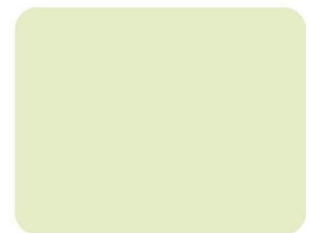
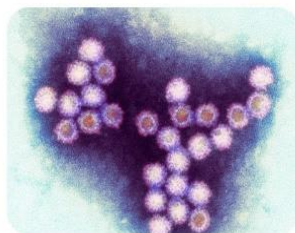
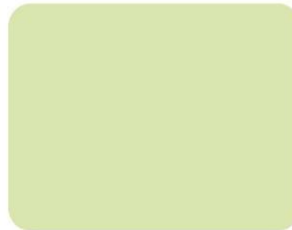
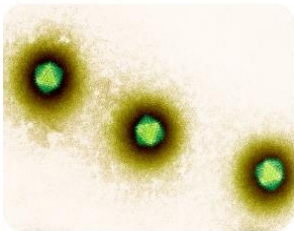
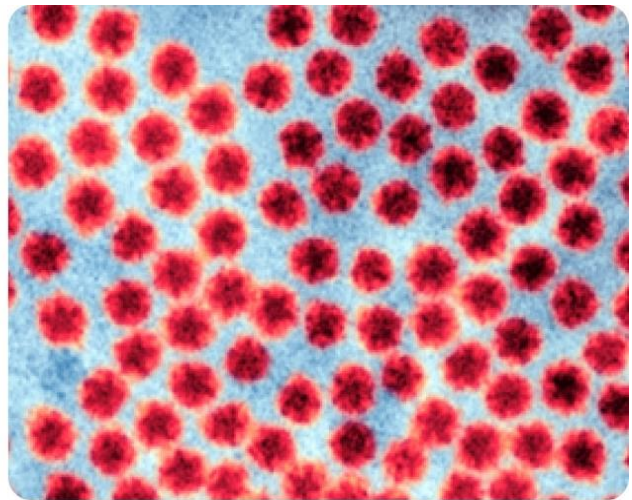
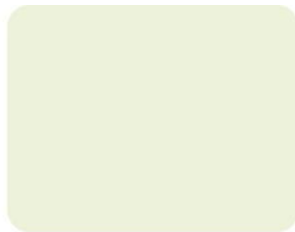




UK Standards for Microbiology Investigations

Laboratory diagnosis of syphilis



Acknowledgments

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Amendment table

Each UK SMI document has an individual record of amendments. The amendments are listed on this page. The amendment history is available from standards@ukhsa.gov.uk.

Any alterations to this document should be controlled in accordance with the local document control process.

Amendment number/date	7/23.05.25
Issue number discarded	3
Insert issue number	3.1
Section(s) involved	Amendment
Whole document	<p>This is an administrative point change.</p> <p>The content of this UK SMI document has not changed.</p> <p>The last scientific and clinical review was conducted on 19.12.2023.</p> <p>Hyperlinks throughout document updated to Royal College of Pathologists website.</p> <p>Public Health England replaced with UK Health Security Agency throughout the document, including the updated Royal Coat of Arms</p> <p>Partner organisation logos updated.</p> <p>Broken links to devolved administrations replaced.</p> <p>References to NICE accreditation removed.</p> <p>Scope and Purpose replaced with General and Scientific information to align with current UK SMI template.</p>
Section 10: Public health responsibilities of diagnostic laboratories	This section has been added to UK SMI templates to highlight the public health responsibilities that diagnostic laboratories have as part of their duties.

Amendment number/date	6/18.12.23
Issue number discarded	2.1
Insert issue number	3.0
Anticipated next review date*	18.12.26
Section(s) involved	Amendment
Title	The title has been changed from syphilis serology to Laboratory diagnosis of syphilis.
Introduction	<p>Included primary, secondary, latent and tertiary stages.</p> <p>Confirmatory treponemal test TPPA has been withdrawn from the UK in 2022 due to regulatory requirements. The diagnostic algorithm has been updated to remove TPPA and gives the option of using either TPHA/TPLA or a second EIA/CLIA as the confirmatory test.</p>
Algorithm/Tables	<p>Treponemal serology screening and confirmation algorithm updated to remove TPPA and gives the option of using either TPHA/TPLA or a second EIA/CLIA as the confirmatory test.</p> <p>All interpreting and reporting tables have been restructured with all the common scenarios.</p>
References	References reviewed and updated.

