

# THE RAPID SYPHILIS TEST TOOLKIT MANAGEMENT 3

Developing Syphilis Testing  
and Treatment Algorithms  
using Rapid Tests



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# Developing Syphilis Testing and Treatment Algorithms using Rapid Tests

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## 1. Introduction

**This document provides guidance on how to develop testing and treatment algorithms for syphilis.** It gives a brief summary of the disease, the rationale for screening, and the type of serological tests for syphilis and their performance and operational characteristics, and suggests several options for testing and treatment algorithms.

Universal screening of pregnant women for syphilis is recommended policy in most countries, in view of the serious adverse effects of syphilis on the foetus. Currently, however, less than 50% of pregnant women with syphilis are screened and treated.

The major barrier to screening is the lack of access to laboratories that can offer screening: women often have to travel long distances to reach a hospital or an antenatal clinic with such services. Whether used alone or in combination with a non-treponemal test, rapid syphilis tests have the potential to help countries improve policy implementation rate and reduce the burden of syphilis.



Rapid syphilis testing at a primary healthcare setting

### 1.1 Syphilis – the disease

Syphilis is caused by a bacterium called *Treponema pallidum*. In the pre-antibiotic era, the disease was a major cause of both ill health and mortality and it has always carried a substantial social stigma. There are two major routes of transmission, through sexual contact and to the unborn foetus by an infected mother.

Classically, the untreated disease proceeds through four stages:

**Primary syphilis** develops around 3 weeks after infection. It is characterized by an ulcer at the site of infection called a chancre; this is often painless. Lymph glands in the groin may be enlarged. During this stage the bacteria disseminate and can be found throughout the body. Typically this stage lasts from 2 to 6 weeks and then the ulcer heals.

**Secondary syphilis** usually begins between 6 weeks and 6 months after infection. Typically it manifests as a non-itchy skin rash affecting the palms of the hands, but there is considerable variation in the type, site, severity and duration of skin lesions. Other common non-specific symptoms include fever and malaise. Generalized lymph gland enlargement may occur. Typically this stage lasts for a few weeks, but it is common for untreated patients to experience relapses for up to 2 years.

**Latent syphilis** follows the resolution of the secondary stage. Bacteria persist and are controlled but not eliminated by host immunity. In the absence of treatment, latent syphilis may be a life-long condition.

**Tertiary syphilis** appears in approximately 30% of those infected, usually decades after infection. It can affect any part of the body. When the central nervous system or cardio-vascular system is involved, it can be fatal.

It is common for patients to be unaware of their infection since the ulcer is often internal in women and men who have sex with men (MSM) and because there are several other causes of genital ulceration. All ulcerative STIs are associated with an increased risk of HIV transmission. The infection can be readily transmitted by those in the primary and secondary stages, and infection can also be transmitted to infants by mothers in the early latent stage.

It is estimated that 30% of pregnant women who have active syphilis lose their babies due to stillbirth, and a further 30% give birth to a live baby with congenital syphilis. Babies born with syphilis have a high rate of mortality in the first few weeks of life, and survivors may suffer from a wide range of disabilities.

Syphilis is completely curable by antibiotic therapy, and penicillin resistance has not been reported. Adverse pregnancy outcomes caused by syphilis can be prevented by treating infected women with a single dose of benzathine penicillin before the third trimester of pregnancy.

#### Syphilis – the burden of infection

- An estimated one million babies die each year from congenital syphilis.
- Two million pregnant women are infected with syphilis each year.
- 1.2 million of these women transmit the infection to their babies, who may be stillborn, born early, born with a low birth weight, or congenitally infected as a result.
- A study in Northern Tanzania showed that among women who had not been screened for syphilis during pregnancy, 51% of stillbirths could be attributed to syphilis.
- Congenital syphilis is entirely preventable at a cost of less than \$2 per unborn child.
- Screening of pregnant women for syphilis is recommended in nearly all countries in the world but is not widely implemented. It is one of the most cost-effective health interventions.

