



PHLN

Public Health Laboratory Network

Syphilis | *Treponema pallidum*

Laboratory case definition

The Public Health Laboratory Network (PHLN) has developed standard case definitions to inform the diagnosis of key diseases in Australia. This document contains the laboratory case definition (LCD) for syphilis.

Version	Status	Authorisation	Consensus Date
1.1	Reviewed recent evidence and guidelines; updated terminology and references; new testing modalities, including genomics; described testing algorithms; added proficiency testing programs and SNOMED CT codes	PHLN	27 July 2025
1.0	Initial PHLN Laboratory Case Definition	PHLN	30 June 2012

1 PHLN summary laboratory definition

1.1. Condition

Acquired active syphilis due to infection with *Treponema pallidum* subsp. *pallidum*

1.1.1. Definitive criteria

- *Detection of T. pallidum* by serology, including reactive non-treponemal test: Rapid Plasma Reagin (RPR) or Venereal Diseases Reference Laboratory (VDRL) or Cardiolipin Wasserman Reaction (CWR) PLUS at least one reactive specific treponemal test such as Treponema pallidum Particle Agglutination/Haemagglutination (TPPA/TPHA); Fluorescent Treponemal Antibody - Absorption (FTA-ABS) test; Chemiluminescent Microparticle Immunoassay (CMIA) or Enzyme Immune Assay (EIA);

OR

- Detection of *T. pallidum* DNA by nucleic acid test (NAT) in a clinically relevant specimen.

1.1.2. Suggestive criteria

- Detection of spirochaetes by dark field microscopy in a clinically relevant specimen;
OR
- Detection of *T. pallidum* by direct fluorescent antibody microscopy; OR
- Detection of *T. pallidum* by special stains e.g., silver stains in a clinically relevant specimen; OR
- Detection of *T. pallidum* by persistently positive treponemal serology but with negative non-treponemal serology tests in an asymptomatic patient who has not been previously diagnosed or treated for syphilis.

1.2. Acquired active syphilis – reinfection or relapse.

1.2.1. Definitive Criteria

- Detection of *T. pallidum* by serology demonstrating a significant increase in non-treponemal test titre (i.e. fourfold or greater) in an individual with previous, laboratory confirmed infection.

- Detection of *T. pallidum* DNA by NAT in a clinically relevant specimen in an individual with previous, laboratory confirmed infection.

1.3. Congenital syphilis

1.3.1. Definitive Criteria

- Detection of antibody titres in infant serum greater than four-fold higher than in maternal serum;
- OR
- Detection of antibody titres in infant serum comparable with those in maternal serum and specific treponemal IgM EIA or immunofluorescent antibody assay (IFA) positive;
- OR
- Detection of *T. pallidum* DNA in normally sterile clinically relevant specimen by NAT.

1.3.2. Suggestive Criteria

- Detection of *T. pallidum* by dark field microscopy; OR
- Detection of *T. pallidum* by special stains; OR
- Detection of *T. pallidum* DNA from non-sterile site by NAT.

2 Introduction

Syphilis is one of the most important diseases in human history and sexually acquired syphilis continues to occur worldwide. It is caused by the spirochaete *Treponema pallidum subspecies pallidum*. Related treponemes cause the non-venereal treponematoses such as bejel, or endemic syphilis (*T. pallidum ssp. endemicum*), yaws (*T. pallidum ssp. pertenue*), and pinta (*T. carateum*). These geographically restricted organisms are closely related genetically and are antigenically similar to *T. pallidum ssp. pallidum* and can be detected by the same laboratory tests.

Syphilis is a multi-stage, multi-system disease which is broadly defined as acquired or congenital and may present in several ways, generally categorised by duration and site of infection (Table 1). The immune response to syphilis (both humoral and cellular) is only partially protective, and the pathology of the late stages of syphilis is associated with chronic inflammation because of ineffective host immune responses. The disease is treatable with

antibiotics at any stage, and fully curable if adequate therapy is given before advanced pathology develops. Reinfections can occur, including following congenital syphilis.

Table 1: Clinical Features of Syphilis Infection

CONDITION TIME POST	EXPOSURE	MAJOR CLINICAL FEATURES
Primary Syphilis	9-90 days (mean 21)	Chancre at site of infection.
Secondary Syphilis	6 weeks - 6 months*	Rash, mucous membrane lesions, condylomata lata, lymphadenopathy, hair loss, malaise, central nervous system (CNS) symptoms including meningitis, ocular disease.
Early latent	<1 year	Generally, no symptoms or signs of syphilis are present. Occasionally secondary symptoms may recur.
Late latent	>1year	No symptoms or signs of syphilis are present. After 2 years of no symptoms people are no longer infectious to sexual partners.
Late, latent syphilis of unknown duration	Time of infection unable to be determined	No symptoms or signs of syphilis are present. Late latent syphilis is able to be transmitted during pregnancy to the fetus.

CONDITION TIME POST	EXPOSURE	MAJOR CLINICAL FEATURES
Late (Tertiary) syphilis	20-40 years after initial infection	<p>Occurs in approximately one-third of untreated patients, although this is less common in the antibiotic era.</p> <p>Complications include but are not limited to destructive skin lesions (gummas), cardiovascular or neurological disease.</p> <p>Tertiary syphilis is able to be transmitted during pregnancy to the fetus.</p>
Neurosyphilis	Can occur at any stage of syphilis infection.	Highly variable: affects small blood vessels in CNS leading to cerebrovascular events, neuropathies, generalized CNS changes, ocular disease.
Cardiovascular syphilis	Can occur at any stage of syphilis infection.	Aortitis, large blood vessel disease.
Gummatous syphilis	Can occur at any stage of syphilis infection.	Granulomatous nodular lesions, mostly affecting skin and bones, but any organ can be affected.
Congenital syphilis	Perinatal exposure	Stillbirth, low birthweight, “snuffles”, secondary like symptoms, eye lesions, long bone lesions, hepatitis, pulmonary haemorrhage. Very high mortality and

CONDITION TIME POST	EXPOSURE	MAJOR CLINICAL FEATURES
		morbidity in both in-utero and neonatal periods.

*in HIV-infected patients especially, secondary features may overlap with primary symptoms.

Syphilis has a high medico-legal profile, and a high degree of duty of care is expected with regard to accuracy of testing, as occurs with blood-borne viral infections. *T. pallidum* cannot be cultured on synthetic media and laboratory diagnosis of syphilis has traditionally relied on serology and direct microscopy of primary lesions, but NAT has largely replaced direct microscopy, particularly in public health laboratories. There is also growing use of rapid NAT at the point of care, particularly in rural and regional areas remote from laboratory facilities. *T. pallidum* subsp. *pallidum* grows well in testes of live rabbits and the rabbit infectivity test (RIT) remains the gold standard detection method but is expensive and slow. A culture model utilising rabbit tissue cells that was previously developed enables long-term *in vitro* culture of *T. pallidum* subsp. *pallidum* but has not been validated for use with clinical isolates (Edmonson *et al*, 2018).

Australia has recently experienced a surge in syphilis infections, with case notifications doubling between 2015 to 2022, and 20 cases of congenital syphilis being reported in 2023 ([National Syphilis Surveillance Quarterly Report – October to December 2024 | Australian Government Department of Health, Disability and Ageing](#)). Highest risk groups include Aboriginal and Torres Strait Islanders in remote regions of Australia, gay, bisexual and other men who have sex with men, and women of reproductive age.

