National Institute for Health and Care Excellence (NICE) has renewed accreditation of the process used by the UK Health Security Agency to produce UK Standards for Microbiology Investigations (UK SMIs). The renewed accreditation is valid until 30 June 2026 and applies to guidance produced using the processes described in ‘UK Standards for Microbiology Investigations Development Process’ (2021). The original accreditation term began on 1 July 2011.
Acknowledgments

UK Standards for Microbiology Investigations (UK SMIs) are developed under the auspices of UKHSA working in partnership with the partner organisations whose logos are displayed below and listed on the UK SMI website. UK SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see the Steering Committee page on GOV.UK).

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the medical editors for editing the medical content.

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

<table>
<thead>
<tr>
<th>Amendment number/date</th>
<th>x/dd.mm.yy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Issue number discarded</td>
<td></td>
</tr>
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<td>Insert issue number</td>
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</tr>
<tr>
<td>Anticipated next review date*</td>
<td>dd.mm.yy</td>
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<tr>
<td>Section(s) involved</td>
<td>Amendment</td>
</tr>
<tr>
<td>Title</td>
<td>The tile has been changed from Syphilis serology to Laboratory diagnosis of syphilis.</td>
</tr>
<tr>
<td>Introduction</td>
<td>Included primary, secondary, latent and tertiary stages. Confirmatory treponemal test TPPA has been withdrawn from the UK in 2022 due to regulatory requirements.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.</td>
</tr>
<tr>
<td>Tables</td>
<td>All interpreting and reporting tables have been restructured with all the possible scenarios.</td>
</tr>
<tr>
<td>References</td>
<td>Some of the references have been updated.</td>
</tr>
</tbody>
</table>

*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: Sexually transmitted infections 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 test
EIA – Enzyme immunoassay
CLIA – Chemiluminescent immunoassay
RPR – Rapid plasma regain
VDRL - Venereal disease research laboratory

5 Introduction

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

Syphilis is transmitted by direct contact with an infectious lesion through genital or extra genital sites (anal, rectal and oral). Transmission occurs during pregnancy, where T. pallidum crosses the placenta. This can occur at any stage of pregnancy (1). Other routes of transmission include injecting drugs and blood transfusion which are rare (1).

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

- Primary stage- ulcer or chancre found at the inoculation site usually the genitals, rectum, tongue, or lips, which occurs 10-90 days after exposure (1)

- 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, headaches, weight
Laboratory diagnosis of Syphilis

loss and fatigue. This occurs 2-10 weeks after the chancre appears

- Latent stage- No signs or symptoms are present. Early latent is within 2 years of infection, and late latent thereafter. Latent syphilis ends with the development of tertiary disease

- Tertiary stage- signs include cardiac, ocular or neurological manifestations and auditory abnormalities. This stage generally occurs 10-20 years after infection

Infectious syphilis (primary, secondary and early latent) is increasing both among gay, bisexual or other men who have sex with men (GBMSM), and heterosexual people (2). In 2022 8,692 diagnosis were reported, which increased by 15.2% compared to 2021 (7,543) and 8.1% compared to 2019. This is the largest annual number of diagnosis since 1948 (3).

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* (4). 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 detects antibody raised in response to endemic treponematoses (4,5). As a precaution an individual with positive treponemal serology should be investigated and treated for syphilis unless previous treatment can be documented (6).

In suspected early primary syphilis a sample should ideally be taken from the lesion for treponemal PCR (7). 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 (6,8).

Most UK laboratories used the Serodia TPPA as the confirmatory treponemal test until this was 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.
6 Treponemal serology

A text description of this algorithm is provided with this document.

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 (6,9).

d. 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 and exclude any false positive results.

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.