## 2. The Diagnosis of Syphilis

*Treponema pallidum* is a bacterium that cannot be grown in the laboratory. The demonstration of treponemes with their characteristic spirochete morphology and motility using dark-field microscopy examination of lesion exudate or tissue is the most specific method for the diagnosis of the early stages of syphilis. The dark-field examination must be performed immediately after specimens are collected from primary chancres, moist secondary lesions or from lymph nodes. Dark-field microscopy requires appropriate training for the technique to be reliable, and the quality of the equipment must be kept in good condition.

As patients seldom present with ulcers, diagnosis of syphilis is more commonly made from detection of antibodies in the blood. Two types of serological tests exist:

### 2.1 Non-treponemal antibody tests

The microscopic Venereal Diseases Research Laboratory (VDRL) and the macroscopic Rapid Plasma Reagin (RPR) tests are non-treponemal tests that may be used as qualitative or quantitative tests. They detect antibodies to lipoidal material released from damaged host cells or cardiolipin-like material from the treponemes.

The most commonly used test is the Rapid Plasma Reagin test. This test contains antigen (cardiolipin plus lecithin and cholesterol) together with carbon particles that become agglutinated when an antibody/antigen complex is formed. The agglutination occurs in a drop on a small card and can be seen with the naked eye. This test is used as the primary screening test in many countries. Rapid Plasma Reagin requires a venous blood sample to obtain plasma or serum. Electricity is needed for centrifugation and for the rotator that mixes the plasma/serum and the particles. The Venereal Diseases Research Laboratory test employs the same antigen with small carbon particles, and detection of the antibody/antigen complexes requires a microscope.

Non-treponemal tests may be negative for up to 4 weeks after the chancre of primary syphilis first appears. To exclude syphilis, repeat tests at 1 and 3 months are recommended where there are suspect lesions with an initial negative test.



Laboratory based syphilis screening in Lima, Peru

There is not a strong immunological memory for cardiolipin antigen. Antibody titres decline markedly and ultimately become negative during the latent phase of syphilis and, in most patients, after successful treatment. This is a useful feature that can be used to monitor response to treatment. Titres will decrease following effective treatment or increase in untreated active infection. For example, a 4-fold change or higher in titre, equivalent to a change of at least two dilutions – from 1:16 to 1:4 for effective positive response to treatment or from 1:8 to 1:32 for continued active infection – would be considered significant between two sequential non-treponemal test results using the same testing method and preferably performed by the same laboratory.

Antibodies to cardiolipin are not syphilis-specific: they may also be found in other diseases and conditions such as acute febrile viral infections, some chronic autoimmune diseases, malaria, hepatitis, intravenous drug abuse, and in some women during pregnancy. Uninfected patients who show anti-cardiolipin antibodies from other underlying causes are known as 'biological false positives'. As a consequence, positive non-treponemal tests are sometimes confirmed with a second assay that uses antigens derived from treponemes.

## 2.2 Treponemal antibody tests.

As mentioned above, *Treponema pallidum* spreads rapidly throughout the body post-infection. The high exposure of the immune system to the invading pathogen leads to an early and vigorous antibody response against a range of antigens from *Treponema pallidum*.

Whole *Treponema pallidum* bacteria are used as antigens in the Fluorescent Treponemal Antibody test (FTA). Antibodies are detected using anti-human immunoglobulin antibodies conjugated to a fluorescent marker. To avoid cross-reactivity with other spirochaetes, the sera are first absorbed with fixed non-treponemal spirochaetes, hence the name, 'FTA-absorbed' (Fluorescent Treponemal Antibody Absorption, FTA-ABS). The test requires both a fluorescence microscope and a skilled and experienced reader.

The other group of tests that use antigens derived from treponemes present the antigens bound to sheep red blood cells or coloured microcapsules in agglutination assays. They are known as the *Treponema Pallidum* Haemagglutination assay (TPHA) and the *Treponema Pallidum* Particle Agglutination assay (TPPA) respectively. These are highly sensitive tests, but they are relatively expensive and require trained staff and laboratory facilities. Together with Fluorescent Treponemal Antibody Absorption, they have been used as the 'gold standard' test in many evaluations.

