**Syphilis (*Treponema pallidum*)**

Laboratory case definition

The Public Health Laboratory Network (PHLN) has developed standard case definitions for the diagnosis of key diseases in Australia. This document contains the laboratory case definition for *treponema pallidum*.

**Authorisation:**  PHLN

**Consensus date:**  June 2012

1 PHLN Summary Laboratory Definition

1.1 Condition:

Acquired active syphilis

1.1.1 Definitive Criteria

* + 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
  + Demonstrated seroconversion of specific treponemal test within 12 months of a negative test; OR
  + Detection of *T. pallidum* by nucleic acid test (NAT) from suitable clinical specimen including cerebrospinal fluid (CSF), tissue and chancre.

1.1.2 Suggestive Criteria

* + Dark field microscopy of lesion exudate or node aspirate smears (not oral lesions) showing characteristic morphology and motility of *T. pallidum*; OR
  + Direct fluorescent antibody microscopy of the same specimens; OR
  + Demonstration of *T. pallidum* in tissues by special (e.g. silver) stains.

1.2 Condition:

Acquired active syphilis - re-infection

1.2.1 Definitive Criteria

* + Significant increase in non-treponemal test titre in previously infected individual.

1.2.2 Suggestive Criteria

* + None.

1.3 Condition:

Congenital syphilis

1.3.1 Definitive Criteria

* + Treponemal-specific antibody titres in infant serum greater than four-fold higher than in maternal serum; OR
  + 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 specimen (CSF, tissue) by NAT.

1.3.2 Suggestive Criteria

* + Dark field microscopy of lesion exudate or node aspirate smears (not oral lesions) to demonstrate characteristic morphology and motility of *T. pallidum*; OR
  + Demonstration of *T. pallidum* in tissues by special (e.g. silver) stains; OR
  + Detection of *T. pallidum* DNA from a non-sterile site by NAT.

1.4 Guide for Use

Treponema specific tests tend to remain positive for life following treponemal infection and should not be used to evaluate response to treatment and are of no value in determining relapse or reinfection in a patient who has had a reactive result. Reactions may be atypical in HIV positive patients and some degree loss of treponemal specific antibodies may occur over time in up to 10% of patents. (DL observation) Non-treponemal (reagin) test titres decline after successful treatment to be weakly reactive or negative after three years in primary and secondary syphilis. This decline may be more gradual in treated latent or late syphilis or in persons who have had multiple episodes of syphilis.  
Particular test combinations are helpful in distinguishing the various clinical presentations, which are discussed further in the Introduction (Section 2) and listed in Appendix 1.

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 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 will be detected by the same laboratory tests.

Syphilis may present in several ways, generally categorised by duration and site of infection.

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| **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, CNS symptoms including meningitis, ocular disease. |
| Early latent | <2 yrs | Nil, occasional recurrent secondary symptoms. |
| Late latent | >2 yrs | Nil. |
| Late (Tertiary) syphilis |  |  |
| Neurosyphilis |  | highly variable: affects small blood vessels in CNS leading to cerebrovascular events, neuropathies, generalized CNS changes, ocular disease. |
| Cardiovascular syphilis |  | aortitis, large vessel disease |
| Gummatous syphilis |  | Granulomatous nodular lesions, mostly affecting skin and bone, but any organ can be affected. |
| Congenital syphilis |  | stillbirth, low birthweight, “snuffles”, secondary like symptoms, eye lesions, long bone lesions, hepatitis, pulmonary haemorrhage. |

\*in HIV patients especially, secondary features may overlap with primary symptoms  
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 at any stage, and fully curable if adequate therapy is given before advanced pathology develops. Reinfections do occur, including following congenital syphilis.

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 and malaria. *T. pallidum* cannot be cultured on synthetic media and laboratory diagnosis of syphilis has traditionally relied on serology and direct microscopy of primary lesions. It grows well in testes of live rabbits and the rabbit infectivity test (RIT) remains the gold standard detection method but is expensive and slow. Nucleic acid tests are available in larger laboratories and may replace direct detection methods although serology should always be performed. Recently, cheap, rapid immunochromatographic point of care (POC) kits for detection of specific treponemal antibodies have revolutionized the diagnosis of syphilis in rural and developing regions remote from laboratory facilities

An algorithm for test selection for syphilis serology follows in Appendix 2. Notes on the diagnosis of neurosyphilis and congenital syphilis follow in Appendix 3 and 4.