3 Laboratory diagnosis

3.1. Serological Diagnosis of Syphilis

Despite advances in early detection, serology remains the cornerstone of syphilis diagnosis and monitoring of response to treatment. Broadly, syphilis serological assays are divided into two types of tests:

- Treponemal Tests, that target specific *T. pallidum* antigens, including agglutination assays (*T. pallidum* haemagglutination (TPHA), Micro haemagglutination Assay for

Antibodies to *T. pallidum* (MHA-TP), *T. pallidum* particle agglutination (TPPA)), Fluorescent Treponemal Antibody – Absorption Test (FTA-ABS), *T. pallidum* EIA and IgM EIA, *T. pallidum* Immunoblot, and Rapid Immunochromatography (ICT), point of care assays).

- Nontreponemal (lipoidal antigen) tests such as Venereal Diseases Research Laboratory (VDRL) and Rapid Plasma Reagin (RPR) assays. These tests are performed on serially diluted serum to detect total antibodies (IgG and IgM) directed against lipoidal antigens which are released from damaged host cells and bacteria. These antibodies are non-specific and usually not detectable until a few weeks after infection (Satyaputra et al, 2021). As opposed to treponemal tests, non-treponemal tests are quantitative and reported in titers.

Serologic tests that measure antibodies to both non-treponemal (lipoidal) and specific treponemal antigens should be used in combination, when the primary test is positive (reactive), to aid in the diagnosis of syphilis.

Syphilis serology testing algorithms typically consist of an initial screening test, followed by confirmatory testing of reactive samples with a second assay. Sole reliance on one positive serological test result can misclassify a patient's syphilis status (27). Both the traditional syphilis screening algorithm (i.e. initial screening with nontreponemal assays) and the reverse syphilis screening algorithm (i.e. initial screening with treponemal immunoassays such as EIA) have been used to provide accurate and reproducible laboratory diagnosis (see [Appendix 1](#) and [Appendix 2](#)). The preferred testing algorithm should be based on laboratory resources and patient populations served (27) however in Australia the “reverse algorithm” is typically used.

Endpoint titres (the highest RPR or VDRL dilution yielding a positive result) should be determined and clearly reported. The UK Guidelines (22) recommended testing of a second specimen to confirm initial positive syphilis result and as close as possible to the day when treatment is commenced so the peak RPR/VDRL endpoint is documented as an accurate baseline. A quantitative RPR/VDRL testing has been recommended for monitoring of serological response to treatment.

Table 2. Sensitivity and specificity of serological tests (adapted from Seña AC *et al* 2010).

Test	Sensitivity during stage of infection, % (range)				Specificity, % (range)
	Primary	Secondary	Latent	Late	
Non-treponemal tests					
VDRL	78 (74-87)	100	96 (88-100)	71 (37-94)	98 (96-99)
RPR	86 (77-99)	100	98 (95-100)	73	98 (93-99)
Treponemal tests					
MHA-TP	76 (69-90)	100	97 (97-100)	94	99 (98-100)
TPPA	88 (86-100)	100	100	NA	96 (95-100)
TPHA	86	100	100	99	96
FTA	84 (70-100)	100	100	96	97 (94-100)
Enzyme immunoassays (IgG EIA, ICE)	77-100	100	100	100	99-100
Immunochemiluminescent assays (e.g., CLIA)	98	100	100	100	99

3.2. Treponema Specific Tests

Treponema-specific tests are generally used as screening assays due to their greater sensitivity in early primary syphilis, lower rate of biological false positive reactions which may outnumber true positives in a low prevalence population and their availability as automated assays. If a treponemal test is used as the screening assay all reactive sera should be retested using a nontreponemal assay and a quantitative, nontreponemal test (RPR or VDRL) for assessing the stage of infection and to establish a baseline for response to treatment.

Antibodies detected by treponemal tests typically persist for life and should not be used to monitor response to therapy. Approximately 15-25% of individuals treated for primary syphilis will become non-reactive to FTA-Abs and MHA-TP, and individuals with advanced HIV infection may serorevert, losing their reactivity to treponemal tests.

3.3. *T. pallidum* haemagglutination (TPHA) and Microhaemagglutination Assay for Antibodies to *T. pallidum* (MHA-TP)

TPHA and MHA-TP are indirect agglutination assays using *T. pallidum* antigens bound to formalinised, tanned avian or sheep erythrocytes. Sera are diluted first with a sorbent/diluent containing non-pathogenic Reiter treponemes (*T. phagedenis*) plus other absorbents. Serum containing specific anti-*T. pallidum* antibodies will cause cross-linking of RBC. Serum without antibody does not cross link RBC MHA-TP is a microplate version of TPHA.

3.3.1. Test sensitivity

MHA-TP: poorer sensitivity than other manual treponemal tests for all stages of syphilis: primary syphilis 45.9-88.6%, secondary 90-100%, early latent 94-100%, late latent 97% (Park et al,2020)

3.3.2. Test specificity

MHA-TP 99.6- 100% (Cole et al), 98.8-99% (Park et al)

3.3.3. Predictive values

False Positive factors: heterophile reactions (anti-red cell) may be seen in MHA-TP, TPHA; non-specific agglutination (should be detectable from control particle reaction); and autoimmune and connective tissue diseases, viral infections, pregnancy

False Negative factors: prozone effect in secondary syphilis.

3.3.4. Suitable test acceptance criteria

According to manufacturer's instructions and expected performance of controls.

3.3.5. Suitable test validation criteria

In accordance with NPAAC guidelines: Requirements for the Validation of In House In vitro Diagnostic Devices.

3.3.6. Suitable Internal Controls

As for VDRL assay.

3.3.7. Suitable external quality assurance program and proficiency testing

RCPA QAP (<https://rcpaqap.com.au/home-page/serology/>), USA CDC USA Proficiency Testing program.

3.4. *T. pallidum* particle agglutination (TPPA)

Passive gelatin particle agglutination assay. Gelatin particles are sensitised with pathogenic *T. pallidum*. Unsensitised control gelatin particles are used to check for anti-gelatin antibodies that would give false positive results. Serum is mixed with the reagent containing the sensitised gelatin particles, and the particles aggregate to form clumps when the patient serum contains specific anti-*T. pallidum* antibodies.

3.4.1. Suitable specimen types

Serum, plasma, or heat inactivated serum can be used. Serum should be stored at 2C-8C and tested within 5 days or frozen at -20C for longer storage (22).

3.4.2. Test sensitivity

Primary syphilis 86.2-100%, secondary 100%, latent 100%, early latent 94-100%, late latent 86.8-100% (Park et al, 2020).

3.4.3. Test specificity

TPPA >99.6-100% (Park et al, 2020)

3.4.4. Predictive values

False Positive factors: non-specific agglutination (should be detectable from control particle reaction); autoimmune and connective tissue diseases, viral infections, pregnancy; or rheumatic heart disease.

False Negative factors: prozone effect in secondary syphilis.

3.4.5. Suitable test acceptance criteria

According to manufacturer's instructions and expected performance of controls.

