Interpretation of syphilis serology

Introduction

Diagnoses of syphilis have been on the rise since the late 1990s, leading to concern about the spread of the infection in the UK (Health Protection Agency, 2009). Syphilis is transmitted through direct innoculation during close physical contact (usually sexual intercourse) or exchange of infected blood or body fluids (such as transfusions or from mother to baby during pregnancy, childbirth and breastfeeding).

Syphilis is more common in developing countries and in the UK in men who have sex with men. Control of syphilis includes sexual health promotion, increasing awareness of the symptoms it causes and encouraging regular testing as part of sexual health screens. While different methods are available for the identification of infection, serological tests remain the mainstay of diagnosis and monitoring of syphilis. Testing for syphilis is an essential skill for all general physicians, but is often perceived as difficult and confusing, and can be a daunting prospect for doctors in training. This article shows that the rational use and interpretation of syphilis serology is straightforward once a few basic principles are understood. This article will not cover congenital syphilis.

The natural history of syphilis

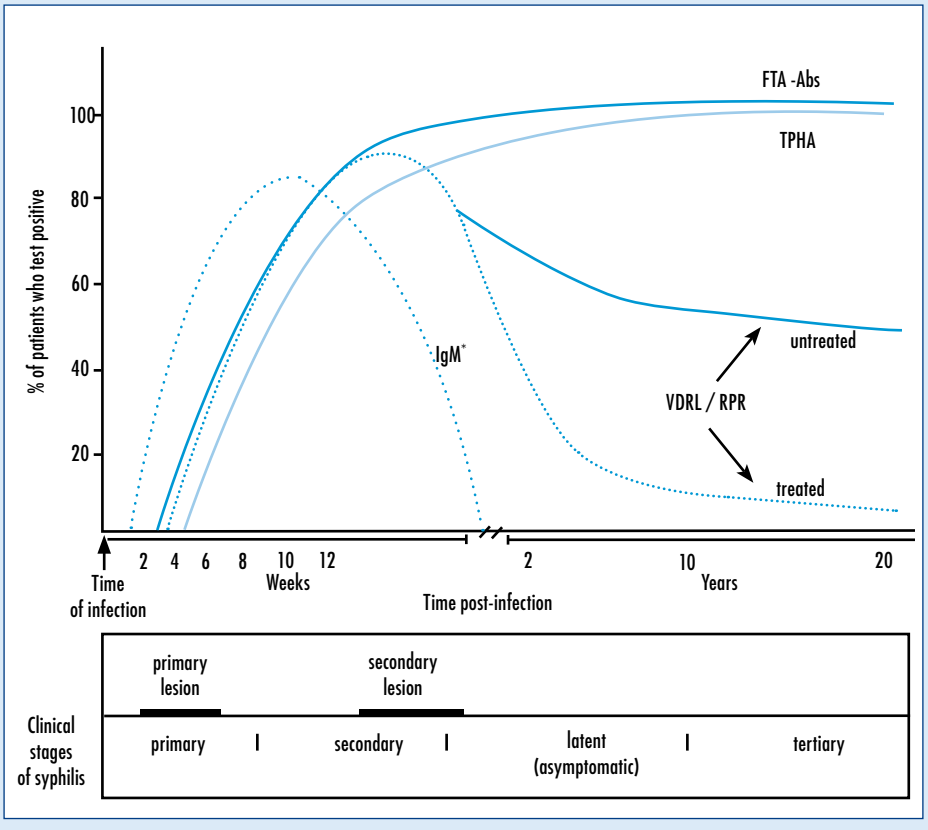
To interpret serological results it is important to understand the natural history of syphilis. Syphilis is caused by *Treponema pallidum* subsp *pallidum*, a spirochaete bacterium transmitted during sexual contact, pregnancy or very rarely via infected blood products. It can be classified into congenital and acquired infections, and further subdivided into clinical stages (*Figure 1*). Many patients with positive serology are asymptomatic, and overlap between the presentation of primary and secondary stages, as well as secondary and tertiary neurological symptoms, can occur.

Primary syphilis presents with a chancre, typically a single painless ulcer (although they may be painful and multiple) with an indurated base (*Figure 2*). This is at the site of inoculation, usually in the ano-genital area or oral cavity, and is accompanied by regional lymphadenopathy.