*Reviews can be extended up to 5 years where appropriate

1 General information

[View general information](#) related to UK SMIs.

2 Scientific information

[View scientific information](#) related to UK SMIs.

3 Scope of document

This UK Standards for Microbiology Investigation (UK SMI) document describes laboratory testing for diagnosis of *Treponema pallidum* infection. It is concerned with diagnosis of syphilis including primary, secondary, latent and tertiary syphilis including central nervous system (CNS) and congenital infections.

Refer to [UK SMI S 6: Infectious syndromes affecting the genitourinary tract and reproductive organs](#) for further information regarding clinical presentations of sexually transmitted infections, and associated tests.

UK SMIs should be used in conjunction with other relevant UK SMIs.

4 Definitions

TPPA – *Treponema pallidum* particle agglutination assay

TPHA – *Treponema pallidum* haemagglutination assay

TPLA – *Treponema pallidum* latex agglutination assay

EIA – Enzyme immunoassay

CLIA – Chemiluminescent immunoassay

RPR – Rapid plasma reagin

VDRL - Venereal disease research laboratory

5 Introduction

Syphilis is a sexually transmitted disease caused by the bacterium *Treponema pallidum* subsp. *pallidum* (1).

Syphilis is typically transmitted by direct contact with an infectious lesion through genital or extra genital sites (anal, rectal and oral). Transmission can occur in utero at any stage of pregnancy, although the risk is increased in the second half of pregnancy, and at the time of birth through contact with maternal lesions in the birth canal (1).

Rare routes of transmission include injecting drugs and blood transfusion (1).

Syphilis is grouped into primary, secondary, latent or tertiary stage. Neurosyphilis can occur at any stage of infection (2).

- primary stage- ulcer (usually painless) or chancre found at the inoculation site usually located on the genitals, rectum, tongue, or lips, which occurs 10-90 days after exposure (1,2)
- secondary stage- signs and symptoms include a skin rash marked by red or reddish-brown macules on the palms and soles or other parts of the body, mucocutaneous lesions, lymphadenopathy, anorexia, fever, sore throat, headaches, weight loss, fatigue, mucous patches, condylomata lata and alopecia. This occurs 2-10 weeks after the chancre appears
- latent stage- No signs or symptoms are present. Early latent is within 1- 2 years of infection, and late latent thereafter. In some patients, latent syphilis ends with the development of tertiary disease
- tertiary stage- signs include gummata, cardiac or neurological manifestations. This stage generally occurs 10-20 years after infection

Currently primary, secondary and early latent syphilis is increasing among gay, bisexual or other men who have sex with men (GBMSM), and heterosexual people (3). In 2022, the number of diagnosed cases reported were 8,692. This increased by 15.2% compared to 2021 where 7,543 diagnosed cases were reported. This is the largest annual number of syphilis diagnoses since 1948 (4).