Enzyme-linked Immunosorbent Assays (EIAs) are treponemal tests in a microplate format, which is high throughput and can be automated. Many laboratories in developed countries reversed the order of the testing and screened with treponemal test, followed by a non-treponemal assay for EIA positive cases. Recent data shows that such tests are highly specific, and their sensitivity is comparable with tests that use other treponeme-derived antigens.

### 2.3 Serological reactivity with related treponemes

In areas in which other closely related treponemal diseases such as yaws, pinta and endemic syphilis are present, interpretation of syphilis serology is problematic. In such locations, treponemal tests are of less value and the results of non-treponemal tests and clinical observations become more important.

### 2.4 Rapid Diagnostic Tests

The availability of a cheap and reliable source of *Treponema Pallidum* antigens has led to the development of low-cost rapid diagnostic tests (RDTs), which require only a small volume of blood and no special laboratory apparatus. These so-called 'immunochromatographic strip' tests are simple to read and can be performed by staff with relatively little training. Most importantly, results are available quickly, in contrast to the long delays often involved with laboratory-based tests. This enables treatment at the first visit. It also makes it possible to perform screening in remote settings where laboratory-based testing is not feasible. The sensitivity of the rapid tests ranges from 85-98% and the specificity from 93-98%, compared against the *Treponema Pallidum* Haemagglutination assay or *Treponema Pallidum* Particle Agglutination assay as reference standards. In general, tests with higher sensitivities tend to have lower specificities and vice versa.

The practical use of rapid syphilis tests is described in [Implementation 2 \(Appendix 3\)](#) and the Standard Operating Procedure for performing finger pricks is given in [Implementation 3 \(Appendix 1\)](#).

Serological tests for syphilis give only a presumptive diagnosis of syphilis: they must be interpreted alongside a good sexual history of the individual, a physical examination, the stage of the disease, any other underlying diseases or infections and the possibility of false positive or false negative reactions. If possible, positive non-treponemal tests should be quantified. Above all, all the tests require rigorous standardization with negative and positive control sera.



Rapid testing for syphilis and HIV at a primary health centre



**Table 1. The advantages and disadvantages of non-treponemal and treponemal tests are summarised in the table below:**

NON-TREPONEMAL TESTS e.g. Rapid Plasma Reagin (RPR) test, or Venereal Diseases Research Laboratory (VDRL) test	TREPONEMAL TESTS e.g. <i>Treponema pallidum</i> Hemagglutination Assay (TPHA) or <i>Treponema pallidum</i> Particle Agglutination Assay (TPPA), Enzyme immunoassays, rapid diagnostic tests
<ul style="list-style-type: none"> <li>■ Can be used to distinguish active from past treated infection and for test of cure</li> <li>■ Serum/plasma</li> <li>■ Needs laboratory facility &amp; trained personnel</li> <li>■ Test only takes 8 minutes but tests are batched requiring patients to return for results (and treatment)</li> <li>■ Reagent needs refrigeration</li> <li>■ False negative results due to prozone effect</li> </ul>	<ul style="list-style-type: none"> <li>■ Treponemal antibodies persist for years – measure of exposure</li> <li>■ Whole blood/serum/plasma</li> <li>■ Rapid diagnostic tests can be done in primary health care settings</li> <li>■ Results in 10-20 minutes and treatment given at same visit (Same Day Testing And Treatment)</li> <li>■ Test kits can be transported and stored at room temperature</li> <li>■ No prozone effect</li> </ul>