If untreated, generalized symptoms and signs of secondary syphilis occur, approximately 4–8 weeks after the primary lesion(s) has healed. Clinical manifestations vary, but usually involve a rash, characteristically but not necessarily including the palms and soles (*Figure 3*), and generalized lymphadenopathy. Condylomata lata (warty-like lesions), lesions in the mucous membranes and symptoms and signs of vasculitis may occur.

A latent phase occurs between secondary and tertiary syphilis, in which serological tests are positive but patients are asymptomatic. This is divided into early latent and late latent syphilis, defined as infection diagnosed within the first 2 years or after 2 years respectively. Tertiary syphilis is the final stage and may result in cardiovascular, neurological or ophthalmic involvement, many years later. Primary, secondary and early latent stages are collectively called early or infectious syphilis, reflecting the period when transmission occurs, with late latent and tertiary syphilis termed late or non-infectious syphilis. Patients may have positive syphilis serology without recalling the presence of clinical symptoms or signs as these may have been mild and unnoticed.

Figure 1. Summary of the clinical stages of syphilis and immune response to syphilis (Peeling and Ye, 2004). IgM = enzyme immunoassay (EIA) tests to detect IgM and IgG; TPHA = Treponema pallidum haemagglutination assay; FTA-Abs = fluorescent treponemal antibody-absorbed test; VDRL/RPR = Venereal Diseases Research Laboratory / rapid plasma reagin. * IgM by ELISA or FTA-ABS 195 or immunoblot.



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Who should I test for syphilis?

Routine screening for syphilis is already incorporated within genitourinary medicine clinics, antenatal departments and blood transfusion services including organ or tissue donors. High-risk groups should also be tested, and include homosexual males, patients with multiple partners, commercial sex workers and those linked with a country where syphilis is known to have a high prevalence. Patients presenting with symptoms or signs suggestive of syphilis should also be tested, particularly in those with ulcers (both in the mouth and ano-genital area), rashes, lymphadenopathy, neurological presentations and aortic root disease. The National Institute for Health and Clinical Excellence guidelines do not recommend routine syphilis testing in patients with dementia unless the history or clinical picture suggests they are at risk (National Institute for Health and Clinical Excellence, 2006).

What tests are available for syphilis?

Several different methods are currently available for the diagnosis of syphilis. It is perhaps easiest to divide them into direct and indirect methods.

Direct detection of syphilis

Direct demonstration of *T. pallidum* is possible by dark ground or fluorescent microscopy of infected lesions or lymph nodes, and polymerase chain reaction testing of tissue samples, vitreous fluid and CSF. Although particularly useful in cases of suspected early syphilis and where immediate diagnosis is needed these investigations are limited to experienced observers and availability respectively (Kingston et al, 2008). In vitro culture of *T. pallidum* is not possible.

Indirect detection of syphilis

Serology allows indirect confirmation of treponemal infection by detection of antibodies produced by the immune response to syphilis. Serology remains the cornerstone of syphilis diagnosis.

Figure 2. Syphilis chancre on penis.

