Diagnostic tools for preventing and managing maternal and congenital syphilis: an overview
Rosanna W. Peeling & Htun Ye

Abstract Syphilis is a major cause of adverse outcomes in pregnancy in developing countries. Fetal death and morbidity due to congenital syphilis are preventable if infected mothers are identified and treated appropriately by the middle of the second trimester. Most pregnant women with syphilis are asymptomatic and can only be identified through serological screening. Non-treponemal tests, such as the rapid plasma reagin (RPR) test, are sensitive, simple to perform, and inexpensive. However, they have often not been available at primary health-care settings because they required cold storage for reagents and electricity to operate a rotator. Additionally, as many as 28% of positive RPR results in pregnant women are biological false positives. Confirmatory assays are usually available only in reference laboratories. Technological advances have resulted in improved serodiagnostic tools for syphilis. New enzyme immunoassays are available for surveillance and for large-scale screening programmes. Decentralized antenatal screening with on-site confirmation is now possible since new RPR reagents that are stable at room temperature have become commercially available, as have solar-powered rotators and simple, rapid point-of-care treponemal tests that use whole blood and do not require electricity or equipment. These will be valuable tools for preventing or eliminating congenital syphilis. The development of a non-invasive rapid treponemal test that distinguishes between active and past infections remains a high priority in areas where syphilis is endemic.

Keywords Syphilis, Congenital. Syphilis/diagnosis/therapy; Syphilis serodiagnosis/utilization; Immunoenzyme techniques/utilization; Treponema pallidum/isolation and purification; Prenatal diagnosis; Evaluation studies (source: MeSH, NLM).

Mots clés Syphilis congénitale/diagnostic/thérapeutique; Séro-diagnostic syphilis/utilisation; Méthode immunoenzymatique/utilisation; Treponema pallidum/isolement et purification; Diagnostic prénatal; Etude évaluation (source: MeSH, INSERM).

Palabras clave Sífilis congénita/diagnóstico/terapia; Serodiagnóstico de la sífilis/utilización; Técnicas para inmunoenzima/utilización; Treponema pallidum/aislamiento y purificación; Diagnóstico prenatal; Estudios de evaluación (fuente: DeCS, BIREME).

Infants born to infected mothers are at risk of acquiring the infection in utero, resulting in serious perinatal morbidity and mortality (4–6). Congenital syphilis can be prevented if infection in the mother is detected and treated by the time of the second trimester. Antenatal screening to prevent congenital syphilis has become the main focus of syphilis control programmes.

Diagnostic tools
A wide range of diagnostic tools is available (Table 1). The underlying principles and characteristics of these tests are well described in laboratory manuals and guidelines and will not be discussed here (7–11). This paper focuses on whether the diagnostic tools are adequate to control maternal syphilis and prevent congenital syphilis as well as on the problems associated with their use for surveillance (Table 2). We also briefly discuss what the future may hold in terms of new tests.

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Identification of *T. pallidum*

The direct identification of *T. pallidum* is not routinely used because most people who have syphilis are asymptomatic. Identification of the treponeme is only possible in people presenting with lesions of primary and secondary syphilis (Fig. 1). Since *T. pallidum* cannot be cultured in the laboratory, darkfield microscopy is used to identify the treponemes in a wet mount of lesion material. The treponemes have a characteristic morphology and motility. Darkfield microscopy has a sensitivity of 74–86% and a specificity approaching 97% depending on the expertise of the reader (8, 9). Its sensitivity may be lowered if the lesion has been wiped with an antiseptic. Direct fluorescent antibody–*T. pallidum* testing (DFA–TP) uses fluorescein-conjugated antibodies to stain the organism in a smear or tissue section (7). DFA–TP does not require motile organisms and is more sensitive and specific than darkfield microscopy, but it is technically demanding and more expensive. The sensitivity of both microscopy techniques can be affected by the age or condition of the lesion and whether the patient has been treated.

Nucleic acid-based amplification assays, such as the polymerase chain reaction (PCR), which can detect as few as 10 treponemes in lesions, placental tissue and other body tissues, have greatly improved the sensitivity and specificity of direct detection methods (12, 13). A multiplex PCR assay which simultaneously detects *T. pallidum*, herpes simplex virus type 2 and *Haemophilus ducreyi* has been used to provide an etiological diagnosis for patients with GUD as well as for validating syndromic management algorithms (14). Since PCR assays for syphilis are not commercially available, these assays have remained research tools.