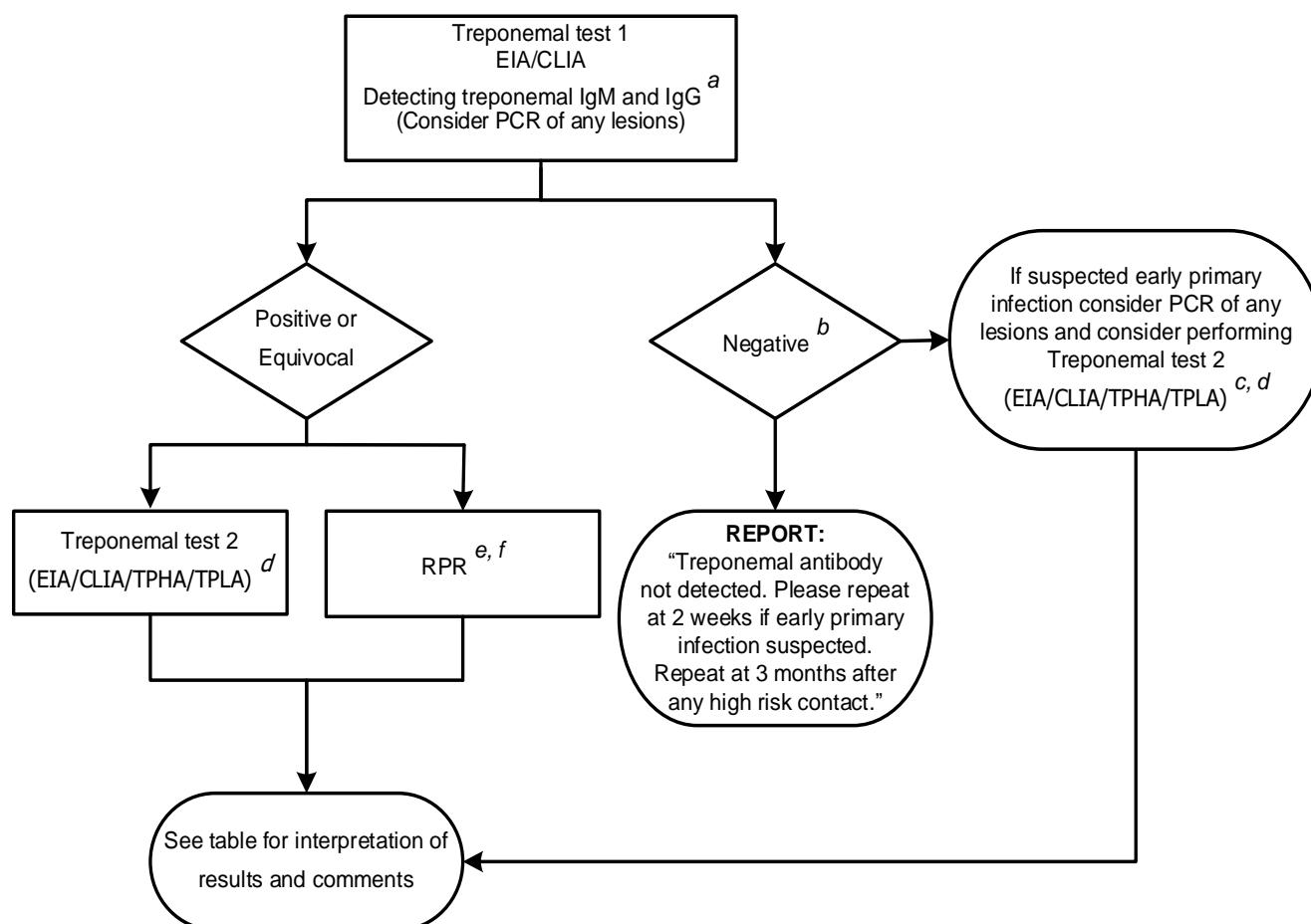
Syphilis shares many clinical features with other treponemal and non-treponemal diseases. *T. pallidum* subsp. *pertenue* (yaws), and *T. pallidum* subsp. *endemicum* (bejel) are morphologically identical subspecies of *T. pallidum* (5). Therefore, laboratory test results must be considered together with the clinical and geographical background of the patient because the serological assays used for syphilis testing also detect antibody raised in response to endemic treponematoses (5,6). As a precaution an individual with positive treponemal serology should be investigated and treated for syphilis unless previous treatment can be documented (7).

In suspected early primary syphilis, a sample should ideally be taken from the lesion for treponemal PCR (8). Examination for treponemes by dark ground microscopy may be undertaken although PCR is preferable when investigating lesions likely to be contaminated with commensal treponemes such as oral lesions (7,9,10).

Most UK laboratories used a *T. pallidum* EIA/CLIA screening test (test 1) followed by TPPA as the confirmatory treponemal test until this was withdrawn from the UK in 2022 due to regulatory requirements. The diagnostic screening algorithm in this UK SMI has been updated to remove TPPA and gives the option of using either TPHA/TPLA or a second EIA/CLIA as the confirmatory test (test 2).

