



World Health
Organization

The use of

Rapid Syphilis Tests



Special Programme for Research & Training
in Tropical Diseases (TDR) sponsored by
UNICEF/UNDP/World Bank/WHO

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Access to syphilis diagnostics is limited in regions of high disease burden

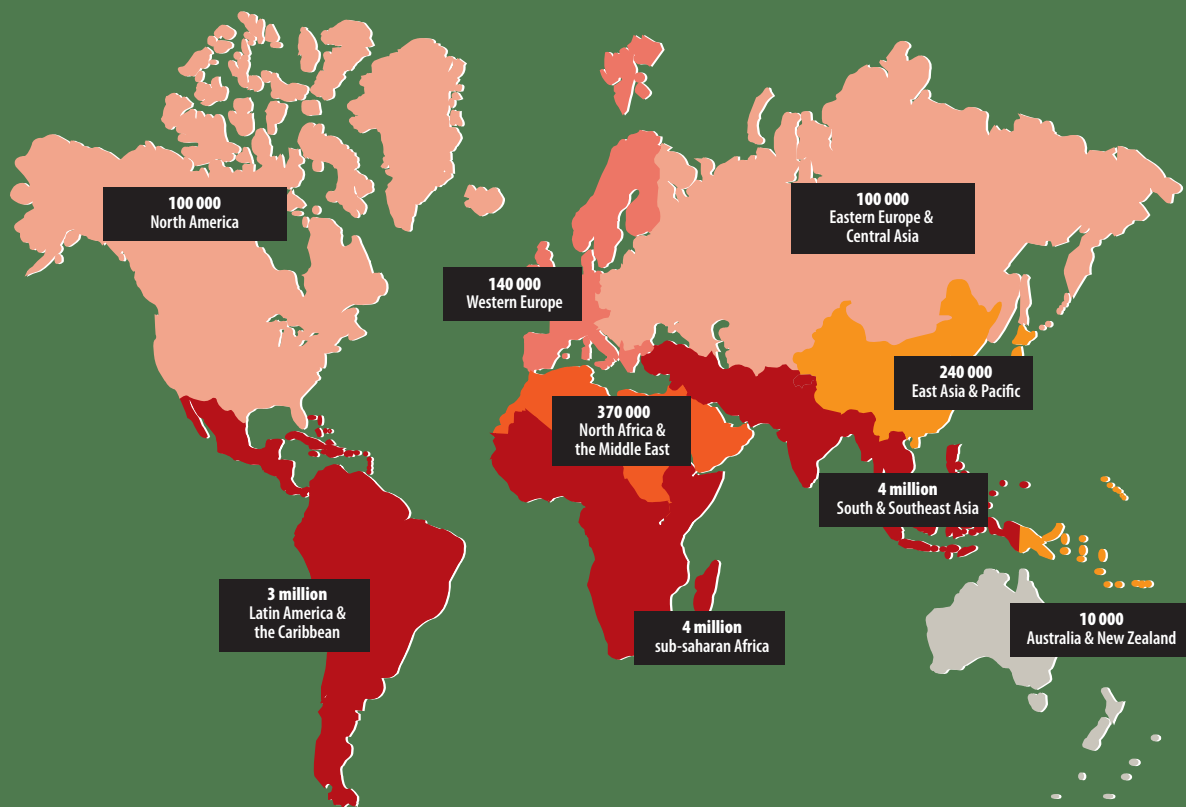


Figure 1 WHO estimates of number of new cases of Syphilis per year

I. What is syphilis and why is it important?

Syphilis is a curable infection caused by a bacterium called *Treponema pallidum*. This infection is sexually transmitted, and can also be passed on from a mother to her fetus during pregnancy. As a cause of genital ulcer disease, syphilis has been associated with an increased risk of HIV transmission and acquisition.

Most persons with syphilis tend to be unaware of their infection and they can transmit the infection to their sexual contacts or, in the case of a pregnant woman, to her unborn child. If left untreated, syphilis can cause serious consequences such as stillbirth, prematurity and neonatal deaths. Adverse outcomes of pregnancy are preventable if the infection is detected and treated before mid-second trimester.

