive Agreement = 100.0% (95% C.I. = 29.2 - 100%) 100.0% (95% C.I. = 54.1 - 100%)
Percent Negative Agreement = 93.6% (95% C.I. = 82.5 - 98.7%) 100.0% (95% C.I. = 92.0 - 100%)

Study Site 1

		RPR		
		pos	neg	Total
Syphilis Health Check	pos	13	15	28
	neg	2	370	372
	Total	15	385	400

TPPA		
pos	neg	Total
21	8	29
6	365	371
27	373	400

Percent Positive Agreement = 86.7% (95% C.I. = 59.5 - 98.3) 77.8% (95% C.I. = 57.7 - 91.4)
Percent Negative Agreement = 96.1% (95% C.I. = 93.7 - 97.8) 97.9% (95% C.I. = 95.8 - 99.1)

It should be noted that two (2) of the false negative samples vs TPPA were only positive for TPPA but negative with the other two reference methods (RPR and FTA). Three samples were positive for TPPA and FTA, but negative for Syphilis Health Check and RPR.

Study Site 2

		RPR		
		pos	Neg	Total
Syphilis Health Check	Pos	2	2	4
	Neg	0	85	85
	Total	2	87	89

TPPA		
pos	neg	Total
4	0	4
0	85	85
4	85	89

Percent Positive Agreement = 100.0% (95% C.I. = 15.8 - 100) 100.0% (95% C.I. = 39.8 - 100%)
Percent Negative Agreement = 97.7% (95% C.I. = 91.9 - 99.7) 100.0% (95% C.I. = 95.8 - 100%)

Study Site 3

		RPR		
		pos	neg	Total
Syphilis Health Check	Pos	6	4	10
	Neg	0	195	195
	Total	6	199	205

EIA		
pos	neg	Total
9	2	11
1	193	194
10	195	205

Percent Positive Agreement = 100.0% (95% C.I. = 54.1 - 100) 90.0% (95% C.I. = 55.5 - 99.7%)
Percent Negative Agreement = 98.0% (95% C.I. = 94.9 - 99.4) 99.0% (95% C.I. = 96.3 - 99.9%)

RETROSPECTIVE STUDIES
Suspected and Known Positive Syphilis Samples

A series of 412 total samples were purchased from serum and blood center suppliers consisting of 149 banked RPR and treponemal reactive serum samples and 28 serum samples that were requested to be Primary or Secondary Patients, treated or untreated, but exhibiting a Syphilitic-type lesion or rash were purchased from a serum supplier, and 138 frozen serum and plasma samples were obtained from a blood center. The samples were found to be RPR and treponemal reactive and having mixed titers. Another series of 97 samples being highly suspected of having a syphilis infection were obtained from a serum supplier that were obtained from various laboratories around the U.S., and were submitted to the laboratories for testing. The samples were tested by RPR, TPPA, and MHA-TP as reference methods for comparison to Syphilis Health Check test.

The samples were further tested by an outside laboratory for TPPA titer and Syphilis Health Check results. Nineteen (19) samples were found to be Negative for TPPA and Syphilis Health Check and Reactive RPR, four samples were negative by TPPA, but Positive by Syphilis Health Check and RPR, one sample was Syphilis Health Check negative and positive RPR and TPPA, and one sample was RPR negative but TPPA and Syphilis Health Check positive. The remaining two hundred eighty-nine samples remained Positive by all four methods. In the Suspected Positive patients two samples were negative with Syphilis Health Check, with one sample low reactive with RPR, and both samples Non-Reactive with TPHA. The results of the testing are shown below.

Known Positives

		RPR		
		pos	neg	Total
Syphilis Health Check	pos	293	1	294
	neg	20	1	21
	Total	313	2	315

TPPA		
pos	neg	Total
290	4	294
1	20	21
291	24	315

Percent Positive Agreement = 93.6% (95%C.I. = 90.3 - 96.1%) 99.7% (95%C.I. = 98.1 - 100.0%)
Percent Negative Agreement = 50.0% (95%C.I. = 1.26- 98.7%) 83.3% (95%C.I. = 62.6 - 95.3%)

Suspected Positive

		RPR		
		pos	Neg	Total
Syphilis Health Check	pos	62	25	87
	neg	0	10	10
	Total	62	35	97