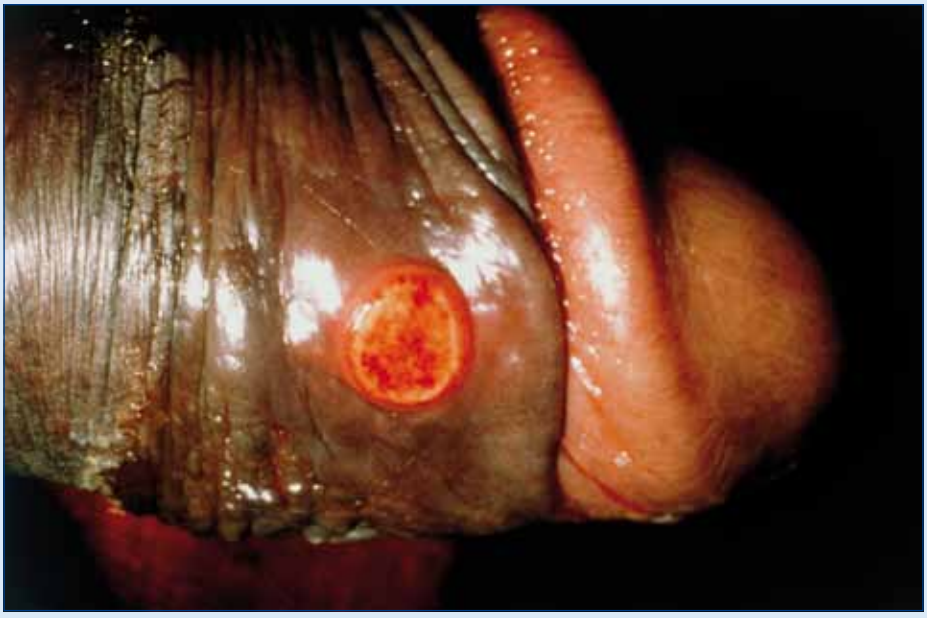
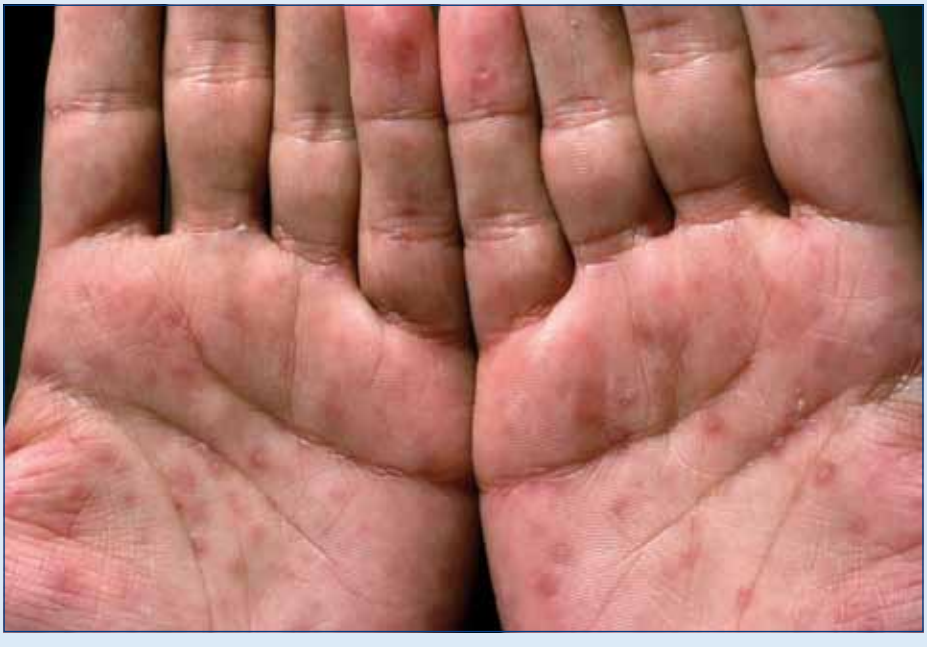


Figure 3. Rash seen on the palm of hands in secondary syphilis.



Serological diagnosis of syphilis

An understanding of the immune response to syphilis is perhaps the first step to fully understanding serological results. The immune system produces non-specific (non-treponemal) and specific (treponemal) antibodies in response to over twenty polypeptide antigens of the treponemal bacterium. IgM-specific antibodies are the first to appear (2–3 weeks after infection), followed by IgG-specific treponemal antibodies 4–5 weeks after initial infection. The non-specific antibodies produced, react against phospholipid, cardiolipin, lecithin and cholesterol antigens, and are generally detectable after IgM appears, declining after treatment and slowly over time without treatment – rising again with reinfection (*Figure 1*).

The two classes of antibodies produced in response to syphilis infection provide the sub-classification of the two groups of serological tests available for syphilis.

Treponemal (specific) serological tests

Treponemal tests detect reactivity of anti-treponemal antibodies against antigens of *T. pallidum*, and include enzyme immunoassay tests to detect IgM and IgG, chemoluminescent microparticle immunoassay, fluorescent treponemal antibody-absorbed test, agglutination assays including the *T.*

pallidum particle or haemagglutination assay and *T. pallidum* recombinant antigen line immunoassay, immunoblot (western) blot. These tests detect specific treponemal antibody, but cannot differentiate between the subspecies of *T. pallidum* which causes (venereal) syphilis and other subspecies of *T. pallidum* which cause non-venereal infections such as bejel, yaws and pinta.

Non-treponemal (non-specific) serological tests

Non-treponemal tests include the Venereal Diseases Research Laboratory (VDRL), and the rapid plasma reagin.