3.4.6. Suitable test validation criteria

In accordance with NPAAC guidelines: Requirements for the Validation of In House In vitro Diagnostic Devices.

3.4.7. Suitable Internal Controls

As for VDRL

3.4.8. Suitable external quality assurance program and proficiency testing

RCPA QAP (<https://rcpaqap.com.au/home-page/serology/>), USA CDC USA Proficiency Testing program.

3.4.9. Special considerations

TPPA shows good correlation with IgM assays in early primary syphilis and does not require complex or expensive laboratory equipment, making it suitable for use as a sole screening assay in resource poor settings.

Some labs may not perform a second treponemal test for samples reactive on non-treponemal testing, particularly with pending discontinuation of TPPA.

3.5. FTA-ABS (Fluorescent Treponemal Antibody - Absorption) Test

Indirect immunofluorescent assay using whole cell pathogenic *T. pallidum* fixed to the slide. Sera are first mixed with a sorbent/diluent to remove antibody activity to antigenically related but non-pathogenic saprophytic treponemes.

3.5.1. Suitable specimen types

Serum, CSF (not contaminated with serum). Plasma not recommended but may be the only specimen available particularly from blood transfusion services.

3.5.2. Test sensitivity

Primary syphilis 78.2-100%, secondary 92.8-100%, latent (overall) 83-100%, early latent (94.4-100%), late latent (84.5-92.6% (Park et al, 2020).

3.5.3. Test specificity

87-100% (Park et al, 2020).

3.5.4. Predictive values

False Positive factors: (~1% of sera): autoimmune and connective tissue diseases including rheumatoid arthritis, systemic and discoid lupus erythematosus (LE), drug induced LE (may get an atypical beading pattern of fluorescence, some due to the presence of anti-DNA antibodies). May be removed by absorption with calf thymus DNA. Elderly patients, pregnancy, intravenous drug use (IVDU), diabetes. Rarely viral infections, Lyme disease.

False Negative factors: problems with FTA slides, conjugate or fluorescent microscopy reducing the sensitivity and discrimination of the test.

3.5.5. Suitable test acceptance criteria

According to manufacturer's instructions and acceptable performance of controls.

3.5.6. Suitable test validation criteria

Many different combinations of antigen, sorbent and conjugate can be used, but each combination should be individually validated against other assays.

3.5.7. Suitable internal controls

As for VDRL above plus a strong positive with and without sorbent, a nonspecific control with and without sorbent and a reactive minimal control, PBS and sorbent control.

3.5.8. Suitable external quality assurance program (proficiency testing)

RCPA QAP (<https://rcpaqap.com.au/home-page/serology/>), USA CDC USA Proficiency Testing program.

3.5.9. Special considerations

This assay requires experience with immunofluorescence microscopy, and careful attention needs to be paid to standardising reading and reporting practices.

3.6. *T. pallidum* immunoassays

An increasing number of commercial immunoassays are available, including chemiluminescent (CLIA), chemiluminescent microparticle (CMIA), enzyme (EIA) and enzyme-linked (ELISA) immunoassays, using either whole *T. pallidum* lysate, or a combination of *T. pallidum*-specific recombinant antigens (e.g., TpN15, TpN17 and/or TpN47 antigens etc). Most detect total antibody against the pathogen. Immunoassays have the advantage of being highly suitable for automation and are favoured as screening assays for large laboratories. If an immunoassay is to be used as the sole screening assay, it must be designed to detect total antibody as IgM may not be detectable in late syphilis and reinfections. Most EIAs are robust tests and have high performance characteristics, based on RCPA QAP data.

3.6.1. Suitable specimen types

Serum is suitable for all assays, with a number of assays also able to utilise plasma (heparinised, EDTA or citrate). Performance with CSF has not been fully evaluated, but available data suggest there is no problem testing these specimen types. The assay has

also performed well in validation studies using dried blood spots. No specimen pre-treatment required.

3.6.2. Test sensitivity

Primary syphilis 94.5-100%; secondary 100%, early latent 95-100%, late latent 91.7-98.5%. Refer to Parks et al for specific assay performance.

3.6.3. Test Specificity

94.5-100%.

3.6.4. Predictive values

False positive factors may be associated with viral or bacterial infection, autoimmune disease, pregnancy, postimmunisation status, diabetes, and old age.

False negative factors: Nil known as yet with this assay.

3.6.5. Suitable test acceptance criteria

According to manufacturer's instructions and acceptable performance of controls.

3.6.6. Suitable Test Validation Criteria

See manufacturer's instructions.

3.6.7. Suitable internal controls

As recommended by manufacturer.

3.6.8. Suitable external quality assurance program and proficiency testing

RCPA QAP (<https://rcpaqap.com.au/home-page/serology/>), NRL proficiency panel, USA CDC USA Proficiency Testing program.

3.6.9. Special Considerations

Occasional false positive results may occur, most likely due to the TpN47kDa protein component (33). This is unlikely to be a problem if positive results are confirmed with a second *T. pallidum* specific assay.

Treponemal tests perform no differently in pregnant persons and should be interpreted in the same manner as for non-pregnant persons. Passively transferred to children maternal non-

treponemal antibodies should decline by three and be negative by six months of age, and treponemal antibodies by 18 months of age (22).

3.7. *T. pallidum* IgM immunoassays

IgM immunoassays are useful for investigating early and congenital syphilis and as for total antibody immunoassays, assay types include chemiluminescent (CIA), chemiluminescent microparticle (CMIA), FTA-based assays and enzyme (EIA) immunoassays. Assays are based on the principle of IgM antibody class "capture". Rabbit antibodies against human IgM are coated on the inner surfaces of microtitre tray wells (solid phase). Diluted patient serum is dispensed into the wells and, during incubation, a proportion of the total serum IgM is "captured" on the solid phase. Surface-bound IgM antibodies to *T. pallidum* are subsequently traced by incubation with the conjugate reagent comprising purified *T. pallidum* antigen, biotinylated monoclonal antibody, and streptavidin-HRP. Unbound conjugate is rinsed away and surface-bound HRP is detected by reaction with a chromogenic substrate. The intensity of the coloured reaction product is directly related to the proportion of the total serum IgM that is *T. pallidum* specific.

3.7.1. Suitable specimen types

Serum is suitable for all assays, with a number of assays also able to utilise plasma (heparinised, EDTA or citrate). Performance with CSF is not fully evaluated.

3.7.2. Test sensitivity

Primary syphilis 86.5% (95% CI 74.2-94.4) (25). Low sensitivity in late syphilis, when IgM is rarely detected (18). IgM has been detected in >95% primary syphilis, and >50% reinfections in previously treated patients who were PCR positive. However, IgM may not rise in reinfections.

3.7.3. Test specificity

Approximately 90% (26).

3.7.4. Predictive values

False positive factors:

- Presence of polyclonal IgM (e.g. in EBV, *M. pneumoniae* infections etc)
- Connective tissue diseases, rheumatoid factor myelomas, malignancies
- Possible low level IgM during pregnancy in previously positive patients.

False negative factors:

- Low negative predictive values in late syphilis when IgM is rarely detectable (18) and in reinfections when an IgM response is variable.

3.7.5. Suitable test acceptance criteria

According to manufacturers' instructions and acceptable performance of controls.

3.7.6. Suitable test validation criteria

As for VDRL above.