Further reading - general aspects of *T. pallidum* biology & syphilis

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| **General overview of syphilis** | **Singh & Romanowski 1999 (27)** |
| *T. pallidum* biology | LaFond & Lukehart 2006 (11) |
| Syphilis & HIV | Lynn & Lightman 2004 (17) Zetola et al 2007 (34) |
| Serodiagnosis (CDC USA) | Larsen et al 1995 (12) |
| CSF examination in syphilis | Marra et al 2004 (19) |
| Congenital syphilis pathogenesis | Wicher and Wicher 2001 (31) |
| CNS infection in congenital syphilis | Michelow et al 2002 (21) |
| Biology of the treponemes (Ch36) | Lukehart SA (16) |
| Clinical manifestations of syphilis (Ch37) | Sparling PF et al (28), In Holmes, K - Sexually Transmitted Diseases (4th Ed) |
| Treponema pallidum (syphilis) (Ch238) | Edmund C Tramont (30), In Mandell, Douglas & BennettPrinciples and Practice of Infectious Diseases, 7th Ed 2010 |

3 Tests

3.1 Direct Detection Assays

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.

3.1.1 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 (0.2uM) 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.

3.1.1.1 Suitable and unsuitable specimens

Fresh serous exudate expressed from chancre, which must be free from blood contamination and bacterial superinfection. Not suitable for specimens derived from mucosal sites 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.

3.1.1.2 Test sensitivity

Low, even with experienced operators, may be improved by examination of multiple slides.

3.1.1.3 Test specificity

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

3.1.1.4 Predictive values and relevant populations

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 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.

3.1.1.5 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.

3.1.1.6 Suitable external (QAP) program(s)

Problematic due to impossibility of transporting live motile treponemes except in liquid N2.

3.1.1.7 Special considerations

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

3.2 Direct Fluorescent Antibody Test (DFA-TP)

This is an immunofluorescence assay using FITC-labeled anti-*T. pallidum* polyclonal antibodies pre-adsorbed with Reiter’s treponemes to remove non-specific anti-treponemal antibodies or labeled 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.

3.2.1 Suitable and unsuitable specimens

Collect specimen as for dark-ground 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 labeled monoclonal antibodies are used. DFA can also be applied to appropriately prepared fixed tissue specimens.

3.2.2 Test sensitivity

Superior to dark-ground microscopy, no adequate comparative studies with PCR as yet.

3.2.3 Test specificity

Superior to dark-ground microscopy, no adequate comparative studies with PCR yet.

3.2.4 Predictive values and relevant populations

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.

3.2.5 Suitable test acceptance criteria

Acceptable staining characteristics obtained in controls.

3.2.6 Suitable internal controls

Fixed positive control slides made in-house or supplied as part of commercial assays.

3.2.7 Suitable original test validation criteria

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

3.2.8 Suitable external (QAP) program(s)

None available.

3.2.9 Special considerations

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

3.3 Nucleic Acid Amplification techniques including PCR

*T. pallidum* PCR is still a developing technology, and is only available as an in-house assay at a few laboratories in Australia. Commercial assays are not yet available. 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. PCR (confirmed with a second NAA) has also identified a small group of patients who have a PCR positive lesion, but completely negative serology in early primary syphilis, and early treatment of these patients may prevent a typical antibody response from developing. Laboratories commonly target the *T. pallidum* 47- kDa protein gene, 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). Newer diagnostic PCR assays often use Real-Time PCR formats, increasing sensitivity and turnaround time and providing a semi-quantitative output.

3.3.1 Suitable and unsuitable specimens

Primary & secondary syphilis lesion swabs, biopsies (including vitreous humour), placental specimens and CSF. Paraffin embedded biopsy specimens may be tested but not tissue in formalin. NB most locally available PCR assays have not been designed for use on blood specimens, and overall reported performance of *T pallidum* PCR assays blood has been poor (9, 29) or highly variable.

3.3.2 Test sensitivity

Sensitivity of PCR assays is generally lower than the rabbit infectivity test which is the gold standard. Early PCR assays targeting the 47 - kDa gene were slightly less sensitive than DFA (~ 81% - 91%) on primary skin lesions but sensitivity has improved over time with better NA extraction and PCR technologies. When used for diagnosing symptomatic congenital disease the sensitivity of the 47- kDa 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 (reviewed by Larsen 1995 (12)).  
PCR assays targeting polA have been shown to be of similar sensitivity to PCR assays targeting the 47 â€“kDa gene (10). Sensitivity can be improved by using real time technology instead of gel-based assays.

The polA TaqMan assay described by Leslie et al (8) has a sensitivity of 80% in early syphilis compared with serology (not yet compared with dark-ground microscopy or DFA). Its performance for detection of *T. pallidum* in CSF specimens in potential cases of neurosyphilis has been generally poor locally. (5).

3.3.3 Test specificity

Specificities of all assays are high (> 97%) (8,10). The VIDRL polA TaqMan assay compared with serology has a specificity of 98.40% - Not yet adequately compared with dark-ground microscopy or DFA.