### 3. Introducing rapid diagnostic tests into a syphilis testing algorithm

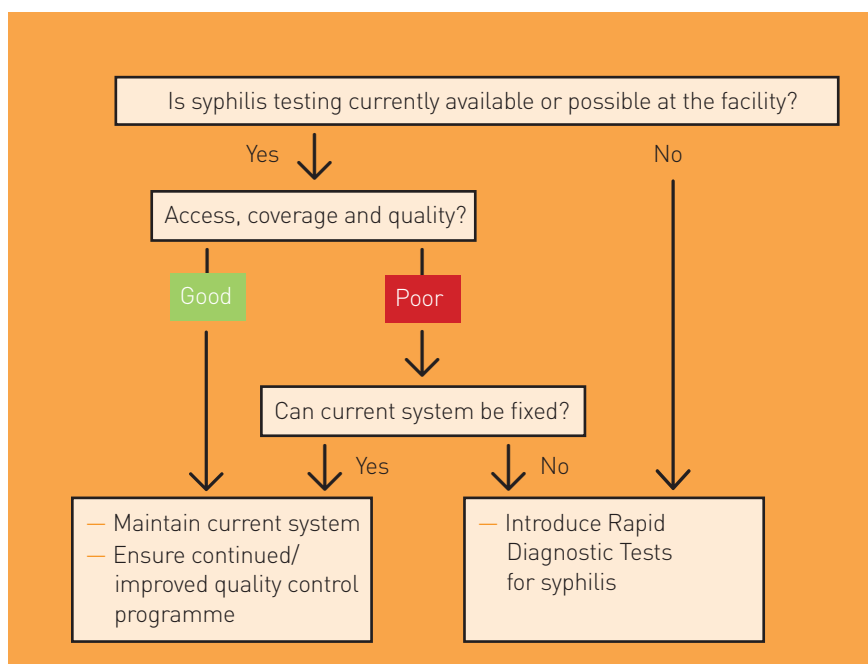
The decision to introduce rapid diagnostic tests into a national system should be based on a careful assessment of the quality, coverage and efficacy of the existing system of testing. Take the following points into consideration:

1. Access: Assess the proportion of persons at risk and pregnant women that has access to syphilis testing.
2. Quality of testing: Assess the quality of testing to ensure accuracy of results.
3. Treatment of sero-reactive individuals: Determine the proportion of persons tested who receive test results and obtain treatment in a timely manner (ideally during the same visit).

If all of the three elements above are working satisfactorily, then changing to a new system may be unjustified. If any of the elements is not working, make efforts to rectify the inadequacies. If the problems cannot be resolved, you should consider introducing a new system.

Once you have decided to introduce the rapid diagnostic test, it is important to be fully conversant with the type of test selected, how it will be used, and at what level of the health-care system the test result will be interpreted. Treponema-based rapid diagnostic tests can be performed in any setting by a suitably trained health care worker. If the existing system is functioning well with the Rapid Plasma Reagin test, there may be no need to replace it with rapid diagnostic tests. The treponema-based rapid diagnostic test could, however, be introduced as the confirmatory test for the Rapid Plasma Reagin. It could either be done at the laboratory or used as a primary point-of-care test ([see Figure 1](#)).

Figure 1. Decision flowchart for introducing rapid diagnostic tests for syphilis



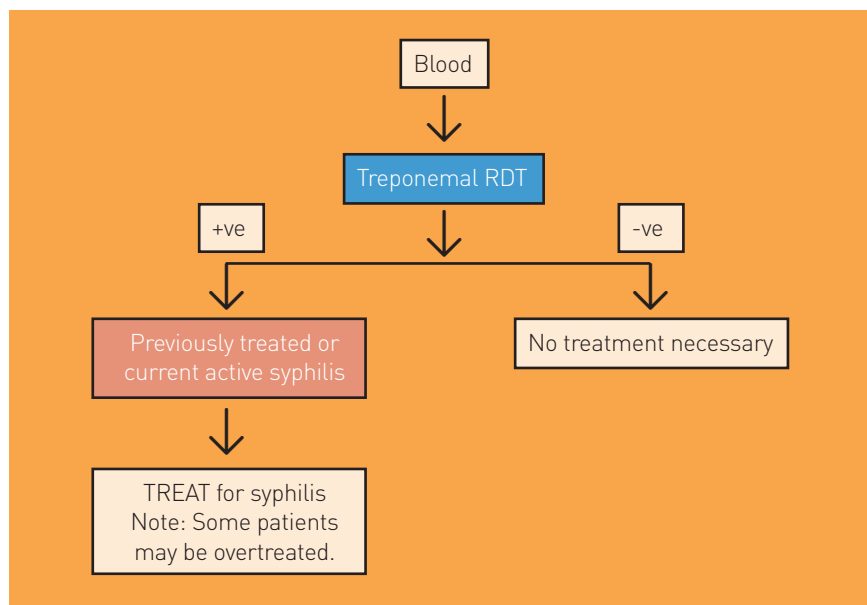
Rapid diagnostic tests can be incorporated into the existing system in three different ways.