Early detection and treatment is also critical in preventing severe long term complications in the patient and onward transmission to sexual partners. Congenital syphilis kills more than one million babies a year worldwide but is preventable if infected mothers are identified and treated appropriately as early as possible. The World Development Report cites antenatal screening and treatment for syphilis as one of the most cost-effective health interventions available:

Health Interventions	Cost per DALY* saved (US\$)
Expansion of Childhood Immunizations	US\$ 2-20
Oral rehydration therapy	US\$ 7-28
Antenatal syphilis screening (8% prevalence)	US\$ 4-18
Prevention of mother to child transmission of HIV (15% prevalence)	US\$ 50-200

* DALY = Disability Adjusted Life Years



Text box 1

Burden of syphilis and its impact in pregnancy

The World Health Organization estimates that 12 million new cases of syphilis occur every year (see Figure 1 for distribution of disease burden)

In developing countries, 3-15% of women of child-bearing age have syphilis. About 30% of pregnant women with syphilis will give birth to a dead baby (stillbirth), and another 30% to a live baby with congenital syphilis, a condition with a mortality of up to 50%.

II. How is syphilis diagnosed?

Patients with symptoms and signs suggestive of syphilis (e.g. genital ulcer, skin rash) may be treated presumptively, unless appropriate investigations can be performed.

However, since most patients with syphilis have no symptoms or signs, diagnosis relies on a blood test for the detection of antibodies (see text box 2).

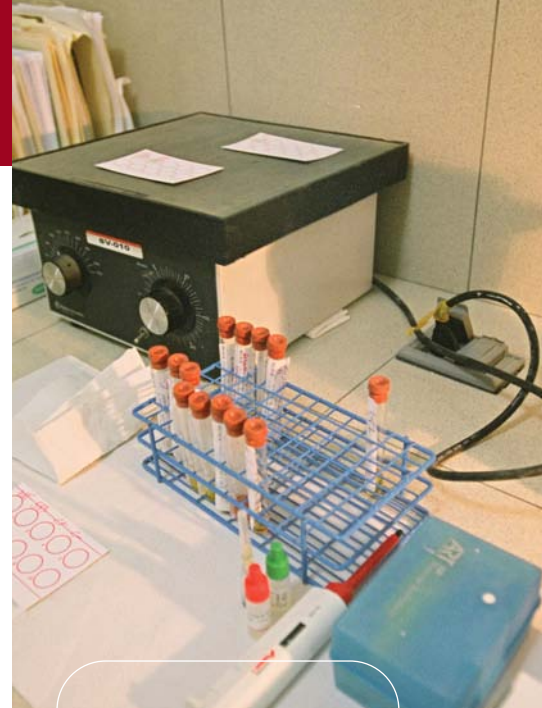
Until recently, serological testing for syphilis has been performed in a laboratory with:

1. Trained personnel;
2. Refrigeration for storage of reagents;
3. Electricity to run equipment: refrigerator, centrifuge and shaker

Since such facilities are generally not available in remote areas, blood or serum samples have to be transported to regional or central facilities for testing. Results are therefore often available only days or weeks after testing. If those who are tested do not return for their results, they are not treated, resulting in a waste of resources, adverse clinical outcomes and continued transmission of infection.

A recent study in Tanzania showed that syphilis was responsible for 50% of stillbirths, yet only 30% of pregnant women are screened for syphilis in sub-Saharan Africa.

Rapid tests for syphilis are now commercially available. These are simple point of care tests and can be performed outside a laboratory setting with minimal training and no equipment using a small amount of whole blood collected by a finger prick. Hence they can address the problems associated with lack of access to a laboratory and the low patient return rates.



Text box 2

Serological tests for syphilis

- A non-specific (non-treponemal) test, such as the Rapid Plasma Reagin (RPR) test or Venereal Disease Reference Laboratory (VDRL) test.
- A specific (treponemal) test, such as the Treponema pallidum Hemagglutination Assay (TPHA) or Treponema pallidum Particle Agglutination Assay (TPPA) or FTA-ABS

Most rapid syphilis tests currently available are treponemal tests but combination non-treponemal/treponemal tests are under development.

III. What is a rapid syphilis test?

For the purposes of this document, a rapid test is a simple point-of-care test that can be used in all health care settings to allow immediate treatment. It is easy to perform and does not require special storage or transport conditions. The result should be easy to interpret and ideally, available in 30 minutes. Most rapid syphilis tests are made in a dipstick or cassette format.

Non-treponemal tests such as the RPR can be considered rapid tests as they can provide a result in less than 10 minutes. However there are important distinctions between currently available non-treponemal test and the rapid treponemal tests as shown in text box 3.