3.7.7. Suitable internal controls

See manufacturer's documentation.

3.7.8. Suitable external quality assurance program (proficiency testing)

RCPA QAP (<https://rcpaqap.com.au/home-page/serology/>)

3.7.9. Special considerations

This test should only be used for the investigation of recently acquired or congenital syphilis and should not be used alone as a screening assay. IgM antibodies may persist for up to a year after infection and treatment, and at low levels for longer periods in some untreated patients. Interpretation of low-level IgM results in patients at high risk of infection can be difficult and require repeat serology. The IgM test should not be used to stage disease or decide the duration of treatment (22).

3.8. Rapid Immunochromatographic Tests (ICT)

These point of care (POC) kits use *T. pallidum* specific antigens to detect specific antibody in a card or strip format with visual readout. The fastest assay can produce a result within 15 minutes. WHO has sponsored programmes to compare commercially available syphilis ICT tests and promote their use in remote regions because they are simple to use, can be transported, stored and performed at room temperature (below 30C) and do not require special laboratory equipment. WHO studies found most kits to have a sensitivity of 85-98% and specificity of 93-98% compared against TPHA or TPPA as reference standards. A few Australian laboratories use them as their screening assay and their performance in RCPA-QAP appears to be acceptable. They can be used in clinics and/or outreach settings, but sera should be formally retested by a NATA accredited laboratory.

3.9. *T. pallidum* Immunoblot

3.9.1. Western Blotting

Western Blotting using lysed whole-cell *T. pallidum* as the antigen source has been an important research tool in the past for identifying immunodominant *T. pallidum*-specific antigens, and the timing of antibody appearance. It is a laborious test, and interpretation can be difficult due to the presence antibodies against non-*T. pallidum* specific antigens (e.g., flagellin protein). It has now been largely replaced by recombinant immunoblotting for diagnostic or confirmatory purposes.

3.9.2. Recombinant Immunoblot assay

Immunoblotting allows for the detection of antibodies to individual *T. pallidum* antigens which are considered to be diagnostic for acquired syphilis. Commercial kits are available and Australian reference laboratories generally use the INNO-LIA Syphilis test (Innogenetics) which is a line immunoassay utilizing three recombinant antigens (TpN15, TpN17, TpN47) and one synthetic peptide (TmpA) derived from *T. pallidum* proteins.

3.10. Non-Treponemal Tests

3.10.1. VDRL (Venereal Diseases Research Laboratory) Test

Historically, reaginic antibodies were measured using a CFT format known as the Cardiolipin Wasserman Reaction (CWR) or, simply Wasserman Reaction (WR). The WR was replaced during the middle of last century by reagin assays using rapid particle agglutination technologies. Both IgG and IgM antibodies are detected. This is a micro-flocculation assay. Sera containing anti-cardiolipin and related antibodies (reagin) will cause flocculation of a liposomal suspension of stabilised cardiolipin. This needs to be observed through a microscope at x100 magnification. The USR (Unheated Serum Reagin) test is a modified VDRL with EDTA to stabilise the antigen and choline chloride is added to serum so heat pre-treatment of serum is unnecessary.

3.10.1.1. Suitable specimen types

Suitable specimens are serum and CSF; specimen preheating is required with some test formats.

3.10.1.2. Specimen collection and handling

Sera are unsuitable if they are haemolysed or lipaemic, contaminated with fungi, bacteria or chemicals, old or multiply freeze/thawed. CSF should not be contaminated with blood or serum.

3.10.1.3. Test sensitivity

Primary syphilis 78%, secondary syphilis 100%, latent syphilis 95%, late syphilis 71% (12) (10).

3.10.1.4. Test specificity

98% (if treponemal tests positive) (12), 98-100% (10).

3.10.1.5. Predictive values

Positive predictive values: Biological false positives (BFP) are seen in sera collected from patients with a large range of co-morbidities. BFP titres may rise transiently, usually to low levels, in response to conditions causing transient polyclonal B cell activation such as immunisation and acute infections including hepatitis A, B, C, measles, EBV, VZV, *Mycoplasma pneumoniae* infection, malaria, upper respiratory tract infections and pregnancy. Longer term (>6 months) rises in BFP titres from low to high levels may be seen in patients with chronic conditions including rheumatic fever, chronic tissue damage, IVDU, extreme age, malignancy especially lymphatic leukaemia, lymphoma and multiple myeloma, autoimmune SLE, gammopathies, diabetes, anti-phospholipid syndrome, major allergic reactions, penicillin allergy; and in patients with chronic infections including leprosy and hepatitis.

Negative predictive values: Prozone may occur (1-2% in secondary syphilis) giving rough, grainy or weak atypical reactions. If suspected retest starting at dilutions up to 1:64.

3.10.1.6. Suitable test acceptance criteria

See manufacturer's protocol. New batches of reagents should give acceptable results when tested in parallel with previous or reference reagents.

3.10.1.7. Suitable test validation criteria

In accordance with NPAAC guidelines: Requirements for the Validation of In House In vitro Diagnostic Devices.

3.10.1.8. Suitable internal controls

See manufacturer's directions. A panel of control sera of reactivity ranging from weakly to strongly reactive should be included in comparative testing of new reagent with reference or previous lot of reagent.

3.10.1.9. Suitable external quality assurance program and proficiency testing

RCPA QAP (<https://rcpaqap.com.au/home-page/serology/>), USA CDC USA Proficiency Testing program.

3.10.1.10. Special considerations

VDRL antigen should be diluted for use in accordance with the manufacturer's instructions each time it is used. All reagents and sera should be allowed to warm to room temperature before use. Other factors that may interfere with test accuracy include rotation time / speed during incubation, volume of antigen added. VDRL may be used in monitoring of disease activity following treatment (see RPR section below).

3.10.2. Rapid Plasma Reagin (RPR)

This is a macro-flocculation (i.e. visible to naked eye, unlike VDRL) assay. Sera containing anti-cardiolipin and related antibodies will cause flocculation of a liposomal suspension of stabilised cardiolipin with charcoal particles added. This can be observed macroscopically due to trapping of the charcoal particles in the cardiolipin antibody clumps (uses USR Antigen and finely ground charcoal particles). The RPR detects IgG & IgM and serial 2-fold dilutions of serum are tested to determine an endpoint titre, expressed as the reciprocal. Titres are frequently 2-4-fold higher than for VDRL.

3.10.2.1. Suitable specimen types

Serum, plasma in the past RPR was not recommended for use with CSF, but studies suggest RPR performs as well as VDRL with CSF. (3)

3.10.2.2. Specimen collection and handling

Samples should be stored at -20C and retrieved for parallel testing.

3.10.2.3. Test Sensitivity

Primary syphilis 86% (can be affected by early treatment), secondary syphilis 100%, latent syphilis 98% late syphilis 73% (12) 86-100% (10).

3.10.2.4. Test specificity

98% (if treponema tests positive), 93-98% (10).

3.10.2.5. Predictive values

Similar to VDRL.

3.10.2.6. Suitable test acceptance criteria

Controls perform according to established criteria.

3.10.2.7. Suitable test validation criteria

In accordance with NPAAC guidelines.

3.10.2.8. Suitable internal controls

See manufacturer's instructions and as for VDRL above.