### 3.1 Option One: Use treponemal rapid diagnostic tests alone

The first option is used in settings in which no Rapid Plasma Reagin testing is being performed and where it would not be feasible to introduce it. This applies in remote areas without the prerequisite facilities for the Rapid Plasma Reagin, such as electricity for refrigeration of reagents or rotator and blood centrifugation. It can also apply in urban settings with a high turnover rate of patients, where Rapid Plasma Reagin testing becomes impractical. In this case the treponemal rapid diagnostic test result is used to direct treatment for syphilis. This is particularly relevant when screening pregnant women in poorly-resourced areas in countries with a high prevalence of syphilis.

While this approach does not distinguish those with active syphilis from those with past treated infections, and will consequently lead to overtreatment of some patients, it has the overwhelming advantage that it will prevent congenital infection in the majority of pregnant women who are at risk of infection with syphilis (see Figure 2).

**Figure 2. Decision flowchart when no Rapid Plasma Reagin testing is available or possible**



Note.

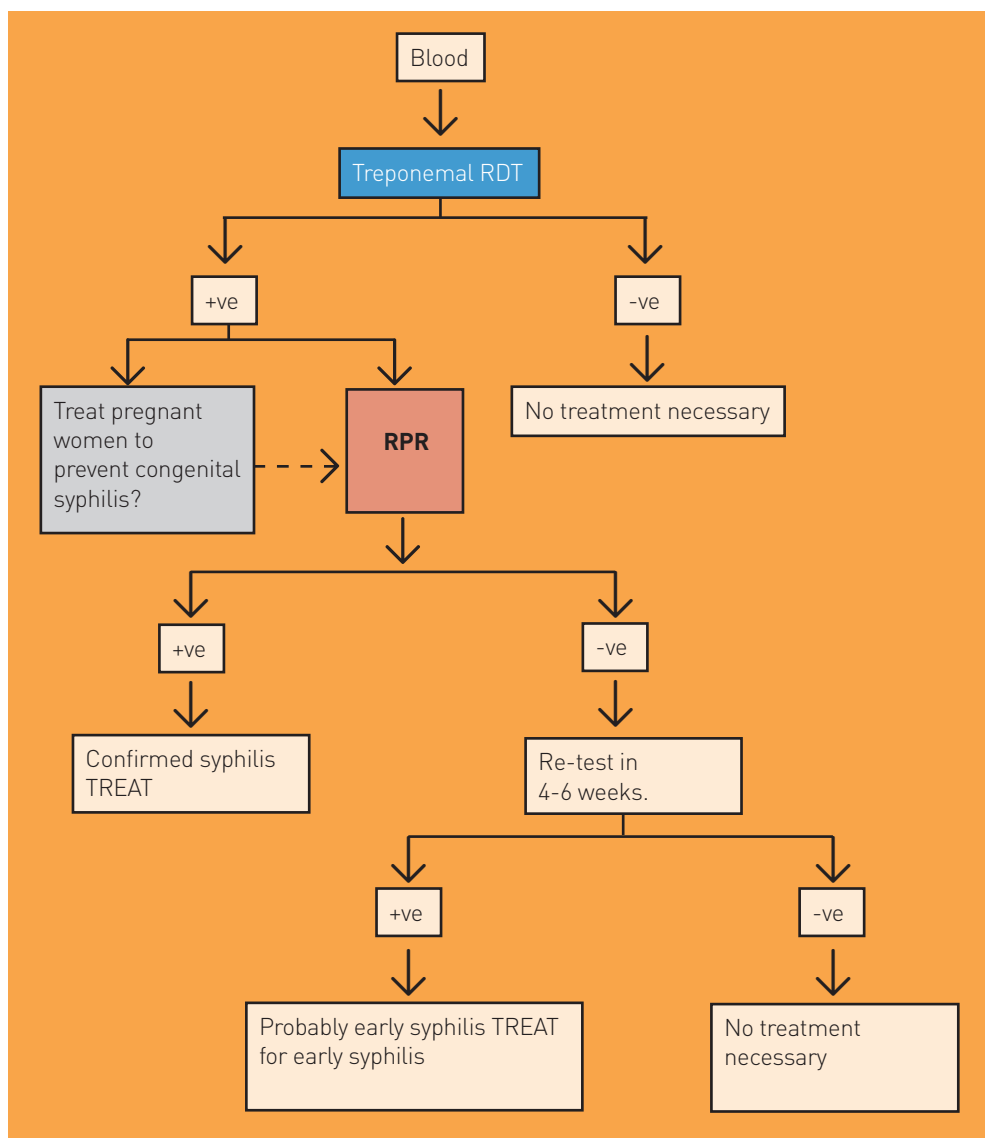
1. That the treponemal test does distinguish between previously adequately treated and untreated syphilis.
2. The sensitivity of treponemal RDTs is reduced with whole blood. Serum performs better.
3. In pregnant women, subsequent testing will likely be still seropositive, therefore, RDT positive women could be treated without re-testing if risk of re-infection is considered high. Alternatively seek quantitative RPR testing.

