Rapid Syphilis Testing Program Guide
2015
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**Intended Use**

Syphilis Health Check is a qualitative rapid membrane immune-chromatographic 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.

**Features of the Syphilis Health Check**

The Syphilis Health Check is a very simple 10 minute, 2-step procedure utilizing a finger-stick; it affords the following features and benefits:

- 98 percent agreement with reference treponemal assays;
- 100 percent agreement with clinically diagnosed samples;
- Detection of both IgG and IgM, enhancing detection with early syphilis;
- Utilization of multiple recombinant syphilis antigens (TP-15, TP-17, and TP-44 for optimized sensitivity and specificity);
- Room temperature kit storage;
- Only rapid syphilis test with FDA Clearance and CLIA-waived; and
- CPT code: 86780.

**Recommended Use**

The Syphilis Health Check is ideal for use in outreach and other venues where syphilis testing is being offered to high-risk individuals and where the follow-up with those individuals who test positive is difficult. Specific venues include:

- Outreach to at-risk women who are pregnant
- Clinics designated for men who have sex with men (MSM);
- Outreach to commercial sex workers;
- Outreach to MSM venues such as gay pride events, gay book stores, bathhouses, and gay campgrounds;
- Testing sites performing rapid HIV testing; and
- Community-based testing in areas of high syphilis morbidity

Please use the Rapid Syphilis Test Risk Assessment form to determine client risk (Appendix B).

**Target Populations - Syphilis Health Check**

- The Syphilis Health Check is not a test that should be offered to everyone.
- It is important that a detailed risk assessment be conducted prior to the offering of the test.
- Because the Syphilis Health Check is a treponemal assay, individuals with a previous known syphilis history should not be offered this test. Individuals with a previous known syphilis history should instead have a routine serologic specimen drawn for the standard lab-based syphilis testing.
When Not to Use
The Syphilis Health Check is not recommended as a screening test for individuals with a past history of syphilis, whether or not they were appropriately treated. It is important to specifically ask individuals if they were previously diagnosed with syphilis prior to using this test.

Adherence to Manufacturer's Instructions
Certain steps need to be taken even before a test is begun to be sure results are accurate. Most importantly, follow the manufacturer’s instructions throughout the testing process. Problems found in testing sites that perform waived tests are most often the result of not following this critical step.

Testing Environment and Preparation
Testing should be performed in an area with adequate space to safely conduct testing while maintaining patient privacy. Testing and storage areas should be monitored to be sure they meet specific environmental requirements described in the manufacturer’s instructions.

Equipment used for testing should be maintained and calibration checks should be performed as directed in the manufacturer's instructions.

1. Check inventory regularly to ensure you will have enough reagents and supplies on hand for testing.
2. Check and record expiration dates of reagents/kits, and discard any reagents or tests that have expired.
3. Check and record temperatures of the testing and reagent storage areas. See Appendix A for samples of daily temperature logs.
4. Check that all kit reagents came from the same kit lot. Do not mix reagents
5. Inspect reagents for damage, discoloration, or contamination, and discard if found.
6. Prepare reagents according to manufacturer’s instructions.
7. Allow time for refrigerated reagents/samples to come to room temperature prior to testing
8. Perform equipment calibration checks, as needed, following the manufacturer’s instructions.
9. Perform testing in a well-lit area.
10. Inspect equipment and electrical connections to be sure they are working.
11. Clean work surfaces before and after testing.

Testing sites that perform testing under a CLIA Certificate of Waiver must follow the current manufacturer’s test instructions. The following steps should be taken to be sure the current test instructions are being followed:

1. Read and understand the manufacturer’s instructions and/or site specific procedure.
2. Keep a copy of the manufacturer’s instructions on hand for easy reference.
3. Check the manufacturer’s instructions with each new lot and shipment of test kits to make sure there are no changes from the test kits being used.
4. File the current manufacturer’s instructions and replace with an update if there are changes.
5. Communicate all changes in the manufacturer’s instructions to other testing personnel and to the person who directs or supervises testing.
7. Practice all tests, while an experienced person watches, before testing patient samples and reporting patient results.
8. Document training on all tests in staff personnel files.

**Syphilis Health Check 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

**Materials Required but not Provided:**

- Timer - 20 min.
- Syphilis Health Check Control Set, order from Trinity Biotech (800) 325-3424.

**Quality Control (QC) Testing**
Quality control (QC) testing gives confidence that your results are accurate and reliable. The manufacturer’s instructions or site specific procedure explain what the controls are checking, the steps for performing QC testing, and when to do QC testing. Incorrect QC results alert the user to potential problems such as reagent/test kit deterioration, equipment failure, adverse environmental conditions, or human error.

**Types of Controls**
Two types of controls are generally found in waived tests:

**Internal Controls** (also referred to as built-in or procedural controls) evaluate whether:
- the test is working as it should,
- enough sample is added,
- the sample is moving through the test strip correctly, and/or
- the electronic functions of the instrument are working correctly.