3.10.2.9. Suitable external quality assurance program (proficiency testing)

RCPA QAP (<https://rcpaqap.com.au/home-page/serology/>), USA CDC USA Proficiency Testing program.

3.10.2.10. Special considerations

Also see VDRL special considerations.

As with all agglutination assays, careful attention needs to be paid to performing the test exactly according to the manufacturer's instructions, including serial dilutions, incubation temperatures and times and agitation of the plates (if recommended). There is a subjective component in reading the endpoint of the assay and the operator should be well trained by experienced staff to ensure reproducibility of results. Different RPR antigen preparations will show different activity, i.e. titres may be generally higher or lower. However, this should not affect the qualitative sensitivity (i.e. reactive or non-reactive) if the test is performing adequately. Any comparison of titres on serial bleeds should be done in parallel - results from different laboratories should not be directly compared.

3.10.3. Monitoring of disease activity following treatment:

Only RPR and VDRL can be used to monitor disease activity and assess response to antibiotic therapy as *T. pallidum* specific serology tests generally remain positive for life following infection. Decline in RPR and VDRL titres following treatment follows a variable course, and the rate of decline of antibodies varies according to the stage and duration of infection, and the initial RPR and VDRL titre. Results should only be compared with those of tests performed with the same antigen, preferably in parallel, and RPR titres should not be directly compared with VDRL. Persistently high RPR/VDRL titres following adequate

treatment, or high titre BFP reactions may occur in patients with underlying autoimmune conditions.

Evidence suggests that approximately 15% of individuals will have a ≥ 4 -fold change in RPR titre between day of presentation and day of treatment (Pandey et al, 2023). Repeating nontreponemal serology on the day of treatment is therefore recommended to document the peak titre for subsequent monitoring of disease.

Following treatment, nontreponemal antibody titres usually decrease at least fourfold in the first 12 months, particularly in patients with primary or secondary infection. Failure of nontreponemal test titers to decrease fourfold within 12 months after therapy for primary or secondary syphilis or 24 months for latent syphilis (inadequate serologic response) might be indicative of treatment failure. However, clinical trial data have demonstrated that 10%–20% of persons with primary and secondary syphilis treated with the recommended therapy will not achieve the fourfold decrease in nontreponemal titre within 12 months after treatment (Sena et al).

3.11. Nucleic Acid Amplification techniques including PCR

T. pallidum PCR assays have proved useful in helping to define the sensitivity of serology assays in early syphilis, demonstration of re-infection in patients with previously positive serology, and confirmation of syphilis infection in placental specimens under investigation for congenital syphilis. There is increasing evidence that DNA is detectable by PCR from multiple sites during early syphilis, including saliva, oropharynx, rectum, urine, semen and plasma, however evidence to date suggest that screening with syphilis PCR does not significantly increase syphilis case finding in high-risk populations.

PCR testing may occasionally identify patients who have a PCR positive lesion, but negative serology in early primary syphilis.

Assays commonly target the *T. pallidum* 47- kDa protein gene (*tp47*), which codes for a putative cell wall protein, and, more recently, the *T. pallidum* polymerase A gene (*polA*) first described by Liu et al (14). Both *tp47* and *polA* will detect *T. pallidum* subspecies (*T. pallidum*, *T. pertunue* and *T. endemicum*). Newer diagnostic PCR assays often use Real-Time PCR formats, increasing sensitivity and turnaround time and providing a semi-quantitative output, as well as multiplexing with targets for other pathogens that cause genital ulcer disease. There is increasing evidence that DNA is detectable by PCR from multiple sites during early syphilis, including saliva, oropharynx, rectum, urine, semen and plasma, however evidence to date suggest that screening with syphilis PCR does not significantly increase syphilis case finding in high-risk populations.

3.11.1. Suitable specimen types

Primary and secondary syphilis lesion swabs, biopsies (including vitreous humour), placental specimens and cerebrospinal fluid (CSF). Paraffin embedded biopsy specimens may be tested but have lower sensitivity. Tissue in formalin is not suitable for NAT.

3.11.2. Specimen collection and handling

NAT can be used for primary and possibly secondary syphilis lesions in seronegative patients.

3.11.3. Test sensitivity

Sensitivity of PCR assays is generally lower than the rabbit infectivity test which is the gold standard (Theel *et al*, 2020). Most PCR assays target the *tp47* (*tp074*) or *poIA* (*tp0105*) genes with varying sensitivity depending on the stage of the disease and specimen type (US CDC, 2024). The sensitivity varies across studies from 72% to 95% on lesion exudate of primary syphilis and from 20% to 86% on secondary lesion swabs depending on lesion type sampled (skin rash versus condylomata lata) (US CDC, 2024). PCR assays targeting *poIA* have been shown to be of similar sensitivity to PCR assays targeting the *tp47* (*tp074*) gene (10). When used for diagnosing symptomatic congenital disease the sensitivity of the 47-kDa gene PCR assay has been shown to approximate 80% in amniotic fluid compared with rabbit infectivity tests but only 60% in CSF and 67% in serum (12).

3.11.4. Test specificity

Specificities of all assays are high (> 97%) (8,10). The VIDRL *poIA* TaqMan assay compared with serology has a specificity of 98.40.% microscopy or DFA.

3.11.5. Predictive values

Very high, particularly when combined with positive serology.

3.11.6. Suitable test acceptance criteria

All controls within expected ranges. Any weak positive results near the cutoff should be retested, recollected or tested with a second NAA assay.

3.11.7. Suitable test validation criteria

In accordance with NPAAC guidelines: Requirements for the Validation of In House In vitro Diagnostic Devices. Refer to published papers for specific data e.g. *poIA* gene PCR (8, 10).

3.11.8. Suitable internal controls

As recommended in NPAAC guidelines: Laboratory Accreditation Standards and Guidelines for Nucleic Acid Detection and Analysis.

3.11.9. Suitable external quality assurance program (proficiency testing)

RCPA QAP and Quality Control for Molecular Diagnostics (QCMD) - an independent International External Quality Assessment (EQA) / Proficiency Testing (PT) organisation (www.qcmd.com).

3.11.10. Special considerations

Serology should always be requested in conjunction with any requests for syphilis PCR. PCR is useful for assisting with the diagnosis of genital ulcer disease. The test is not affected by the presence of saprophytic spirochaetes, or concurrent bacterial or viral infections. Although positive PCR results are helpful in establishing a diagnosis, a negative result, especially in blood, CSF, vitreous or amniotic fluid, does not rule out infection. PCR can be considered as an adjunct test in amniotic fluid, neonatal CSF, or neonatal blood in cases of suspected congenital infection, however, PCR is not usually recommended for whole blood or blood fractions because of low sensitivity.

3.12. Direct Detection Assays and histology features

Direct detection assays include direct microscopy using dark field or direct fluorescent antibody (DFA) techniques and nucleic acid assays. All have the advantage of becoming positive one to 3 weeks before serology and are most useful in primary, secondary and early congenital syphilis when specimens from infectious lesions contain large numbers of treponemes and can be easily obtained.

