

Syphilis Immunochromatographic Assay Rapid Diagnostic Test for Detecting Syphilis Infection

Name and Intended Use

Syphilis Immunochromatographic Strip Assay is a visual, qualitative immunoassay for *in vitro* detection of antibodies to *Treponema pallidum* in serum, plasma or whole blood. The test is intended as an aid to diagnosis of syphilis infection in humans.

Summary and Explanation of the Test

Treponema pallidum is the causative agent of syphilis. Multiple clinical stages and long period of latent, asymptomatic infection are characteristic of syphilis.

Diagnosis of syphilis depends on the correlation of clinical data with the results of serologic (non-treponemal and treponemal) assays. Non-treponemal tests (VDRL, RPR, etc) are generally used for screening and treponemal tests (TPHA, FTA-ABS) are used as confirmatory tests. Persons in whom both assays are reactive are considered infected.

Principles of the Procedure

The Syphilis Immunochromatographic Strip (ICS) assay is a membrane-based immunoassay for the detection of *Treponema pallidum* antibodies in serum, plasma or whole blood. In the test procedure, recombinant *Treponema pallidum* antigen is coated on the test band region. Patient sample, when placed on the specimen pad, reacts with the antigen-colloidal gold conjugate. Running buffer facilitates the movement of the sample and antigen-colloidal gold conjugate along the length of the membrane to the test region. If antibody to *Treponema pallidum* is present in the sample, an antigen-antibody-antigen complex will form. This is shown by a red colored line in the test area. To serve as a procedural control, a red colored line will always appear at the control region if the test has been performed correctly.

Materials Provided

Syphilis ICS kit contains the following components to perform the assay:

1. Test Strip, either individually packaged or in multi-strip cylinder
2. Running buffer (Phosphate Buffered Saline – PBS)
3. Sample Loop (5 µL)

Accessories (required, but not provided):

1. Micropipette (if loop is not used)
2. Test tube
3. Timer

Storage and Stability

The test strips are sealed in a moisture-proof foil laminate pouch, containing desiccant, or in a multi-strip container with desiccant cap. These should be stored in the coolest and driest area available, preferably at 5 - 35°C. The kit has a shelf life of 24 months from the date of manufacture. The strips should not be frozen and must be protected from exposure to humidity; once a strip is removed, it should be used within one hour. Do not use beyond expiration date.

Precautions

To obtain reproducible results, the following instructions must be followed:

1. For *in vitro* diagnostic use only with serum, plasma or whole blood.
2. Examine strip after removal from pouch or container. If a faint blue line is not visible in the Control Region, discard the strip.
3. Use disposable gloves while handling potentially infectious material and performing the assay.
4. Do not eat, smoke, or drink while handling specimens.
5. Do not combine reagents from different batch numbers as the components are optimized for individual batch to give best results.
6. Clean up spills thoroughly using an appropriate disinfectant.
7. Do not open the foil pouch until it attains room temperature to prevent formation of condensation.
8. For best results, follow the test procedure and storage instructions strictly.

Specimen Collection and Storage

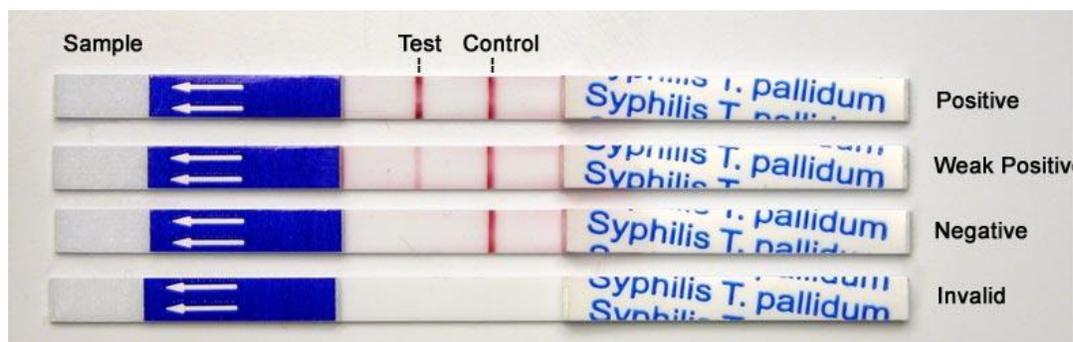
1. Collect whole blood in a clean container containing anti-coagulant (EDTA, citrate or heparin) by venipuncture. Fresh samples are preferred for testing. If samples are not immediately tested, they should be stored at 4-8°C for not more than 3 days. Do not use hemolyzed samples.
2. Fresh blood from a finger prick may also be used as a test sample.
3. Samples with microbial contamination must not be used.

Test Procedure

1. Bring the test strip and sample to be tested to room temperature prior to testing.
2. Label the strip with the patient's identifier.
3. Dispense 3 drops (approximately 100 microliters) of running buffer (PBS) directly into the bottom of a clean, dry test tube.
4. Using the sample loop provided, or a suitable micropipette, place one loop of serum or plasma (5 microliters) onto the test strip in the area just below the arrows. If testing a whole blood sample, use two loops (10 microliters).
5. Alternately, the end of the test strip with the arrows may be dipped into the sample for 1 second, only to the level of the edge of blue tape with arrows (about 7 mm).
6. Drop the strip, with arrows pointing down, into the test tube containing the running buffer. Keep the tube vertical so that the running buffer comes in contact with only the portion of the assay strip below the arrows.
7. After 15 minutes, read the results. Do not read test results after 30 minutes.
8. Record the number of lines observed.

Interpretation of Results

As shown below, a reactive test shows as two colored lines, one in the test area, and one in the control area. The test should be considered reactive if any visible line is evident in the test area, even if it is very faint. A non-reactive test shows only one colored line in the control area. If no lines are visible, the test is invalid.



Performance Characteristics

The test has been evaluated with positive and negative clinical samples with results as follows:

Comparison Test	Total Number	ICS Positive	ICS Negative	Sensitivity (%)	Specificity (%)
RPR Negative	251	13	238	----	94.82
RPR Positive	25	25	0	100.00	----
TPHA Negative	2	0	2	----	100.00
TPHA Positive	87	86	1	98.85	----
VDRL+FTA Negative	81	2	79	----	97.53
VDRL+FTA Positive	57	54	3	94.74	----

Limitations of the Test

1. The Syphilis Immunochromatographic strip (ICS) assay is designed to detect antibodies to *Treponema pallidum* in human serum, plasma or whole blood. Other body fluids or pooled specimens may not give accurate results.
2. The test procedure, precautions and interpretation of results for the test must be followed strictly.
3. No test provides complete assurance that a sample does not contain low levels of antibodies to *Treponema pallidum* such as those present in very early stages of infection. A negative result at any time does not preclude the possibility of infection with syphilis.
4. As with all diagnostic tests, the test result must always be consistent with clinical findings.
5. Persons with a past or current non-venereal *Treponema* infection (Yaws, Pinta) may give positive reactions with this test.
6. Persons with history of *Treponema pallidum* infection, even though cured, will give positive reactions. A non-treponemal test (VDRL, RPR) should be used to assess if the infection is active.