Step by step interpretation of syphilis serology

The testing strategy for diagnosing syphilis uses both types of serological tests, but should be interpreted with clinical findings, including details of any previous diagnosis and treatment of syphilis, and risk of re-infection. It is important to bear in mind that the test used in your lab will vary depending on available resources. The tests most widely used across the UK are described. Interpretation of syphilis serology can be divided into three steps: screening, confirmation and disease activity (*Figure 4*).

Screening

If syphilis is suspected the first step is to perform a screening test, the enzyme immunoassay (IgG/IgM) treponemal specific test. The enzyme immunoassay IgG/IgM test usually takes 3–4 weeks to become positive after infection so may need to be repeated if there has been a risk within this time. If positive, the result must be confirmed. Patients who have had syphilis in the past (even if fully treated) will usually have a positive screening test.

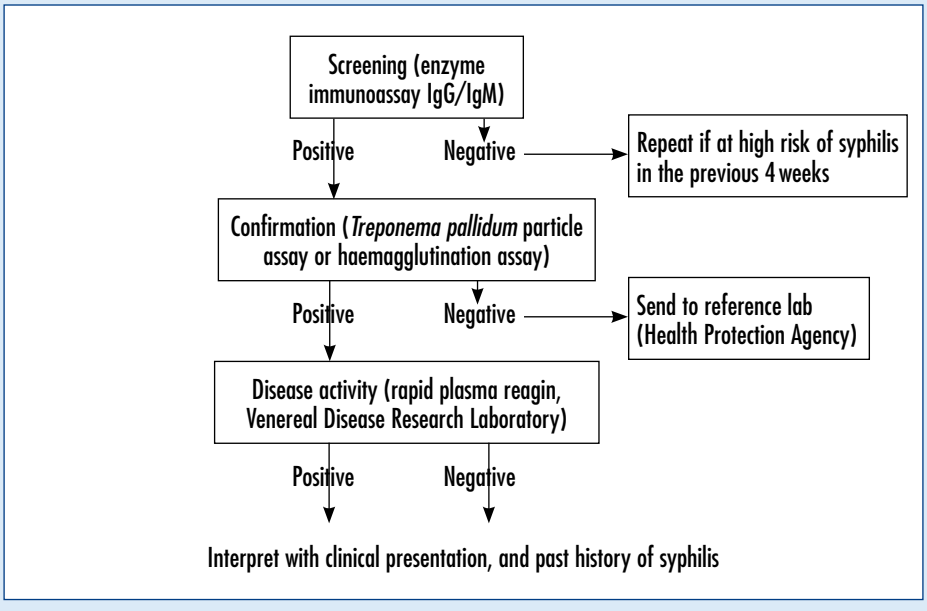
Confirmation

Confirmation with a treponemal specific test that is different to the one that was used as the screening test should be performed next. *T. pallidum* particle assay is currently the most widely used confirmation test. Laboratories often automatically perform the confirmatory test if the screening test is reactive. All patients with positive treponemal serology should have a repeat sample taken, but this should not delay treatment if indicated. Patients who have had syphilis in the past (even if fully treated) will usually have a positive *T. pallidum* particle assay test.

Disease activity

Following a positive confirmatory result, the next stage is to perform a quantitative non-treponemal rapid plasma reagin/VDRL test which may help to assess the stage or activity of disease, determine whether treatment is needed and act as a baseline to allow monitoring of response to treatment. Again, this is usually performed automatically with the confirmatory test.

Figure 4. Interpretation of syphilis serology.



A reactive result is titred in serial dilution and reported as reactive at the highest dilution that gives a reactive result. Secondary syphilis is the most active stage of disease, with titres usually greater than 1:16. Over time, after early syphilis, the titres will gradually fall in late syphilis, up to 30% of untreated patients will have a negative rapid plasma reagin test. In patients with a past history of syphilis, a four-fold or greater rise in titre indicates reinfection.

Staging disease

The results need to be correlated with clinical assessment. The characteristic feature of primary syphilis is an ulcer or ulcers at the site of innoculation. Secondary syphilis can only be diagnosed if symptoms or signs of this stage are present. In both stages, dark ground microscopy or *T. pallidum* polymerase chain reaction tests of any lesions will further support the diagnosis. Early latent syphilis is asymptomatic, and can be diagnosed providing patients have no clinical evidence of disease and serological tests were known to have been negative within the last 2 years. Late latent syphilis is also asymptomatic, with serological tests not known to have been negative within the last 2 years.