TPHA		
pos	neg	Total
87	0	87
0	10	10
87	10	97

MHTP		
pos	neg	Total
87	0	87
0	10	10
87	10	97

Percent Positive Agreement = 100.0% (95% C.I. = 94.2 -100%) 100.0% (95% C.I. = 95.8 -100%) 100.0% (95% C.I. = 95.8 - 100%)
Percent Negative Agreement = 28.6% (95% C.I. = 14.6 - 46.3%) 100.0% (95% C.I. = 69.2 -100%) 100.0% (95% C.I. = 69.2 -100%)

Clinically Diagnosed

A panel of one hundred sixty-four (164) well-characterized clinically diagnosed serum samples from treated and untreated patients with primary, secondary, and latent syphilis infections were obtained from a clinic serving a population of individuals with a variety of infectious diseases. The samples were tested with reference assay tests for RPR, TPPA, and FTA-ABS. The samples were then tested with Syphilis Health Check and the results are summarized below.

Known Clinical Status		RPR	TPPA	FTA-ABS	Syphilis Health Check	No.	% Agree-ment	95% C.I.
Untreated	Primary	React	React	React	React	23	100	85.2 - 100%
	Secondary	React	React	React	React	25	100	86.3 - 100%
	Latent	React	React	React	React	22	100	84.6 - 100%
		NR	React	React	React	3	100	29.2 - 100%
Treated	Primary	React	React	React	React	28	100	87.8 - 100%
	Secondary	React	React	React	React	26	100	86.8 - 100%
	Latent	React	React	React	React	18	100	81.5 - 100%
		NR	React	React	React	19	100	82.4 - 100%
					Total	164	100	97.8 - 100%

Interference Study

Interference testing was conducted using serum. Concentrates of the compounds were prepared and diluted to multiple concentrations into eight sera with different levels of syphilis reactivity. The following results were obtained:

Hemoglobin: No effect was observed up to 1000 mg/dL of hemoglobin;

Bilirubin (total): No effect was observed up to 40 mg/dL of total bilirubin;

Triglycerides: No effect was observed up to 3000 mg/dL of triglycerides.

Cholesterol (total): No effect was observed up to 400mg/dL of cholesterol

Albumin: No effect was observed up to 1000 mg/dL of albumin

Gamma-globulin: No effect as observed up to 5000 mg/dL of gamma-globulin

Potential Cross Reactors:

Panels of samples were obtained to evaluate potential interference from different disease conditions confirmed positive and containing different concentrations of potentially cross-reactive antibodies and were analyzed with the Syphilis Health Check test. From 151 (138 Males) prospectively collected drug users eleven (7%) were found syphilis positive by reference methods and Syphilis Health Check, with two additional samples found positive for Syphilis Health Check. Two samples from Lyme disease and HSV showed a positive result with Syphilis Health Check but could not be confirmed by reference methods. One sample each from CMV positive and heterophile positive patients were found Syphilis Health Check positive, but non-reactive by reference methods. Two HPV patients were found Syphilis Health Check positive with one confirmed by reference methods. Two co-infected Chlamydia/GC patients were found Syphilis Health Check positive and one confirmed positive by TPPA. The number of disease condition categories and reactive results obtained is listed in the following table.

Cross-reactor	Number	# positive by Syphilis Health Check	# positive by reference method(s)
Self-reported Drug Users	151	13	11
ANA Positive	24	0	
RF Positive	40	0	
U.S. Lyme Disease IgG & IgM	25	1	Not confirmed
HSV	24	1	Not confirmed
CMV	10	2	1
EBV	21	0	
HAV	25	0	
HIV 1 & 2	14	1	1
HTLV	14	1	1

Heterophile	32	1	0
HCV	24	1	1
Anti-HBs	25	2	2
Other STD – GC, Chlamydia, HPV, Trichomonas,	78	4	2

Syphilis Positive HIV / HCV / HBV Patients

A series of 13 patient EDTA Plasma samples from a blood center were identified that were screened RPR positive in patients that were also confirmed positive for HIV, HCV and/or Hepatitis B virus. Three of these patients were further identified as previously testing positive for syphilis and treated at that time. All of the samples were tested with TPPA and Syphilis Health Check to evaluate syphilis reactivity. All 13 samples were positive for RPR, TPPA, and Syphilis Health Check.