6 Treponemal serology screening and confirmation algorithm

Refer to section 8 for the neonatal algorithm.



6.1 Footnotes

- a. At least one test should be performed using the primary tube.
- b. False negative screening results may be seen in immunocompromised individuals. Negative results within 3 months of infection cannot exclude early syphilis.
- c. Treponemal IgM tests lack sensitivity and specificity and should not be used to stage disease, diagnose reinfections or determine the duration of treatment. A positive IgM result may be useful if primary syphilis is suspected. Results can only be interpreted in association with other treponemal and non-treponemal antibody test results and clinical information. True positive results may reflect recent or active infection but note that IgM reactivity can persist for 12 - 18 months even after adequate treatment of infection (7).
- d. A second treponemal test is used to confirm screen positive results and identify false positives. Most CLIA/EIAs use one or more recombinant treponemal antigens. They are sensitive but may have poor specificity. Where possible, laboratories should use a second treponemal assay that uses different antigen targets to the screening assay to

inform the likelihood of false positive results. Conversely, TPHA/TPLA may be less sensitive than some CLIA/EIAs resulting in true screen positive results which fail to confirm. It is important to request a second sample 2 weeks later if early syphilis is suspected.

- e. Prozone effect (high antibody titres) leading to false negative results may be observed in secondary syphilis or early latent syphilis.
- f. RPR should also be repeated on the day of commencing treatment so that the highest titre is documented.

6.2 Interpreting and reporting laboratory results for treponemal serology and NAAT testing

Note that the table of comments is a guide, and that clinical details and previous serological results should always be considered when interpreting treponemal serology results.

The table cannot cover all serological profiles but should cover most of those encountered in clinical practice. A full repertoire of tests for final interpretation may include referral tests, depending on the local laboratory test repertoire.

Note that the quotation marks indicate the report comment.

Treponemal test 1 (EIA/CLIA)	Treponemal test 2 (EIA/CLIA/TPHA/TPLA)	RPR	Report comment	Notes
Positive	Positive	≤1:16 or including negative	<p>“Consistent with treponemal infection at some time. Active infection is not excluded. Please send a further sample if this is a new diagnosis. Serology results should be interpreted according to clinical presentation.” Refer to notes section.</p> <p>If first sample: “Result may be due to non-specific cross-reactivity or early infection.” Please send a further sample in two weeks to exclude early syphilis. Serology results should be interpreted according to clinical presentation and history of risk.</p> <p>If same profile on repeat sample (at least two weeks later): “Persistent reactivity in treponemal tests may be non-specific. Serology results should be interpreted according to clinical presentation and history of risk.”</p>	<p>This would be consistent with a recent infection if seroconversion, or a four-fold rise in RPR titre was seen in comparison to an earlier sample, or if there were clinical signs suggesting early syphilis.</p> <p>If BOTH treponemal tests used are EIA/CLIAs (and RPR is negative) consider reviewing level of reactivity in treponemal tests.</p> <p>Laboratories need to establish what constitutes a low level reactive result with each test in use, according to local data.</p> <p>Low level reactivity in both treponemal assays may be consistent with treponemal infection but could possibly be due to non-specific cross-reactivity. Interpret in the context of clinical presentation and history of risk. If the same profile is seen on repeat testing and the patient has clinical features or risk factors for syphilis, consider treponemal IgG immunoblot testing.</p>

Treponemal test 1 (EIA/CLIA)	Treponemal test 2 (EIA/CLIA/TPHA/TPLA)	RPR	Report comment	Notes
Positive	Positive	Positive at >1:16	"Consistent with recent or active treponemal infection. Please send a further sample if this is a new diagnosis. Serology results should be interpreted according to clinical presentation."	An RPR of >1:16 is suggestive of active or recent infection, or re-infection. If this is a follow-up sample, review previous results and report changes in RPR titre. Follow-up RPR testing should be according to BASHH guidelines (1).
Negative	Negative	Positive (any titre)	"Isolated RPR reactivity is likely to reflect non-specific reactivity. Please send a repeat sample in two weeks to exclude recent infection. Serology results should be interpreted according to clinical presentation."	None.
Negative	Negative	Negative (if done)	"No serological evidence of treponemal infection. In suspected primary syphilis, consider testing a further sample taken at least two weeks after onset of symptoms to account for the possible seronegative window in early cases. In cases of recent contact, retest after 3 months or earlier if compatible symptoms develop."	Antibody responses may be reduced in the immunosuppressed.