3.3.4 Predictive values and relevant populations

Very high when combined with positive serology.

3.3.5 Suitable test acceptance criteria

All controls within expected ranges. Any weak positive results near the cutoff should be retested, or tested with a second NAA assay. The TaqMan assay of Leslie et al (8) has a cutoff set at cycle threshold (CT) 38.

3.3.6 Suitable internal controls

As recommended in NPAAC guidelines: Laboratory Accreditation Standards and Guidelines for Nucleic Acid Detection and Analysis. Controls should be designed to detect sample inhibitory activity.

* + e.g. Positive control: DNA fragment from *T. pallidum* target sequence e.g. cloned polA, or *T. pallidum* suspension if available
  + Negative controls: no-template negative control, reagent-only negative control.
  + Inhibition control: TaqMan exogenous internal positive control DNA and mix in each well
  + Specimens and controls tested in duplicate.

3.3.7 Suitable original 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. polA (8, 10)

3.3.8 Suitable external (QAP) program(s)

Problematic - nil available at present due to a lack of available cultured *T pallidum* in Australia and multiple PCR targets being used.

3.3.9 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.

3.4 Culture of *T. pallidum*

**Rabbit Infectivity Test (RIT)**  
Inoculation of fresh clinical material (< 1 hr old) from syphilitic lesions or CSF into the testes of live rabbits remains the only method for isolating *T. pallidum*. Inoculation may also be intradermal, ocular, intravenous or scrotal. This test is highly sensitive and specific but not available outside research centres. Some ethical problems exist with the use of live animals now that good alternative tests are available. PCR assays are expected to replace this test for diagnostic purposes, however animal inoculation is still required for the generation of large amounts of live pathogenic *T. pallidum* for kit manufacture and research purposes.

3.4.1 Suitable and unsuitable specimens

Any source of specimen as long as it is less than 1 hr old or was flash-frozen immediately after collection and maintained in liquid nitrogen or at temperatures below -78oC.

3.4.2 Test sensitivity

Gold standard - highly sensitive ~ 100% if the number of organisms inoculated exceeds 23 and the patient has not received antibiotic treatment.

3.4.3 Test specificity

Specificity is 100%.

3.4.4 Predictive values and relevant populations

Positive predictive value is 100%. Negative predictive value depends on the adequacy of specimen collection.

3.4.5 Suitable Test Acceptance Criteria

Detailed methods are available from CDC, Atlanta.

3.4.6 Suitable external (QAP) program(s)

None available.

4 Serology Assays

Serological methods are of two types - non-treponemal and specific treponemal tests. Non-treponemal tests are those which do not detect antibodies to *T. pallidum* but instead detect antibodies to reagin, a combination of cardiolipin, lecithin and cholesterol which forms a serologically reactive antigen. Reagin is not specific to *T pallidum* infection, but is generated as a response to spirochaete-induced damage to cellular membranes, and is a useful indicator of disease activity.

In contrast, treponemal tests detect antibody to *T. pallidum* and are available in a variety of formats.

Non-Treponemal Tests

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.

4.1 VDRL (Venereal Diseases Research Laboratory) Test

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.

4.1.1 Suitable and unsuitable specimens

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

4.1.2 Test sensitivity

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

4.1.3 Test specificity

* + 98% (if treponemal tests positive) (12)
  + 98-100% (10)

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, intra-venous drug use (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 (2Âº) syphilis) giving rough, grainy or weak atypical reactions. If suspected retest starting at dilutions up to 1:64.

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4.1.5 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.

4.1.6 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.

4.1.7 Suitable original test validation criteria

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

4.1.8 Suitable external (QAP) program(s)

RCPA QAP, CDC USA.

4.1.9 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).

4.2 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.

4.2.1 Suitable and unsuitable specimens

Serum, plasma in the past RPR was not recommended for use with CSF, but a recent re-evaluation suggests RPR performs at least as well as VDRL with CSF. (3)

4.2.2 Test sensitivity

Primary syphilis 86%, secondary syphilis 100%, latent syphilis 98% late syphilis 73% (12) 86-100% (10).

4.2.3 Test specificity

98% (if treponemal tests positive) 93-98% (10)

4.2.4 Predictive values and relevant populations

Similar to VDRL test above.

4.2.5 Suitable test acceptance criteria

Controls perform according to established criteria.

4.2.6 Suitable internal controls

See manufacturer’s instructions and as for VDRL above.

4.2.7 Suitable original test validation criteria

See published reports and kit information leaflet.

4.2.8 Suitable external (QAP) program(s)

RCPA QAP, CDC USA.