To further evaluate the influence of HIV on Syphilis Health Check results, a series of 24 banked serum containing high levels of viral load for HIV were tested with Syphilis Health Check. The HIV positive patient samples were known to be RPR and/or Treponema positive, and were purchased from a commercial serum supplier. The samples were tested by an outside lab with the Syphilis Health Check test to assess reactivity. Six of these samples were Nonreactive by RPR but reactive with TPHA. One sample was RPR positive, but TPHA nonreactive. All 24 samples were Syphilis Health Check positive.

Pregnant Women

A series of sixty-nine (69) pregnant female samples with known trimester, age, and ethnicity were purchased from a vendor. An additional set of 93 pregnant women serum samples were obtained from a commercial source that were identified as syphilis positive by RPR screening and further tested with TPPA and Syphilis Health Check. Age and trimester were known, but ethnicity was not identified. Three samples out of the 162 total samples were RPR low positive but nonreactive by the treponemal test method.

Pregnant Women
Summary

		RPR		
		pos	neg	Total
Syphilis Health Check	pos	91	0	91
	neg	3	68	71
	Total	94	68	162

TPPA		
pos	neg	Total
94	0	94
0	68	68
94	68	162

Percent Positive Agreement = 96.8% (95% C.I. =91.0 - 99.3%) 100.0% (95% C.I. = 96.2 - 100%)
Percent Negative Agreement = 100.0% (95% C.I. =94.7 - 100%) 100.0% (95% C.I. = 94.7 - 100%)

PRECISION and REPRODUCIBILITY

Studies were performed to demonstrate the Intra-Assay, Inter-day, and Inter-Lot reproducibility of the Syphilis Health Check test kit. The within-run and between-day reproducibility of the Syphilis Health Check test were evaluated at three laboratory sites. Two trained technicians at each site performed the testing using a panel of six pooled samples. Each testing site conducted reproducibility studies using a supplied panel of samples ranging from non-reactive to highly reactive, i.e. one nonreactive, one high (borderline) nonreactive, one low (borderline) reactive, one moderate reactive, and two mid-high to high reactive in addition to the kit controls. For the between-day reproducibility evaluation, each site ran these panel member samples for at least five days, twice per day per operator. For the within-run reproducibility evaluation, each operator at the three testing sites performed ten tests on each of the panel member in one day. The one negative and three low to high positive samples had 100% agreement with expected results. The two critical borderline samples near the cut-off yielded the following results:

Within-Run

Panel D (Borderline Reactive)

	Positive	Negative	Expected	% Agree	Discrepant
Site 1 (2 operators)	57	3	60 positive	95.0	3
Site 2 (2 operators)	58	2	60 positive	96.7	2
In-house (2 operators)	59	1	60 positive	98.3	1

Panel E (Borderline Nonreactive)

	Positive	Negative	Expected	% Agree	Discrepant

Site 1	1	59	60 negative	98.3	1
Site 2	2	58	60 negative	96.7	2
In-House	0	60	60 negative	100.0	0

Inter-Day (5 days)

Panel D (Borderline Reactive)

	Positive	Negative	Expected	% Agree	Discrepant
Site 1 (2 operators)	57	3	60 positive	95.0	3
Site 2 (2 operators)	58	2	60 positive	96.7	2
In-house (2 operators)	57	3	60 positive	95.0	3

Panel E (Borderline Nonreactive)

	Positive	Negative	Expected	% Agree	Discrepant
Site 1	2	58	60 negative	96.7	2
Site 2	2	58	60 negative	96.7	2
In-House	1	59	60 negative	98.3	1

Inter-Lot

Studies were performed to demonstrate the Inter-Lot assay reproducibility of the Syphilis Health Check test kits. The Inter-Lot study used four dilutions of a treponemal antibody positive pool and a treponemal antibody negative sera sample. These five samples were run in duplicate on five different lots to demonstrate lot-to-lot reproducibility run on the same day by the same technician.

The following table demonstrates the reproducibility of the Syphilis Health Check Test over 5 lots of kits.