Syphilis causes histologic features of the affected tissue depending on the type of lesion and the stage of the disease. Primary syphilis is characterised by a chancre at the infection entry point associated with ulceration of epidermis, lymphocytic and plasma cell infiltration, and endothelial swelling. Spirochetes are usually detected within or around blood vessels. Secondary syphilis can present histologically in multiple ways, including a lichenoid tissue reaction, normal or ulcerated epidermis and changes in the dermis resembling primary syphilis. Tertiary syphilis can present local inflammation or necrotising granulomatous lesions (gummas) in the affected tissues (Satuaputra *et al*, 2021). Silver and other special stains can be used to highlight spirochetes in formalin-fixed paraffin-embedded tissues.

3.12.1. Dark-ground (dark-field microscopy)

Principle - light microscopy using a special condenser that excludes directly transmitted light, and only allows oblique light to fall on the specimen. This partially overcomes the resolution limits of light microscopy (0.2µm) and allows *T. pallidum* to be seen as bright motile spirochaetes on a dark background. *T. pallidum* can be partially differentiated from other spirochaetes by size, tightness & regularity of the spiral, and characteristic motility.

3.12.1.1. Suitable specimen types

Fresh serous exudate expressed from chancre, which must be free from blood contamination and bacterial superinfection, collected by a sterile bacteriological loop or touch preparation onto a microscope slide. The site should be gently cleaned before collection. Specimens derived from mucosal sites are not suitable due to the presence of saprophytic spirochaetes. The test must be done on site soon after specimen collection, as the specimen must not be allowed to dry out.

3.12.1.2. Test sensitivity

Low, even with experienced operators. May be improved by examination of multiple slides from the same infection site.

3.12.1.3. Test specificity

High when done on specimen from cutaneous lesions in patients with suggestive clinical history.

3.12.1.4. Predictive values

Positive predictive value (PPV) is high when performed on cutaneous lesions by highly experienced microscopist in a clinic which sees symptomatic patients and often performs the test. PPV is reduced when performed by inexperienced microscopist or on specimens from a mucosal site (oral, genital, anorectal), which may contain saprophytic spirochaetes (*T. denticola* is morphologically indistinguishable from *T. pallidum*). Negative predictive value depends on the specimen quality and freshness that determines the number and motility of the spirochaetes. Superinfection with other bacteria or the presence of blood or pus may prevent spirochaetes being seen.

3.12.1.5. Suitable test acceptance criteria

Fresh sample and absence of superinfection.

3.12.1.6. Suitable internal controls

Routinely, controls are not used, due to difficulty of maintaining viable *T. pallidum*. A suspension of fresh gingival scrapings in saline may be used to confirm that the settings of the microscope are correct prior to examining the specimen.

3.12.1.7. Suitable external quality assurance program (proficiency testing)

Problematic due to impossibility of transporting live motile treponemes except in liquid nitrogen.

3.12.1.8. Special considerations

This test should only be performed by experienced staff working adjacent to specimen collection room.

3.12.2. Fluorescent Antibody Test (DFA-TP)

This is an immunofluorescence assay using FITC-labelled anti-*T. pallidum* polyclonal antibodies pre-adsorbed with Reiter's treponemes to remove non-specific anti-treponemal antibodies or labelled monoclonal antibodies specific to pathogenic *T. pallidum*. Specimens collected as for dark-ground microscopy are incubated with the conjugated-antibody then washed. If *T. pallidum* is present in the specimen it can then be visualized under an immunofluorescence (IF) microscope with a dark-ground condenser, or by epifluorescence microscopy.

3.12.2.1. Suitable specimen types

Fresh serous exudate expressed from chancre, which must be free from blood contamination and bacterial superinfection. The test is less reliable in examining rectal and oral lesions or other specimens derived from mucosal sites due to the presence of commensal spirochaetes.

3.12.3. Specimen collection and handling

Collect specimen as for dark-field microscopy, but allow to dry in air. Specimens are usually fixed by methanol, acetone or heat before IF staining. In theory specimens from mucosal lesions can also be tested if fluorescently labelled monoclonal antibodies are used. DFA can also be applied to appropriately prepared fixed tissue specimens.

3.12.4. Test sensitivity and specificity

Superior to dark-ground microscopy, as DFA-TP does not require motile organisms to detect *T. pallidum*. No adequate comparative studies with PCR as yet.

3.12.5. Predictive values

Positive predictive value is high when performed by an experienced microscopist on a well-collected specimen. Negative predictive value is influenced by the quality of the specimen collection. Superinfection with other bacteria or the presence of blood or pus may prevent spirochaetes being seen.

3.12.6. Suitable test acceptance criteria

Acceptable staining characteristics obtained in controls.

3.12.7. Suitable test validation criteria

In accordance with NPAAC guidelines Requirements for the Validation of In House In vitro Diagnostic Devices. Fixed positive control slides made in-house or supplied as part of commercial assays can serve as suitable internal controls.

3.12.8. Suitable external quality assurance program and proficiency testing

None available.

3.12.9. Special considerations

This test should only be performed by laboratories with relevant experience.

4 Laboratory diagnosis of neurosyphilis

Symptomatic neurosyphilis (NS) may occur at any stage of infection, and diagnosis is critical to ensure appropriate therapy. Neuro-invasion is often asymptomatic and can be detected in up to 30% of individuals with early (primary and secondary) infection. No single test can diagnose neurosyphilis, nor can a negative result definitively exclude CNS infection.

Diagnosis requires consideration of the history and clinical findings, and CSF parameters including microscopy and protein measurement, and treponemal serology results.

Hamill *et al* (Clin Infect Dis 2024) outline an approach to the diagnosis of neurosyphilis, including indications for CSF examination (see Appendix 2 for summary). The CSF findings of neurosyphilis include pleocytosis, elevated protein, and a reactive CSF-VDRL.

Indications for CSF examination in individuals with confirmed syphilis infection include:

- Presence of neurological signs or symptoms*, OR
- Presence of gummatous or cardiovascular syphilis, OR

- Sustained, (persisting beyond 2 weeks) fourfold increase in nontreponemal serological titres following stage appropriate therapy, in the absence of reinfection.

Additionally, CSF examination should be considered in individuals whose nontreponemal serological titres remain serofast, that is they fail to decline fourfold after stage-appropriate therapy *and* after an appropriate period post therapy (12 months for primary and secondary, and 24 months for latent syphilis).

*CSF examination is not recommended for individuals with otic or ocular syphilis, as approximately 30% will have normal CSF parameters. An exception is when neurological signs or symptoms are present.

4.1. CSF Testing algorithms and interpretation

Serological analysis requires a CSF sample without blood contamination, referred with a serum sample collected at the time of lumbar puncture. CSF white cell count and protein analysis should be performed.

VDRL has been the gold standard for diagnosis of neurosyphilis due to its high specificity (78.2-100%) but with a lower sensitivity of 66.7-88.3% and is not widely available in Australia. CSF RPR is frequently used as an alternative due to its availability and high specificity (82.6-100%), however it is an insensitive test for neurosyphilis being positive in only about 50% of cases.

CSF FTA-Abs test performance varies according to the gold standard applied, however in studies of definitive neurosyphilis, sensitivity was 90.9%–100% and in two studies of presumptive neurosyphilis CSF FTA-ABS demonstrated 100% sensitivity.

CSF-TPHA is highly sensitive for neurosyphilis but lacks specificity. 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.