4.2.9 Special considerations

See also VDRL special considerations.

As with all agglutination assays, careful attention needs to be paid to performing the test exactly according to the manufacturers 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.

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 speed of decline of antibodies varies according to the stage and duration of infection, and the initial RPR and VDRL titre

In early syphilis (primary & secondary) there should be a roughly fourfold decline in RPR/VDRL titre by 4 months, eight-fold decline by 8 months, and RPR/VDRL should become negative or serofast at low titre within a year.

In latent or late syphilis, or following multiple episodes of infection there is a more gradual decline in RPR/VDRL titre, and in many cases will stabilise at a low titre (RPR usually <=16 and VDRL at <8).  
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.

Treponema Specific Tests

Any specimen found to be reactive with a non-treponemal screening test should also have specific treponemal tests performed to confirm the diagnosis. Many laboratories prefer to screen using treponemal tests due to their greater sensitivity in early primary syphilis and lower rate of biological false positive reactions which may outnumber true positives in a low prevalence population. If a treponemal test is used as the screening assay all reactive sera should be retested using another type of treponemal assay and a non-treponemal test. A quantitative RPR or VDRL is also essential for assessing the stage of infection and to establish a base line for response to treatment. Referral to a reference laboratory for further analysis of the humoral response by immunoblot may also be useful.

4.3 *T. pallidum* haemagglutination (TPHA), Microhaemagglutination Assay for Antibodies to T pallidum (MHA-TP) & *T. pallidum* particle agglutination (TPPA) TPHA, MHA-TP

These are passive haemagglutination assays using formalinised, tanned sheep erythrocytes sensitised with ultrasonicated extract of pathogenic *T. pallidum*. 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 and a smooth mat will form in the base of a U shaped microtitre plate well. Serum without antibody does not cross link RBC and they fall into the base of the U well and form a button.

Serodia 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. Otherwise, as for MHA-TP, but slightly more sensitive and fewer problems with various confounding anti-red cell reactions.  
Please see reference (22) for individual kit comparisons.

4.3.1 Suitable and unsuitable specimens

Serum, Plasma, Heat inactivated serum can be used, but is not essential.

4.3.2 Test sensitivity

* + MHA-TP primary 76%, secondary 100% latent 97% late 94% (12)
  + TPPA >99% (22)
  + TPHA / TPPA 85-100% (10)

4.3.3 Test specificity

* + MHA-TP 99% (12)
  + TPPA >99% (22)
  + TPHA / TPPA 98-100% (10)

4.3.4 Predictive values and relevant populations

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).
    - Autoimmune and connective tissue diseases, viral infections, pregnancy.
    - TPPA - rheumatic heart disease.

False Negative factors:

* + - Prozone effect in secondary syphilis.

4.3.5 Suitable test acceptance criteria

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

4.3.6 Suitable internal controls

As for VDRL.

4.3.7 Suitable original test validation criteria

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

4.3.8 Suitable external (QAP) program(s)

RCPA QAP, CDC USA.

4.3.9 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.

4.4 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.

4.4.1 Suitable and unsuitable specimens

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

4.4.2 Test sensitivity

* + Primary 86%, secondary 100% latent 98% late 73% (12)
  + 70-100% (10)

4.4.3 Test specificity

* + 97% (12)
  + 94-100% (10)

4.4.4 Predictive values and relevant populations

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, IV Drug use, 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.

4.4.5 Suitable test acceptance criteria

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

4.4.6 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.

4.4.7 Suitable original test validation criteria

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

4.4.8 Suitable external (QAP) program(s)

RCPA QAP, CDC USA.

4.4.9 Special considerations

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

4.5 Treponema pallidum EIA

There are a number of EIA assays available, using either whole *T. pallidum* lysate, or a combination of *T. pallidum* recombinant antigens. For an assessment of the various assays available, please see refs (25) & (23). Most detect total antibody. EIA assays have the advantage of being highly suitable for automation and are favoured as screening assays for large laboratories. If EIA 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.

The information below relates to the Trepanostika TP recombinant EIA (rEIA), which uses recombinant *T. pallidum*-specific 15 and 17 kDa antigens fixed to the solid phase (microtitre well). Serum samples are added, then a conjugate consisting of horseradish peroxidase (HRP)-labeled recombinant antigens. If *T. pallidum* specific antibodies are present in the serum (both IgG and IgM), they will bind to both the solid phase and the conjugate, and colour will develop in the well following the addition of a TMB (3,3’,5,5’-Tetramethylbenzidine) substrate. Other manufacturers’ kts (eg Murex-ICE) may include other recombinant antigens such as TpN47 Ag, which is frequently included to increase sensitivity in early syphilis. Most EIAs are robust tests and have high performance characteristics, based on RCPA QAP data.