<table>
<thead>
<tr>
<th>Treponemal test 1 (EIA/CLIA)</th>
<th>Treponemal test 2 (EIA/CLIA/TPHA/TPLA)</th>
<th>RPR</th>
<th>Report comment</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>≤1:16 or negative</td>
<td>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.</td>
<td>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.</td>
</tr>
</tbody>
</table>

If both treponemal tests used are EIA/CLIAs (and RPR is negative) consider reviewing level of reactivity in treponemal tests. If there is low level reactivity in both tests:

- 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

Laboratories need to establish what constitutes a low level reactive result with each test in use, according to local data.

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...
| Laboratory diagnosis of Syphilis | according to clinical presentation and history of risk.  
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. | 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. |
|---|---|---|
| Positive | Positive | >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. |
| 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. |
| 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. |

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).
### Laboratory diagnosis of Syphilis

<table>
<thead>
<tr>
<th>Positive or equivocal</th>
<th>Negative</th>
<th>Negative</th>
<th>Negative or equivocal</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Negative</strong></td>
<td>Positive or equivocal</td>
<td>Negative</td>
<td>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 perform or referral of treponemal IgG immunoblot testing.</td>
<td></td>
</tr>
<tr>
<td><strong>Positive or equivocal</strong></td>
<td>Negative</td>
<td>Positive or equivocal</td>
<td>Positive or equivocal</td>
<td>Any titre</td>
</tr>
<tr>
<td><strong>Negative</strong></td>
<td>Positive or equivocal</td>
<td>Positive or equivocal</td>
<td>Positive or equivocal</td>
<td>Any titre</td>
</tr>
</tbody>
</table>

**NAAT**

<table>
<thead>
<tr>
<th>NAAT</th>
<th>Report comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detected</td>
<td><em>T. pallidum</em> detected, consistent with active syphilis infection</td>
</tr>
<tr>
<td>Not detected</td>
<td><em>T. pallidum</em> not detected. Review syphilis serology in light of clinical presentation</td>
</tr>
</tbody>
</table>
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 (10). In the preantibiotic era some 20% of infected individuals developed symptomatic neurosyphilis. Early treatment with penicillin markedly reduces the risk of progression of asymptomatic to symptomatic CNS infection.

No single test can diagnose neurosyphilis and similarly no CSF result can definitively exclude a diagnosis of neurosyphilis. Diagnosis of neurosyphilis requires consideration of the history (including risk factors, treatment history and HIV status), clinical findings, and CSF microscopy and protein, 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 x 10^6/L is found in tabes dorsalis and general paresis. However, the CSF is acellular in 10% of cases of tabes dorsalis.

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 (1). Blood contamination of CSF should be minimised. A matched serum sample should be taken to compare antibody levels with CSF. Non-treponemal test results on peripheral blood can help to predict, or exclude, neurosyphilis: A negative RPR virtually excludes neurosyphilis, whereas RPR ≥1:32 increases the likelihood of neurosyphilis approximately 11-fold in patients without concurrent HIV infection and 6- fold (in the HIV-infected individual) (11,12).

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 Serodia TPPA assay from the UK, TPHA maybe performed alongside RPR. If CSF RPR is negative, consider performing TPHA if available.

- CSF RPR is an insensitive test for neurosyphilis being positive in only about 50% of cases (1,13). 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.
Laboratory diagnosis of Syphilis

- The evidence base 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 varies between 40% and 70% and specificity between 60% and 100%. (14)

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

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 (15).

A text description of this algorithm is provided with this document.
8.1 Footnotes relating to early congenital syphilis

a. Symptomatic baby with risk factors 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 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

Passively transferred maternal non-treponemal antibodies should decline by three and be negative by six months of age, and treponemal antibodies 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 (16).