<table>
<thead>
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<th>Table 1. Comparison of syphilis tests (7–9)</th>
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<td><strong>Criteria</strong></td>
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<td><strong>Comments</strong></td>
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DFA–TP = direct fluorescent antibody–*Treponema pallidum* testing; PCR = polymerase chain reaction; RPR = rapid plasma reagin test; VDRL = Venereal Diseases Research Laboratory test; EIA = enzyme immunoassay; TPHA–TPPA = *T. pallidum* haemagglutination assay–*T. pallidum* particle agglutination test; FTA-ABS = fluorescent treponemal antibody absorption test.
Menstrual and congenital syphilis

Direct detection methods to confirm congenital syphilis are useful in disease surveillance programmes (10, 15). In an infant presenting with symptoms or born to an infected mother who has not been treated adequately, the diagnosis of congenital syphilis can be confirmed using microscopy or molecular techniques by identifying T. pallidum in lesions, ulcers, nasopharyngeal secretions, cerebrospinal fluid, umbilical cord blood, placental tissues or other body tissues. Since microscopy techniques lack sensitivity, a positive finding by microscopy confirms the diagnosis of syphilis in the infant while a negative result does not imply absence of disease (10).

Serological tests for screening and case detection

Serological tests remain the method of choice for diagnosing syphilis. Infection with T. pallidum produces different patterns of serological response (Fig. 1). Antibodies to phospholipid antigens on the surface of the treponeme cross-react with mammalian cardiolipin antigens. Flocculation assays to detect these phospholipid antibodies have been developed as non-specific screening tests, such as the rapid plasma reagin (RPR) test and the Venereal Diseases Research Laboratory (VDRL) test. These tests are simple, rapid, inexpensive and have excellent sensitivity, especially early in infection (7, 8). The major disadvantages are that they cannot be used on whole blood; they require a rotator or microscope for processing; and they may yield false-positive results. In pregnant women, as many as 28% of positive RPR results may be biological false positives (6). False-negative results can occur when there is an excess of antibody (known as the prozone phenomenon). Cross-reactivity may occur with other treponemal infections including yaws and pinta.

Non-treponemal tests can be qualitative or semiquantitative. RPR and VDRL titres are raised in patients with acute infection, reinfection or reactivation of a past infection that had not been adequately treated. About 72–84% of patients with primary or secondary syphilis show a four-fold decrease in their RPR or VDRL titre 6 months after completing appropriate treatment. The rate of seroreversion depends on the pretreatment titre and stage of disease (5, 16). Individuals with their first episode of infection are more likely to serorevert than those with repeat infections. Non-treponemal tests are therefore useful not only in identifying active infection but also in monitoring the effectiveness of treatment (Fig. 1).

Treponemal tests, such as the T. pallidum haemagglutination assay (TPHA), the T. pallidum particle agglutination test (TPPA) and the fluorescent treponemal antibody absorption test (FTA-ABS), are used to confirm positive screening tests. The microhaemagglutination assay (MHA–TP), which was also used to confirm positive tests, is no longer commercially available. Treponemal tests are expensive, require equipment and technical expertise, and are therefore not widely available only in reference laboratories. Immunoblotting has been proposed as an alternative to FTA-ABS as a confirmatory test. It is more sensitive than FTA-ABS but less sensitive than TPPA (17, 18). False-positive rates are slightly lower for treponemal tests than non-treponemal tests. Treponemal assays are not generally used for screening because they are not as sensitive as non-treponemal tests in the first 2–3 weeks of the primary stage of syphilis. Treponemal

Table 2. Tools for preventing and controlling maternal and congenital syphilis in different settings