4.5.1 Suitable and unsuitable specimens

Serum. Performance with CSF or plasma is not fully evaluated, but available data suggest there is no problem testing these specimen types. No specimen pre-treatment required.

4.5.2 Test sensitivity

VIDRL validation series compared with TPPA / FTA consensus 100%  
Data from similar recombinant assays indicate superior performance (both sensitivity and specificity) to TPPA / FTA for post-primary syphilis. Sensitivity in early primary syphilis (with PCR positive chancre present) slightly less than TPPA

* + >99% (23)
  + 82-100% (10) (includes older lysate based assays)

4.5.3 Test specificity

VIDRL validation series compared with TPPA / FTA gave 100% consensus. In use,

* + specificity approaches 100% (23)
  + Published values of 97-100% (10)

4.5.4 Predictive values and relevant populations

False positive factors:

* + Nil known as yet with this assay but some laboratories have reported EIA positive sera which do not react in other assays and react with only one antigen band when investigated by immunoblot (L. Hueston, personal communication).
  + Isolated rEIA only positive results have been seen in patients with known old treated syphilis.

False negative factors:

Nil known as yet with this assay.

4.5.5 Suitable test acceptance criteria

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

4.5.6 Suitable internal controls

As recommended by manufacturer.

4.5.7 Suitable original test validation criteria

See manufacturer’s documentation.

4.5.8 Suitable external (QAP) program(s)

RCPA QAP, CDC USA.

4.5.9 Special considerations

**Abbott Architect Syphilis TP Chemiluminescent Microparticle Assay (CMIA).**  
Chemiluminescent microparticle assays are a modification of the sandwich EIA principle where light production, rather than color change, is used as an indicator when the sought antibodies are present in the test serum. The Abbott architect instrument is capable of performing a wide range of diagnostic infectious disease serology assays, and is becoming increasingly popular in the hospital setting. The Syphilis TP assay uses recombinant antigens TpN15, TpN17 and TpN47 (from the same source as the murex ICE assay), but has been tweaked to optimize sensitivity during early syphilis. Overall, reported performance of the assay has been very good to date (2), but a few 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.

4.6 Treponema pallidum IgM EIA

IgM EIA assays are useful for investigating early and congenital syphilis.  
Current tests available are based on the principle of IgM antibody class "capture". Rabbit antibodies against human IgM (Âµ chain specific) 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. Unbound serum components are rinsed away. 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.

4.6.1 Suitable and unsuitable specimens

Serum. Performance with CSF is not fully evaluated.

4.6.2 Test sensitivity

* + Primary syphilis 86.5% (95% CI 74.2-94.4) Ref (25).
  + Low sensitivity in late syphilis, when IgM is rarely detected.(18). IgM may not rise in reinfections.

4.6.3 Test specificity

approximately 90% Ref (26)

4.6.4 Predictive values and relevant populations

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.

4.6.5 Suitable test acceptance criteria

According to manufacturers’ instructions and acceptable performance of controls.

4.6.6 Suitable internal controls

As for VDRL above.

4.6.7 Suitable original test validation criteria

See manufacturer’s documentation.

4.6.8 Suitable external (QAP) program(s)

RCPA QAP, CDC USA.

4.6.9 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 has been detected in >95% primary syphilis, and >50% reinfections in previously treated patients who were PCR positive (D. Leslie, personal communication) IgM antibodies may persist for up to 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.

There is a very limited range of *T pallidum* IgM serology kits currently available in Australia since the Mercia Syphilis M kit (Microgen Bioproducts Ltd) was withdrawn late 2011 due to an increase in false positive results in some lot numbers.

4.7 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 and developing regions because they are simple to use, can be transported, stored and performed at room temperature (below 30 o C) and do not require microscopic or electrical 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 but sera should be retested by a NATA accredited laboratory

Furtherinformation can be found at [ref (18)](https://www1.health.gov.au/internet/main/publishing.nsf/Content/cda-phln-syphilis.htm#ref-18) and:

* + [WHO - The use of Rapid Syphilis Tests](http://www.who.int/tdr/publications/tdr-research-publications/use-rapid-syphilis-tests/en/index.html) (www.who.int/tdr/publications/tdr-research-publications/use-rapid-syphilis-tests/en/index.html)
  + [WHO - Diagnostics Evaluation Series No.1 - Laboratory-based evaluation of rapid syphilis diagnostics](http://www.who.int/tdr/publications/tdr-research-publications/sdi/en/index.html) (www.who.int/tdr/publications/tdr-research-publications/sdi/en/index.html)

4.8 Other Serology Assays

4.8.1 The Treponema pallidum immobilization (TPI) test

The TPI is highly specific for syphilis but requires a source of fresh, motile *T. pallidum* and is no longer available in Australia. It has been replaced by EIA.