Text box 3

Comparison of non-treponemal vs rapid treponemal tests

	Advantages	Disadvantages
Non-treponemal tests: RPR or VDRL	<ul style="list-style-type: none">- simple to perform- can distinguish between active and past treated infection (antibodies wane after effective treatment except for a small number of sero-fast individuals)	<ul style="list-style-type: none">- require electricity for refrigerator to store reagent, and for a rotator and centrifuge- cannot be used with whole blood- false negative results can occur with excess antibody (prozone effect)
Rapid treponemal tests	<ul style="list-style-type: none">- simple to perform- can be used with whole blood, serum or plasma- can be transported and stored at temperatures below 30°C- no prozone effect	<ul style="list-style-type: none">- cannot distinguish between active and past treated infection (antibodies to treponemal antigens are retained for years)

IV. How is a rapid syphilis test performed?

More than 20 rapid syphilis tests are commercially available. Although the specific instructions for processing differ, most tests are performed with only 3-4 steps. Figure 2 illustrates the simplicity of these tests:

1. Remove the test from the wrapper and place on a flat surface;
2. Add a specified amount of patient sample (whole blood, plasma or serum) to the sample well S;
3. Add a specified amount of diluent buffer to sample well S;
4. Read the results after a specified time (usually 15-20 mins) as shown in Figure 2: (C=control line; T=test line).

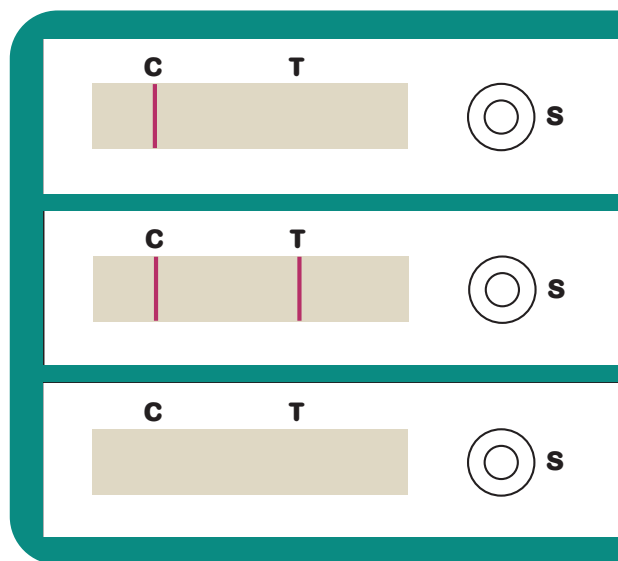


Figure 2
How rapid syphilis
test are read?

- Negative:
only 1 line below C
- Positive:
lines below C & T
- Indeterminate:
no lines below C or T

V. How effective is a rapid test in detecting syphilis?

When used according to the manufacturers' directions, most rapid syphilis tests are effective at detecting syphilis. The Sexually Transmitted Diseases Diagnostics Initiative (SDI), based in the Special Programme for Research and Training in Tropical Diseases (TDR), has evaluated a number of these tests and found them to be appropriate for use in various settings.

The sensitivity of these tests, which is the proportion of people with syphilis that have a positive test result and is hence a measure of the ability of a test to detect infection, ranges from 85-98% compared to a laboratory-based reference standard test such as the TPHA or TPPA. The specificity of these tests, which is the proportion of people without syphilis that have a negative test result and hence a measure of the ability of a test to exclude infection, range from 93-98% (see Appendix 2 for more details on these issues and the predictive value of these tests). More information is available at the SDI website:

www.who.int/std_diagnostics

Results of the rapid tests are reproducible only if the tests are stored and performed according to manufacturer's instructions, including special attention to expiry dates.



Visit the SDI website:
www.who.int/std_diagnostics

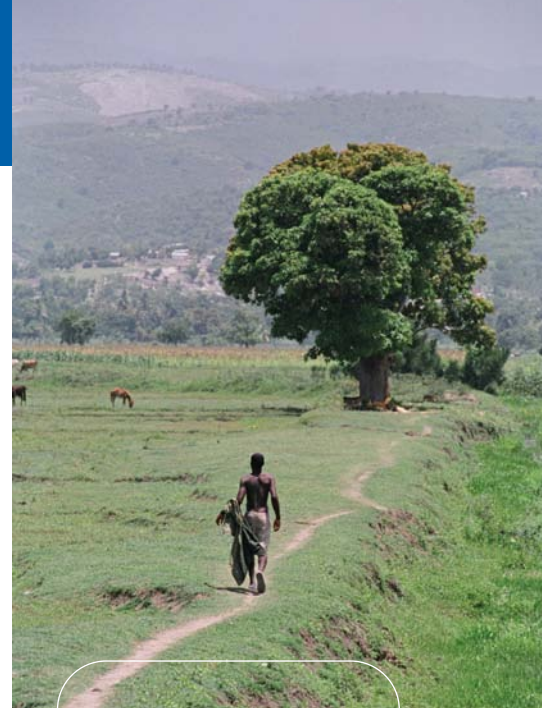
VI. When is a rapid syphilis test useful?