Dilution	Results after 10 min.					
	Expected results	**Lot I**	**Lot II**	**Lot III**	**Lot IV**	**Lot V**
Negative serum	-	-	-	-	-	-
$1/10^4$	-	-	-	-	-	-
$1/10^3$	+/-	+/-	+/-	+/-	+/-	+/-
$1/10^2$	+	+	+	+	+	+
1/10	+	+	+	+	+	+

CLIA WAIVER STUDIES

The performance of the Syphilis Health Check (SHC) test was evaluated in a prospective study conducted over four months at three geographically diverse sites located in Florida, Tennessee, and Illinois. At each site, Syphilis Health Check testing was conducted by operators who had no laboratory experience and were representative of users at CLIA waived testing sites (intended use). The 12 operators (intended users) who participated in the study were not given training on the use of the test. There were 417 subjects enrolled in the study. Fingerstick whole blood from each subject was tested with Syphilis Health Check by the untrained operators at each site. Syphilis patient infected status for each subject was determined by a composite comparator method (Comparator) testing a subject-matched venous serum specimen in a reference laboratory. The composite comparator method consisted of an FDA-cleared treponemal antibody chemiluminescent immunoassay (CIA), an FDA-cleared rapid plasma reagin (RPR) test, and an FDA-cleared *T. pallidum* particle agglutination (TP-PA) assay, following a pre-determined algorithm presented in the table below.

Treponemal Antibody Chemiluminescent Immunoassay (CIA)	**Rapid Plasma Reagin (RPR) Test**	***T. pallidum* Particle Agglutination (TP-PA) Assay**	**Syphilis Patient Infected Status (PIS)**
Negative	N/A	N/A	Negative
Positive	Positive	N/A	Positive
Positive	Negative	Positive	Positive
Positive	Negative	Negative	Negative

The result of the Syphilis Health Check test was compared to the syphilis patient infected status (PIS) of the subject. The positive percent agreement and negative percent agreement between the Syphilis Health Check result and the PIS for the 415 analysable study specimens are presented in the table below. There was one invalid Syphilis Health Check test result reported and the specimen was excluded from performance calculations. An additional specimen was also excluded from performance analysis due to inability to assign a PIS result by the composite comparator method following the pre-determined algorithm presented in the table above (CIA Indeterminate; RPR Negative).

All Three Study Sites Combined		PIS		
		Positive	Negative	Total
SHC	Positive	197	6	203
	Negative	4	208	212
	Total	201	214	415
Positive Percent Agreement: 98.0% (197/201) (95% CI: 95.0% - 99.2%) Negative Percent Agreement: 97.2% (208/214) (95% CI: 94.0% - 98.7%)				

Percent of invalid results was 0.2% (1/417) with 95% CI: 0.0% to 1.3%

Additionally, a study was conducted to evaluate the ability of untrained operators to detect treponemal antibodies using the Syphilis Health Check test in weakly reactive samples. Randomly coded panels consisting of two contrived weakly reactive whole blood samples were tested with Syphilis Health Check test at three intended use sites by nine untrained operators (72 tests in total per sample). The testing was done over three weekdays per week for four weeks with samples integrated into daily workflow at each site. The samples were prepared by diluting a characterized syphilis antibodies positive plasma sample with a known RPR titer in whole blood, and represented weakly reactive samples that are slightly above (Low Positive sample) and slightly below (High Negative sample) the cut-off of the Syphilis Health Check test. The same panel was also tested by trained laboratory professionals to verify that the samples gave the expected reactivity results.

The table below shows performance of the Syphilis Health Check test with samples near the cut-off of the test in the hands of intended users (across all sites).

Sample	**Untrained Users (Intended Users)**						**P-Value***
	POC Site 1		**POC Site 2**		**POC Site 3**		
	% Agreement with Expected Result	95% C.I.	% Agreement with Expected Result	95% C.I.	% Agreement with Expected Result	95% C.I.	
High Negative	95.8% (23/24)	79.8%, 99.3%	97.1% (22/24)	74.2%, 97.7%	97.1 % (22/24)	74.2%, 97.7%	1.000

Low Positive	100% (24/24)	86.2%, 100%	95.8% (23/24)	79.8%, 99.3%	95.8% (23/24)	79.8%, 99.3%	1.000

* (Fisher-Freeman-Halton Test)

Using risk analysis as a guide, analytical flex studies were conducted to evaluate the operational limits of the Syphilis Health Check test. The studies demonstrated that the Syphilis Health Check test is insensitive to stresses of environmental conditions and potential user errors.

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