4.8.2 Immunoblotting

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 (eg flagellin protein). It has now been largely replaced by recombinant immunoblotting for diagnostic or confirmatory purposes.

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 (including ICPMR) 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.

5 Typing and Subtyping Method

Two laboratories in Australia now have molecular based *T pallidum* typing systems under development but these are for research purposes only at present. Although it has been possible to demonstrate that a number of *T pallidum* strains similar to those seen in other English - speaking countries are circulating here, the method is relatively insensitive in that a full typing profile is achievable from only a minority of PCR positive DNA extracts, and the method is not yet adequately discriminatory to be of use in contact tracing at this stage(1). For an overview of current tying methods, see ref (20).

6 Laboratory Nomenclature for National Data Dictionary

* + Organism Name(s) List
  + Typing/Subtyping Nomenclature List(s)
  + Other assays

Appendix 1: Guide for Use of Assays to Investigate Clinical Presentations

Primary and secondary syphilis

* + Demonstration of *T. pallidum* in lesions or biopsy specimens by:
  + Nucleic acid amplification (NAA) methods including PCR
  + Dark ground (dark field) microscopy (not applicable to mucous membrane lesions)
  + *T. pallidum* specific direct fluorescent antibody staining (DFA)
  + Histopathology with appropriate stains

with

* + Demonstration of seroconversion to *T. pallidum* specific tests (TPPA, TPHA, FTA-Abs, EIA); OR
  + Detection of *T. pallidum* specific IgM; OR
  + A fourfold or greater rise in RPR antibodies with positive *T. pallidum* specific serology

Latent syphilis

* + Demonstration of positive *T. pallidum* specific tests (TPPA, TPHA, FTA-Abs, EIA)
  + IgM is usually negative.
  + The RPR is usually positive (although low titres may be reported) in a patient with no history of syphilis treatment.

NB it may not be possible to distinguish between latent syphilis and old, treated serofast syphilis on serology testing of a single bleed. Supportive clinical information or declining RPR following treatment may be needed to support the diagnosis of latent infection. Many patients with latent syphilis may be unaware they have ever been exposed to the disease.

*syphilis*

* + Positive serum *T. pallidum* specific tests and RPR. IgM may be present.

with

* + Clinical findings consistent with late syphilis and suggestive radiological or histopathology findings

or

Demonstration of *T pallidum* in tissue biopsies by

* + Direct fluorescent antibody staining (DFA)
  + NAA methods including PCR

*Neurosyphilis*

* + Positive serum *T. pallidum* specific tests (TPPA, TPHA, FTA-Abs, EIA)

with

* + Positive CSF *T. pallidum* specific tests (FTA-Abs still recommended, performance of other tests with CSF is not fully established)
  + Clinical or radiological findings consistent with neurosyphilis

and

Other supportive factors including

* + The presence of a raised CSF WCC (usually lymphocytes predominate)
  + Raised CSF protein, low CSF glucose
  + Reactive CSF VDRL (RPR is not fully validated for use with CSF, but anecdotal evidence suggests it performs adequately(3))
  + High ratio of CSF: serum markers
  + Positive NAA in CSF, neural or ocular tissues (in most small studies performed to date, CSF PCR is rarely positive in neurosyphilis)

NB the diagnosis of neurosyphilis is complex and requires evaluation of several clinical, radiological and laboratory findings. For further guidance, please see appendix 3

*Neonatal / congenital syphilis*

* + Positive maternal syphilis serology

and

* + Positive neonatal *T. pallidum* specific serology and raised RPR (at similar or increased titre compared with maternal serology) and positive IgM

and/or

Clinical features suggestive of congenital syphilis, demonstration of *T pallidum* in tissues (including placenta, cord & membranes) or neonatal secretions by histopathology, DFA or PCR.  
NB. Babies born to mothers with positive syphilis serology will usually have lower levels of the same antibodies detectable in serum due to maternal IgG antibodies crossing the placenta. IgM does not normally cross the placenta, and a positive neonatal IgM result is strongly indicative of neonatal infection.

For further information, please see appendix 3.

Congenital syphilis may present early (<2 years after birth) or late (persists >2 yrs after birth) with a range of clinical syndromes.

*Old, treated or “burnt out” syphilis*

* + Positive *T. pallidum* specific serology, low or negative RPR - stable pattern on repeat testing with or without treatment. Clinically asymptomatic.