Treponemal test 1 (EIA/CLIA)	Treponemal test 2 (EIA/CLIA/TPHA/TPLA)	RPR	Report comment	Notes
Positive or equivocal	Negative	Negative	If first sample: "Result may be due to non-specific cross-reactivity or early infection. Please send a further sample in two weeks to exclude early syphilis. Serology results should be interpreted according to clinical presentation and history of risk." If same profile on repeat sample (at least two weeks later): "Persistent reactivity in one treponemal test is probably non-specific. Serology results should be interpreted according to clinical presentation and history of risk."	Evaluate level of reactivity in treponemal test. Laboratories need to establish what constitutes a low level reactive result with each test in use, according to local data. If the same profile is seen on repeat testing and the patient has clinical features or risk factors for syphilis, or high level of reactivity, consider performing or referral for treponemal IgG immunoblot testing.
Negative	Positive or equivocal	Negative		
Positive or equivocal	Negative	Positive (any titre)	If first sample: "Result may be due to non-specific cross-reactivity or early infection. Please send a further sample in two weeks to exclude early syphilis. Serology results should be interpreted according to clinical presentation and history of risk."	This is an unusual profile. Evaluate level of reactivity in treponemal test and RPR titre. If RPR titre is high, consider treating. If the same profile is seen on repeat testing, perform or refer for treponemal IgG immunoblot testing for resolution of antibody status.
Negative	Positive or equivocal	Positive (any titre)		

NAAT	Report comment
Detected	<i>T. pallidum</i> detected consistent with active syphilis infection
Not detected	<i>T. pallidum</i> not detected. Review syphilis serology in light of clinical presentation

7 Diagnosis of neurosyphilis

T. pallidum commonly invades the central nervous system at an early stage of infection and may or may not produce symptoms. The diagnosis is based on clinical findings with positive serological tests.

Symptomatic infection can present early as aseptic meningitis or later as meningovascular syphilis or parenchymal late neurosyphilis including general paresis and tabes dorsalis (11). In the preantibiotic era some 20% of infected individuals developed symptomatic neurosyphilis. Early treatment with penicillin markedly reduces the risk of progression from asymptomatic to symptomatic CNS infection.

No single test can diagnose neurosyphilis and similarly no CSF result can definitively exclude a diagnosis of neurosyphilis. Local validation must be performed for treponemal and non-treponemal serology tests performed on CSF. Diagnosis of neurosyphilis requires consideration of the history (including risk factors, treatment history and HIV status), clinical findings, and CSF microscopy and protein level, together with blood and CSF treponemal serology results. CSF protein is variably raised in neurosyphilis depending on the stage of infection. CSF pleocytosis, when present, is lymphocytic. An average of 25-75 cells $\times 10^6/L$ is found in tabes dorsalis and general paresis. However, the CSF is acellular in 10% of cases of tabes dorsalis.

Consider the following for CSF samples:

- if the peripheral blood is negative for treponemal antibodies there is no need to test a CSF sample
- testing of CSF should be considered in patients with treponemal infection and neurological signs or symptoms (1). Blood contamination of CSF should be minimised. A matched serum sample should be taken to compare antibody levels with CSF levels

7.1 Treponemal serology in neurosyphilis

Much of the original work on serological diagnosis of syphilis was performed using VDRL as the non-treponemal test for CSF. However, changes in practice now mean that RPR is more commonly used. Following the withdrawal of the TPPA assay from the UK, TPHA may be performed alongside RPR. If CSF RPR is negative, consider performing TPHA if available.