<table>
<thead>
<tr>
<th>Maternal syphilis</th>
<th>Tests for diagnosis or screening</th>
<th>Tests for surveillance</th>
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<tr>
<td>Symptomatic</td>
<td>RPR or VDRL; rapid treponemal tests</td>
<td>Molecular tests; quantitative RPR or VDRL; TPHA–TPPA or FTA-ABS</td>
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<td>Darkfield microscopy; DFA–TP; EIA-IgM; quantitative RPR or VDRL; rapid treponemal tests</td>
<td>Molecular tests; quantitative RPR or VDRL; TPHA–TPPA or FTA-ABS</td>
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<td>Asymptomatic</td>
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<td></td>
<td>Quantitative RPR or VDRL; EIA-IgM; Rapid treponemal tests</td>
<td>EIA-IgM</td>
</tr>
<tr>
<td>Congenital syphilis</td>
<td>Symptomatic</td>
<td>Molecular tests; quantitative RPR or VDRL; Rapid treponemal tests</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>Molecular tests; EIA-IgM; quantitative RPR or VDRL</td>
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<td></td>
<td>Quantitative RPR or VDRL</td>
<td>EIA-IgM</td>
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<td></td>
<td>None</td>
<td>Rapid treponemal tests</td>
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<tr>
<td>Asymptomatic</td>
<td>None</td>
<td>Quantitative RPR or VDRL</td>
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RPR = Rapid plasma reagin test; VDRL = Venereal Diseases Research Laboratory test; DFA–TP = direct fluorescent antibody–Treponema pallidum testing; EIA = Enzyme immunoassay; TPHA–TPPA = T. pallidum haemagglutination assay–T. pallidum particle agglutination test; FTA-ABS = fluorescent treponemal antibody absorption test.

a The lack of sensitive and specific tests for diagnosing congenital syphilis means that all infants born to mothers with syphilis should be treated regardless of test results.

b Serodiagnosis of an infant born to an infected mother who is reactive in treponemal tests is not recommended because of the passive transfer of IgG antibodies through the placenta; suspected congenital syphilis can be confirmed by an RPR titre in the infant > 4 times that of the maternal titre. However a negative result does not exclude syphilis in the infant. EIA-IgM is not recommended for asymptomatic infants due to low sensitivity.

antibodies often persist for life, making these tests less useful as confirmatory tests in areas where prevalence is high; they are also not useful for monitoring treatment effectiveness (Fig. 1).

Serological tests for congenital syphilis

Many infected infants are asymptomatic at birth. Serodiagnosis of congenital syphilis in infants younger than 15 months is confounded by the passive placental transfer of maternal immunoglobulin G (IgG) to the fetus and is not recommended in infants born to seropositive mothers (10, 19, 20) (Table 2). Infants born to mothers with untreated syphilis should be treated regardless of their test results. Infants born to mothers with reactive nontreponemal and treponemal test can be evaluated with the same quantitative nontreponemal test as that used for their mother, preferably in the same test run. An RPR titre in the infant that is four times greater than that of the mother strongly suggests congenital syphilis, and the infant should be treated appropriately (10, 11). However, studies of serum pairs from infected mothers and infants show that less than 30% of infants have higher titres than their mothers (21). Congenital syphilis cannot be excluded in infants who do not have a four-fold or higher increase in their RPR titre since infants treated early in the course of the disease may not develop sufficiently high levels of antibodies for detection. Immunoglobulin M (IgM) antibodies can be detected in more than 80% of symptomatic infants but data on its sensitivity for asymptomatic infants are limited (19, 20). Rheumatoid factor in fetal serum can cause false-positive results, especially with the FTA-ABS IgM assay (22). Using immunoblot techniques to determine IgM response yields higher sensitivity than the 19S (IgM) FTA-ABS or enzyme immunoassays (EIAs) but results must be interpreted with caution (18–21). Because IgM responses take time to develop in infants and may be diminished with early treatment, a negative IgM result should not be used to exclude congenital infection.

Serodiagnosis of syphilis in pregnant women

Prenatal screening using non-treponemal serological tests is feasible and affordable in most developing countries. In pregnant women non-treponemal tests are prone to giving false-positive results so results should be confirmed with a treponemal test. False-positive results are usually associated with low titres. Hence, in high-prevalence areas where there is limited or no access to confirmatory testing, a woman with a non-treponemal titre ≥8 should be treated (23, 24). However, given the serious morbidity
and potential mortality associated with congenital infection and the lack of quantitative RPR testing in many settings, all pregnant women with a positive non-treponemal or treponemal test result should be treated immediately. The risks associated with untreated syphilis in pregnancy far outweigh the risk of overtreatment of mothers with a false-positive result.