4.2. CSF Testing by nucleic acid tests

T. pallidum PCR may be used as an adjunct to diagnosis. Performance is poor compared to swab and tissue samples, with a sensitivity of 40-70% and they cannot be used as a primary

test to diagnose neurosyphilis. Neurosyphilis is not excluded by negative *T. pallidum* NAT (Satuaputra et al, 2021).

In summary, in the absence of gold standard diagnostic criteria for the diagnosis of neurosyphilis, laboratories may use a staged approach to diagnosis using multiple assays based on test performance characteristics and test availability.

5 Laboratory diagnosis of congenital syphilis

Syphilis infection can be transmitted to the fetus at any stage of maternal disease. As a result, Australia's National Response to Syphilis has focussed on enhanced testing in women of reproductive age and during pregnancy, including opportunistic and repeat testing.

The rate of transmission to the fetus is approximately 70% during primary and secondary syphilis and decreases with later stages of maternal infection; approximately 40% in early latent infection and 10% in late latent infection.

Treatment of early maternal syphilis at least 30 days before delivery is the most important factor reducing the risk of congenital infection, with 70 - 100% of infants born to untreated mothers will be infected compared to 1% - 2% of those born to women adequately treated during pregnancy. Treatment in pregnancy is considered adequate if recommended treatment is completed more than 30 days before delivery and there is a four-fold drop in RPR titre. Laboratory definitive and suggestive criteria for congenital syphilis are outlined in Section 1.3, however if these criteria are not met, clinical criteria are required to confirm the case definition for reporting. Diagnosis of congenital syphilis relies on clinical suspicion and physical examination of the infant, documentation of maternal antenatal serology, treatment history and treatment response, and post-natal laboratory testing of the mother, infant and placental tissue. It may be difficult to distinguish between congenital and acquired syphilis in a seropositive child after infancy. Follow-up of the infant for at least two years is required to detect late-onset congenital syphilis. In the event of stillbirth or neonatal death in a mother with syphilis, fetal examination and postmortem tissue including placental histology and NAAT testing are required to confirm the diagnosis. Transplacentally acquired nontreponemal (VDRL or RPR) antibody will be present in most infants, but it disappears in uninfected infants by six months of age. A *syphilitic stillbirth* is defined as a fetal death in which the mother had untreated or inadequately treated syphilis⁴ at delivery of a fetus after a 20-week gestation or of 500 g (Arnold & Ford-Jones, 2000).

6 Typing and Subtyping Methods

Typing and next generation sequencing (NGS) of *T. pallidum* has been available in Australia as a research application. This includes culture-independent or metagenomic sequencing of the pathogen in different samples. The multi-locus sequence typing scheme for *T. pallidum* included four targets (TP0136, TP0548, TP0705, and 23S rRNA gene), however, results from a single-locus typing of the TP0548 allele correlated with genomic subgroups derived from whole genome sequencing data (Taouk et al, 2022).

Culture-independent sequencing of *T. pallidum* genomes in primary syphilis lesions identified four major sub-lineages circulating in Australia and globally, two belonging to the SS14 lineage, and two representing the Nichols lineage. Most Australian *T. pallidum* genomes also appeared to be genomically macrolide resistant (Taouk et al, 2022).

The low evolutionary rate of *T. pallidum* means that there is relatively limited genetic diversity between genomes across space and time. Accordingly, whole genome sequencing has limited capacity to infer in real-time direction of transmission in sexual networks (Taouk et al, 2022).

7 Laboratory nomenclature for national data dictionary

SNOMED CT code	Concept name	Description
76272004	Disorder	Syphilis
72904005	Organism	Treponema pallidum
708462008	Substance	Treponema pallidum DNA
120723002	Substance	Treponema pallidum antibody
818821000000104	Procedure	Treponema pallidum detection by nucleic acid amplification test
40675008	Procedure	Serologic test for syphilis
19869000	Procedure	Rapid plasma regain test
401262008	Procedure	Treponema pallidum particle agglutination test
7786006	Procedure	Venereal Disease Reference Laboratory test
9232009	Procedure	Fluorescent treponemal antibody absorption test
82191002	Procedure	Syphilis test, qualitative
104334001	Procedure	Treponema pallidum antibody confirmatory test

8 References

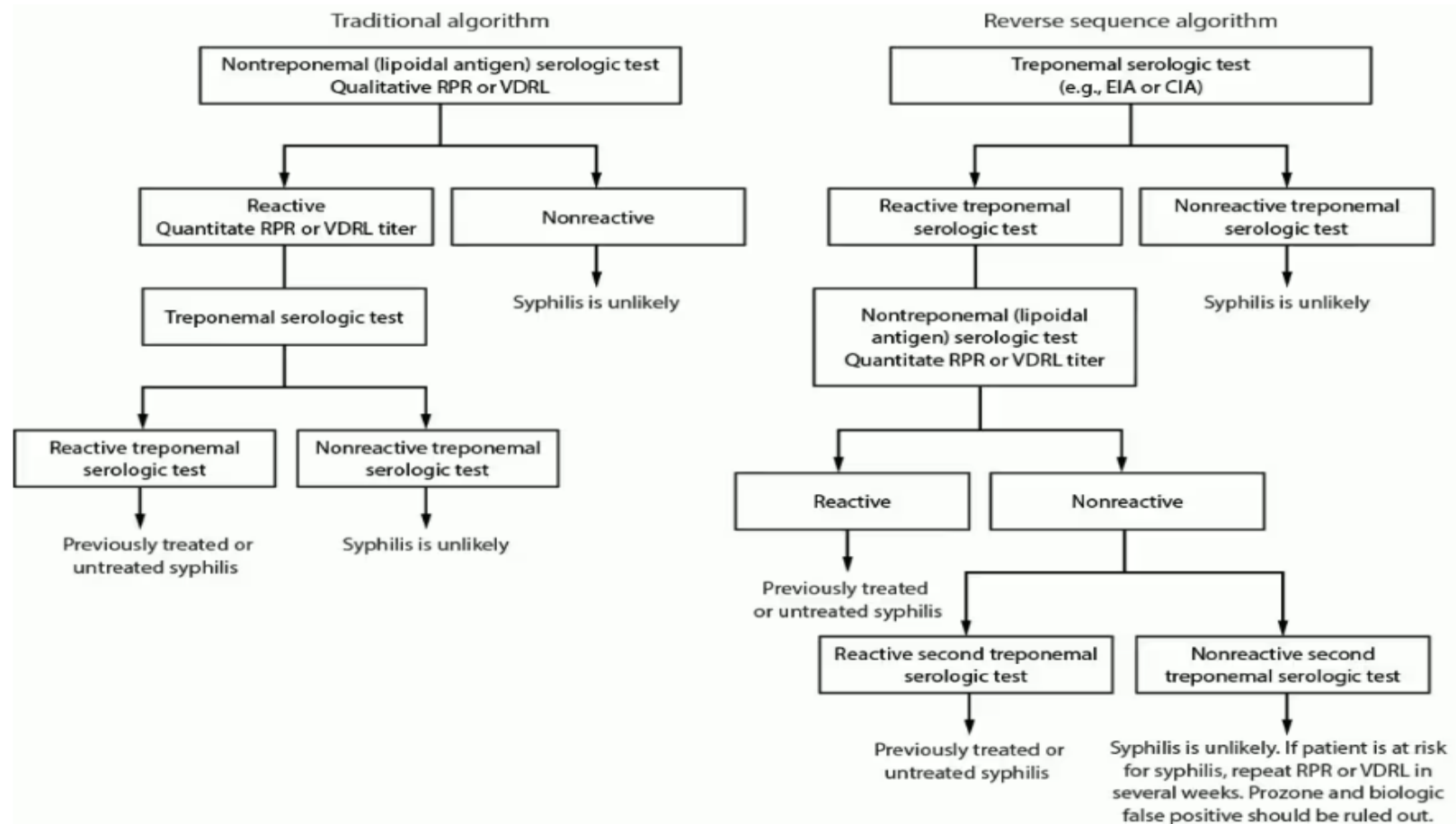
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9 Appendix 1

Source: US CDC Guidelines 2024 (27).