Consider the following for neurosyphilis:

- CSF RPR is an insensitive test for neurosyphilis being positive in only about 50% of cases (1,12). A positive RPR, in the absence of evidence of blood contamination of the CSF sample, is diagnostic of neurosyphilis (1)
- a negative CSF TPHA makes a diagnosis of neurosyphilis unlikely. A positive CSF TPHA test is highly sensitive for neurosyphilis but lacks specificity because reactivity may be caused by transudation of immunoglobulins from the serum into the CSF. CSF TPHA titres can help to distinguish between higher antibody levels associated with neurosyphilis due to intrathecal antibody production and lower levels due to passive transfer from the blood. A CSF TPHA titre $>1:320$ is sensitive

and specific for neurosyphilis and may be helpful in supporting the diagnosis of neurosyphilis when the CSF RPR is negative

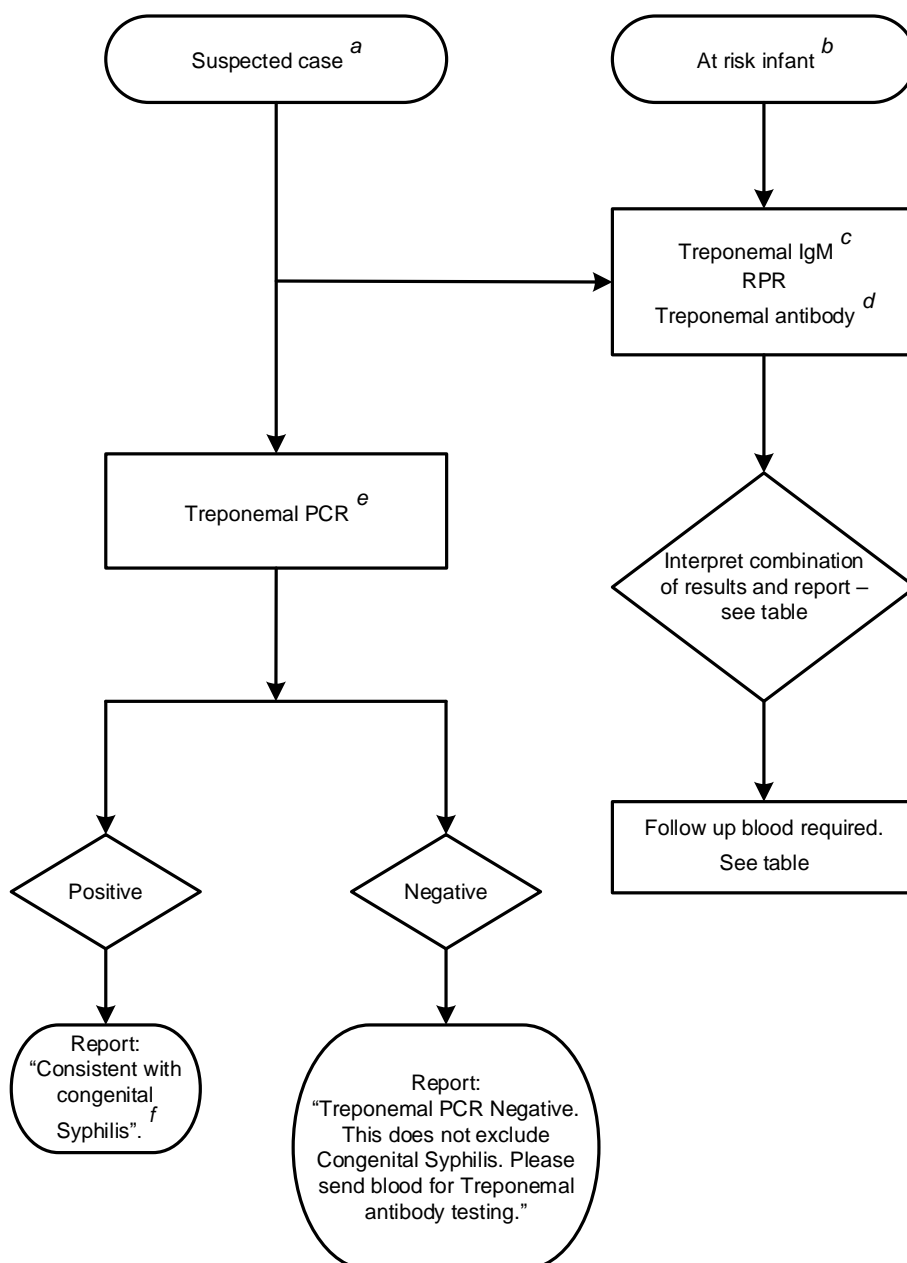
- non-treponemal test results on peripheral blood can help to predict, or exclude, neurosyphilis: a negative RPR virtually excludes neurosyphilis, whereas RPR $\geq 1:32$ increases the likelihood of neurosyphilis (approximately 11-fold in patients without concurrent HIV infection and 6-fold in the HIV-infected individual) (13,14)
- supporting evidence for the use of *T. pallidum* PCR for diagnosing neurosyphilis is weak; studies are generally small and heterogeneous due to lack of a diagnostic gold standard. In studies using a positive CSF VDRL to diagnose neurosyphilis, the sensitivity of the PCR varied between 40% and 70% and specificity between 60% and 100% (15)