Guidelines in the United States and Europe recommend prenatal screening during the first and third trimester and at delivery (10, 11). For a variety of reasons prenatal screening often occurs far less frequently in developing countries. For mothers who have been treated, a drop of less than four-fold in non-treponemal titre suggests that treatment has failed or the mother has been reinfected. Unfortunately, for most of the women who are diagnosed during prenatal screening, there is often insufficient time for confirmation of successful treatment through a four-fold decrease in titre before delivery.

Serodiagnosis of syphilis in HIV-infected mothers
The interpretation of serological test results is complicated in patients who are also infected with human immunodeficiency virus (HIV). There are insufficient data to determine whether serological tests for syphilis in pregnant women or neonates perform adequately if women are infected with both syphilis and HIV (24, 25). The alteration of B-cell function in HIV patients can result in biological false-positive RPR results or raised serum IgM levels, especially in injecting drug users. False-negative serological tests have also been reported (5, 25).

Tests as surveillance tools
The complexity of the natural history of syphilis and the lack of specific clinical presentation in infected individuals impose major barriers to implementing comprehensive surveillance programmes in developing countries. Seroprevalence data from antenatal screening programmes allow trends to be monitored. Syphilis seroprevalence among pregnant women age 15–24 has been selected as one of the proxy indicators for monitoring the prevalence of sexually transmitted infections in the general population. Treponemal tests such as the TPHA or EIAs are useful tools for prevalence surveys and surveillance, but they are often available only at reference laboratories or large regional centres in developing countries. Simple, rapid treponemal-specific tests are needed for collecting surveillance data from remote areas. Periodic quality assurance validation of surveillance data will also need to be implemented.

The United States Centers for Disease Control and Prevention surveillance case-definition for congenital syphilis includes any infant born to a mother who had untreated or inadequately treated syphilis at the time of delivery (10). The low specificity of this definition may lead to uninfected infants being included, but current tests are inadequate to confirm most infections in infants. There is no WHO surveillance system to estimate the rates of syphilis among those having late trimester abortions, stillbirths or for cases of neonatal death (15).

Problems with access to testing
Implementation of screening programmes to prevent congenital syphilis varies significantly between countries and between rural and urban areas within countries. The success of screening programmes depends on many factors including political commitment, the existence of a robust health-care infrastructure to implement programmes, and the availability of screening tools and drugs. In settings where resources are limited, health-service providers must prioritize scarce monetary and human resources to accommodate competing demands. Even where resources are available, there are often operational difficulties, such as a lack of transport or equipment and a lack of access to reagents; these difficulties may cause providers to feel unmotivated and unwilling to implement and scale-up screening services.

In primary health-care settings there are often technical difficulties associated with performing serological tests, such as retaining trained personnel and assuring that supplies are adequate and tests are of good quality (26). When screening is centralized, patients often fail to return for laboratory results or specimens or results are lost in transit, resulting in treatment being delayed or missed (27, 28).

Decentralized screening services result in significant declines in the prevalence of maternal and congenital syphilis (29–32). In Haiti, introducing solar-powered generators to operate centrifuges and rotators, and training field workers to perform RPR tests in rural health centres have resulted in a 75% decline in the incidence of congenital syphilis in two years (33). These results are encouraging but decentralization programmes must be sustainable if they are to have any impact. In the United Kingdom, universal antenatal screening for syphilis is cost effective even at the current prevalence of 0.06 per 1000 live births (34).

For all these reasons, there is an urgent need for simple, rapid, affordable and effective tests that will improve the coverage of syphilis screening in resource-limited settings. Simple tests that can be performed in 20–30 minutes so that treatment can be started immediately are likely to be more effective than those that require samples to be sent to a laboratory.

New tools for controlling and treating maternal and congenital syphilis
In the past decade, a number of improved serodiagnostic tools have become available. These offer exciting opportunities for developing innovative screening programmes (12).

Non-treponemal tests
In the past, RPR and VDRL tests often could not be used at primary health-care settings because they required cold storage for reagents and electricity to operate a rotator. However, new RPR and VDRL reagents that can be stored at room temperature and solar-energy powered rotators are now available for use in settings with limited or no electricity.