Targeted screening of syphilis for at risk individuals is cost-effective. Given the potentially serious sequelae of syphilis and increased risk of HIV transmission, the following groups should be targeted for screening:

- Pregnant women (to prevent congenital syphilis)
- Individuals with or at risk of STIs
- Sex workers
- Clients of sex workers
- Men who have sex with men
- Injection drug users

ALMOST EVERY COUNTRY HAS A POLICY FOR ANTENATAL SCREENING BUT ACCESS TO TESTING IN MOST AFFECTED COUNTRIES range between 30-38% (Gloyd et al 2001)

Once it has been decided that the introduction of rapid tests would be useful in a country or a specific area, it has to be decided to whom this test should be offered and under what circumstances.



Text box 4

Mean distance to the nearest health facility for the poorest fifth of the population

(source: World Development Report 2004)

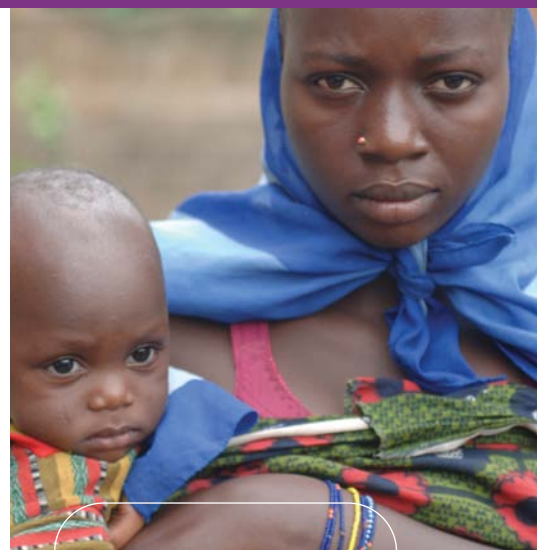
Country	distance in km
Benin	7.5
Bolivia	11.8
Chad	22.9
Haiti	8.0
Madagascar	15.5
Niger	26.9
Tanzania	4.7
Uganda	4.7
Zimbabwe	8.6

VII. How to decide if you need rapid testing for syphilis?

Countries that have already established effective syphilis control programmes, including screening for antenatal and high risk populations, may prefer to maintain their program rather than introduce rapid tests. This decision should be based on a careful assessment of the quality, coverage and efficacy of the current programme.

Points to consider in such an assessment, including the decision to introduce rapid testing for syphilis, are:

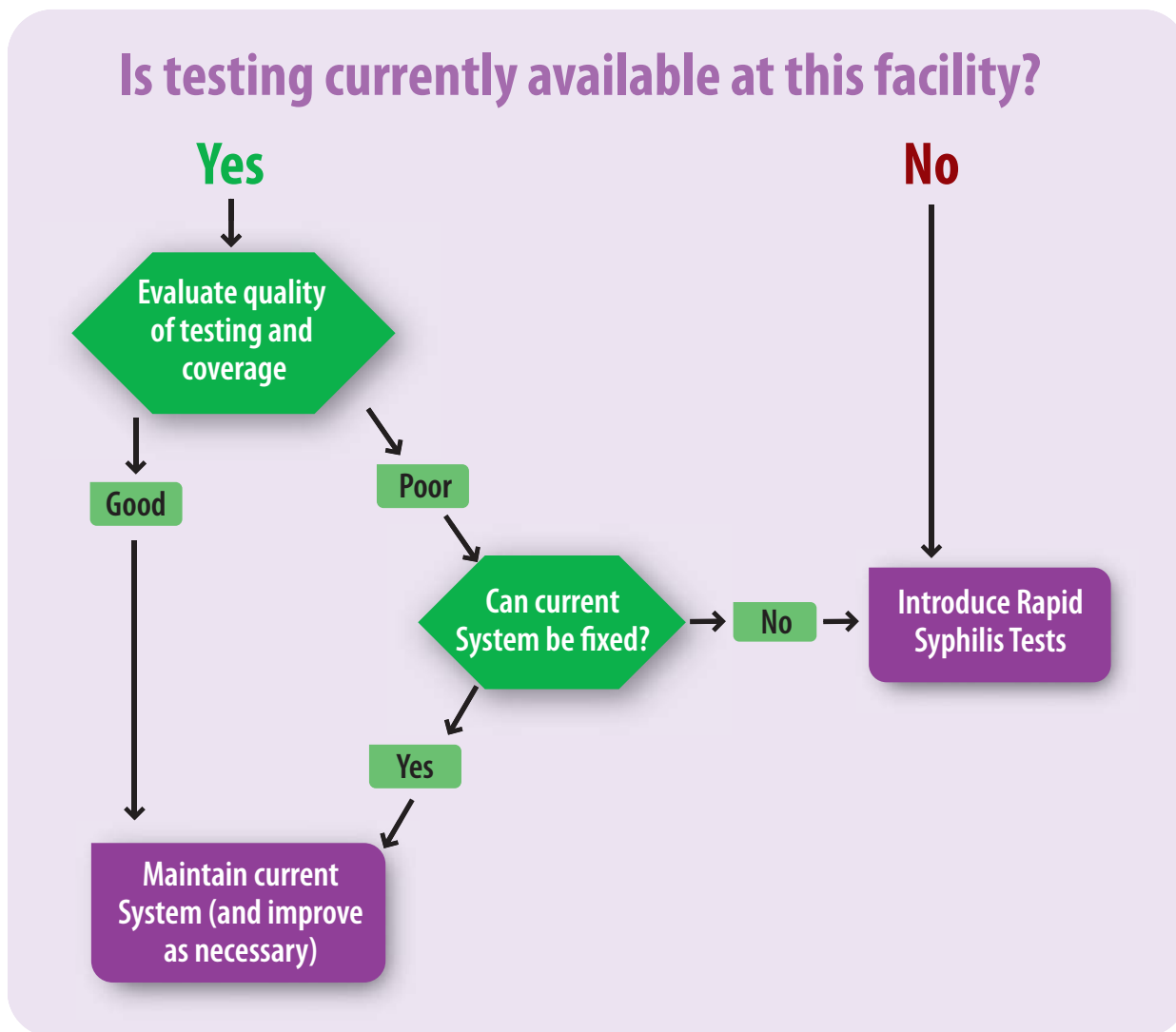
- **Access:** what is the proportion of persons at risk and pregnant women that have access to syphilis testing? Rapid point of care tests enable countries to increase access to antenatal screening.
- **Quality of testing:** what is the quality of the testing? Is there an on-going quality control program to monitor and to ensure accurate results?
- **Treatment of sero-reactive case:** what proportion of persons tested receive their test results and have access to treatment in a timely manner (ideally during the same visit)?
- **Rapid tests:** will introducing rapid testing help improve coverage/access and programme efficacy?



Consider the following steps for a facility-based approach to introduction of rapid tests for syphilis screening (see Figure 3 on the next page):

1. Decide which population groups are to be targeted for screening
2. Decide which facilities are to be involved to cover the population groups to be tested.
3. Assess the current capacity to conduct syphilis testing.
4. Decisions regarding rapid test introduction will depend on type of facility, capability, and case load.
5. Introduction of rapid tests includes procurement, logistics, training, and quality assurance.

Figure 3 Sample flow chart for this decision making process



VIII. What do rapid test results mean and when do you treat?

Since rapid treponemal tests cannot be used to distinguish between active and past treated infection, treatment of all rapid test positive individuals will result in over-treatment. However, given the serious consequences of missed treatment, the benefits of treatment far outweigh the harm of over-treatment.

Suggested testing algorithms and treatment decisions are shown in Figure 4 (see page 17).

If RPR is not available, test with a rapid test and treat all patients with a positive result.

Where RPR is available and performed well, rapid tests may also have a role:

- **Treatment** can be provided on the basis of a positive RPR result alone
- **Rapid treponemal** tests can be used to confirm a positive RPR result, and treatment would only be given to patients with a confirmed positive result
- **Rapid tests** can also be used for screening; for patients with a positive rapid test result, RPR can be used to confirm active infection.

In patients with discrepant non-treponemal and rapid test result, if confirmation with a reference standard test is not an option or re-test in 6 weeks, treatment should be given.