### 3.2 Option Two: Screen with treponemal rapid diagnostic test and confirm active infection with Rapid Plasma Reagin

Since treponemal rapid diagnostic tests can provide increased access to screening and can detect syphilis earlier than non-treponemal tests, they can be used as a screening test for syphilis. A test that is negative on the treponemal rapid diagnostic test can then be interpreted as negative for syphilis. If the test is reactive, this can be interpreted as past treated or active syphilis. A first dose of penicillin can be given at this point. If non-treponemal testing, such as the Rapid Plasma Reagin, is available, blood can be taken for Rapid Plasma Reagin to confirm whether or not the individual has active syphilis. If the Rapid Plasma Reagin is reactive, two further doses of penicillin should be given. If the Rapid Plasma Reagin is negative, it can be repeated after about a month to exclude persons with primary syphilis whose Rapid Plasma Reagin test may still be negative. Such patients will become Rapid Plasma Reagin-positive about a month after the onset of the primary chancre. Thus, a repeat test in four to six weeks is advisable. Treatment should be given as for early syphilis, if the patient found to be positive at that stage.

In pregnant women, however, immediate treatment should be given at the first positive test to prevent adverse outcomes of pregnancy. A single dose of benzathine penicillin will be sufficient to prevent such a tragic event. The woman can proceed to further testing with the Rapid Plasma Reagin and treated appropriately for syphilis in her own right according to the determined stage of her infection (see Figure 3).