Indeterminate results

If indeterminate results occur, samples should be sent to the reference lab for additional testing and confirmation. Tests used by the Health Protection Agency include the fluorescent treponemal antibody-absorbed test. A false positive result occurs when only the screening test is positive, but this can be found in very early primary syphilis. It is important to be aware of biological false positive results of non-treponemal tests, which are found in acute febrile illnesses following vaccinations and pregnancy, but disappear within 6 months. They can persist for longer than 6 months in chronic infections such as leprosy, autoimmune conditions and drug addiction.

IgM testing

Some laboratories use an IgM enzyme immunoassay test after quantitative testing if early primary syphilis is suspected. This may help with determination of the stage of disease.

Monitoring

Follow up of patients diagnosed with syphilis is essential to ensure re-infection and relapse has not occurred. *T. pallidum* has a stable genome, and resistance to penicillin and doxycycline does not occur, but macrolide resistance is well described. Non-treponemal quantitative tests are the method of choice for follow-up testing. A decline in the titre should be demonstrated to confirm successful treatment.

Early stages of syphilis require rapid plasma reagin titres to be checked 1 month after commencing treatment and then at months 2, 3, 6 and 12. Rapid plasma reagin titres should decline 4-fold (two dilutions) at 6 months (Kingston et al, 2008). Thereafter it should be checked at 3-monthly intervals until negative or serofast (identical titres 3 months apart). Late syphilis requires 3-monthly testing until serofast. Ideally titres should decline 4-fold at 12 months (Romanowski et al, 1991), but often they do not despite adequate treatment.

Interpretation of CSF serology

Interpretation of CSF serology requires careful clinical assessment and plasma serological results. CSF examination is only indicated if neurological or ophthalmic symptoms or signs are present, or in cases of treatment failure. A computed tomography head scan should be arranged to exclude any contraindications before performing the lumbar puncture. When performing the lumbar puncture, care should be taken to prevent contamination with macroscopic blood, as this can affect the accuracy of the tests (Izzat et al, 1971). Samples should be collected for protein, white cell count and syphilis serology, in addition to the 'usual' tests. Neurosyphilis should be suspected in any individual with compatible symptoms and signs, positive CSF serology, raised white cell count (>5 cells/mm^3) and a raised protein (>0.4 g/litre). Interpretation of specific treponemal CSF serological tests can be tricky. For further details please refer to the British Association for Sexual Health and HIV syphilis guidelines (Kingston et al, 2008).

Management of syphilis

Syphilis has been effectively treated with parenteral penicillin since 1943 (Marshall and Selbie, 1946), with doxycycline reserved for those allergic to penicillin. Treatment regimens vary according to the stage of disease. All cases should be managed by or in conjunction with a genitourinary medicine specialist. It is important that pregnant patients with positive serology are treated promptly with obstetric input and paediatric follow-up of the baby. Patients should be offered screening for other sexually transmitted infections including HIV, and partner notification needs to be carried out in all patients diagnosed with syphilis. Appropriate follow up must be arranged as outlined earlier, and can be done at a genitourinary medicine clinic. For further details about the general management and recommended treatment regimens please refer to Kingston et al (2008).

Conclusions

Syphilis was termed the 'great imitator' by Sir William Osler because of its varied presentations. Clinical awareness of this curable infection is important for prevention of its potentially serious complications and onward transmission. Understanding the basic principles of serological testing in syphilis, as outlined in this article, will make interpretation of syphilis serology easier. BJHM

KEY POINTS

- Know who and when to test for syphilis.
- Understand the clinical stages of syphilis.
- A basic understanding of the immune response to syphilis will aid understanding.
- Follow the three key steps needed to interpret syphilis serology.
- All patients need appropriate monitoring and follow up with a genitourinary medicine specialist.

Conflict of interest: none.

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Further reading

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TOP TIPS

- When interpreting syphilis serology, take each result in turn and apply the three steps: screening, confirmation and disease activity. Think about the clinical stage of disease and how this fits with the serological results.
- CSF serology can be tricky! Undertake CSF examination after brain scans in all patients with neurological symptoms or signs who have positive syphilis serology. Suspect neurosyphilis in patients with compatible symptoms, a raised CSF white cell count and positive serological tests.
- If a patient has a past history of syphilis, find out when and where they were originally diagnosed, previous results (particularly the rapid plasma reagin/venereal disease research laboratory result), treatment details and ascertain whether there has been a risk of re-infection.