Special considerations

Pregnant women:

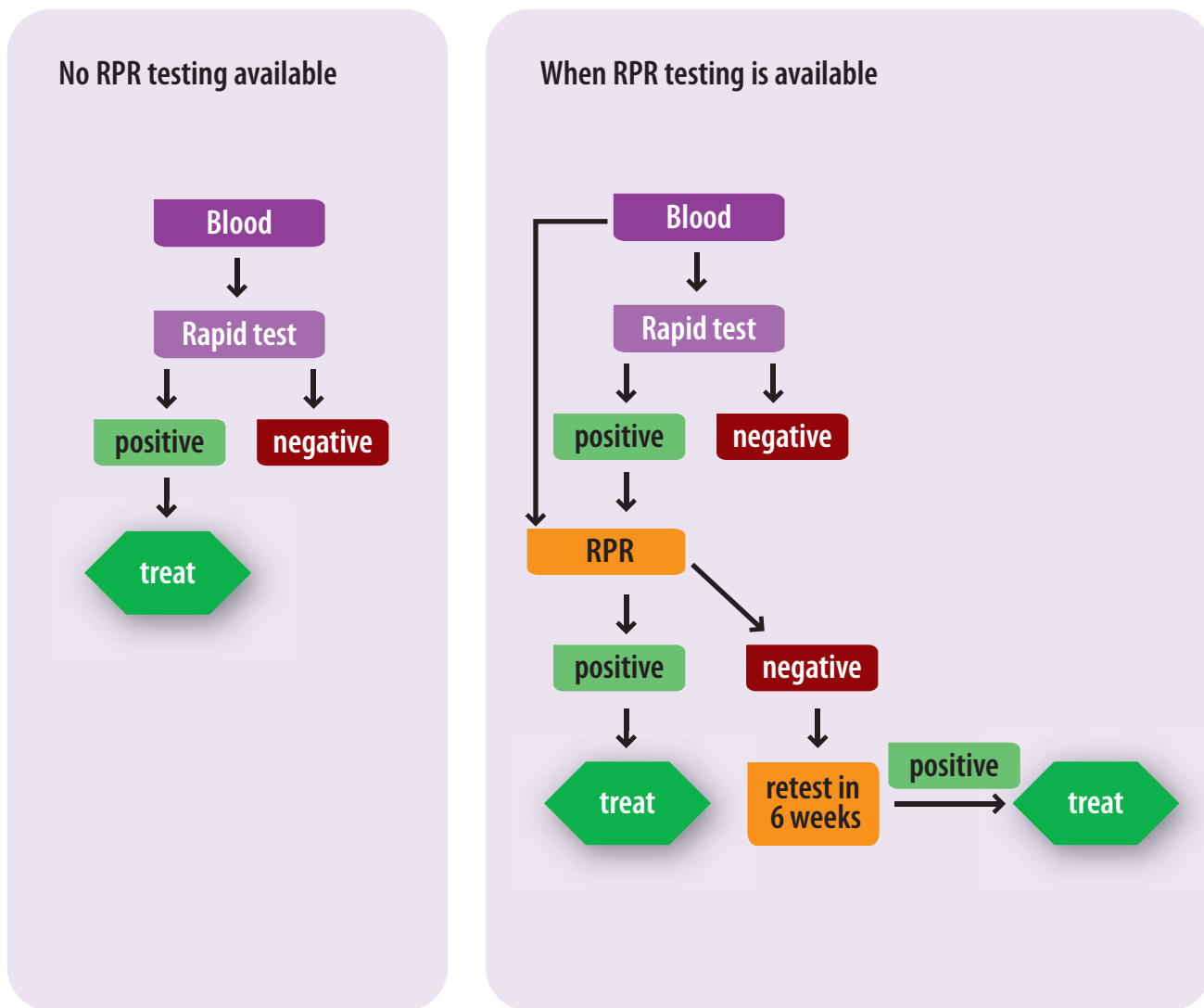
In pregnancy, all patients with a positive test should be treated, regardless of treatment history in a previous pregnancy. This is important because of the possibility of a new infection since the last pregnancy, and of the very severe consequences of untreated infection in this group.

High risk/vulnerable populations:

In high risk populations, all patients with a positive rapid treponemal test should be treated. The most important and unresolved issue in this group is the frequency of testing and treatment in patients that already have had a positive test. Further studies are needed in this area.

Rapid point-of-care tests are currently under development that would be better indicators of active infection and should help resolve this issue with regard to treatment decisions.