This is probably the most common pattern seen in Australia, and is difficult to differentiate from the pattern seen in latent syphilis. Again, many patients will not be aware that they have ever been exposed to syphilis. In some cases patients may spontaneously clear infection, and with the common use of antibiotics in the community, many active cases of syphilis may have been inadvertently cured. Similarly, with its extreme sensitivity to heat, prolonged high fever (eg in malaria) may also kill *T. pallidum*.

*Reinfections*

Reinfections with *T. pallidum* are not uncommon in high-risk populations (especially HIV-infected MSM). Reinfection can be demonstrated by:

* + A fourfold rise in RPR on parallel testing with an earlier stored serum specimen with or without detectable IgM

or

* + Known old treated serology pattern, but with Demonstration of *T. pallidum* in lesions or biopsy specimens by:
    - * Dark ground microscopy (not applicable to mucous membrane lesions)
      * *T. pallidum* specific direct fluorescent antibody staining (DFA)
      * Histopathology with appropriate stains
      * NAA methods including PCR

NB recent data indicates more than 50% of reinfections show an IgM response

***Treatment failure***

True treatment failure in patients treated with the correct dose and formulation of parenteral penicillin for the stage of the disease are very rare, regardless of HIV status. *T pallidum* has no known genetic resistance mechanisms against penicillin, and is unable to accept lateral genetic transfer. Seeing an increase in RPR titre a week or two after treatment has been commenced is not unusual and is caused by the increase in stimulation of the immune system by antigens released from dead and dying organisms. In adequately treated patients, a rising RPR more than month after treatment is likely to be due to reinfection.

Treatment failures have been documented following macrolide (Azithromycin) therapy. This is most commonly caused by a spontaneous mutation in the 23SrRNA gene. This mechanism of macrolide resistance also occurs in several other STI agents, including C trachomatis, N gonorrhoeae and M genitalium, but is not transferrable between bacteria. Rates of macrolide resistance have risen rapidly to over 50% in centres where azithromycin has been used extensively for syphilis Rx, and azithromycin should no longer be used for syphilis Rx unless there is no alternative and the treatment outcome is closely followed.

Treatment failures following Rx with Doxycycline or Ceftriaxone are less well documented

Biological false positive (BFP) results in non-treponemal tests

A positive RPR or VDRL in a serum negative by *T. pallidum* specific assays occurs in up to 2% of sera tested. These BFP results do not indicate *T. pallidum* infection, and may be transient or stable. High titre BFP reactions may indicate other underlying disease (see VDRL section for causes of false positive reactions)

NB: uncommonly BFP reactions can occur in the setting of an old, “burnt out” serology pattern, and can be easily confused with reinfection or reactivation.

Isolated single *T. pallidum* specific serology assay reactivity.

Uncommonly, a single assay only may be positive. This is usually noticed only by reference laboratories that use multiple test types.

In older patients, this generally reflects a gradual loss of antibodies over time, and usually presents as an isolated EIA or FTA result. (24) Such patterns are more frequent in HIV infected persons, and cases of loss of all antibodies following proven infection & response to treatment have been observed in 5-10% of HIV patients (DE Leslie personal observation).

Isolated TPHA / TPPA positive alone results in at-risk patients need to be treated with caution, as they may represent a very early serological response to primary infection. This can be confirmed by IgM testing, testing of a later serum or by direct detection methods if lesions are present. In some cases of very early syphilis (PCR pos primary), patients may develop.

In low risk patients, an isolated TPHA / TPPA positive result is more likely to be false positive.

Appendix 2. Suggested syphilis serology screening algorithm

(see attached file syph\_test\_ alg.doc)

Notes to go with algorithm

1. Follow up of sero-negative patients at recent risk of acquiring a sexually transmitted infection is essential because of the sero-negative window in early primary syphilis. This window is much reduced if an IgM assay is available.  
2. A treponemal test alone (EIA IgG, total AB or TPPA/TPHA), or a combination of a non-treponemal test and a treponemal test (VDRL/RPR and TPPA / TPHA / EIA), is appropriate for screening in Australia. If a laboratory screens sera with only a treponemal assay, use of TPPA or Architect EIA is preferred due to their superior sensitivity in early syphilis.  
3. Screening with a non-treponemal test alone is not recommended because of the potential for false negative results.  
4. Ideally specimens that are reactive on screening should be confirmed with a different treponemal test of equal sensitivity to that used for screening and ideally, greater specificity.  
5. Specimens giving discrepant treponemal test results on confirmatory testing should be referred to a reference laboratory for further testing.  
6. All confirmed reactive specimens should be reported to the relevant health department to allow collection of surveillance data  
7. In treponemal infection a quantitative non-treponemal test and/or a test for specific treponemal IgM should be performed as part of the assessment of the stage of infection and to monitor the efficacy of treatment. Comparison of non-treponemal test results is only valid if the testing has been performed in parallel.  
8. All laboratories performing syphilis serology in Australia should be participating in a regular quality control program covering all tests used.