Abbreviations: CIA = chemiluminescence immunoassay; EIA = enzyme immunoassay; RPR = rapid plasma regain; TPPA = *Treponoma pallidum* particle agglutination; VDRL = Venereal Disease Research Laboratory.

10 Appendix 2

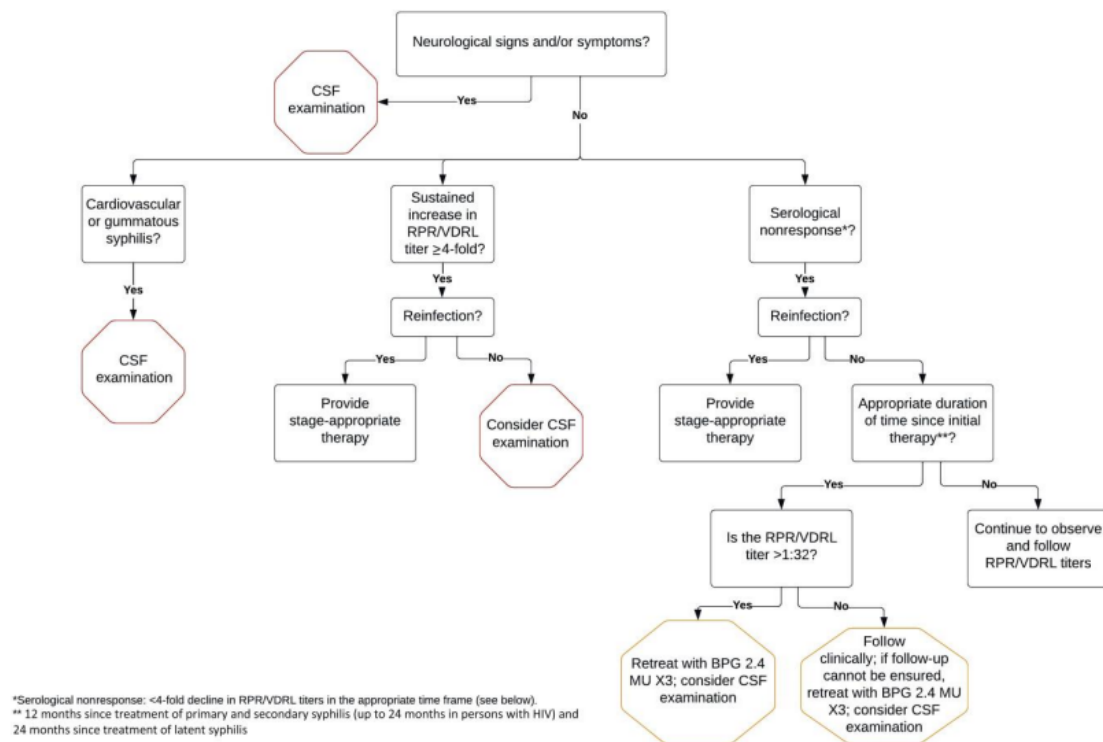


Figure 2. When to perform a CSF examination. Algorithm that summarizes the current guidelines for performing a CSF examination in persons with serological or clinical evidence of syphilis. These recommendations are based on the 2021 Centers for Disease Control and Prevention STI Treatment Guidelines [14]. Abbreviations: BPG, long-acting benzathine penicillin G; CSF, cerebrospinal fluid; HIV, human immunodeficiency virus; RPR, rapid plasma regain; VDRL, Venereal Disease Research Laboratory.

11 Glossary

Ag/Ab – Antigen/Antibody

AMR – Antimicrobial resistance

ARTG – Australian Register of Therapeutic Goods

Biotype – Strain distinguished from other microorganisms of the same species by its physiological properties or a group of organisms with the same genotype

(US) CDC – Centers for Disease Control and Prevention

CDNA – Communicable Diseases Network Australia

CDS – Calibrated dichotomous susceptibility

CIA – Chemiluminescent immunoassay

Clade – Group of organisms composed of a common ancestor and all its lineal descendants

CLSI – Clinical and Laboratory Standards Institute

CSF – Cerebrospinal fluid

Ct – Cycle threshold

DFA – Direct fluorescent antibody

DNA – Deoxyribonucleic acid

EDTA – Ethylenediaminetetraacetic acid

EIA – Enzyme immunoassay

ELISA – Enzyme linked immunosorbent assay

EUCAST – European Committee on Antimicrobial Susceptibility Testing

ICT – Immunochromatographic test

IFA – Immunofluorescent antibody

IgA – Immunoglobulin A

IgG – Immunoglobulin G

IgM – Immunoglobulin M

IVD (device) – In vitro diagnostic medical device

In vitro – performed in a test tube, culture dish, or elsewhere outside a living organism

In vivo – performed or taking place in a living organism

ITS – Inter-genic spacer region

LAMP – Loop-mediated isothermal amplification

LPS – Lipopolysaccharide

MALDI-TOF – Matrix-assisted laser desorption ionization-time of flight

MAT – Microscopic agglutination test

MDR – Multidrug resistant

MIA – Microsphere immunoassay

MLST – Multilocus sequence typing

NAAT – Nucleic acid amplification testing

NATA - National Association of Testing Authorities, Australia

NGS – Next generation sequencing

NPAAC – National Pathology Accreditation Advisory Council

NRL – National Serology Reference Laboratory

PCR – Polymerase chain reaction

PC2 laboratory – Physical containment level 2 laboratory

PC3 laboratory – Physical containment level 3 laboratory

PC4 laboratory – Physical containment level 4 laboratory

PFGE – Pulsed field gel electrophoresis

POC – Point-of-care

QAP – Quality assurance program

QC – Quality control

RCPA – Royal College of Pathologists of Australasia

RFLP – Restriction fragment length polymorphism

RNA – Ribonucleic acid

RT – Reverse transcriptase

RT-PCR – Reverse transcription polymerase chain reaction

SBT – Sequence based typing

Serotype – Pathogens of the same species that are antigenically different

SNT – Serum neutralisation

SSBA – Security sensitive biological agent

STI – Sexually transmitted infection

Strain – Variant that possesses unique and stable phenotypic characteristics

SQAP – Serology quality assurance program

Test sensitivity – Ability of a test to correctly identify patients with a disease

Test specificity – Ability of a test to correctly identify people without the disease

TGA – Therapeutic Goods Administration

UTM – Universal transport medium

WGS – Whole genome sequencing

WHO – World Health Organization

WHO CC – WHO Collaborating Centre

XDR – Extensively drug resistant