**External Controls** evaluate whether:
- the entire testing process is performed correctly, and
- the control results are in the expected ranges or values as found in the manufacturer’s instructions.

**Built-in Quality 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 Trinity Biotech at (800) 325-3424. For any other concerns regarding Syphilis Health Check please call the STD and Viral Hepatitis Section at (850) 245-4303.

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 non-treponemal 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.

**Tracking of Quality Control**

Documenting and tracking quality control results can show whether a test is being performed correctly and if the test is working correctly. A periodic review of QC records can show whether the QC results are changing over time. This information can help identify problems that may be affecting patient testing and need to be addressed.

**Actions for unexpected QC results:**

If controls do not give the expected results, patient results should not be reported until the problem is identified and corrected.

- Check to see if the manufacturer’s instructions were followed correctly.
- Look for possible sources of error such as outdated reagents or test devices.
- Check to see if reagents were stored correctly.
- Make sure controls or reagents were not cross-contaminated by accidentally switching caps.
- Follow the troubleshooting steps in the manufacturer’s instructions or site specific procedure.
- For additional assistance, contact the manufacturer, technical representative, and/or the person(s) who directs or supervises the testing.

Once the problem is identified and corrected, repeat QC testing. If the QC results are acceptable, re-test patient sample(s) and report the final acceptable results.

**Storage:**

All Syphilis Health Check kit components should be stored at (4º - 30°C). Test cassettes should be stored in their sealed pouch.

All Syphilis Health Check Control sets should be stored at (2º - 8°C). The Syphilis Health Check kit is stable until the expiry date stated on the package label.

**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 non-treponemal 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 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 screened for colorblindness before performing the test.

Collection and Storage of Specimens

For Finger stick 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.

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°C 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 - 8°C) 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.
- 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.
- Remove tube cap and touch the end of the 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.
- 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) are 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.
- Replace cap on tube.

Assay Procedure

- Allow samples and the Syphilis Health Check test devices to come to room temperature prior to testing.
- Remove the reaction device from its protective wrapper by tearing along the notch.
- Label the device with the patient’s name or control number.
- Fill the pipette with specimen (whole blood, serum or plasma).
- 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.
- Allow sample to be absorbed into the pad.
- 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.
- Set the cassette on a flat surface and incubate at room temperature (20 - 26ºC) for 10 minutes.
- Read the results after 10 minutes. The result can be read up to 15 minutes. 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 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.

Positive: 2 colored bands in test area AND control area,

Negative: 1 colored band in control area.

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.

A positive SHC result is not diagnostic of syphilis without additional non-treponemal serologic testing and a full clinical evaluation. A new venous whole blood specimen must be obtained for further testing.
Safety

- Wear appropriate personal protective equipment (PPE) such as gloves.
- Clean hands and change gloves between patients

Follow work practices that reduce the risk of exposure including:
- handle all blood and body fluids as if they are infectious,
- use required PPE and safety devices,
- do not eat, drink, or apply cosmetics in the testing area,
- be cautious of exposure to mucous membranes such as eyes, nostrils, and mouth,
- wear goggles or face shields,
- avoid the use of needles and lancets if safe and effective alternatives are available,
- never re-use single-use devices such as needles and lancets,
- avoid recapping needles, transferring a body fluid between containers, and opening blood tubes,
- dispose of used sharps properly in puncture-proof sharps containers,
- report all occupational exposures promptly to ensure that you receive appropriate follow-up care,
- report any real or potential hazards you observe to the person who directs or oversees testing,
- participate in training related to infection prevention, and get hepatitis B vaccination.

Biohazardous Waste:
During the testing process, the biohazard bags and sharps containers used for disposal of contaminated materials should be:
- As close as possible to the immediate testing area,
- Upright throughout use,
- Replaced routinely, and
- Not overfilled.

Containers for contaminated waste must be:
- Constructed to contain all contents and prevent leakage of fluids during handling, storage, transport and/or shipping,
- Labeled or color-coded to indicate biohazard material, and
- Closed prior to removal to prevent spillage or protrusion of contents during handling.

OSHA Requirements
All sites that collect blood samples for traditional and/or rapid testing must meet the OSHA standards for blood-borne pathogens. Providers must establish a written Exposure Control Plan designed to eliminate or minimize employee exposures to occupational risks. Providers must provide PPE to employees at no cost. Examples of PPE are latex or vinyl gloves, eye protectors, and lab coats. If a problem arises with an article of PPE, the provider must repair or replace it at no cost to the employee.
Providers must develop an Exposure Control Plan, which must be readily accessible to all employees who may encounter occupational exposure. Providers must provide hand-washing facilities, which are readily accessible to all employees. If hand washing facilities are not feasible, the provider must provide either an appropriate antiseptic hand cleanser or antiseptic towelettes.