Following treatment for neurosyphilis, any CSF pleocytosis should have decreased within six months and CSF should be normal within two years (except for persistent positive treponemal specific antibody tests) (1).

8 Early congenital syphilis testing algorithm

The diagnosis of congenital syphilis can be very difficult; most infected neonates appear normal at birth and passive transfer of maternal syphilis antibodies may cause reactive neonatal serology in the uninfected infant.

A recent review of congenital syphilis cases in England found that most had been born to mothers who had negative antenatal screening results, meaning they became infected with syphilis later during their pregnancy and were undiagnosed and untreated before giving birth. The majority of mothers were diagnosed following their symptomatic infant's diagnosis (16).



8.1 Footnotes relating to early congenital syphilis

- a. Symptomatic baby with signs suggesting possibility of congenital syphilis. Early congenital syphilis manifests within two years of birth. Symptoms might include: snuffles, perioral fissures, hepatomegaly and jaundice, cataracts, growth retardation, rash, mucous patches and condylomata lata.

It is advisable to take and compare a contemporaneous blood from the mother when investigating the baby's serum.

- b. All children born to mothers with confirmed positive treponemal serology require clinical evaluation and syphilis serology tests, with the following exceptions:
- maternal biological false-positive serology
 - maternal syphilis cured prior to this pregnancy

Women with positive syphilis serology who have been adequately treated prior to this pregnancy may have ongoing risk factors for infection. Review of cases of vertical transmission of syphilis have shown that some women acquire syphilis and develop primary infection after an initial negative screening result. This highlights the importance of offering repeat testing to women at risk of exposure to syphilis and/or who have relevant symptoms at any stage of pregnancy.

Passively transferred maternal non-treponemal antibodies should decline by three and be negative by six months of age. Passively transferred treponemal antibodies should decline by 18 months of age.

Infants should be tested at birth and at three months of age, and then the RPR repeated at three monthly intervals until negative (1). If titres remain stable or increase evaluate and treat for congenital syphilis (1).

Note that when maternal syphilis is acquired late in the pregnancy antibodies might not be present in mother or baby at birth (17).

- c. Serological tests should be performed on baby's blood (not the cord blood). Treponemal IgM test should be the priority on small volume samples.
- d. Treponemal antibody test can be EIA, CLIA, TPLA or TPHA. There is no need to confirm with a 2nd treponemal test.
- e. Suitable samples for PCR include nasal discharge, naso-pharyngeal aspirate, throat swabs, lesion swabs, blood and CSF. If placental tissue is available this may also be tested by PCR. In the case of foetal loss where a post-mortem is performed, suitable samples for PCR (in addition to those above) include liver, lung and spleen tissue samples. NAAT testing maybe performed locally or can be discussed with a reference laboratory.
- f. Testing of siblings should be carried out when a maternal or a congenital syphilis diagnosis is made (1).