**Figure 3. Rapid Plasma Reagin (RPR) testing available**



**A treponemal positive but non-treponemal negative result can be interpreted in the following ways:**

1. Patients with early syphilis. Treponemal tests do become positive slightly before the non-treponemal tests. The treponemal test result is likely to be a weak positive in such patients. They will become Rapid Plasma Reagin positive after a short period, which is why retesting after a short time is recommended. However, the 'window' to produce this result is short, so such patients will be relatively infrequent. Clinical examination can identify some patients in this class if they develop a classical chancre.
2. Patients with late latent syphilis. The non-treponemal titres can reach negativity in late latent syphilis but this takes some time and is unlikely in younger patients. Such patients are not infectious by sexual transmission and women are unlikely to transmit the disease to a foetus. However, they will require treatment to prevent possible progression to the tertiary phase.
3. Patients with cured syphilis. Such patients should be aware of their status.
4. Patients with suppressed syphilis. Many patients infected with syphilis are unaware of their infection. Many antibiotics, although not optimal for syphilis treatment, do have anti-treponemal activity. Thus some patients may have inadvertently treated their infection while taking antibiotics for another disease. This treatment is likely to be sub-optimal and the safest course is to treat such patients.
5. Patients with a false positive treponemal result. Such patients are infrequent since the specificity of treponemal tests, including that of rapid diagnostic tests, is high (see above).
6. Patients with a false negative non-treponemal test result. Agglutination tests are prone to pro-zone effects in patients with very high titres and it is important to re-test a dilution of serum, usually 1/8. (This requirement for double testing with tests such as Rapid Plasma Reagin is another advantage of using a treponemal test for the primary screen). Random laboratory errors can also produce this result and reading errors are possible, since agglutination test assessment requires experience.

It should be noted that patients in classes 1, 2, 4 and 5 above will all require treatment. The status of such patients can be resolved by re-testing a new serum/plasma sample in combination with taking a history and a clinical examination. Where it is either unlikely that the patient will return or it is impracticable for the patient to return for these procedures, the safest course is to treat such patients.

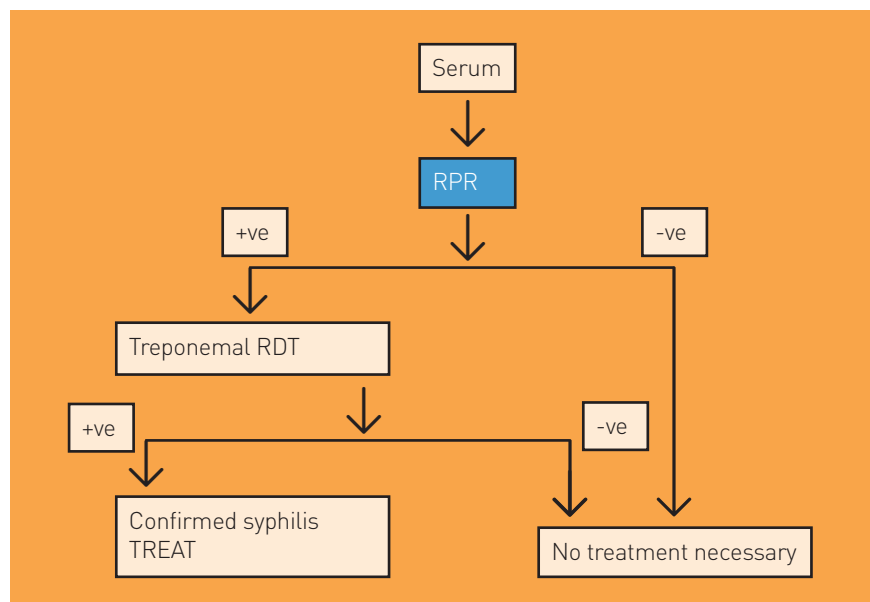
While this algorithm is the one now in use in most developed countries, it can now be used in less well-resourced settings through the use of rapid diagnostic tests as the primary screen where Rapid Plasma Reagin is available in an accessible laboratory or facility. It has the advantage of considerably reducing the number of Rapid Plasma Reagin tests required and allows the primary screen to be performed in low resource or outreach settings in which Rapid Plasma Reagin testing would be difficult or unfeasible.

### 3.3 Option three: Rapid Plasma Reagin confirmed by treponemal rapid diagnostic test.

This can be used in settings in which the Rapid Plasma Reagin test is being successfully implemented. The treponema-based rapid diagnostic test can be introduced as a rapid method of confirming Rapid Plasma Reagin seropositive tests, either in the laboratory or at the same facility where the Rapid Plasma Reagin is being performed (see Figure 4).