Areas where there is a reasonable likelihood of occupational exposure include eating, drinking, smoking, applying cosmetics or lip balm, and handling contact lenses. Food and drinks must not be kept in refrigerators, freezers, shelves, cabinets or on countertops or bench tops where blood or other potentially infectious materials are present.

The employer must ensure that all medical evaluations and procedures including the hepatitis B vaccination series, post-exposure evaluation and follow-up, including prophylaxis, are made available at no cost to employees. Following a report of an exposure incident, the employer shall immediately make available to the exposed employee a confidential medical evaluation and follow-up. Providers must contain and dispose of biohazardous waste in accordance with applicable regulations and develop a plan to ensure proper biohazardous waste and sharps disposal.


Clinical Laboratory Improvement Amendments (CLIA) Requirements

There is an important distinction between traditional syphilis testing and rapid testing. Sites that conduct traditional syphilis testing obtain a specimen and send that sample to a laboratory where testing is performed. Sites conducting rapid syphilis testing are considered to be a clinical laboratory and are held to laboratory standards. Rapid test sites must possess a CLIA waiver which designates the facility as authorized to perform waived rapid syphilis testing.

Prior to initiating a rapid testing program, sites must be issued a CLIA waiver and number from the U.S. Centers for Medicare and Medicaid Services (CMS). Applications are available from and must be submitted to the Florida Agency for Health Care Administration (AHCA). A CLIA Certificate of Waiver allows these sites to perform FDA approved waived rapid tests. For special events, and on a limited basis, sites may offer rapid testing at locations not on the CLIA waiver with the written approval of the area STD Program Manager. Email exchange for approval is acceptable. All rapid testing sites must adhere to the standards of a waived rapid testing venue.

The CLIA of 1988 established quality standards for all laboratory testing to ensure the accuracy, reliability and timeliness of patient test results. A laboratory is defined as any facility that performs testing on specimens derived from humans for the purpose of providing information for the diagnosis, prevention, treatment of disease, or impairment of, or assessment of health. Federal law requires that all laboratories performing testing, no matter what type, must obtain a CLIA certificate and number.

For more information about the CLIA waiver application process, visit CMS CLIA website.
Appendix A - TEMPERATURE LOG for Syphilis Health Check Controls

Refrigerator/freezer Location ____________________________  Month/Year

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Acceptable temperature range: (2º -8ºC)

Corrective Action for Out of Range Temperature

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Reviewed by: ________________________________________ Date: __/__/__
Appendix B- Rapid Syphilis Test Risk Assessment

A risk assessment must be completed on all clients to determine whether they are at risk, or have a history of syphilis. Completed copies of this form are to be sent to the STD and Viral Hepatitis Section, including for persons who were not tested.

Today’s Date: ___/___/____  County: __________________________________________

Test Site: ☐ STD Clinic ☐ HIV Pt. Care ☐ Jail ☐ Outreach ☐ CBO ☐ Prenatal
☐ Family Planning  ☐ Other

Last Name: ______________________________       First Name: ____________________

Address: __________________________________________________________________

City: _______________________   State: ______   Zip: __________ County: ______________

Phone: (___) _____-_________ Date of Birth (mm/dd/yyyy) ___/___/____   Age: __________

Sex: ☐ Male   ☐ Female
Race: ☐ White  ☐ Black ☐ Am. Indian/Alaskan ☐ Asian/Pacific Islander ☐ Other ☐ Unknown
Ethnicity: ☐ Hispanic  ☐ Non-Hispanic ☐ Haitian  ☐ Other

History
1. Have you ever been diagnosed with syphilis? ☐ Yes  ☐ No  ☐ Not Sure
   If yes, stop here! Do not perform a rapid test!

2. In the past 12 months have you experienced any of the following? (Check all that apply)
   ☐ Genital sore/lesion  ☐ Body rash  ☐ Sudden hair loss  ☐ Palmar/plantar rash
   ☐ Sore(s) in mouth/lips  ☐ Swolen lymph nodes (groin)  ☐ Condyloma lata

   *If client claims a history of any of the above symptoms, perform test.

Risks in Past 12 Months (Check all that apply)

☐ MSM   ☐ Multiple sex partners   ☐ Sex for money/drugs   ☐ Known syphilis exposure
☐ Commercial sex   ☐ Illicit drug use   ☐ HIV positive   ☐ History of STD(s)   ☐ Pregnant with no prenatal care   ☐ Female sex with an MSM

If client has at least one of the above risks checked, perform test.

If client claims none of the above risks, do not perform test.

Test results: ☐ Negative   ☐ Positive   ☐ Inconclusive   ☐ Not Tested

Mail or fax all risk assessment forms to:
STD and Viral Hepatitis Section
4052 Bald Cypress Way, Bin A-19
Tallahassee, Florida 32399-1716
Fax: (850) 414-8103