8.2 Interpreting and reporting laboratory results for early congenital syphilis

IgM	RPR	Treponemal test (EIA/CLIA/TPLA/TPHA)	Report comment	Notes
Positive	Positive (any titre)	Positive/ equivocal	“Consistent with congenital infection. Please repeat to confirm. Consider treponemal PCR on suitable samples.”	Note that IgM false positives and false negatives may occur, so results must always be interpreted in conjunction with the maternal serology results and clinical history.
Positive	Negative	Negative	<p>If mother has acquired syphilis late in pregnancy it is possible that the baby may be treponemal antibody negative around the time of birth:</p> <p>“Possible congenital syphilis. Please repeat to confirm and send samples for treponemal PCR.”</p> <p>In other situations:</p> <p>“No conclusive evidence of congenital syphilis. The IgM reactivity is likely to be false. Please test a further sample to confirm status. Verify maternal treponemal antibody. Consider treponemal PCR on suitable samples.”</p>	Note that IgM false positives and false negatives may occur, so results must always be interpreted in conjunction with the maternal serology results and clinical history.

IgM	RPR	Treponemal test (EIA/CLIA/TPHA/TPHA)	Report comment	Notes
Negative	Negative	Negative	<p>"No serological evidence of congenital syphilis."</p> <p>If baby <3 months old: "Repeat sample at 3 months if mother acquired syphilis late in pregnancy."</p>	If baby is >3 months old at time of testing, repeat sample is not necessary.
Negative	Positive with a titre ≥ 4 times that of mother	Positive/ equivocal	"Consistent with congenital syphilis. Please repeat to confirm. Consider treponemal PCR on suitable samples."	<p>If no recent maternal RPR result is available for interpretation, add the comment:</p> <p>RPR result should be interpreted in comparison with maternal RPR titre. Please contact the laboratory to discuss.</p>
Negative	Positive with a titre <4 times that of mother	Positive/ equivocal	"Serology profile suggestive of passively transferred maternal antibody. However, this must be interpreted in comparison with maternal serology and in a clinical context. Advise repeat RPR at 3 monthly intervals to monitor for changes in titres, or until RPR becomes negative."	Four-fold (or greater) difference in RPR titre has high sensitivity for the diagnosis of congenital syphilis. Note a lower RPR titre does not exclude the diagnosis; most infants with congenital syphilis have an RPR titre that is the same or one or two dilutions less than the maternal titre (18,19). If a mother acquires syphilis and seroconverts late in pregnancy the baby may be delivered prior to a mature antibody response. This results in a low RPR titre and negative IgM, even in the presence of congenital infection.

IgM	RPR	Treponemal test (EIA/CLIA/TPLA/TPHA)	Report comment	Notes
Negative	Negative	Positive/ equivocal	<p>If sample taken around the time of birth: "Serology profile suggestive of passively transferred maternal antibody. Advise repeat at 3 months to confirm negative RPR and exclude early congenital syphilis."</p> <p>Sample from 3 months of age: "Passively transferred maternal antibody. No further testing is necessary."</p>	Note if RPR remains negative at 3 months of age it is unnecessary to repeat samples until treponemal tests are negative, which may persist until 12 months of age.
Negative	Positive (any titre)	Negative	Repeat RPR on original sample and if repeat reactive report as "Probable false positive RPR. Repeat serology in 3 months"	

9 Safety considerations

The section covers specific safety considerations (20-39) related to this UK SMI, and should be read in conjunction with the general [safety considerations](#).

10 Public Health responsibilities of diagnostic laboratories

Diagnostic laboratories have public health responsibility as part of their duties. Amongst these are additional local testing, or referral, to further characterise the organism, as required, primarily for public health purposes e.g. routine cryptosporidium detection; serotyping or microbial subtyping; and a duty to refer appropriate specimens and isolates of public health importance to a reference laboratory.

Diagnostic laboratory outputs inform public health intervention, and surveillance data is required to develop policy and guidance, forming an essential component of healthcare. It is recognised that additional testing and referral of samples may entail some costs that has to be borne by the laboratory but in certain jurisdictions these costs are covered centrally.

Diagnostic laboratories should be mindful of the impact of laboratory investigations on public health and consider requests from the reference laboratories for specimen referral or enhanced information.

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An explanation of the reference assessment used is available in the [scientific information section on the UK SMI website](#).

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