Figure 4 Suggested testing algorithms and treatment decision points



IX. What should you consider in purchasing rapid syphilis tests?

The performance and reproducibility of some commercially available rapid treponemal tests have been evaluated by the WHO. Details and results of the evaluation can be found at the SDI website:

www.who.int/std_diagnostics

- **Test Performance:** given the serious consequences of a missed diagnosis, and rarity of adverse side effects of over-treatment, test sensitivity is more important than specificity for screening. If the rapid tests are to be used as confirmatory tests, then high specificity is important.
- **Ease of use:** the number of processing steps, whether it can use whole blood, and need for accurate timing will influence extent of training and supervision required.
- **Conditions of use:** in humid conditions, the selection of rapid tests individually packaged in moisture-proof pouches is strongly recommended.
- **Conditions of storage:** most rapid syphilis specify storage temperatures between 4-30°C. If the clinic temperature is above 30°C, periodic quality control checks to ensure the ongoing validity of the tests are important.
- **Shelf life:** longer shelf life reduces the pressure on the supply chain and the probability of wastage of expired tests; a minimum of 18 months is recommended in remote, poorly resourced areas.
- **Price:** rapid tests can be purchased through the WHO Bulk Procurement Scheme at between US\$ 0.19 to US\$1.0.

Choosing an appropriate rapid syphilis tests

Major considerations in test selection include:

- test performance
- ease of use, including format of the test (e.g. cassette, dipstick, card)
- shelf-life and temperature stability in intended conditions of storage and use
- requirement for additional supplies such as pipettes
- cost (including transport, training)

Rapid test products and manufacturers

WHO does not certify or recommend any specific product, but makes data on the performance and utility of rapid tests available to all UN member states and on its website.

Tendering and the availability of product information

Together with considerations of the sensitivity and cost of a product, it is useful to know the quality of manufacturing processes, the long-term viability of a company, and the consistency of production. This will influence the ability of a company to replace a product should the received lot fail the quality control process, and will ensure long-term supply of a product to minimize the need for re-training.

Purchasers should request the following information from manufacturers during the tendering process:

1. Real-time temperature stability data on the product, and accelerated data on the purchased lot;
2. Evidence of successful operational use, or good quality field data on the product;
3. Long-term viability of manufacturer (to ensure continuity of supply);
4. Availability of product support;
5. Provision of sample products for assessment and testing for ease of use;
6. Agreement for replacement of products which fail agreed quality control procedures;
7. Box sizes appropriate to the rate of use of tests in the intended area, to minimize storage time in poor conditions and limit the need to split boxes.

Point 3 implies that the place of manufacture of rapid tests should be disclosed to the purchaser if rapid tests are re-labeled.

Purchasers should also consider evidence of good manufacturing practice (e.g. GMP or ISO certification; ISO13485:2003 is a standard specific for medical devices).

Clarity of packaging of the end product is essential to allow identification of product type, production lots and expiry date.



X. How do you transport and store rapid tests?

Exposure to high temperatures is probably a major contributor to poor performance, particularly during transport and storage. Transfer from the manufacturer, and road transport within a country, are particularly vulnerable times. High humidity can rapidly degrade rapid tests, including prolonged exposure to humidity after removal from the envelope or if the envelope is damaged.

Most manufacturers recommend rapid tests storage between 4°C and 30°C. Expiry dates are generally set according to these conditions. If kits are stored at temperatures exceeding the recommended limits, it is likely that the shelf life of the rapid tests will be reduced and sensitivity lost prior to the expiry date. The maintenance of temperatures between 4°C to 30°C for shipment of rapid tests is essential. Transport of rapid tests from manufacturers and within countries should be monitored as follows:

Shipping from manufacturers

1. Before shipping, the manufacturer contacts consignees with details of air waybill numbers, airline carrier, flight number, numbers of containers, and expected arrival time. These details should be sent by e-mail and followed up by facsimile.
2. The shipper (air carrier) is notified of temperature storage requirements by the manufacturer in writing and by clear markings on cartons and related documents. (Stowage of the shipment close to the skin of some aircraft may result in freezing.)
3. The manufacturer initiates shipment only when the consignee confirms the shipping notification is received.
4. Consignees then arrange to have customs agents or other personnel on site to receive materials – shipments are moved immediately to moderate temperature storage (less than 30° C if possible). Avoid leaving materials on airport tarmacs, in customs sheds or in vehicles.





Ground transportation

5. Ground transportation during any stage of delivery is carried out without delay and with attention to ambient temperature while the vehicle is moving and if parked. Avoid leaving rapid tests in vehicles parked in the sun.

