SYPHILIS HEALTH CHECK
VSC-11-01

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 non-treponemal 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 the specific antibody 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 an 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 pipettes : 20
- Diluent in a dropper bottle containing saline buffer, detergent and sodium azide (NaN_3, 0.1%) : 5 mL
- Package insert 1

STORAGE

All SYPHILIS HEALTH CHECK kit components should be stored at room temperature (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.

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 before performing Syphilis Health Check.
4. Lay device on 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 confirmed using a laboratory non-treponemal syphilis assay.
9. Clinical judgment is necessary for interpreting the test results
10. A positive result is not useful for establishing a diagnosis of syphilis. In most situations, such a result may reflect a prior treated infection; a negative result can exclude a diagnosis of syphilis except for incubating or early primary disease
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 contains 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. **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 2 drops.

For Venous Whole Blood Collection:

The serum or plasma specimen should be collected under the standard laboratory conditions (aseptically in such a way as to avoid 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 end of 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 blood into the end 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 samples and Syphilis Health Check test devices to come to room temperature prior to testing.
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 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) in 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 colored band 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 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 band 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 with 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 non-trepomemal 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.

Non-Treponemal 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. 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 that 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 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.

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