Treponemal tests
Important recent developments in treponemal tests are the availability of automated high-throughput EIAs, and simple, rapid tests that use whole blood and do not require equipment other than a dipstick or syringe.

EIAs that use recombinant treponemal antigens to detect IgM and IgG antibodies in a 96-well plate have equivalent sensitivity to non-treponemal tests and specificity equivalent to TPHA, FTA-ABS tests (35–41). They have the potential to replace the traditional combination of non-treponemal and treponemal tests for the definitive diagnosis of syphilis (42). The EIA IgM tests are more sensitive than the FTA-ABS 195 IgM test and are suitable for the diagnosis of early or congenital syphilis. However, these tests are unlikely to be available in primary health-care settings because of their costs and requirement for technical expertise and equipment. In laboratories in urban
centres, EIAs are useful surveillance tools because they are automated and so can manage high-volume testing. More than 20 companies manufacture rapid, simple treponemal tests that can use whole blood, serum or plasma. Most use immunochromatographic strips coated with antigens of \( T. pallidum \). Antigen–antibody reactions appear as a coloured line or spot on the membrane. Some use a format similar to RPR where latex particles are coated with treponemal antigens. Most of these rapid tests are appropriate for use in primary health-care settings as they require no equipment; can be performed in three to four steps; require minimal training; and give a visual readout in 8–20 minutes. They can be stored at room temperature for 6–12 months and cost US$ 0.40–2.00. Preliminary evaluation suggests that some have comparable performance to laboratory-based confirmatory tests (43–45).

**New tools for control and prevention**

Innovative strategies should take advantage of the affordability and portability of these rapid treponemal tests. In areas where the prevalence is low, these tests can be used for screening. Active infection can then be confirmed with quantitative RPR. In areas where non-treponemal tests are not available, and the patient does not have a history of syphilis, presumptive treatment should be considered for anyone with a positive rapid test result. However, their use in high-prevalence settings will be limited since a high proportion of those at risk will have antibodies as a result of past infection. Rapid tests are suitable for rapid assessment of trends or situation analysis in settings where laboratory services are not available.

For areas that have access to laboratory testing, screening with EIAs can largely replace the RPR–VDRL and TPHA–TPPA combinations (42). For large-scale screening and surveillance, the use of EIAs should prove to be cost effective, less labour intensive, and less subject to bias than conventional tests. Commercially available immunoblot strips, although costly, are highly sensitive and specific and can be used for confirmation testing (46).

**A vision for the future**

The Sexually Transmitted Diseases Diagnostics Initiative (SDI), in the UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, recently initiated a programme for the development, evaluation and application of tests that meet criteria known by the acronym ASSURED (47). This acronym is used to ensure that tests are:

- **Affordable**
- **Sensitive**
- **Specific**
- **User-friendly** (can be performed in a few simple steps with minimal training)
- **Rapid and robust**
- **Equipment-free and**
- **Deliverable to developing countries.**

The SDI currently is focusing on ASSURED tests that are appropriate for detecting syphilis and genital chlamydial and gonococcal infections in primary health-care settings in developing countries (45, 47, 48). Syphilis tests are being evaluated in laboratories in diverse locations using archived samples. Promising tests are then selected for further evaluation using whole blood and for their utility, acceptability and sustainability of use in primary health care settings. SDI plans to develop mathematical models to assess cost-effectiveness and the impact of introducing these new tools in developing countries. Studies will be conducted to estimate the coverage of screening programmes required for reducing the reservoir of infection in a community to a level at which disease transmission cannot be sustained.

The development of a rapid test that can distinguish between infections that are active or that have been treated, and that ideally uses specimens taken by a non-invasive means, such as saliva or urine, remains an urgent priority. Exciting opportunities exist for the discovery of novel diagnostic targets as markers of active infection, for example by using the genome sequence of \( T. pallidum \) and studies of sera from infants with congenital syphilis (49, 50).

**Conclusion**

The early detection and treatment of infection in pregnant women and their sexual partners are important strategies for preventing and controlling maternal and congenital syphilis. Since most people with syphilis are asymptomatic, the availability of sensitive and specific tools for diagnosis and screening is a top priority. ASSURED tests that can use whole blood are being evaluated for use in settings where resources are limited. Although the diagnosis of congenital syphilis remains difficult, promising tools are available that allow on-site screening and confirmation of maternal syphilis. Resources to make these tools and treatment widely available for decentralized antenatal screening programmes will be important in controlling maternal syphilis and averting congenital syphilis. In areas where syphilis is endemic, the priority remains the development of a non-invasive rapid treponemal test that distinguishes between active infections and those that have been treated in the past.