Storage

6. Storage at central and final field facilities should be within the manufacturer's specifications.
7. Maximize the time rapid tests are stored in centralized, controlled conditions; minimize uncontrolled storage in remote areas. Smaller box sizes may help achieve this.
8. Select a cool peripheral storage location; thatch roofing may be cooler than iron, maximize shade, consider evaporative cooling cabinets.

Transport and storage at temperatures above 30°C is sometimes unavoidable, as in many remote locations where rapid tests are intended for use. Monitoring of sensitivity of rapid tests at appropriate intervals is therefore essential. WHO is developing recommendations for quality assurance to address these issues.

XI. How do you evaluate the quality of your testing programme?

The quality of the testing programme depends on the quality of the test kits and the proficiency of the end-users at performing the tests. A number of steps should be considered in the evaluation of the testing programme:

Quality Control on validity of test kits

Since temperatures that the test kits are subject to during transport may affect the sensitivity of the tests, the sensitivity of rapid tests should be checked at a central laboratory with a well-characterized quality control panel on receipt from the manufacturer, and periodically throughout the recommended shelf life. Users of test kits sent to peripheral health centres should be alerted of any depreciation of test quality during that time.

Proficiency of users

Adequate training and supervision of end-users of rapid tests should be integrated as far as possible into existing health worker training and quality assurance schemes.

Concise clear Standard Operating Procedures (SOPs) should be prepared in local languages for the health workers trained to perform the test. Instructions for processing and interpretation should be clear, including biosafety issues associated with finger pricking procedures.

Health workers using the tests should be trained and assessed, and systematically monitored on test processing and interpretation.



Appendixes

Appendix 1. Non-treponemal and treponemal tests for syphilis

Since it is not possible to culture *Treponema pallidum* on artificial media, the detection of serum antibodies is often used as surrogate markers of *T. pallidum* infection. As mentioned previously, these tests fall into 2 categories:

Non-treponemal tests

Non-treponemal tests such as the VDRL (Venereal Disease Research Laboratory) or the RPR (Rapid Plasma Reagin) detect antibodies to a lipoidal antigen, resulting from the interaction of the host with *T. pallidum* or from *T. pallidum* itself. These tests are widely used because of their low-cost and are relatively easy to perform. However, non-treponemal tests may give false positive results. Hence positive treponemal test results may need to be confirmed using treponemal tests. Some common conditions associated with false-positive test results are listed below:

Potential causes of cross-reactivity

Infectious causes

- Malaria
- Tuberculosis
- Viral fevers
- Trypanosomiasis
- Leprosy
- Other treponemes

Non-infectious causes

- Drug addiction
- Connective tissue disease
- Pregnancy
- Advanced age

Treponemal Tests

The most frequently used tests are the TPHA (*T. pallidum* hemagglutination), the TPPA or the FTA-ABS. When positive, these tests are considered evidence of a previous or current infection with *T. pallidum*. These are laboratory-based tests that require equipment and trained staff to perform. They will remain positive for life in a person that previously had syphilis. A new generation of these tests in an ELISA format is now commercially available. These tests can be batched for high throughput testing.

Appendix 2. Test sensitivity and specificity and the importance of prevalence in the estimation of predictive value of diagnostic tests.

Sensitivity and specificity of a test are not the only parameters of importance in evaluating its appropriateness in a given population. The prevalence of the disease is important in assessing its positive and negative predictive value. The predictive value of the same test can differ between countries and between different populations in the same country as showed in the following table.

Given a population of 10,000 with the prevalence of syphilis estimated at 1, 5, 10, and 15% and at a test sensitivity of 85, 90 and 95% and specificity of 95%, the positive predictive value (PPV) and negative predictive value (NPV) and the number of false negative and false positive tests are as follows:

Prevalence	Sensitivity	Specificity	PPV*	NPV*	#False negatives	#False positives
1%	85%	95%	15	100	15	495
1%	90%	95%	15	100	10	495
1%	95%	95%	16	100	5	495
5%	85%	95%	47	99	75	475
5%	90%	95%	49	99	50	475
5%	95%	95%	50	100	25	475
10%	85%	95%	65	98	150	450
10%	90%	95%	67	99	100	450
10%	95%	95%	68	99	50	450
15%	85%	95%	75	98	225	425
15%	90%	95%	76	98	150	425
15%	95%	95%	77	99	75	425

The results presented in the presented in the preceding table are not specific to rapid tests but can be applied to all diagnostic tests.

* PPV = positive predictive value

* NPV = negative predictive value



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