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## SYPHILIS HEALTH CHECK

### 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. NOTE: This is a CLIA-waived test for finger-stick whole blood specimens only.

### 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) 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.

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. 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. 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 whole blood (finger-stick).

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 by Diagnostics Direct LLC (VSC-11-01):	20
- Disposable plastic fixed volume (25 uL) pipettes :	20
- Diluent in a dropper bottle containing saline buffer, detergent and sodium azide (NaN <sub>3</sub> , 0.1%):	5 mL
- Package insert	1

### STORAGE

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

#### **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 by Diagnostics Direct LLC (VSC-11-02)

### STORAGE OF CONTROLS

Store refrigerated at 2 to 8°C until expiration date printed on label if unopened. Do not use after the expiry date. Allow to warm to room temperature before use in the assay. Refrigerate after use. Controls are stable for 12 months after first opening (or by the expiry date if it is earlier) when stored at 2 to 8°C between each use. Write the open date on the vials. Do NOT freeze.

### 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 a laboratory nontreponemal syphilis assay with titer information (RPR).
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 specimen 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.

### 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.

#### Quality Control Testing Procedure:

1. The Syphilis Health Check Positive and Negative controls are liquid and are ready to use.
2. Bring to room temperature before use.
3. Add ONE drop (25  $\mu$ L) of the control (Positive or Negative) into the sample well of the cassette and proceed in the same way as for a patient's sample by adding 4 drops of the Buffer Diluent.
4. Read the result after 10 minutes. The result can be read up to 15 minutes.

**PLEASE NOTE: Do not read before 10 minutes or after 15 minutes.**

#### External Controls:

The Positive and Negative Controls should be run like an unknown patient specimen with:

- 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

NOTE: If the controls do not give expected results (Positive or Negative), patient results must NOT be reported, and the test should be re-run once the controls pass.

Record quality control results on the QC Results Log.

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).

## COLLECTION AND STORAGE OF SPECIMENS

### For Finger-stick Whole Blood Collection:

1. Prior to collection, using two patient identifiers, verify the patient's identity, and explain the procedure to patient.
2. Observe universal precautions; wear gloves and other personal protective equipment as appropriate.
3. Rub the chosen finger towards the tip and wipe the end of the finger with an alcohol wipe and a sterile pad.
4. Alcohol will affect the test. Let dry thoroughly.
5. **Two drops of whole blood** (50  $\mu$ L) is required to perform the test.
6. Stick fingertip with a lancet.
7. 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.
8. Rub the finger towards the tip for two more drops of blood.
9. Using the fixed volume pipette provided in the kit, touch the end of the pipette to the drop of blood.
10. 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.

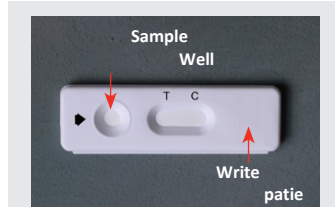


11. It may be necessary to rub the finger for an additional drop of blood to get two drops.
12. Whole Blood samples collected by finger-stick should be used on the Syphilis Health Check test devices immediately after collection.

## ASSAY PROCEDURE

**NOTE: Quality Control results must pass prior to patient testing.**

- 1- Allow the Syphilis Health Check test device 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 patient's unique identifier.



- 4- Collect the specimen as described above and fill the pipette with specimen (whole blood (50 µL),



- 5- Hold the pipette vertically; dispense two drops of whole blood (50 µl) into the sample well.



- 6- Allow sample to be absorbed into the pad.
- 7- Add 4 full drops of Diluent (200 µl), one drop at a time and allow each to soak in, 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 (15 - 30°C) for 10 minutes.
- 9- Read the results after 10 minutes. The result can be read up to 15 minutes.

**PLEASE NOTE: Do not read before 10 minutes or 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.

### **B. 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 pink / red line in the test area is considered a negative result.

### **C. Positive**

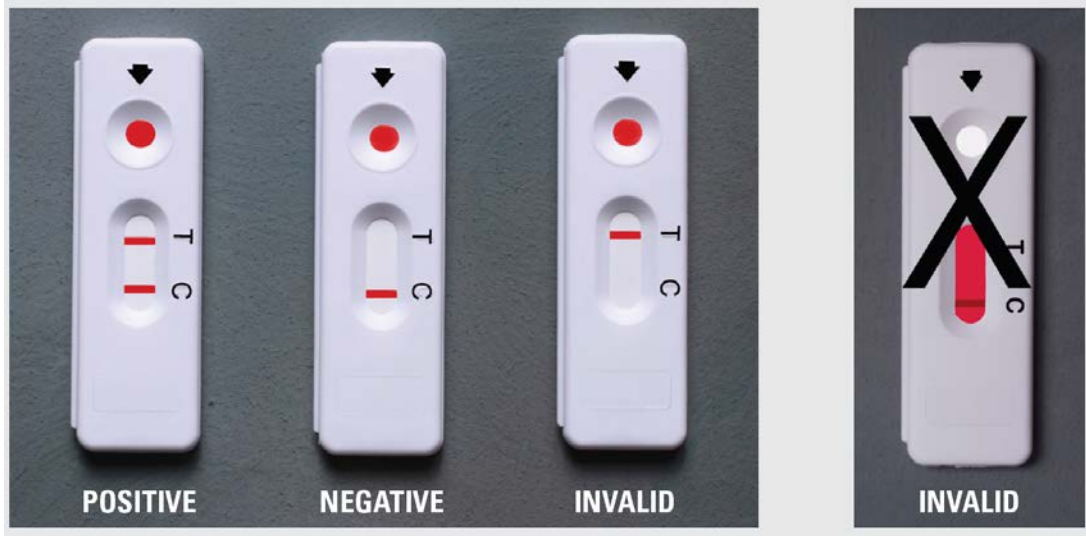
A pink/red line of any intensity appears in the device window line adjacent to "T" Test and a second line of any intensity appears adjacent to "C" Control. This indicates a Reactive result that is interpreted as Presumptive Positive for Syphilis antibodies. Any visible pink/red only line is considered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

### **D. 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. If repeat testing results in an invalid result again, then collect a gold top tube, order a Syphilis test (RPR) and send it to the Clinical Laboratory.

Contact Diagnostic Direct Technical Services at 866-358-9282 if you are unable to produce a valid result upon repeat testing. Also, report the issue to the POCT Service Team.

**IMPORTANT:** In addition to the pink / red line by the Control mark ANY pink/red only 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.



#### CONFIRMATORY TESTING

**A positive SHC result is not diagnostic of syphilis without additional nontreponemal serologic testing and a full clinical evaluation.  
A new blood specimen (gold top tube) must be obtained for further testing (Syphilis Antibody/RPR Serology test).**

#### EXPECTED RESULTS

Normal Range: Negative

#### 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 (IgG and IgM) 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.

#### **RECORD KEEPING**

- 1- All records for QC Testing, Patient Testing, and staff Initial Orientation & Training and Competency will be kept on file for at least three years.
- 2- Patient results are documented in an electronic medical record system as part of the patient's medical record for perpetuity. During downtime, use a Result form.

#### **REFERENCES**

- 1- Syphilis Health Check Package Insert. Rev. Q, 05/18

#### **DISTRIBUTION**

1. XXXXXXXXXXXXX - ZSFG Laboratory Procedure Manual