**Conflicts of interest:** none declared.

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**Résumé**

Outils de diagnostic pour la prévention et la prise en charge de la syphilis maternelle et congénitale : vue d’ensemble

La syphilis est une cause majeure d’issue défavorable de la grossesse dans les pays en développement. Les morts fœtales et la morbidité due à la syphilis congénitale peuvent être évitées si les mères infectées sont identifiées et traitées vers le milieu du deuxième trimestre de la grossesse. La plupart des femmes enceintes atteintes de syphilis sont asymptomatiques et ne peuvent être identifiées que par dépistage sérologique. Les tests ne reposant pas sur la recherche des tréponèmes, comme le test RPR (rapid plasma reagin) sont sensibles, faciles à exécuter et peu coûteux. Mais dans bien des cas ils n’étaient jusqu’ici pas disponibles au niveau des soins de santé primaires car les réactifs devaient être conservés au froid et une alimentation électrique était nécessaire pour l’agitateur rotatif. De plus, jusqu’à 28 % des résultats RPR positifs chez la femme enceinte sont des faux positifs dus à des causes biologiques, et les tests de confirmation ne peuvent en général être réalisés que dans un laboratoire de
Maternal and congenital syphilis

Référence. Les progrès de la technologie ont permis d’améliorer les tests de sérodiagnostic de la syphilis. De nouveaux tests immunoenzymatiques sont maintenant disponibles pour la surveillance et pour les programmes de dépistage à grande échelle. Le dépistage anténatal décentralisé avec confirmation sur place est désormais possible depuis que de nouveaux réactifs pour le test RPR, stables à la température ambiante, sont commercialisés, de même que des agitateurs rotatifs fonctionnant à l’énergie solaire et des tests tréponémiques simples et rapides, réalisables sur place sur le sang total et ne nécessitant ni électricité ni matériel. Ces tests seront des outils précieux pour la prévention et l’élimination de la syphilis congénitale. Le développement d’un test tréponémique rapide et non invasif permettant de distinguer les infections actives des infections passées reste hautement prioritaire dans les régions où la syphilis est endémique.

Resumen

Medios diagnósticos para prevenir y tratar la sífilis materna y congénita: panorama general

La sífilis es una causa destacada de resultados adversos en el embarazo en los países en desarrollo. La muerte fetal y la morbilidad por sífilis congénita se pueden prevenir si se consigue identificar y tratar apropiadamente a las madres infectadas para la mitad del segundo trimestre. La mayoría de las mujeres embarazadas aquejadas de sífilis son asintomáticas y sólo pueden identificarse mediante cribado serológico. Las pruebas no treponémicas, como la prueba de reagina rápida en plasma (RRP), son sensibles, sencillas y baratas. Sin embargo, a menudo no es posible aplicarlas en los entornos de atención primaria debido a que requieren sistemas de refrigeración para los reactivos, así como electricidad para utilizar un rotador. Además, hasta un 28% de los resultados RRP positivos detectados en mujeres embarazadas eran falsos positivos biológicos. Generalmente los análisis confirmatorios sólo se pueden realizar en laboratorios de referencia. Los adelantos técnicos han permitido obtener mejores instrumentos serodiagnósticos para la sífilis. Existen nuevos inmunoensayos enzimáticos, usados con fines de vigilancia y en los programas de cribado en gran escala. El cribado prenatal descentralizado con confirmación in situ es ya posible gracias a la comercialización de reactivos de RRP estables a temperatura ambiente, así como de rotadores de energía solar y de pruebas treponémicas sencillas, rápidas y aptas para el lugar de consulta que usan sangre entera y no requieren ni electricidad ni equipo especial alguno. Estas herramientas serán de gran utilidad para prevenir o eliminar la sífilis congénita. El desarrollo de una prueba treponémica rápida no invasiva que permita diferenciar las infecciones activas y las antiguas sigue siendo una alta prioridad en las zonas con sífilis endémica.

References