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 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. Note laboratories may consider testing these and adding this as a non-validated specimen type. If they have these samples already being extracted for other PCRs.

f. Contact 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

<table>
<thead>
<tr>
<th>IgM</th>
<th>RPR</th>
<th>Treponemal test (EIA/CLIA)</th>
<th>Report comment</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Positive (any titre)</td>
<td>Positive/equivocal</td>
<td>Consistent with congenital infection. Please repeat to confirm. Consider treponemal PCR on suitable samples.</td>
<td>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.</td>
</tr>
</tbody>
</table>
| Positive | Negative | Negative                     | If mother has acquired syphilis late in pregnancy and is treponemal antibody negative around the time of birth:  
‘Possible congenital syphilis. Please repeat to confirm and send samples for treponemal PCR’.  
In other situations:  
‘No conclusive evidence of congenital syphilis. The IgM reactivity is likely to be false. Please repeat to confirm status. Verify maternal treponemal antibody. 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. |
### Laboratory diagnosis of Syphilis

<table>
<thead>
<tr>
<th>Negative</th>
<th>Negative with a titre ≥4 times that of mother</th>
<th>Positive/equivocal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>No serological evidence of congenital syphilis.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>If baby &lt;1 month old: Repeat sample at 3 months if mother acquired syphilis late in pregnancy.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>If baby is &gt;1 month old at time of testing, repeat sample is not necessary.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Negative</th>
<th>Positive with a titre ≥4 times that of mother</th>
<th>Positive/equivocal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Consistent with congenital syphilis.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Please repeat to confirm. Consider treponemal PCR on suitable samples.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>If no recent maternal RPR result is available for interpretation, add the comment:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RPR result should be interpreted in the context of maternal RPR titre. Please contact the laboratory to discuss.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>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 (17,18).</td>
<td></td>
</tr>
<tr>
<td></td>
<td>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.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Negative</th>
<th>Positive with a titre &lt;4 times that of mother</th>
<th>Positive/equivocal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Probably passively transferred maternal antibody. However, this must be interpreted in parallel 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.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>If no recent maternal RPR result is available for interpretation, add the comment:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RPR result should be interpreted in the context of maternal RPR titre. Please contact the laboratory to discuss.</td>
<td></td>
</tr>
</tbody>
</table>

**Note:** RPR titre should be interpreted in the context of maternal RPR titre.
### 9 Safety considerations

The section covers specific safety considerations (19-38) related to this UK SMI, and should be read in conjunction with the general safety considerations on GOV.UK.

<table>
<thead>
<tr>
<th>Negative</th>
<th>Negative</th>
<th>Positive/equivocal</th>
<th>If sample taken around the time of birth: Probably passively transferred maternal antibody. Advise repeat at 3 months to confirm negative RPR and exclude early congenital syphilis.</th>
<th>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.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Positive (any titre)</td>
<td>Negative</td>
<td>Repeat RPR and if repeat reactive report as “Probable false positive RPR. Repeat serology in 3 months”</td>
<td></td>
</tr>
</tbody>
</table>

**Negative**
- If sample taken around the time of birth: Probably passively transferred maternal antibody. Advise repeat at 3 months to confirm negative RPR and exclude early congenital syphilis.
  - Sample from 3 months of age: Passively transferred maternal antibody. No further testing is necessary.

**Positive**
- If sample taken around the time of birth: Probably passively transferred maternal antibody. Advise repeat at 3 months to confirm negative RPR and exclude early congenital syphilis.
  - Sample from 3 months of age: Passively transferred maternal antibody. No further testing is necessary.

**Positive/equivocal**
- If sample taken around the time of birth: Probably passively transferred maternal antibody. Advise repeat at 3 months to confirm negative RPR and exclude early congenital syphilis.
  - Sample from 3 months of age: Passively transferred maternal antibody. No further testing is necessary.
References

An explanation of the reference assessment used is available in the scientific information section on the UK SMI website.


3. GOV.UK. Gonorrhoea and syphilis at record levels in 2022 2023.


13. Marra CM, Tantalo LC, Maxwell CL, Ho EL, Sahi SK, Jones T. The rapid plasma reagin test cannot replace the venereal disease research laboratory


15. GOV.UK. ISOSS syphilis report 2022 2022.


18. Cooper JM, Sánchez PJ. Congenital syphilis. Semin Perinatol 2018;42:176-84. 10.1053/j.semperi.2018.02.005