Appendix 3: Investigation of neurosyphilis.

The diagnosis of neurosyphilis is complex and requires evaluation of several clinical, radiological and laboratory findings.

Relatively early following primary infection, *T. pallidum* can cross the blood-brain barrier and invade the CNS, where the spirochaetes may be partially protected from antibiotic therapy, particularly if antibiotic levels are suboptimal, and any degree of immunosupression may slow spirochaete clearance. In recent times in Australia the patients most at risk for neurosyphilis are HIV-infected persons, and occasionally HIV-infected patients will develop neurosyphilis following apparently adequate therapy for primary or secondary syphilis.

The essential findings are abnormalities in one or more of several CSF parameters in the presence of peripheral positive syphilis serology. CSF examination is not necessary if peripheral syphilis serology is completely negative

Parameters examined in CSF:

* + CSF WCC & differential count, protein, glucose
  + CSF VDRL (RPR is not fully validated for use with CSF)
  + CSF *T. pallidum* specific serology (TPPA, TPHA, FTA-abs, EIA)
  + CSF IgM serology
  + *T. pallidum* PCR (if available)
  + Ratio of CSF to peripheral serum titres.

The serological diagnosis of neurosyphilis is made by demonstrating that an antibody response has occurred in the central nervous system. Syphilis serology in CSF specimens is only interpretable if the CSF is free of any blood contamination, and CSF cell counts, glucose and protein estimation need to be available to the laboratory performing the serology to assist with result interpretation. Various attempts have been made to develop calculated indices to demonstrate local antibody production in the CSF and control for serum contamination of CSF or problems with the blood-brain barrier, however these do not appear to be widely used in Australia. These indices include measuring the amount of adenovirus antibody in the CSF . For a further discussion of the diagnostic utility of such indices see Luger et al 2000. (15) Some authors have attempted to set predictive quantitative cutoffs for CSF serology assays (TPPA, TPHA, VDRL), but the numbers studied have been small and not all laboratories provide a quantitative result for TPHA / TPPA assays

A positive CSF VDRL (with positive peripheral serology) is the most predictive parameter for neurosyphilis. However it is relatively insensitive (estimated to be as low as 27%) and a negative VDRL does not exclude the diagnosis.

FTA in CSF has shown to be 100% sensitive in the diagnosis of neurosyphilis (13).  
HIV infection itself may cause a CSF pleocytosis, and cell counts should be interpreted with caution in this setting, however a WCC of > 20 cells/mm3 in the setting of positive serology is likely to be due to *T. pallidum* infection (ref Chan 2005).

The role of PCR in the diagnosis of neurosyphilis is not fully established - even though invasion of the CNS occurs relatively early in infection, spirochaetes may be spontaneously cleared without progression to neurosyphilis, and the rate of clearance of *T. pallidum* DNA from the CNS following therapy is unknown.

The value of CSF examination in immunocompetent patients with asymptomatic late latent syphilis has been questioned in one study, as using CSF examination results as part of the diagnostic algorithm did not appear to significantly improve outcome when compared to empirical therapy without LP, given that the LP procedure itself carries a risk of complications. (32)

Appendix 4: Investigation of neonatal / congenital syphilis

* + Any suspected active syphilis during pregnancy should be fully investigated, and if active infection seems likely, the mother should be treated, the response to treatment monitored, and the neonate tested on delivery.
  + Maternal non-treponemal test titres need to be interpreted with caution in patients with a history known treated syphilis, as small non-specific rises in residual RPR / VDRL titres may occur, and may be difficult to distinguish from rising titres due to relapse or re-infection.
  + Post delivery, both a maternal and neonatal serum specimen should be tested in parallel.
  + Cord blood should not be used due to the possibility of contamination with maternal blood
  + Both treponemal and non-treponemal tests should be used on both sera, and the neonatal serum tested with IgM assay.
  + In most cases, positive serology in the neonate represents passive transfer of maternal antibody, but a positive IgM assay indicates neonatal infection.
  + Passively acquired antibodies decline over time, and become undetectable at 12 to 18 months, however decline in antibody titres should be seen by 3 months in un-infected children.
  + Ideally, neonates from mother with active syphilis in pregnancy should be followed at 1, 2, 3, 6 and 12 months post delivery (6).
  + Lumbar puncture should be considered if there are any CNS symptoms and the child treated if there are any CSF abnormalities in the setting of positive peripheral serology.
  + If indicated, placenta and membranes, and respiratory secretions from the neonate can be tested by PCR to demonstrate the presence of *T. pallidum*.

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