A seropositive Rapid Plasma Reagin accompanied by a seropositive treponemal rapid diagnostic test confirms the diagnosis of syphilis. This allows for confirmatory testing or treatment to be initiated at the first visit – same day testing and treatment. The use of the treponemal rapid diagnostic test at the primary point-of-care also avoids the need for transportation of samples to a laboratory and saves on laboratory time and costs. Additionally, in a few areas where current diagnosis depends on non-treponemal testing alone, the addition of rapid diagnostic tests to the clinical algorithm avoids the treatment of persons with biological false positive results.

**Figure 4. Rapid Plasma Reagin testing available, Treponemal rapid diagnostic test is used as confirmatory test**



## 4. Treatment of syphilis

Many countries have their own recommendations for syphilis treatment. General guidance is provided in the World Health Organization (WHO) [Guidelines for the Management of STIs](#). This distinguishes two regimens: one for 'early syphilis', defined as syphilis of less than 2 years duration; and the second for syphilis of over 2 years duration with indicative serology but no symptoms or signs. The two regimens differ only in the duration of treatment, as late syphilis is more difficult to cure. Early syphilis cases should receive a single dose, and late cases should receive three doses at weekly intervals. Penicillin is the antibiotic of choice, with doxycycline or tetracycline for penicillin-allergic non-pregnant patients and erythromycin for allergic pregnant patients. (However, treatment failures with erythromycin and other macrolides have been reported.) Many of the patients diagnosed as infected in antenatal care (ANC) or sexually transmitted infection clinics will have early syphilis and can be treated in a single visit with one intramuscular injection of 2.4 million units of benzathine benzylpenicillin.

Serious adverse reactions to penicillin are extremely rare, occurring in less than 1 in 10,000 patients. Anaphylactic shock, in which the patient collapses with a very low blood pressure, is even rarer. In clinics where very large numbers of patients are treated, an ampoule of adrenaline should be kept to treat such cases. In pregnant women, the extremely small risk of a serious drug reaction must be balanced against the very high risk of damage to the foetus if the woman is not treated.

An immunological reaction, called the Jarisch-Herxheimer reaction, may occur following treatment, especially in patients with secondary syphilis. It is caused by the rapid death of treponemes in the body. Patients should be warned that they may suffer from fever, headaches and malaise after treatment, but that the symptoms will not last more than a few hours and can be treated with aspirin. In pregnant women, there is a small risk that this reaction can precipitate premature labour.

The second class of adverse effect are those common to any diagnosis of a sexually transmitted infection, namely risks to patients personal relationship(s) when sexual partners become aware of the infection. Pregnant women may be particularly at risk of domestic violence if they have a partner who is liable to become abusive, and this has been observed in a trial of syphilis rapid diagnostic tests. This poses a dilemma here: tracing of contacts of those with sexually transmitted infections is necessary and desirable, both to prevent re-infection and to reduce prevalence. Staff responsible for the screening programme should be trained to counsel patients sensitively regarding these matters.

## 5. Links to Syphilis Rapid Diagnostic Test Resources

1. WHO. The global elimination of congenital syphilis: rationale and strategy for action.  
Available at: [www.who.int/reproductivehealth/publications/rtis/9789241595858/en/index.html](http://www.who.int/reproductivehealth/publications/rtis/9789241595858/en/index.html)
2. WHO Guidelines for the management of sexually transmitted infections.  
[www.who.int/std\\_diagnostics/publications/guidelines.htm](http://www.who.int/std_diagnostics/publications/guidelines.htm)
3. WHO-SDI booklet. The use of rapid syphilis tests.  
[www.who.int/tdr/svc/publications/tdr-research-publications/use-rapid-syphilis-tests](http://www.who.int/tdr/svc/publications/tdr-research-publications/use-rapid-syphilis-tests)



Rapid testing for syphilis and HIV at a primary health centre in Zambia



## 6. Further Reading

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